Infectious Diseases in an Age of Change: The Impact of Human Ecology and Behavior on Disease Transmission

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National Academy of Sciences

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Preface

… it is not inconsistent with respect to personal freedom to attempt to create an environment which encourages people to do what is good for them and to avoid what is bad.

Thomas McKeown The Origins of Human Disease Blackwell, Oxford, United Kingdom, 1988 p. 215.

The two key factors which affect the spread of infectious diseases in the human community other than the nature of the infectious agent are human ecology and behavior. Although some aspects of the impact of the resulting infections are similar since both result in disease, the processes by which these human activities lead to increased risk of infectious disease are quite different.

The agents of infectious diseases do not arise *de novo* in each infected individual by spontaneous generation. For the most part, they are transmitted from one species to another or from one individual of a species to another member of the same species. To survive, infectious agents must be successful in maintaining themselves in their chosen, susceptible population. There are many determinants of success, but undoubtedly contact between the infected and the susceptible populations is a major determinant of the frequency of transmission. Furthermore, when a human population comes in contact with a new transmissible agent, or with a previously isolated human population, new

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infectious agents are likely to enter the human community. These simple rules have been known for many years. They accounted for the spread of measles, smallpox, influenza, and bubonic plague in the centuries past. The principle that human ecology affects the incidence and patterns of infectious diseases is not only accepted but has been credited with playing a major role in the rise and fall of nations in human history (1). The focus of attention of the articles in this volume is on two aspects of changes in ecology. The first concerns the spread of infectious agents resulting from the human migrations from regions of high disease incidence to low incidence, from cities to forested suburban areas, etc. The second concerns the rise and spread of agents resistant to drugs designed to arrest their multiplication.

Only recently, however, have we become fully aware of the global impact of the changes in human behavior on infectious diseases. At first glance, "changes in human behavior" implies changes in sexual behavior, and in fact, increases in the number of sexual partners accounts for the increased incidence of sexually transmitted diseases. We may add failure to self administer available drugs to arrest the development and spread of drug-resistant, non-sexually transmitted agents such as *Mycobacterium tuberculosis*, and the role of immunosuppressive medical treatments which may predispose patients to serious infections with agents considered relatively innocuous for immunocompetent individuals.

The increase in the incidence or severity of infectious maladies has led to the recognition of emerging infectious agents and diseases; these have been described in numerous reports and publications (e.g., 2–4). It is relevant to note that some 10 to 15 years ago it was thought that most infectious agents of diseases had been identified and that the few agents still unknown played a role in diseases, like cancer, which manifested themselves many years after infection. Two events discredited this view. Foremost, the technology for identification of the presence of an infectious agent underwent dramatic developments and led to the identification of several hitherto unknown, but not novel agents of disease. No less important, however, was the fact that changes in human ecology and behavior increased the incidence or severity of diseases resulting in their recognition and subsequent investigation. The significance of the latter factor cannot be overstated. Improved surveillance to ensure a timely response to the effects of changes in ecology and behavior on human infectious disease will play a major role in reducing the human burden of infectious diseases (5). Control of infectious diseases is our goal. The lesson of history is that prevention of infectious diseases by prophylaxis or immunization will be only partially effective

in the absence of changes in human behavior and ecology. Syphilis, for example, is still rampant notwithstanding availability of inexpensive and effective therapy.

Effecting changes in human behavior and ecology pits the rights of the individual against the best interests of the society. The issues are not new. Carlo M. Cipolla's studies (6) of archival documents dealing with the plague which ravaged Tuscany in the middle of the seventeenth century are of particular interest. The plague was endemic in seventeenth-century Europe and its causes were not well understood. Diseases which spread through the population were thought to be caused by miasmas characterized by foul odors which clung to the environment and spread from person to person. The Florentine authorities established Health Magistrates whose task was to monitor the pesthouses to which the ill were brought, and to enforce quarantines. The quarantines were commonly disliked because they interfered with everyday life. By and large the Church assisted the Health Magistrates in enforcing procedures that were found empirically to minimize the spread of the plague. In a few instances conflicts arose. While the Health Magistrates had little doubt that religious processions kindled the spread of plague, the men of the Church fervently believed that processions and similar ceremonies had exactly the opposite effect. Cipolla recounts that in December of 1630 the Prior of the Monastery of St. Marco pressed for a religious procession in the face of opposition of the Health Magistrates. A compromise was finally worked out. The procession was held, but it was restricted to chosen members of the clergy, the Grand Duke and his court, and the Senators in their purple. The populace was excluded and access to the procession was blocked by horse guards. Even so, as Cipolla recounts, the streets were strewn with fragrant herbs, presumably to mask the odors through which the plague spread.

The Florentines of the mid-seventeenth century did not know of the role of bacilli, rats and fleas in the dissemination of the plague. While the basic premise was in error, they learned empirically of the need to control human behavior to reduce the impact of the epidemics. Over three centuries later, as this collection of scientific reports illustrates, we know the etiology of most infectious diseases affecting humans but we are far from being able to deal effectively with the factors which facilitate their transmission. Continuous research and dissemination of facts concerning the role of human behavior and ecology on the incidence and spread of infections may ultimately bring about the kind of environment sought by Thomas McKeown.

BERNARD ROIZMAN AND JAMES M. HUGHES

REFERENCES

1. McNeill, W. H. 1976. *Plagues and Peoples*. Garden City, N. Y.: Anchor Press/Doubleday.
2. Krause, R. M. 1992. The origin of plagues: old and new. Science **257:**1073-1078.
3. Lederberg, J., Shope, R. E., and Oaks, S. C., eds. 1992. *Emerging Infections: Microbial Threats to Health in the United States*. Washington, D.C.: National Academy Press.
4. Morse, S. S., ed. 1993. *Emerging Viruses*. New York: Oxford University Press.
5. Centers for Disease Control and Prevention. 1994. *Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States*. Atlanta: U.S. Department of Health and Human Services.
6. Cipolla, C. M. 1981. *Faith, Reason, and the Plague in Seventeenth Century Tuscany*. New York: W.W. Norton & Co.

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BERNARD ROIZMAN

Contents

INFECTIOUS DISEASES IN AN AGE OF CHANGE

Lyme Disease: A Growing Threat to Urban Populations

ALLEN C. STEERE

Lyme disease was recognized as a separate entity in 1975–1976 because of geographic clustering of children in Lyme, Connecticut, who were thought to have juvenile rheumatoid arthritis (1). The rural setting of the case clusters, the usual onset of illness in the summer and early fall, the onset in members of the same family in different years, and the recognition that erythema migrans was a feature of the illness suggested that the disorder was transmitted by an arthropod. Epidemiologic studies of patients with erythema migrans implicated certain ixodes ticks as vectors of the disease (2, 3). Prospective studies of these patients showed that the illness could affect multiple systems over a period of years, including the skin, nervous system, heart, or joints (4).

Erythema migrans also linked Lyme disease in the United States with certain syndromes described previously in Europe. Early in this century, Afzelius in Sweden (5) and Lipschutz in Austria (6) described a characteristic expanding skin lesion, which they called erythema migrans or erythema chronicum migrans. Many years later, it was recognized that erythema migrans could be followed by a chronic skin disease, acrodermatitis chronica atrophicans, which had already been described as a separate entity (7). In the 1940s, Bannwarth (8) defined a syndrome that consisted of radicular pain followed by

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chronic lymphocytic meningitis and sometimes cranial or peripheral neuritis. In a few cases, the neurologic syndrome was preceded by an erythema.

These various syndromes were brought together conclusively in 1982 when a previously unrecognized spirochete, now called *Borrelia burgdorferi*, was recovered from *Ixodes dammini* ticks (also called *Ixodes scapularis*) (9). The spirochete was then isolated from patients with Lyme disease in the United States (10, 11) and from those with erythema migrans, Bannwarth's syndrome, or acrodermatitis in Europe (12–14). In addition, the immune responses of patients were linked conclusively with infection with this organism.

Although the basic outlines of the illness are similar worldwide, regional variations have been noted (15). Lymphocytoma, acrodermatitis, and encephalomyelitis have been seen primarily in Europe (16, 17), whereas widely disseminated early infection, secondary annular skin lesions, and arthritis have been found more commonly in America (18, 19). Recent work in the classification of *B. burgdorferi* has begun to clarify the issue of geographic differences in Lyme disease. Using a variety of methods, three genomic groups of *B. burgdorferi* have now been identified (20, 21). To date, all North American strains have belonged to the first group, *B. burgdorferi sensu stricto*. Although all three groups have been found in Europe, most isolates have been group 2 or 3 strains. Group 2 strains have been renamed *Borrelia garinii* (21), and group 3 strains have been renamed *Borrelia afzelii* (81).

VECTOR AND ANIMAL HOSTS

Lyme disease is transmitted in all of these locations by several closely related ixodid ticks that are part of the *Ixodes ricinus* complex. These include *I. dammini* (also named *I. scapularis*) in the northeastern and midwestern United States (3, 22), *Ixodes pacificus* in the western United States (82), *I. ricinus* in Europe (23), and *Ixodes persulcatus* in Asia (24). The endemic cycle of *B. burgdorferi* varies among geographic locations. In the northeastern and midwestern United States, the preferred host for both the larval and nymphal stages of *I. dammini* is the white-footed mouse *Peromyscus leucopus* (22). It is critical that this rodent host is tolerant to infection with *B. burgdorferi* and that both of the tick's immature stages feed upon this host, since the life cycle of the spirochete depends upon horizontal transmission between immature ticks and mice (25). The preferred host for the adult stage of *I. dammini* is the white-tailed deer *Odocoileus virginianus* (26). Although deer are not involved in the life cycle of the spirochete, they seem to be critical for

survival of the ticks (27). The endemic cycle of *B. burgdorferi* is different in the western United States. There, the spirochete is maintained in nature in a horizontal cycle between the dusky-footed woodrat *Neotoma fuscipes* and *Ixodes neotomae*, a tick that does not feed on humans (28). Only the relatively few larval and nymphal *I. pacificus* that feed upon infected woodrats, rather than lizards, are then responsible for transmitting the spirochete to humans. For this reason, only about 1% of nymphal *I. pacificus* are infected with *B. burgdorferi* (28), whereas infection rates in nymphal *I. dammini* in the northeast range from 20% to 50% (10, 29).

FIGURE 1 Worldwide locations of Lyme disease: Areas in North America, Europe, and Asia that are affected by Lyme disease. These areas are along the terminal moraine of the glaciers 15,000 years ago.

EVOLUTION OF ENVIRONMENT

The primary areas now affected by Lyme disease in the United States, Europe, and Asia are sites near the terminal moraine of the glaciers 15,000 years ago (Figure 1) (30, 31). At that time, the northeastern and upper midwestern parts of what is now the United States were covered by tundra. As the glaciers retreated, forests grew in these areas, which eventually became populated with large numbers of deer. Early descriptions of colonial New England include comments about the abundance of deer and annoying ticks (32). During the 18th and 19th centuries, forests were destroyed in New England

to make farms, and deer were hunted practically to extinction. Deer are thought to have survived only in a few isolated locations, such as Naushon Island near Cape Cod and on the eastern end of Long Island, New York (31). Analysis of museum tick collections by polymerase chain reaction (PCR) has documented the presence of *B. burgdorferi* -infected *I. scapularis* on Montauk Point, Long Island 50 years ago (33).

The emergence of Lyme disease in the United States in the last several decades is thought to have occurred primarily because of ecological conditions favorable for deer (22, 34). As farmland in the northeast reverted to woodland, the habitat for deer improved: their natural predators were gone, the number of deer increased dramatically, they migrated to new areas, and federal programs protected them. With the advent of the automobile and superhighways, rural and suburban areas, where deer now lived, became populated with large numbers of susceptible suburbanites who had never been exposed to the spirochete. Lyme disease and the deer tick do not occur in all areas where deer are found. Other factors such as temperature, vegetation, and rodent populations must also play an important role in the ecology of this disease.

EPIDEMIOLOGY OF LYME DISEASE

Lyme disease is now the most common vector-borne infection in the United States (35). The infection is usually acquired when nymphal ticks feed between May and July, but adult ticks occasionally transmit the

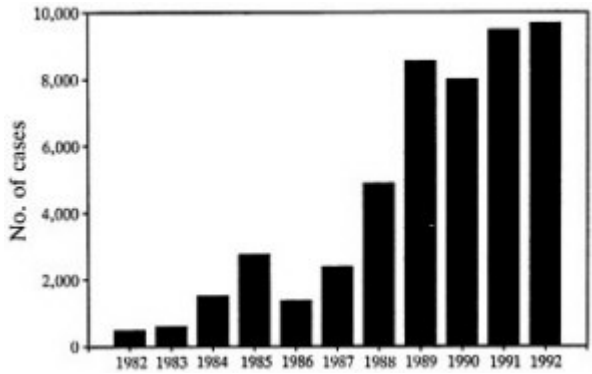

FIGURE 2 Cases of Lyme disease reported to the Centers for Disease Control from 1982 through 1992 (data from ref. 36).

disease when they feed in autumn. People of all ages and both sexes are affected. From 1982 through 1991, 40,195 cases occurring in 47 states were reported to the Centers for Disease Control (Figure 2) (35). In 1992, 45 states reported a provisional total of 9677 cases, a 19-fold increase over the number of cases reported in 1982. However, enzootic cycles of *B. burgdorferi* have been identified in only 19 states, and 94% of the cases reported in the last 2 years have come from these states (35). Three distinct areas are most affected: the northeast from Massachusetts to Maryland, the upper midwest in Wisconsin and Minnesota, and the west in northern California and Oregon (3, 35). As would be anticipated from the infection rate in ticks, the frequency of the disease is much greater in the northeast and midwest than in the west. The infection also occurs in most European countries, particularly in northern parts of the continent, including Germany, Austria, and Sweden (36). In the former Soviet Union, a central area is affected from the Baltic Sea to the Pacific Ocean (24). Cases have also been reported in China (37), Japan (38), and Australia (39).

During the last several decades, Lyme disease has spread and has caused focal epidemics, particularly in the northeastern United States. In 1980, in Ipswich, Massachusetts, a community close to Boston, *I. dammini* became established in a nature preserve that had many deer (40). During the next 7 years, clinical symptoms of Lyme disease developed in 35% of the 190 residents of the area adjacent to the preserve. In other outbreaks, 16% of the 162 permanent residents of Great Island, Massachusetts, had the illness, in most instances between 1972 and 1979 (41); and 7.5% of the 200 people who participated in a study on Fire Island, New York, had the disorder during a 5-year period (42). Between 1985 and 1989, the number of counties in New York State with documented *I. dammini* ticks increased from 4 to 22, and the number of counties endemic for Lyme disease increased from 4 to 8 (43). In addition to the eastern end of Long Island, Westchester County, a heavily populated area close to New York City, became a hyperendemic site of the disease. In recent years, suburban communities in Morris and Monmouth Counties in New Jersey, which are also close to New York City, have become heavily affected (44), and suburban areas near Philadelphia are now endemic for the disease. *I. dammini* continues to invade new areas, and the ultimate limit of this spread is not known.

The fear of Lyme disease in these suburban communities has become marked (45). Residents in these areas regularly see deer in their yards. Protective measures, such as long clothing, sprays, and tick checks, are difficult to keep up throughout the summer. Because of the small size of nymphal *I. dammini*, tick bites often go unnoticed.

TABLE 1 Stages of Lyme disease

Early infection
Stage 1 (localized infection)
Erythema migrans
Stage 2 (disseminated)
Skin
Musculoskeletal
Nervous system
Lymphadenopathy
Heart
Eyes
Liver
Respiratory
Genitourinary
Late infection
Stage 3 (persistent infection)
Chronic arthritis
Late neurologic involvement
Acrodermatitis

CLINICAL PICTURE

Lyme disease or Lyme borreliosis is most like syphilis in its multisystem involvement, occurrence in stages, and mimicry of other diseases (15). Early infection (stage 1) consists of localized erythema migrans (Table 1). Within days to weeks (stage 2), the spirochete often disseminates to multiple sites, manifested most commonly by secondary annular skin lesions (18), meningitis, facial palsy, radiculoneuritis (46), atrioventricular nodal block (47), or migratory pain in joints, tendons, bursae, muscle, or bone (19). Even in untreated patients, these early manifestations usually resolve or improve within weeks or months. Late or persistent infection (stage 3) usually begins months to years later and typically consists of intermittent or chronic arthritis (19), chronic neurologic involvement (48–51), or acrodermatitis chronica atrophicans (52).

Late neurologic abnormalities of Lyme disease are still being defined, and diagnosis of this manifestation of the disorder has created the most difficulty. From months to years after disease onset, sometimes following long periods of latent infection, patients may develop a subtle encephalopathy, primarily manifested as memory deficit, irritability, or somnolence (49). Neuropsychological tests of memory are often abnormal, and, in some cases, white matter lesions are found on magnetic resonance imaging scans of the brain. Cerebrospinal fluid may show elevated total protein, intrathecal antibody production to *B. burgdorferi* (53), or a positive PCR test result for borrelial DNA sequences (54, 55).

Most patients with subacute encephalopathy also have an axonal polyneuropathy manifested by spinal radicular pain or by numbness and tingling in the hands or feet (50, 51). Electromyography generally shows extensive abnormalities of proximal and distal nerve segments (51). Leukoencephalitis, a rare manifestation of Lyme disease, is a severe neurologic picture that may include spastic paraparesis, upper motor neuron bladder dysfunction, and lesions in the periventricular white matter (16, 49). These patients have been distinguished from those with multiple sclerosis on the basis of selective concentration of antibody to *B. burgdorferi* in cerebrospinal fluid. Since patients with late neurologic abnormalities of Lyme disease usually respond to antibiotic therapy, active spirochetal infection is thought to be important in the pathogenesis of these syndromes.

CHRONIC FATIGUE OR FIBROMYALGIA FOLLOWING LYME DISEASE

Chronic fatigue syndrome or fibromyalgia, which may be variants of the same disorder, share certain symptoms with the encephalopathy or polyneuropathy of Lyme disease. The distinction among these entities has been further confused by the fact that a small percentage of patients have developed chronic fatigue or fibromyalgia in association with or soon after Lyme disease (56), suggesting that *B. burgdorferi* is one of the infectious agents or stressful events that may trigger these syndromes. Compared with Lyme disease, chronic fatigue syndrome or fibromyalgia tends to produce more generalized and disabling symptoms. They include marked fatigue, severe headache, diffuse musculoskeletal pain, multiple symmetric tender points in characteristic locations, pain and stiffness in many joints, diffuse dysesthesias, difficulty with concentration, or sleep disturbance. These patients lack evidence of joint inflammation; they have normal neurologic test results; and they usually have a greater degree of anxiety and depression (57). In the author's experience, the symptoms of fibromyalgia, even when triggered by infection with *B. burgdorferi,* continue to wax and wane for years, despite treatment with antibiotic therapy. Therefore, chronic fatigue syndrome or fibromyalgia associated with Lyme disease would not appear to require the presence of a live spirochete for continued symptoms of the illness.

MISDIAGNOSIS OF CHRONIC LYME DISEASE

A recent phenomenon is that a number of poorly understood conditions, such as fibromyalgia or chronic fatigue syndrome, are misdiagnosed as "chronic Lyme disease." Of the 788 patients seen in the

author's Lyme disease clinic during a 4.5-year period, 180 (23%) had active Lyme disease, usually arthritis, encephalopathy, or polyneuropathy (58). One hundred and fifty-six patients (20%) had previous Lyme disease and another current illness, most commonly chronic fatigue or fibromyalgia; and in 49 patients, these symptoms began soon after objective manifestations of Lyme disease. The remaining 452 patients (57%) did not have Lyme disease. The majority of these patients also had the chronic fatigue syndrome or fibromyalgia; the others usually had rheumatic or neurologic diseases. Prior to referral, 409 of the 788 patients had been treated with antibiotic therapy. In 322 of these patients (79%), the reason for lack of response was incorrect diagnosis. Thus, only a minority of the patients referred to the clinic met diagnostic criteria for Lyme disease, and the most common reason for lack of response to antibiotic therapy was misdiagnosis.

DIAGNOSTIC TESTS

Part of the reason for misdiagnosis is due to problems associated with diagnostic tests. In most instances, culture has yielded positive results only from biopsy samples of erythema migrans skin lesions (59, 60). Therefore, serologic testing is currently the only practical laboratory aid in diagnosis. Serologic testing for Lyme disease can be done with a high degree of sensitivity and specificity, and after the first weeks of infection, almost all patients have an elevated antibody response to *B. burgdorferi* (61). However, *B. burgdorferi* contains a number of cross-reactive antigens, the test is insensitive early in the infection, and test procedures are not standardized. For these reasons, false-negative and, more commonly, false-positive results have been a considerable problem (62, 63). In the author's laboratory, antibody determinations are performed by ELISA, using sonicated whole spirochetal lysates as the antigen preparation (61). The results are divided into three groups: negative, indeterminate, or positive (Table 2). In patients with indeterminate responses by ELISA, Western blotting is done to determine whether the response is positive or negative. In the future, serologic tests that employ several recombinant antigens will probably replace the current antigen preparations, which use whole cell lysates.

Regardless of the antigen preparation, serologic tests do not distinguish active from inactive infection. Patients who have had late manifestations of Lyme disease usually remain seropositive for years after treatment. If these patients develop other illnesses, particularly those with joint or neurologic symptoms, the positive test for Lyme disease may cause diagnostic confusion. It has been hoped that the PCR might have a role in the diagnosis of active Lyme disease equivalent to culture

in common bacterial infections. In a recent study, *B. burgdorferi* DNA was detected in the joint fluid of 75 of 88 patients with Lyme arthritis (85%) and in none of 64 control patients (83). Borrelial DNA sequences have also been detected in blood, cerebrospinal fluid, urine, or skin of patients with Lyme disease (54, 55, 64, 65), but the value of PCR as a reliable diagnostic test is still being researched.

TABLE 2 Criteria for positive serologic tests for Lyme disease*

	ELISA			
	SD above mean of normal subjects	Aborbance value	Units	Western blot
IgM				
Negative	<5	<0.17	<100	
Indeterminate	5–15	0.17–0.4	100–200	
Positive	>15	>0.4	>200	Any 2 of 8 bands at 18, 21, 28, 37, 41, 45, 58, and 93 kDa
IgG				
Negative	<2	<0.1	<200	
Indeterminate	2–8	0.1–0.26	200–400	
Positive	>8	>0.26	>400	Any 5 of 10 bands at 18, 21, 28, 30, 39, 41, 45, 58, 66, and 93 kDa

*Ref. 61.

TREATMENT

The various manifestations of Lyme disease can usually be treated successfully with oral antibiotic therapy, except for objective neurologic abnormalities, which seem to require intravenous therapy (15, 66). For early Lyme disease, doxycycline, 100 mg twice a day, or amoxicillin, 500 mg four times a day, is effective therapy (67, 68). For patients with infection localized to the skin, 10 days of therapy is generally sufficient, but for patients with disseminated infection, longer courses of 20–30 days are usually needed. For objective neurologic abnormalities, with the possible exception of facial palsy alone, intravenous ceftriaxone, 2 g/day, is most commonly used (49, 69, 70), but intravenous cefotaxime, 2 g three times a day (70), or intravenous sodium penicillin G, 5 million units four times a day, may also be effective (71). Treatment failures have occurred with any of these regimens, and the course of antibiotic therapy may need to be repeated.

PREVENTION

Most vector-borne diseases are prevented through vector control, but this has proved difficult with tick-borne diseases. In one study, eradication of deer on a small island greatly reduced the number of deer ticks during a 5-year period (26). Aerial application of carbaryl during the fall has been reported to be successful at reducing the number of ticks through the following spring (72). Habitat destruction by burning has long been considered an effective alternative to synthetic insecticides as a means of reducing tick populations (73). Another method involves the distribution of permethrin-treated cotton balls, intended as rodent nesting material, around individual residences during the summer and fall (74, 75). However, with each of these methods, fear of environmental consequences or lack of efficacy has limited their use.

Consequently, reduction of the risk of Lyme disease has been limited primarily to personal protection measures. The risk of tick bites can be reduced by wearing long clothing and by checking for ticks after exposure in wooded areas. Insecticides containing *N,N*-diethylmetatoluamide (DEET) or permethrin effectively deter ticks (76), but permethrin can only be applied on clothing, and DEET may cause serious side effects when excessive amounts are applied directly to the skin (77). Should patients with tick bites receive prophylactic antibiotic therapy? In one recent study, the risk of acquiring Lyme disease from a recognized tick bite was only 1.2%, perhaps because 24–48 hours of tick attachment is often required for transmission of the spirochete (78). Although amoxicillin or doxycycline therapy for 10 days will probably prevent the occurrence of Lyme disease in these patients, many patients must be treated to prevent one case. Thus, personal protection measures may help the city dweller who takes a long walk in the woods, but such measures are of limited benefit in areas where people have constant exposure to large numbers of ticks.

It is hoped that a vaccine can be developed for Lyme disease to provide protection in high-risk areas. Although reinfection may occur in patients treated early in the course of the illness, those with an expanded antibody response to the spirochete appear to have protective immunity. In an animal model of Lyme disease, mice vaccinated with recombinant outer-surface protein A (OspA) have been shown to be protected from infection with *B. burgdorferi*, both by antibody-mediated killing of the spirochete within the host and by destruction of the organism within the tick prior to disease transmission (79, 80). Trials are now under way to determine the efficacy and safety of OspA immunization in human subjects.

SUMMARY

Lyme disease or Lyme borreliosis, which is caused by three groups of the spirochete *Borrelia burgdorferi*, is transmitted in North America, Europe, and Asia by ticks of the *Ixodes ricinus* complex. The primary areas around the world that are now affected by Lyme disease are near the terminal moraine of the glaciers 15,000 years ago. The emergence of Lyme disease in the United States in this century is thought to have occurred because of ecological conditions favorable for deer. From 1982 through 1991, 40,195 cases occurring in 47 states were reported to the Centers for Disease Control, but enzootic cycles of *B. burgdorferi* have been identified in only 19 states. During the last several decades, the disease has spread to new areas and has caused focal outbreaks, including locations near Boston, New York, and Philadelphia. Lyme disease is like syphilis in its multisystem involvement, occurrence in stages, and mimicry of other diseases. Diagnosis of late neurologic abnormalities of the disorder has created the most difficulty. A recent phenomenon is that a number of poorly understood conditions, such as chronic fatigue syndrome or fibromyalgia, are misdiagnosed as "chronic Lyme disease." Part of the reason for misdiagnosis is due to problems associated with diagnostic tests. The various manifestations of Lyme disease can usually be treated successfully with oral doxycycline or amoxicillin, except for objective neurologic manifestations, which seem to require intravenous therapy. Vector control of tick-borne diseases has been difficult and, therefore, reduction of the risk of infection has been limited primarily to personal protection measures.

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REFERENCES

1. Steere, A. C., Malawista, S. E., Snydman, D. R., Shope, R. E., Andiman, W. A., Ross, M. R. & Steele, F. M. (1977) *Arthritis Rheum.* **20,** 7–17.
2. Steere, A. C., Broderick, T. F. & Malawista, S. E. (1978) *Am. J. Epidemiol.* **108,** 312–321.
3. Steere, A. C. & Malawista, S. E. (1979) *Ann. Intern. Med.* **91,** 730–733.
4. Steere, A. C., Malawista, S. E., Hardin, J. A., Ruddy, S., Askenase, P. W. & Andiman, W. A. (1977) *Ann. Intern. Med.* **86,** 685–698.
5. Afzelius, A. (1921) *Acta Derm. Venereol.* **2,** 120–125.
6. Lipschutz, B. (1923) *Arch. Dermatol. Syph.* **143,** 365–374.
7. Herxheimer, K. & Hartmann, K. (1902) *Arch. Dermatol. Syph.* **61,** 57–76, 255–300.
8. Bannwarth, A. (1944) *Arch. Psychiatr. Nervenkrankh.* **117,** 161–185.

9. Burgdorfer, W., Barbour, A. G., Hayes, S. F., Benach, J. L., Grunwaldt, E. & Davis, J. P. (1982) *Science* **216,** 1317–1319.
10. Steere, A. C., Grodzicki, R. L., Kornblatt, A. N., Craft, J. E., Barbour, A. G., Burgdorfer, W., Schmid, G. P., Johnson, E. & Malawista, S. E. (1983) *N. Engl. J. Med.* **308,** 733–740.
11. Benach, J. L., Bosler, E. M. Hanrahan, J. P., Coleman, J. L., Habicht, G. S., Bast, T. F., Cameron, D. J., Ziegler, J. L., Barbour, A. G., Burgdorfer, W., Edelman, R. & Kaslow, R. A. (1983) *N. Engl. J. Med.* **308,** 740–742.
12. Ackermann, R., Kabatzki, J., Boisten, H. P., Steere, A. C., Hartung, S. & Runne, U. (1984) *Dtsch. Med. Wochenschr.* **109,** 92–97.
13. Preac-Mursic, V., Wilske, B., Schierz, G., Pfister, H. W. & Einhaupl, K. (1984) *Eur. J. Clin. Microbiol.* **3,** 564–565.
14. Asbrink, E., Brehmer-Andersson, E. & Hovmark, A. (1986) *Am. J. Dermatopathol.* **8,** 209–219.
15. Steere, A. C. (1989) *N. Engl. J. Med.* **321,** 586–596.
16. Ackermann, R., Rehse-Kupper, B., Gollmer, E. & Schmitt, R. (1988) *Ann. N.Y. Acad. Sci.* **539,** 16–23.
17. Weber, K., Schierz, G., Wilske, B. & Preac-Mursic, V. (1984) *Yale J. Biol. Med.* **57,** 13–21.
18. Steere, A. C., Bartenhagen, N. H., Craft, J. E., Hutchinson, G. J., Newman, J. H., Rahn, D. W., Sigal, L. H., Spieler, P. H., Stenn, K. S. & Malawista, S. E. (1983) *Ann. Intern. Med.* **99,** 76–82.
19. Steere, A. C., Schoen, R. T. & Taylor, E. (1987) *Ann. Intern. Med.* **107,** 725–731.
20. Marconi, R. T., Lubke, L., Hauglum, W. & Garon, C. F. (1992) *J. Clin. Microbiol.* **30,** 628–632.
21. Baranton, G., Postic, D., Saint Girons, I., Borelin, P., Piffaretti, J. C., Assous, M. & Grimont, P. A. (1992) *Int. J. Syst. Bacteriol.* **42,** 378–383.
22. Levine, J. F., Wilson, M. L. & Spielman, A. (1985) *Am. J. Trop. Med. Hyg.* **34,** 355–360.
23. Krampitz, H. E. (1986) *Zentralbl. Bakteriol. Mikrobiol. Hyg.* **263,** 21–28.
24. Dekonenko, E. J., Steere, A. C., Berardi, V. P. & Kravchuk, L. N. (1988) *J. Infect. Dis.* **158,** 748–753.
25. Matuschka, F. R. & Spielman, A. (1986) *Exp. Appl. Acarol.* **2,** 337–353.
26. Wilson, M. L., Adler, G. H. & Spielman, A. (1986) *Ann. Entomol. Soc. Am.* **78,** 172–176.
27. Wilson, M. L., Telford, S. R., III, Piesman, J. & Spielman, A. (1988) *J. Med. Entomol.* **25,** 224–228.
28. Brown, R. N. & Lane, R. S. (1992) *Science* **256,** 1439–1442.
29. Bosler, E. M., Ormiston, B. G., Coleman, J. L., Hanrahan, J. P. & Benach, J. L. (1984) *Yale J. Biol. Med.* **57,** 651–659.
30. Davis, M. B. (1983) in *Late Quaternary Environments of the U.S.,* ed. Wright, H. E., Jr. (Univ. of Minn. Press, Minneapolis), Vol. 2, pp. 166–179.
31. Spielman, A., Telford, S. R., III, & Pollack, R. J. (1993) in *Ecology and Environmental Management of Lyme Disease*, ed. Ginsberg, H. S. (Rutgers Univ. Press, New Brunswick, NJ), pp. 83–96.
32. Severinghaus, C. W. & Brown, C. Y. (1983) in *Changes in the Land: Indians, Colonists, and the Ecology of New England*, ed. Cronon, W. (Hill & Wang, New York), p. 241.
33. Persing, D. H., Telford, S. R., Rys, P. N., Dodge, D. E., White, T. J., Malawista, S. E. & Spielman, A. (1990) *Science* **249,** 1420–1423.

34. Mather, T. N., Ribiero, J. M. C. & Spielman, A. (1987) *Am. J. Trop. Med. Hyg.* **36,** 609–614.
35. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rep.* **42,** 345–350.
36. Stanek, G., Pletschette, M., Flamm, H., Hirschl, A. M., Aberer, E., Kristoferitsch, W. & Schmutzhard, E. (1988) *Ann. N.Y. Acad. Sci.* **539,** 274–282.
37. Ai, C., Hu, R., Hyland, K. E., Wen, Y., Zhang, Y., Qui, Q., Liu, X., Shi, Z., Zhao, J. & Cheng, D. (1990) *Int. J. Epidemiol.* **19,** 1061–1065.
38. Kawabata, M., Baba, S., Iguchi, K., Yamaguti, N. & Russell, H. J. (1987) *J. Infect. Dis.* **156,** 854.
39. Steward, A., Glass, J., Patel, A., Watt, G., Cripps, A. & Clancy, R. (1982) *Med. J. Aust.* **1,** 139.
40. Lastavica, C. C., Wilson, M. L., Berardi, V. P., Spielman, A. & Deblinger, R. D. (1989) *N. Engl. J. Med.* **320,** 133–137.
41. Steere, A. C., Taylor, E., Wilson, M. L., Levine, J. L. & Spielman, A. (1986) *J. Infect. Dis.* **154,** 295–300.
42. Hanrahan, J. P., Benach, J. L., Coleman, J. L., Bosler, E. M., Morse, D. L., Cameron, D. J., Edelman, R. & Kaslow, R. A. (1984) *J. Infect. Dis.* **150,** 489–496.
43. White, D. J., Chang, H. G., Benach, J. L., Bosler, E. M., Meldrum, S. C., Means, R. G., Debbie, J. G., Birkhead, G. S. & Morse, D. L. (1991) *J. Am. Med. Assoc.* **266,** 1230–1236.
44. Goldstein, M. D., Schwartz, B. S., Friedmann, C., Maccarillo, B., Borbi, M. & Tuccillo, R. (1990) *Am. J. Public Health* **80,** 1225–1229.
45. Barbour, A. G. & Fish, D. (1993) *Science* **260,** 1610–1616.
46. Pachner, A. R. & Steere, A. C. (1985) *Neurology* **35,** 47–53.
47. Steere, A. C., Batsford, W. P., Weinberg, M., Alexander, J., Berger, H. J., Wolfson, S. & Malawista, S. E. (1980) *Ann. Intern. Med.* **93,** 8–16.
48. Halperin, J. J., Luft, B. J., Anand, A. K., Roque, M. D., Alvarez, O., Volkman, D. J. & Dattwyler, R. J. (1989) *Neurology* **39,** 753–759.
49. Logigian, E. L., Kaplan, R. F. & Steere, A. C. (1990) *N. Engl. J. Med.* **323,** 1438–1444.
50. Halperin, J. J., Little, B. W., Coyle, P. K. & Dattwyler, R. J. (1987) *Neurology* **37,** 1700–1706.
51. Logigian, E. L. & Steere, A. C. (1992) *Neurology* **42,** 303–311.
52. Asbrink, E. & Hovmark, A. (1988) *Ann. N.Y. Acad. Sci.* **539,** 4–15.
53. Steere, A. C., Berardi, V. P., Weeks, K. E., Logigian, E. L. & Ackermann, R. (1990) *J. Infect. Dis.* **161,** 1203–1209.
54. Lebech, A. M. & Hansen, K. (1992) *J. Clin. Microbiol.* **30,** 1646–1653.
55. Keller, T. L., Halperin, J. J. & Whitman, M. (1992) *Neurology* **32,** 32–42.
56. Dinerman, H. & Steere, A. C. (1992) *Ann. Intern. Med.* **117,** 281–285.
57. Kaplan, R. F., Meadows, M. E., Vincent, L. C., Logigian, E. L. & Steere, A. C. (1992) *Neurology* **42,** 1263–1267.
58. Steere, A. C., Taylor, E., McHugh, G. L. & Logigian, E. L. (1993) *J. Am. Med. Assoc.* **269,** 1812–1816.
59. Berger, B. W., Johnson, R. C., Kodner, C. & Coleman, L. (1992) *J. Clin. Microbiol.* **30,** 359–361.
60. Melski, J. W., Reed, K. D., Mitchell, P. D. & Barth, G. D. (1993) *Arch. Dermatol.* **129,** 709–716.
61. Dressler, F., Whalen, J. A., Reinhardt, B. N. & Steere, A. C. (1993) *J. Infect. Dis.* **167,** 392–400.

62. Bakken, L. L., Case, K. L., Callister, S. M., Bourdeau, N. J. & Schell, R. F. (1992) *J. Am. Med. Assoc.* **268,** 891–895.
63. Schwartz, B. S., Goldstein, M. D., Ribeiro, J. M. C., Schulze, T. L. & Shahied, S. I. (1989) *J. Am. Med. Assoc.* **262,** 3431–3434.
64. Goodman, J. L., Jurkovich, P., Kramber, J. M. & Johnson, R. C. (1991) *Infect. Immun.* **59,** 269–278.
65. Liebling, M. R., Nishio, M. J., Rodriguez, A., Sigal, L. H., Jin, T. & Louis, J. S. (1993) *Arthritis Rheum.* **36,** 665–675.
66. Rahn, D. W. & Malawista, S. E. (1991) *Ann. Intern. Med.* **114,** 472–481.
67. Dattwyler, R. J., Volkman, D. J., Conaty, S. M., Platkin, S. P. & Luft, B. J. (1990) *Lancet* **336,** 1404–1406.
68. Massarotti, E. M., Luger, S. W., Rahn, D. W., Messner, R. P., Wong, J. B., Johnson, R. C. & Steere, A. C. (1992) *Am. J. Med.* **92,** 396–403.
69. Dattwyler, R. J., Volkman, D. J., Conaty, S. M., Platkin, S. P. & Luft, B. J. (1990) *Lancet* **336,** 1404–1406.
70. Pfister, H. W., Preac-Mursic, V., Wilske, B., Schielke, E., Sorgel, F. & Einhaupl, K. M. (1991) *J. Infect. Dis.* **163,** 311–318.
71. Steere, A. C., Pachner, A. R. & Malawista, S. E. (1983) *Ann. Intern. Med.* **99,** 767–772.
72. Schulze, T. L., Taylor, G. C., Vasvary, L. M., Simmons, W. & Jordan, R. A. (1992) *J. Med. Entomol.* **29,** 544–547.
73. Mather, T. N., Duffy, D. C. & Campbell, S. R. (1993) *J. Med. Entomol.* **30,** 642–645.
74. Mather, T. N., Ribeiro, J. M. C. & Spielman, A. (1987) *Am. J. Trop. Med. Hyg.* **36,** 609–614.
75. Stafford, K. C., III (1991) *J. Med. Entomol.* **28,** 611–617.
76. Schreck, C. E., Snoddy, E. L. & Spielman, A. (1986) *J. Med. Entomol.* **23,** 396–399.
77. Oransky, S., Roseman, B., Fish, D., Gentile, T., Melius, J., Cartter, M. L. & Hadler, J. L. (1989) *Morbid. Mortal. Wkly. Rep.* **38,** 678–680.
78. Shapiro, E. D., Gerber, M. A., Holabird, N. B., Berg, A. T., Feder, H. M., Jr., Bell, G. L., Rys, P. N. & Persing, D. H. (1992) *N. Engl. J. Med.* **327,** 1769–1773.
79. Fikrig, E., Barthold, S. W., Kantor, F. S. & Flavel, R. A. (1990) *Science* **250,** 553–556.
80. Fikrig, E., Telford, S. R., III, Barthold, S. W., Kantor, F. S., Spielman, A. & Flavell, R. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5418–5421.
81. Canica, M. M., Nato, F., duMerle, L., Mazic, J. C., Baranton, G. & Postic, D. (1993) *Scand. J. Infect. Dis.* **25,** 441–448.
82. Burgdorfer, W., Lane, R. S., Barbour, A. G., Gresbrink, R. A. & Anderson, J. R. (1985) *Am. J. Trop. Med. Hyg.* **34,** 925–930.
83. Nocton, J. J., Dressler, F., Rutledge, B. J., Rys, P. N., Persing, D. H. & Steere, A. C. (1994) *N. Engl. J. Med.* **330,** 229–234.

Working Parents: The Impact of Day Care and Breast-Feeding on Cytomegalovirus Infections in Offspring

SERGIO STAGNO AND GRETCHEN A. CLOUD

Human cytomegalovirus (CMV) is of medical importance because it is the leading cause of congenital infection in most parts of the world and because it can cause significant disease in immunocompromised patients. CMV is highly species specific, and humans are believed to be its only reservoir (1).

THE PROBLEM

Seroepidemiologic surveys have found CMV infection in every population that has been tested (2). Transmission occurs by direct or indirect person-to-person contact. Known sources of the virus include urine, oropharyngeal secretions, cervical and vaginal secretions, semen, milk, tears, and blood (3). CMV is not very contagious, and the spread of infection requires close or intimate contact with infected secretions. Infected children excrete virus for much longer periods of time (months to years) and in larger quantities than adults. Under special circumstances fomites may also play a role, since CMV has been shown to retain infectivity for hours on plastic surfaces and has been isolated from randomly selected toys and surfaces in day-care centers (4, 5). Restriction

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enzyme analyses of CMV DNA have been used to demonstrate route of transmission of the virus in situations in which close contact occurs, such as breast-feeding, sexual activity, day care, and interaction between parents or caretakers and infected toddlers.

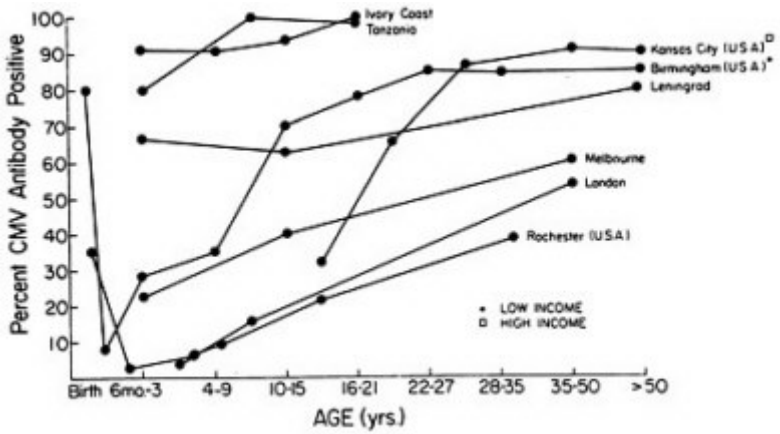

FIGURE 1 Age-related prevalence of antibody to CMV in various populations. [Reproduced with permission from ref. 6 (copyright 1981, C. A. Alford).]

The prevalence of CMV infection increases with age, but, according to geographic, ethnic, and socioeconomic backgrounds, the patterns of acquisition of this infection vary widely among populations (6). As illustrated in Figure 1, CMV is acquired earlier in life in developing countries and among the lower socioeconomic strata of developed countries. Differences between populations can be particularly striking during childhood. Presumably, these significant differences are the reflection of factors that account for increased exposure to CMV such as crowding, breast-feeding, sexual practices, and certain rearing practices.

Studies carried out in the past 15 years indicate that breast-feeding and child-rearing practices singly or in combination are two of the most powerful factors influencing the rate of acquisition of CMV in the various populations (7, 8). Breast-feeding is the major factor during the first year of life with CMV excretion rates of >50% observed in countries where the majority of women are seropositive and breast-feed their infants. The rapid increase in the rate of infection that generally takes place after the first year of life is the result of close contact and exposure to children who acquired CMV infection from a maternal source (i.e., *in utero*, from exposure to genital secretions at birth, or from breast milk). In our view, the profound alterations that have occurred in breast-feeding

and child-rearing practices during the past several decades are largely responsible for the current regional differences in seroprevalence and will continue to exert a powerful effect in CMV-related morbidity in those areas where these practices undergo significant changes. Because of the public health importance of breast-feeding and child-rearing practices in the epidemiology of CMV this article will focus on their roles and the implications for the future. The data presented here cannot be extrapolated to all continents, or even across national boundaries. The situation we are reviewing applies principally to the United States and to a lesser extent to Canada and parts of Western Europe. However, it is likely that in many other countries a similar situation already exists or is likely to develop if these modifications take place. More studies are needed to better define the epidemiology of CMV on an international scale.

ROLE OF BREAST-FEEDING

Diosi *et al.* (9) were the first to report the isolation of CMV from human milk. The virus was found in 1 of 49 milk specimens collected from parturient women. Hayes *et al.* (10) subsequently reported the isolation of CMV from 14 milk specimens (27%) collected from 63 seropositive women. In addition, they found that virolactia was more common in samples collected >1 wk postpartum (50%) than in those collected during the 1st week (11%). Studies in our laboratory provided compelling evidence that CMV is indeed transmitted from mother to baby through breast milk and that the ensuing infection is almost always without clinical consequence (8, 11). Among seropositive women sampled serially, milk was the most common site for recovery of CMV, with 13 of 41 women (32%) having at least one positive sample. It is noteworthy that women excreting CMV from other sites and mothers of congenitally infected infants were more likely to have virolactia. The rate of CMV excretion changed during the course of lactation, with only 2 of 40 women (5%) shedding CMV in colostrum while 13 of 31 (42%) had positive milk specimens when sampled between 2 and 12 wk postpartum. As illustrated in Table 1, the acquisition of CMV by the infants of these seropositive women was significantly related to the duration of breast-feeding and the presence of detectable virus in the breast milk. In sharp contrast, no bottle-fed babies or infants born to women who shed CMV only in saliva or urine become infected. From this study, we can conclude that nearly 40% of all infants nursed for a month or longer by seropositive mothers acquire CMV postnatally (12) (Table 1). Since in most populations of the world the prevalence of CMV infection among women of childbearing age is very high (80–98%) and most women

breast-feed their infants for a period of 2–6 months, transmission through breast milk is certainly quite prevalent and accounts for the majority of infants infected in early life. A few years ago we reported that, of a cohort of 154 predominantly Black infants of low socioeconomic background who were prospectively followed for a 2-yr period to establish the rate of postnatal CMV infection, only 8% had acquired the infection by age 6 mo (8). The rate of seropositivity among mothers was 85%, yet only 8% of the infants had been breast-fed. As illustrated in Table 2, this is one of the lowest rates of perinatal transmission ever reported (13). What makes it more remarkable is that this rate is far

TABLE 1 Breast-feeding and infant CMV infection from prospectively studied mother–infant pairs

Maternal status	No.	No. of infants infected (%)	*P* value
Seronegative	17	0	0.006
Seropositive	41	12 (30)	
Breast-fed <1 mo	10	0	0.015
Breast-fed ≥1 mo	31	12 (39)	
CMV excreted in milk	13	9 (69)	0.0007
CMV not excreted in milk	28	3 (10)	

Data are from ref. 11.

TABLE 2 Breast-feeding patterns and prevalence of CMV infection in young children from various nations

	Breast-feeding rate		% seropositive	
Nation	Ever	At 3 mo	Mothers	Children (age)
Solomon Islands	100	97	100	100 (5 mo to 4 yr)
India (Vellore)	96	64	98	80 (1 yr)
India (Pondicherry)			97	67 (1–5 yr)
Barbados	96	?	77	62 (1–5 yr)
Guatemala	95	?	98	47 (6 mo to 1 yr)
Chile	89	?	92	42 (1–2 yr)
Japan (Sapporo)	?	56	67	42 (6 mo to 2 yr)
Japan (Sendai)			85	38 (1 yr)
Finland (Helsinki)	95	50	55	28 (1 yr)
USA (Houston)	46	?	48	15 (1 yr)
USA (Birmingham)	8	?	85	8 (1 yr)
France (Paris)	85	?	56	10 (10 mo)
Canada (Nova Scotia)	49	26	34	12 (6–12 mo)
UK (Manchester)	51	13	59	12 (3–11 mo)

Data are from ref. 13.

below the rates seen in other populations in which the seroprevalence of CMV among mothers is much lower.

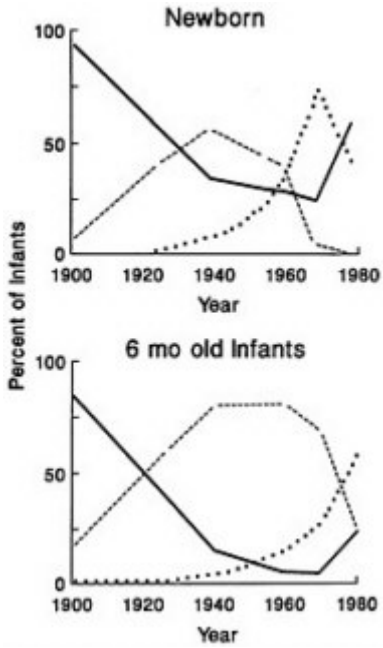

FIGURE 2 Feeding practices in the United States of newborns and infants 5–6 mo old from 1900 to 1980. Solid line, breast feeding; dotted line, combined feeding of formulas prepared from evaporated milk or whole cow's milk; dashed line, commercially prepared formulas. [Reproduced by permission of *Pediatrics*, Vol. 60, p. 654, copyright 1982 (12).]

The popularity of breast-feeding in the industrialized world and particularly in the United States has changed dramatically during this century in terms of both frequency and duration (12). Trends in infant feeding practices within the United States since 1900 are shown in Figure 2. Breast-feeding within the hospital setting reached a nadir in 1970, when fewer than one in five mothers elected to breast-feed. Exclusive breast-feeding was reported by 19% of White and 9% of Black mothers. Abandonment of breast-feeding in the earlier part of this century started first within the upper socioeconomic levels and spread downward on the socioeconomic scale. After 1970, the resurgence of breast-feeding began at the upper socioeconomic levels, and it is spreading along the various strata in much the same manner as the abandonment of breast-feeding occurred.

In 1980 the National Center for Health Statistics reported a significantly higher percentage of both Black and White women exclusively breast-feeding their infants than in 1969 (14). For Whites, the percentage had increased to 51%, while for Black women it had increased only to

25%. The differences by race persist when education and parity are controlled. It is interesting to note that among White women a significantly higher percentage of breast-feeding was observed with increasing maternal education in 1969 as well as in 1980. In sharp contrast, among Black women with newborns in 1969 the rate of breast-feeding was significantly lower among those with a higher level of education. However, in 1980, a relationship similar to that observed in Whites appeared.

It is our belief that these changes in the popularity of breast-feeding have had a major effect on the epidemiology of CMV. The much lower prevalence of CMV infection that we and others have documented in the United States among women of the upper middle class when compared to women of low socioeconomic background living in the same geographic area is to some extent the result of this phenomenon. As fewer White, largely upper and middle class seropositve women elected to breast-feed their infants in the first half of this century, the perinatal transmission of CMV declined progressively. As the low socioeconomic sector followed in this trend a similar drop in perinatal transmission is now evident in this group.

IMPORTANCE OF DAY-CARE CENTERS

With the changing composition of the American family, many children live in single parent families in which the mother is the primary source of economic support (15). It is estimated that nearly 60% of the women with children 5 yr of age and under are employed. The National Center for Health Statistics reported that in 1988 two-thirds of children in the United States 5 yr of age and under (68% or 13.3 million) had been in child-care arrangements at some point in their lives (15). Two-thirds of them were cared for in a nursery, preschool, or day-care center. Twenty-four percent of children in formal child-care arrangements were cared for in groups of seven or more children. The number of family day-care or group home and day-care centers increased dramatically between 1977 and 1985 (15). The report from the Center for Health Statistics demonstrates that receipt of child care is strongly associated with socioeconomic status. Women of low socioeconomic strata are significantly less likely to use a child-care arrangement.

In 1971, Weller suggested that the high rate of seropositivity among Swedish children was probably due to the frequent use of day-care centers (1). Swedish children had a rate of infection that was 3–4 times higher than that observed in London or Rochester, New York. Similar high rates of CMV infection in young children were noted in the Solomon Islands and Israeli kibbutzim, where high hygienic standards

were maintained (16, 17). As illustrated in Table 3, high rates of CMV infection among children attending day-care centers were later confirmed in Sweden and have been reported in several studies in the United States (7, 18–26). The studies, which included a control group of children, confirmed that the rate of CMV infection was substantially higher among those in day care than in those who stayed at home (20, 21). The highest rates have been found consistently in children between 1 and 3 yr old. In the study of Pass *et al.* (7), in a group of 70 children of middle- to upper-income background whose ages ranged from 3 to 65 mo, the rate of CMV excretion in urine and saliva was 51%. The lowest rate of excretion (9%) occurred in infants <1 yr old, and the highest rate (88%) was among toddlers in their second year of life. Infants younger than 12 mo in group day care who excrete CMV are more likely to have acquired CMV congenitally or perinatally from maternal cervical secretions or breast milk. Twelve children whose mothers were seronegative excreted CMV, which indicated that their infection was not perinatally acquired. The findings of Pass *et al.* have been subsequently confirmed by Adler (18, 20, 23) and by Murph *et al.* (24). There is consensus that CMV infection acquired by young children is generally asymptomatic. No specific syndrome has been temporarily associated with CMV infection acquired in day-care centers. The high rates of infection combined with the large quantities of infectious virus excreted by these children create a potential for exposure that is difficult to encounter under any other circumstances.

TABLE 3 Prevalence of CMV excretion among children in day-care centers

Investigator	Year	Location	% infected
Strangert	1976	Stockholm	35 (7/20)
Strom	1979	Stockholm	72 (13/18)
Pass	1982	Birmingham, AL	51 (36/70)
Adler	1985	Richmond, VA	24 (16/66)
Hutto	1985	Birmingham, AL	41 (77/188)
MMWR	1985	Birmingham, AL	29 (66/231)
Jones	1985	San Francisco, CA	22 (31/140)
Murph	1986	Iowa City, IA	22 (9/41)
Adler	1987	Richmond, VA	53 (55/104)

MMWR, *Morbidity and Mortality Weekly Report*. Data are from ref. 18.

There is now compelling evidence that the high rate of CMV infection among children in group day care is caused by horizontal transmission from child to child. The route of transmission that appears most likely is the transfer of virus that occurs through saliva on hands and toys. The

survival of CMV on fomites such as toys, diapers, and hands of day-care personnel also suggests a role for environmental contamination (4, 5, 27). No data have indicated CMV transmission via respiratory droplets.

The strongest evidence supporting child-to-child transmission was obtained by analysis of the restriction enzyme digestion patterns of CMV DNA of the isolates obtained from infected children attending day care. Adler (20) examined the restriction endonuclease patterns of isolates obtained from 16 children at a single day-care center. Four children older than 28 mo who shared a common room were shedding a common strain. A second strain was shed by seven children younger than 28 mo who had little contact with the older group but played with one another daily. Only five of the infected children were shedding unique strains. In a subsequent study, Adler (28) identified by endonuclease analysis 14 different strains of CMV among 104 children in a single day-care center who were monitored at 4-mo intervals for >26 mo. Three of these 14 strains infected 44 children, all of whom were <3 yr old. Of 75 initially seronegative children, 34 acquired day-care-associated strains, whereas 4 were infected with unique isolates. These findings have been confirmed in subsequent studies (24-26, 28, 29). These findings demonstrate that CMV is very efficiently transmitted from child to child in the day-care setting and that it is not unusual to find excretion rates as high as 20-80% in young toddlers. In many instances these rates of infection are substantially higher than the seroprevalence rates for the parents of the children and young adults in the cities where the studies were done.

COMBINED EFFECT OF BREAST-FEEDING AND DAY-CARE CENTERS

In the low socioeconomic population, the combined effects of a low rate of breast feeding and low utilization of child-care arrangements may result in a lowering of the prevalence of CMV. Over the past 20 years, as part of an ongoing study of the natural history of maternal CMV infection, we have surveyed the seroprevalence of CMV infection among nearly 6000 pregnant women attending a single prenatal public health clinic in the city of Birmingham, Alabama. All these women were enrolled at the time of the first prenatal visit. All deliveries took place at University Hospital. As illustrated in Figure 3*A*, in the past 2 decades the seroprevalence of CMV infection among these predominantly Black women of low socioeconomic background has significantly declined across all ages. The lower curve represents the last 6 yr of involvement (1986-1992), while the upper curve reflects the rate of seropositivity during the first 6 yr of involvement (1971-1977). It is particularly striking that in the 15- to 24-yr-old group, the rate of seropositivity has dropped

nearly 10 percentage points. Since 1980 we have been screening this population for congenital CMV infection with a sensitive tissue culture method. As illustrated in Table 4 the rate of congenital infection has remained constant during the past 12 years. However, we have noticed an increase in the rate of symptomatic congenital infection from 4.3% to 10.2% (P = 0.09). This provocative observation may well be the result of more primary, as opposed to recurrent, infections experienced by young seronegative mothers in this socioeconomic group.

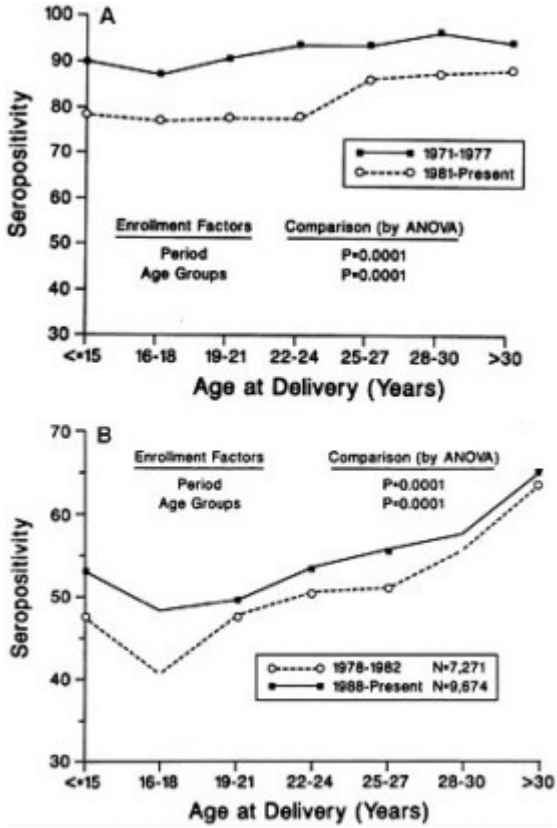

FIGURE 3 (*A*) Rate of seropositivity to CMV of two enrollment periods for women of low socioeconomic extraction. (*B*) Rate of seropositivity to CMV of two enrollment periods for middle and upper-middle income women.

TABLE 4 Congenital CMV infection in low socioeconomic population

		Congenital infection		Symptomatic	
Interval	Total newborn	Total	%	Total	%
1980-1983	6,128	70	1.14	3	4.3
1984-1987	5,845	83	1.42	7	8.4
1988-1992	12,393	166	1.34	17	10.2

[2] test for trend of percentage of symptomatic infection ($P = 0.09$).

With the dramatic resurgence of breast-feeding in the middle and upper middle classes and a greater utilization of child-care arrangements, we anticipated the opposite phenomenon. As part of our ongoing studies of CMV in pregnant women since 1978, we have enrolled nearly 24,000 women of upper socioeconomic background from a single private obstetrical practice in Birmingham, Alabama. All women were enrolled and tested for CMV antibodies at the time of the first prenatal visit. All deliveries took place at a single private hospital. As predicted, the seroprevalence of CMV infection in women of middle and upper middle class has significantly increased, particularly in the 15- to 18-yr-old group (Figure 3*B*).

RISK TO CARE GIVERS

Several lines of evidence indicate that children excreting CMV often become the source of infection for serosusceptible parents and child-care personnel, particularly women of childbearing age. Using restriction endonuclease analysis, Spector and Spector (30) and Dworsky *et al.* (31) demonstrated transmission of CMV from infants with perinatal infections to a parent or other adult family members. Yeager (32) reported in 1983 that 7 (47%) of 15 seronegative mothers of infants who had acquired CMV in a nursery seroconverted within 1 yr. The same year, Dworsky *et al.* (33) reported that the rate of seroconversion for women with at least one child living at home was 5.5%, significantly higher than the 2.3% rate for women from the same clinic who were pregnant for the first time or the rates of susceptible nursery nurses and for physicians in training. Taber *et al.* (34) monitored the acquisition of CMV in 68 Houston families observed for a mean of 3.5 yr per family. The study showed a significant association between seroconversion among children and seroconversion among susceptible parents. Pass *et al.* (26, 35, 36) and Adler (23, 28, 37) have presented more compelling evidence linking the acquisition of CMV by children in day care with subsequent infection in their mothers and care givers. Pass *et al.* (35) did a

longitudinal serologic follow-up study of seronegative parents whose children attended a day-care center and of seronegative parents whose children did not attend day care. The groups were followed for a mean of 17 and 21 mo, respectively. The study revealed that 14 of 67 seronegative parents with children in day-care centers acquired CMV, compared with 0 of 31 serosusceptible parents whose children did not attend day care. More significant, all 14 parents of the day-care group who seroconverted had a child who was shedding CMV in saliva or urine. In fact, seroconversion occurred in 14–48 parents of children who shed CMV, compared with 0 of 21 whose children did not excrete CMV. The highest risk of seroconversion (45%) was for parents with a child shedding CMV who was 18 mo old or younger at enrollment. In 2 of the 14 cases, DNA analysis indicated the child as the source of CMV infection. In a subsequent study, this group of investigators also demonstrated by means of restriction enzyme analysis that infections acquired by a mother from a child can be transmitted to her fetus (26). In a very similar study, Adler (23) observed that of 18 seronegative mothers whose children shed CMV strains associated with day care, 6 seroconverted and excreted CMV strains identical to the strains shed by their children. On an average these mothers acquired the infection within 4.2 mo (range, 3–7 mo) after their children became infected.

In assessing the risk to day-care workers Adler (37) and Pass (38) have recently reported annual seroconversion rates of 11% and 20% among seronegative day-care workers. The risk could be different in smaller centers or in day-care homes where supervision and infection control measures are not well enforced. These rates are significantly higher than the 2–2.5% expected incidence from studies of large numbers of pregnant women or hospital workers of similar socioeconomic background (38). The risk of infection appears to be significantly higher among those caring for children who are <3 yr old. Results of restriction endonuclease analyses of CMV DNA from strains recovered from children attending day-care centers, from their parents, and from day-care workers provide compelling evidence that children transmit CMV to their care givers. Using this methodology, Adler (23) analyzed strains recovered from children, parents, and care givers at a single day-care center. He found that nine mothers, four fathers, and two child-care workers excreted strains of CMV that were identical to those excreted by children at the center. The same investigator in a more extensive study found that seven care givers from three different centers excreted CMV strains that by restriction enzyme analyses were identical to the strains isolated from one or more of the children they cared for.

From the data generated by these studies it is reasonable to expect that `50% of susceptible children between the ages of 1 and 3 yr who

attend group day care will acquire CMV from their playmates and become an important potential source of infection for susceptible parents and care givers. Of particular concern is the risk to seronegative mothers who have children in group day care and who become pregnant. As part of our ongoing investigations of CMV infection in pregnancy, we have prospectively studied >2200 seronegative women from a high socioeconomic group during more than one pregnancy and have been able to establish the risk of seroconversion in successive pregnancies. In general, as illustrated in Table 5, the annualized rate of seroconversion increases with time from 2.0% for the first studied pregnancy to 4.6% during the fourth studied pregnancy. These observations combined with the results of the studies in day care underline the fact that susceptible pregnant women may acquire CMV infection introduced into the household by their young children. Given these figures and the data presented in the preceding paragraphs, it is obvious that the increased utilization of day-care centers is having a significant effect on the epidemiology of CMV.

TABLE 5 Rate of seroconversion during first and subsequent pregnancies in women of middle to upper socioeconomic background

Order of studied pregnancy	Mean age, yr	Seroconversions/no. seronegatives	Annualized rate*
Pregnancy 1†	25.4	97/8194 (1.2%)	2.0%
Between 1 and 2		260/2236 (11.6%)	4.5%
Pregnancy 2	28.0	29/1597 (1.8%)	3.1%
Between 2 and 3		64/485 (13.2%)	6.3%
Pregnancy 3	29.3	5/352 (1.4%)	2.4%
Between 3 and 4		8/97 (8.3%)	5.2%
Pregnancy 4	30.0	2/73 (2.7%)	4.6%

* Based on an average gestational age of 2 mo at enrollment.
† Does not equate with primigravida.

CONCLUSIONS

In the United States between one-half and one-third of all women of childbearing age are susceptible to CMV infection. The rate of seronegativity is higher for middle to upper-middle class women than for women of low socioeconomic extraction. However, these differences appear to be narrowing and may reverse themselves if the current trends in breast-feeding practices and utilization of day-care centers continue. If 20–50% of all mothers elect to breast-feed their newborn infants, and considering that those who are seropositive transmit CMV

to their infants through breast milk at a rate of 40%, then somewhere between 10% and 15% of all infants can be expected to start shedding CMV before the age of 6 mo. In addition, the pool of infected infants must be expanded to include the estimated 0.5–2.0% of infants born with congenital infection and 2–4% of infants who acquire natal CMV infection from exposure to maternal genital tract secretion at delivery. Since a characteristic of CMV infections acquired *in utero* and early infancy is chronic viral shedding of large quantities of virus, grouping children for care will inevitably lead to horizontal spread to other children, parents, and other adults who have daily close contact with children. From the studies reviewed here, it is reasonable to anticipate that between one-half and two-thirds of all children who attend day care will become infected within 2 yr of enrollment and that these children will also shed CMV from urine and saliva for extended periods of time. The data also indicate that 30–50% of the parents of these children and those who care for them are susceptible to CMV and the evidence is persuasive that they are at increased risk for infection. In day-care centers of moderate size, the risk of transmission to parents is 15–30% and the risk to workers is 10–20% per yr. Because many mothers become pregnant while a previous child is still in day care, those that are susceptible to CMV are at a significant risk of acquiring CMV. When primary infection occurs during pregnancy, the risk of *in utero* transmission is `40% (6). Prospective studies have demonstrated that between 10% and 20% of congenitally infected infants whose mothers acquired a primary CMV infection during pregnancy develop sequelae (39). Fortunately, the risk of adverse effects for the fetus is extremely low for women who are seropositive before pregnancy.

For the immediate future, the implications of the data reviewed in this manuscript are quite serious for seronegative women of childbearing age who as parents or for work-related reasons are exposed to young children who receive group care outside their homes.

It is reasonable to anticipate that, over the next decade or so, many seronegative women of middle and upper-middle class background will become infected from exposure to children who acquired CMV infection in group day-care centers. This risk will be particularly serious for those women who become infected while pregnant as they will face the threat of transmission of virus to the fetus *in utero*, which carries a risk, albeit low, of serious fetal morbidity. In the more distant future, as more infants and young children acquire CMV in early life, the pool of seronegative individuals will decline progressively and the risk of primary infection for women of childbearing age will subside. The situation in the low socioeconomic sector is quite different. Few women in this group breast-feed their infants and the utilization of day-care

arrangements is also relatively low. Then it is to be expected that as there is less transmission of CMV the pool of seronegative individuals will actually increase and many more women will reach their reproductive age without the benefit of immunity to CMV.

If the risk of primary CMV infection during gestation increases, it is logical to anticipate more cases of severe congenital infection, as seems to be occurring in our community.

The sequence of these changes in the various socioeconomic groups will differ within any given geographic region principally in relation to the popularity of breast-feeding and the utilization of day-care centers. Since the data analyzed in this review were obtained from a limited number of studies carried out in only a few urban settings in the United States, we should not extrapolate to other settings without considering the many variables that obviously affect the epidemiology of CMV.

SUMMARY

The increase in the popularity of breast feeding and utilization of child care arrangements are having a major effect on the epidemiology of cytomegalovirus infections. The impact is greater for women of upper socioeconomic background who send their toddlers to day-care centers and for day-care workers. If primary cytomegalovirus infections occur during pregnancy it is logical to anticipate more cases of severe congenital infections.

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REFERENCES

1. Weller, T. H. (1971) *N. Engl. J. Med.* **285,** 203–214.
2. Krech, U., Jung, M. & Jung, F. (1971) *Cytomegalovirus Infections of Man* (Karger, Basel), p. 28.
3. Stagno, S. (1980) in *Infectious Diseases of the Fetus and Newborn Infant*, 3d Ed., eds. Remington, J. S. & Klein, J. O. (Saunders, Philadelphia), Vol. 6, pp. 241–281.
4. Hutto, S. C. & Pass, R. F. (1985) *Pediatr. Res.* **19,** A202.
5. Faiz, R. G. (1973) *J. Pediatr. (St. Louis)* **106,** 649–752.
6. Alford, C. A., Stagno, S., Pass, R. F. & Huang, E. S. (1980) in *The Human Herpesviruses: Interdisciplinary Perspective*, eds. Nahmias, A., Dowdle, W. & Schinazi, R. (Elsevier, New York), pp. 159–171.
7. Pass, R. F., August, A. M., Dworsky, M. E. & Reynolds, D. W. (1982) *N. Engl. J. Med.* **307,** 477–479.

8. Stagno, S., Reynolds, D. W., Pass, R. F. & Alford, C. A. (1980) *N. Engl. J. Med.* **302,** 1073–1076.
9. Diosi, P., Babusceac, L., Nevinglovschi, O. & Kum-Stoleu, G. (1967) *Lancet* **1,** 1063.
10. Hayes, K., Danks, D. M., Gibas, H. & Jack, I. (1972) *N. Engl. J. Med.* **287,** 177–178.
11. Dworsky, J., Yow, M., Stagno, S., Pass, R. F. & Alford, C. (1983) *Pediatrics* **72,** 295–299.
12. American Academy of Pediatrics, Task Force Report (1982) *Pediatrics* **60,** 654–661.
13. Pass, R. F. (1986) in *Human Milk in Infant Nutrition and Health* , eds. Howell, R. R., Morris, F. H., Jr., & Pickering, K. (Springfield, IL), pp. 205–224.
14. Setterly, K. & Graubard, B. (1984) *Morbid. Mortal. Wkly. Rep.* **33,** 153–154.
15. Dawson, D. A. & Cain, V. S. (1990) Vital & Health Statistics of the National Center for Health Statistics 187, (Natl. Cent. Health Statistics, Rockville, MD), No. 187, pp. 1–12.
16. Lang, D. J., Garruto, R. M. & Gajdusek, D. C. (1977) *Am. J. Epidemiol.* **105,** 480–487.
17. Sarov, B., Naggan, L., Rosenzveig, R., Katz, S., Haikin, H. & Sarov, I. (1988) *J. Med. Virol.* **10,** 195–201.
18. Adler, S. P. (1988) *Pediatr. Infect. Dis. J.* **7,** 279–285.
19. Strangert, L., Carlstrom, G., Jeansson, S. & Nord, C. E. (1976) *Acta Paediatr. Scand.* **65,** 455–463.
20. Adler, S. P. (1985) *J. Infect. Dis.* **152,** 760–768.
21. Hutto, C., Ricks, R., Garvie, M. & Pass, R. F. (1985) *Pediatr. Infect. Dis.* **4,** 149–152.
22. Jones, L. A. & Duke-Duncan, P. M. (1985) *J. Infect. Dis.* **151,** 953–955.
23. Adler, S. P. (1988) *J. Pediatr. (St. Louis)* **112,** 366–372.
24. Murph, J. R., Bale, J. F., Murray, J. C., Stinsky, M. F. & Perlman, S. (1986) *J. Pediatr. (St. Louis)* **109,** 35–39.
25. Grillner, L. & Strangert, L. (1986) *Pediatr. Infect. Dis.* **5,** 184–197.
26. Pass, R. F., Little, E. A., Stagno, S., Britt, W. J. & Alford, C. (1987) *N. Engl. J. Med.* **316,** 1366–1370.
27. Schupfer, P. C., Murph, J. R. & Bale, J. F., Jr. (1986) *Pediatr. Infect. Dis.* **5,** 677–679.
28. Adler, S. P. (1986) *Pediatr. Infect. Dis. J.* **5,** 315–318.
29. Hutto, C., Little, E. A., Ricks, R., Lee, J. D. & Pass, R. F. (1986) *J. Infect. Dis.* **154,** 527–530.
30. Spector, S. A. & Spector, D. H. (1982) *Pediatr. Infect. Dis.* **1,** 405–409.
31. Dworsky, M., Lakeman, A. & Stagno, S. (1984) *Pediatr. Infect. Dis.* **3,** 236–238.
32. Yeager, A. S. (1983) *Pediatr. Infect. dis.* **2,** 295–297.
33. Dworsky, M. E., Welch, K., Cassady, G. & Stagno, S. (1983) *N. Engl. J. Med.* **309,** 950–953.
34. Taber, L. H., Frank, A. L., Yow, M. D. & Bagley, A. (1985) *J. Infect. Dis.* **151,** 948–952.
35. Pass, R. F., Hutto, C., Ricks, R. & Cloud, G. (1986) *N. Engl. J. Med.* **314,** 1414–1416.
36. Pass, R. F. & Hutto, C. (1986) *Rev. Infect. Dis.* **8,** 599–605.
37. Adler, S. P. (1989) *N. Engl. J. Med.* **321,** 1290–1300.
38. Pass, R. F. (1990) *Semin. Pediatr. Infect. Dis.* **1,** 245–251.
39. Fowler, K. B., Stagno, S., Pass, R. F., Britt, W. J., Boll, T. J. & Alford, C. A. (1992) *N. Engl. J. Med.* **326,** 663–667.

Changes in Human Ecology and Behavior in Relation to the Emergence of Diarrheal Diseases, Including Cholera

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In a broad sense, one can divide the world into two distinct ecologies with respect to the occurrence of enteric diseases, with a developing country ecology at one extreme and an industrialized world ecology at the other. Between these extremes, one encounters gradations and exceptions. In each of these settings, changes in human ecology and behavior are having an impact on enteric infections. In developing areas, some of the sweeping changes in demographics and population distribution that are underway are creating environments of amplified transmission of enteric pathogens or are attenuating the protective features of some traditional practices. In industrialized areas, the changes underway to meet the ever-increasing demands of consumption-based economies are creating opportunities for the importation of pathogens that improvements in infrastructure were believed to have eliminated. Finally, even the gains in infrastructure and commercialization of food production, food distribution, and food retailing, with all of their positive impact on health, have by their very nature created environments in which we have seen the emergence of enteric pathogens.

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DEVELOPING WORLD ECOLOGY

Much of the developing world's population lives in substandard housing, under crowded conditions, without piped water or sanitation. Under these conditions of pervasive fecal contamination, the various bacterial, protozoal, and viral agents that cause diarrheal illness are readily transmitted (1–6). In low-socioeconomic-level populations in the developing world, infants and toddlers experience from 4–10 episodes per child per yr during the first 2 yr of life (1–3). Up to 20% of life experience of infants may be spent suffering from diarrheal illness (1–3) and up to one-third of deaths among children <2 yr of age are due to diarrheal dehydration, persistent diarrhea, and other complications of diarrheal illness (7).

A striking feature of pediatric diarrheal illness in developing countries is the large proportion associated with bacterial enteropathogens. Among the most important agents are enterotoxigenic *Escherichia coli* , enteropathogenic *E. coli*, and *Shigella* (1–6, 8–11). Enteroaggregative *E. coli*, a recently described category of diarrheagenic *E. coli* has been incriminated as an important cause of persistent diarrhea in developing countries (12–15). Consequent to clinical infections caused by the various agents and their antigenic varieties, infection-derived immunity is acquired (4, 16–18); this is reflected in markedly lower incidence rates in older children and adults and in increased prevalence of antibody with age (18).

INDUSTRIALIZED WORLD ECOLOGY

Quite distinct patterns of diarrheal disease are encountered in industrialized regions of the world, where there is access to microbiologically monitored drinking water, flush toilets, wastewater treatment, and adequate housing with little or no crowding. In these relatively affluent settings, viral agents of diarrhea predominate in pediatric populations (19). Nevertheless, some notable exceptions occur in specific settings in which hygienic conditions are less adequate (e.g., pediatric day care, custodial institutions for the mentally retarded), with the net effect of creating environments similar to those observed in less developed countries (20, 21).

CHANGES IN HUMAN BEHAVIOR AND ECOLOGY THAT PROMOTE THE EMERGENCE AND/OR AMPLIFIED TRANSMISSION OF ENTERIC INFECTIONS

Developing Areas

(*i*) *Urbanization*. At one extreme, we may consider the diarrheal disease problem among the most isolated and primitive populations

alive today (such as the Yanomami Indians in the Orinoco River basin) as an example of the burden of diarrheal disease in the absence of any urbanization. A lack of crowding, relatively small population, and prolonged breast-feeding of infants and young children for several years are believed to account for this. Even among the Mayan Highlanders in Guatemala, a much less isolated traditional society, the incidence of diarrheal disease does not peak until the second year of life (1), likely due to breast-feeding patterns. This timing is in contrast to the pattern observed in periurban slums in many developing areas where diarrheal disease incidence peaks in the second semester of the first year of life.

As another example, we can consider the concurrent emergence of typhoid fever as a public health problem and the rise of cities during the Industrial Revolution of Europe in the 1800s (22). Not to despair, typhoid fever also serves as an example of how a single change in infrastructure can rapidly and markedly diminish the incidence of enteric infections (22–24).

In most large cities of Western Europe and North America in the last quarter of the 19th century typhoid fever was highly endemic with incidence rates of 200–500 cases per 100,000 population being common (23, 24). In the late 19th and early 20th centuries, the introduction of a single intervention, treatment of municipal water supplies (with chlorine, sand filtration, or both) drastically and precipitously reduced the incidence of typhoid fever wherever these interventions were applied (22–24).

(*ii*) *Decreased breast-feeding*. Breast-feeding constitutes one of the most effective interventions to diminish the occurrence of diarrheal illness in young infants, particularly with respect to bacterial diarrheas (25–28). Sociologic changes that are increasingly encountered in urban areas of developing countries lead women to abandon breast-feeding early, as they must return to work long hours in situations where they cannot nurse their infants. In the 1960s and 1970s, aggressive advertising by some manufacturers of infant formulas, inadvertently or otherwise, led many mothers of low socioeconomic level to abandon breast-feeding and to administer diluted formula to their infants. Often these formulas were diluted with contaminated water, and a lack of refrigeration fostered heavy bacterial contamination. Ultimately, the World Health Assembly attempted to deal with this problem by passing a resolution that provides a restrictive code and guidelines on advertising to be followed by artificial formula manufacturers (29).

(*iii*) *Shifting agricultural patterns toward cash crop production*. As developing countries attempt to industrialize and become a part of the global economy, they are forced to generate large sums of foreign exchange to finance their development. As a result, many farmers have switched

from growing an array of crops for their own needs to cultivation of a single cash crop (e.g., sugar, rice, coffee), which may be of little or no nutritional value. In many cases, children have suffered nutritionally from this switch (30). The adverse consequences and the interaction of malnutrition and diarrheal disease are well described (1).

(*iv*) *Wars and political upheavals*. No human behavior so impacts the public's health as war. The impact of war on diarrheal disease is no exception. Former U.S. President Jimmy Carter estimates that there are no less than 30 wars currently under way worldwide (31). With the proliferation of advanced weapons during recent decades and their distribution among much of the developing world, the potential for devastation of infrastructure and health resources is at an unprecedented level.

The impact of war and political unrest on diarrheal disease is illustrated all too clearly by the current situations in Somalia and Bosnia–Herzegovina. In Somalia, where large segments of the population have been displaced and forced to live in refugee camp settings, mortality rates among children <5 yr as high as 69.4 per 10^5 per day have been reported (32); dysentery caused by multiresistant *Shigella dysenteriae* was one of the two most important causes of mortality during this period (32).

Civil turmoil can revert an industrialized country to developing country status with respect to inadequate provision of piped water supplies and sanitation. This abrupt change in ecology has occurred in Bosnia–Herzegovina, for example, where epidemics of diarrheal illness and typhoid fever have resulted.

Industrialized Areas

(*i*) *Commercialization of food production and food service*. In industrialized countries, agriculture is characterized by massive farms where vegetables are cultivated, extensive ranches where herd animals are raised, and enormous animal husbandry operations where poultry are raised. Additionally, a uniquely Western phenomenon has arisen: enormous fast-food chains have come to exist that serve millions of meals daily in the United States and Europe with all outlets in each chain adhering to uniform food-preparation techniques. While such commercialization of food production and food service have achieved impressive economies of scale, it has also been observed how such large-scale standardization can unwittingly foster and facilitate the emergence of certain diarrheal pathogens such as *Salmonella enteritidis* and enterohemorrhagic *Escherichia coli*.

Recent epidemics of infection with *Sa. enteritidis* through raw or

partially cooked eggs in restaurants provide one example to illustrate this point (33). Through molecular epidemiologic techniques, a recent outbreak of *Sa. enteritidis* was traced to a single egg-producing farm that housed >400,000 egg-laying hens in five henhouses (a veritable poultry city!) (34, 35).

Infections due to *Sa. enteritidis* have been increasing throughout Europe and the United States so rapidly that by 1990, *Sa. enteritidis* surpassed the venerable *Salmonella typhimurium* as the most commonly isolated serotype from humans in the United States (36, 37). With 80,000 hens per henhouse, often with cages stacked one upon another, commercial egg-producing farms create a permissive environment for the rapid transmission of enteric pathogens such as *Salmonella* and *Campylobacter jejuni* among animals, thereby creating a reservoir of infection for humans.

Part of the appeal of fast-food chains is the consumer's expectation that the food prepared in all outlets will conform to uniform procedures, using comparable ingredients (often from the same source). Thus, a high level of consistency is demanded and expected by the consumers from each fast-food chain, irrespective of the state or country in which a particular outlet is found. This integral part of the appeal of fast food also makes it capable of disseminating infection widely through a common vehicle. Recent outbreaks of hemorrhagic colitis and hemolytic uremic syndrome due to enterohemorrhagic *E. coli* of serotype O157:H7 provide an excellent example (38–42).

Enterohemorrhagic *E. coli* first came to be recognized in the U.S. as an enteric pathogen in 1982 when a multistate outbreak of an unusual clinical syndrome was seen in several midwestern and western states associated with the consumption of undercooked hamburgers (38). The clinical syndrome observed, hemorrhagic colitis, consisted of watery bloody diarrhea, without fever or fecal leukocytes (38). An unusual serotype of *E. coli*, O157:H7, was isolated from cases that had not previously been incriminated as a cause of diarrheal illness. Examination of the O157:H7 organism by various investigators revealed that this pathogen had amassed a fascinating array of virulence properties, including elaboration of phage-encoded potent Shiga-like toxins (43), a 60-MDa virulence plasmid associated with expression of novel fimbriae (44), and a chromosomal gene (45) that induces attaching and effacing lesions of intestinal mucosa (46). It was subsequently shown that serotype O157:H7 is a prototype within a new category of diarrheagenic *E. coli*, referred to as enterohemorrhagic *E. coli*; multiple other O:H serotypes also fall within this category, most notably O26:H11 and O111:NM (42), among others.

It rapidly came to be recognized that ` 1–2% of individuals with

bloody diarrhea caused by enterohemorrhagic *E. coli* go on to develop the hemolytic uremic syndrome characterized by the triad of hemolytic anemia, acute renal failure, and thrombocytopenia (42). Finally, it is now appreciated that cattle serve as the main reservoir of enterohemorrhagic *E. coli* infection that can be passed to humans by consumption of improperly cooked beef products or by close contract with infected cattle, particularly calves (as in a petting zoo for children) (42, 47, 48).

Two large multistate outbreaks of enterohemorrhagic *E. coli* disease have resulted from the consumption of undercooked beef hamburgers served by large fast-food chains. In the original outbreak of 1982, as well as in some recent outbreaks, epidemic investigations have shown that the restaurants' cooking practices were insufficient to kill the bacteria (38, 42, 49, 50). Enterohemorrhagic *E. coli* is becoming increasingly important as an enteric pathogen in North America and Europe, while it remains distinctly uncommon in developing countries.

(*ii*) *Importation of food from developing countries*. Occasionally, imported foods may lead to outbreaks of unexpected bacterial diarrheal illness. For example, an outbreak of cholera in Maryland followed the ingestion of frozen coconut milk imported from Thailand (51). Investigation of the packaging process in Thailand indicated a high degree of fecal contamination of the environment with several possible opportunities for contamination of the food. Taylor *et al*. (51) point out that "In a global economy, the control of cholera and other food-borne diseases will require globally recognized regulatory standards for manufacturers of imported products."

(*iii*) *Increase in day-care center attendance*. In the United States, there are more single-parent families than ever. In addition, more women than ever are pursuing careers that require them to seek day care for their children. As the number of young children and infants in day care has grown, we have seen the re-emergence of diarrheal diseases caused by certain bacterial and protozoal pathogens among these children. The reason for the emergence of these enteric pathogens that are so common to developing areas is the compromised hygienic conditions found in these day-care settings. Infections due to *Shigella* and the protozoa *Giardia lamblia*, for instance, are common in day-care center attenders (21, 52, 53), where children may be crowded together sharing toys and sanitary facilities.

Shigella sonnei is by far the predominant serotype of *Shigella* isolated in day-care settings and is the main serotype found in industrialized countries, in general. A relationship has been noted between level of development and the serogroup of *Shigella* that is prevalent (54). *Sh. dysenteriae* is found in the least developed ecologies, and *Shigella flexneri* is found in somewhat further developed environments; *Sh. sonnei*

predominates in those niches where shigellosis persists in industrialized settings (54). A possible explanation has been proposed to explain these observations (4). O antibodies are believed to mediate protection against *sonnei* shigellosis (55). Some strains of *Plesiomonas shigelloides*, an autochthonous bacterial species of surface waters (56), express a polysaccharide O antigen identical to that of *Sh. sonnei* (57). Among populations living in less-developed ecologic conditions, the repeated antigenic stimulation by *Plesiomonas* bacteria consequent to ingestion of untreated surface waters may stimulate cross-protection against *Sh. sonnei*.

(*iv*) *Institutions that care for the aged*. In industrialized countries the proportion of the population that is elderly is steadily increasing, as is life expectancy. Improved geriatric health care has resulted in the survival of many elderly patients with chronic illnesses, many of whom live in institutions for the aged. The result is an unusual ecologic niche where there exist many elderly chronically infirm individuals with diminished host defenses (e.g., hypochlorhydria) who are fed institutional food from large-scale kitchens. Should there occur a breakdown of food hygiene in such a setting, large outbreaks of gastrointestinal disease may ensue with high attack rates and fatalities (58, 59).

(*v*) *International travel*. Approximately 30–50% of individuals from industrialized areas who travel to developing areas experience at least one episode of diarrhea due to enterotoxigenic *E. coli* during a 2-week stay (60). Potentially, travelers may transport highly virulent enteric pathogens back with them, either knowingly—e.g., the outbreak of cholera due to crabs smuggled in luggage from South America (61)—or unwittingly—e.g., the outbreak of cholera among passengers on a transcontinental airline flight from South America (62).

CHANGES IN THE EPIDEMIOLOGIC BEHAVIOR OF CHOLERA,IN PART DUE TO CHANGES IN HUMAN BEHAVIOR

One of the characteristics of cholera is its propensity for pandemic spread, wherein many countries come to be affected over many years. The seventh pandemic of cholera, due to the El Tor biotype of *Vibrio cholerae* O1 began in Sulawesi in 1961 and progressively spread to other continents over many years. The last large developing world populations that had so far escaped the ravages of cholera were finally touched when the enormous and explosive epidemic began in South America in 1991 (63).

In recent years it has become increasingly recognized that an important reservoir for *V. cholerae* O1 exists in the environment in brackish waters where the vibrios adsorb to zooplankton and other chitinous fauna (64). After the onset of the South American outbreak of cholera, it

came to be recognized that cargo ships can inadvertently disseminate *V. cholerae* O1 to new receptive brackish water environments through their practice of using ballast water to balance the vessel. Thus, ballast water of ships from South America approaching U.S. ports was found to contain the outbreak strain of *V. cholerae* O1 (65).

In late 1992 epidemic cholera due to a newly recognized serogroup of *V. cholerae* (O139) broke out in India and swiftly spread to Bangladesh (66, 67). The high rates of illness in adults in these cholera-endemic areas suggest that prior immunity to *V. cholerae* O1 does not afford protection against the new serogroup. Preliminary analysis of virulence properties of the O139 serogroup shows virtual identity to El Tor strains (68). What is remarkable about this new strain is its rapidity of spread. O139 serogroup has already caused cholera in Thailand, China, Pakistan, Kazakhstan, and Malaysia, and cases have been imported into the United States (69) and the United Kingdom (70). Moreover, one indigenous case has been observed in the United Kingdom in an individual who did not travel. This extraordinarily rapid spread is being attributed to high-volume air travel between the subcontinent and developing as well as industrialized areas of the world.

CONCLUSIONS

Changes in human behavior and ecology clearly impact on the emergence or disappearance of enteric infections. We have limited our attention to certain changes in ecology and human behavior that we believe to be of primal importance but have failed to mention others. Nevertheless, the picture from those presented is clear. With each advance in human development there arise consequences. The commercialization of food production and service, the shift in the role of women from home to work force, the increase in pediatric populations attending day-care centers and of elderly populations housed in institutions for the aged, the linking of distant places by rapid intercontinental travel, and the interdependence of economies have all impacted to modify the epidemiology of diarrheal disease. In some instances the net result is a diminution in the transmission of diarrheal diseases; in other cases the effect is a fostering of the emergence of certain enteric infections.

SUMMARY

Human populations throughout the world can be found in diverse conditions. A proportion of the population of developing countries lives in deprived conditions characterized by ramshackle housing, lack of piped water and sanitation, and widespread fecal contamination of the

environment. Enteric infections, particularly due to bacterial pathogens, are readily transmitted under these circumstances. In contrast, the majority of inhabitants of industrialized countries live in a sanitary environment that generally discourages the transmission of enteric pathogens, particularly bacteria. In both these ecologic niches, changes in human ecology and behavior are leading to the emergence of certain enteric infections. Relevant factors in developing areas include urbanization (leading to periurban slums), diminished breast-feeding, and political upheaval that results in population migrations. In industrialized areas, large-scale food production (e.g., enormous poultry farms), distribution, and retailing (e.g., fast-food chains) create opportunities where widespread and extensive outbreaks of food-borne enteric infection can ensue if a breakdown in food hygiene occurs.

REFERENCES

1. Mata, L. J. (1978) *The Children of Santa Maria Cauque: A Prospective Field Study of Health and Growth* (MIT Press, Cambridge, MA).
2. Black, R. E., Brown, K. H., Becker, S., Abdul Alim, A. R. M. & Huq, I (1982) *Am. J. Epidemiol.* **115,** 315–324.
3. Guerrant, R. L., Kirchhoff, L. V., Shields, D. S., Nations, M. K., Leslie, J., de Sousa, M. A., Araujo, J. G., Correia, L. L., Sauer, K. T., McClelland, K. E., Trowbridge, F. L. & Hughes, J. M. (1983) *J. Infect. Dis.* **148,** 986–997.
4. Ferreccio, C., Prado, V., Ojeda, A., Cayazzo, M., Abrego, P., Guers, L. & Levine, M. M. (1991) *Am. J. Epidemiol.* **134,** 614–627.
5. Levine, M. M., Ferreccio, C., Prado, V., Cayazzo, M., Abrego, P., Martinez, J., Maggi, L., Baldini, M. M., Martin, W., Maneval, D., Kay, B., Guers, L., Lior, H., Wasserman, S. S. & Nataro, J. P. (1993) *Am. J. Epidemiol.* **138,** 849–869.
6. Cravioto, A., Reyes, R. E., Ortega, R., Fernandez, G., Hernandez, R. & Lopez, D. (1988) *Epidemiol. Infect.* **101,** 123–134.
7. Nichols, B. & Soriano, H. (1977) *Am. J. Clin. Nutr.* **30,** 1457–1472.
8. Black, R. E., Lopez de Romana, Brown, K. H., Bravo, N., Bazalar, O.-G. & Kanashiro, H. C. (1989) *Am. J. Epidemiol.* **129,** 785–799.
9. DeMol, P., Brasseur, D., Hemelhof, W., Kalala, T., Butzler, J. P. & Vis, H. L. (1983) *Lancet* **i,** 516–518.
10. Bhan, M. K., Bhandari, N., Sazawal, S., Clemens, J., Raj, P., Levine, M. M. & Kaper, J. B. (1989) *Bull. W.H.O.* **67,** 281–288.
11. Levine, M. M., Prado, V., Robins-Browne, R. M., Lior, H., Kaper, J. B., Moseley, S., Gicquelais, K., Nataro, J. P., Vial, P. & Tall, B. (1988) *J. Infect. Dis.* **158,** 224–228.
12. Bhan, M. K., Raj, P., Levine, M. M., Kaper, J. B., Bhandari, N., Srivastava, R., Kumar, R. & Sazawal, S. (1989) *J. Infect. Dis.* **159,** 1061–1064.
13. Bhan, M. F., Khoshoo, V., Sommerfelt, H., Raj, P., Sazawal, S. & Srivastava, R. (1989) *Pediatr. Infect. Dis. J.* **8,** 499–502.
14. Cravioto, A., Tello, A., Navarro, A., Ruiz, J., Villafan, H., Uribe, F. & Eslava, C. (1991) *Lancet* **337,** 262–264.

15. Wanke, C., Schorling, J. B., Barrett, L. J., DeSouza, M. A. & Guerrant, R. L. (1991) *Pediatr. Infect. Dis. J.* **10,** 746–751.
16. Glass, R. I., Becker, S., Huq, I., Stoll, B. J., Khan, M. U., Merson, M. H., Lee, J. V. & Black, R. E. (1982) *Am. J. Epidemiol.* **116,** 959–970.
17. Clemens, J. D., Van Loon, F., Sack, D. A., Rao, M. R., Ahmed, F., Chakraborty, J., Kay, B. A., Khan, M. R., Yunus, M., Harris, J. R., Svennerholm, A.-M. & Holmgren, J. (1991) *Lancet* **337,** 883–884.
18. Mosley, W. H., Beneson, A. S. & Barui, R. (1968) *Bull W.H.O.* **38,** 327–334.
19. Kotloff, K. L., Wasserman, S. S., Steciak, J. Y., Tall, B. D., Losonsky, G. A., Nair, P., Morris, J. G. & Levine, M. M. (1989) *Pediatr. Infect. Dis. J.* **7,** 753–759.
20. DuPont, H. L., Gangarosa, E. J., Reller, L. B., Woodward, W. E., Armstrong, R. W., Hammond, J., Glaser, K. & Morris, G. K. (1970) *Am. J. Epidemiol.* **92,** 172–179.
21. Pickering, L., Evans, D. G., DuPont, H. L., Vollet, J. J., III & Evans, D. J., Jr. (1981) *J. Pediatr.* **8,** 539–547.
22. Levine, M. M. (1993) in *Proceedings of Eastern Pennsylvania Branch–American Society for Microbiology Symposium on the Migration of Infectious Diseases: Five Hundred Years After Columbus,* ed. Evangelista, A. T. (Plenum, New York), in press.
23. Wolman, A. & Gorman, A. (1931) *The Significance of Water-Borne Typhoid Fever Outbreaks* (Williams & Wilkins, Baltimore).
24. Anonymous (1919) *J. Am. Med. Assoc.* **72,** 997–999.
25. Glass, R. I., Svennerholm, A.-M., Stoll, B. J., Khan, M. R., Hossain, K. M. B., Huq, M. I. & Holmgren, J. (1983) *N. Engl. J. Med.* **13,** 89–92.
26. Levine, M. M. & Edelman, R. (1984) *Epidemiol. Rev.* **6,** 31–51.
27. Clemens, J. D., Stanton, B., Stoll, B., Shahid, N. S., Banu, H. & Chowdhury, A. K. M. A. (1986) *Am. J. Epidemiol.* **123,** 710–720.
28. Gunn, R. A., Kimball, A. M., Dutta, S. P., Matthew, P. P., Mahmood, R. A., Pollard, R. A., Feeley, J. C., Levine, M. M. & Feldman, R. A. (1979) *Lancet* **ii,** 730–732.
29. World Health Assembly (1981) *International Code of Marketing of Breast Milk Substitutes* (W.H.O., Geneva), WHA Resolution 34.22.
30. Kennedy, E., Bouis, H. & von Braun, J. (1992) *Soc. Sci. Med.* **35,** 689–697.
31. Carter, Jimmy (1993) *Talking Peace: A Vision for the Next Generation* (Dutton Children's Books, New York).
32. Centers for Disease Control (1992) *Morbid. Mortal. Wkly. Rep.* **41,** 913–917.
33. Mishu, B., Griffin, P. M., Tauxe, R. V., Cameron, D. N., Hutcheson, R. H. & Schaffner, W. (1991) *Ann. Intern. Med.* **115,** 190–194.
34. Vugia, D. J., Mishu, B., Smith, M., Tavris, D. R., Hickman-Brenner, F. W. & Tauxe, R. V. (1993) *Epidemiol. Infect.* **110,** 49–61.
35. Alterkruse, S., Koehler, J., Hickman-Brenner, F., Tauxe, R. V. & Ferris, K. (1993) *Epidemiol. Infect.* **110,** 17–22.
36. Rodrigue, D. C., Tauxe, R. V. & Rowe, B. (1990) *Epidemiol. Infect.* **105,** 21–27.
37. Centers for Disease Control (1991) *Salmonella Surveillance Report 1990* (U.S. Public Health Service, Atlanta).
38. Riley, L. W., Remis, R. S., Helgerson, S. D., McGhee, H. B., Wells, J. G., Davis, B. R., Herbert, R. J., Olcott, E. S., Johnson, L. M., Hargrett, N. T., Blake, P. A. & Cohen, M. L. (1983) *N. Engl. J. Med.* **308,** 681–685.
39. Spika, J., Parsons, J., Nordenberg, D., Wells, J. G., Gunn, R. A. & Blake, P. A. (1986) *J. Pediatr.* **109,** 287–291.

40. Neill, M., Tarr, P., Clausen, C., Christie, D. L. & Hickman, R. O. (1987) *Pediatrics* **80,** 37–40.
41. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rep.* **42,** 85–86.
42. Griffin, P. M., Ostroff, S. M., Tauxe, R. V., Greene, K. D., Wells, J. G., Lewis, J. H. & Blake, P. A. (1988) *Ann. Intern. Med.* **109,** 705–712.
43. Strockbine, N., Marques, L., Newland, J., Smith, H. W., Holmes, R. K. & O'Brien, A. D. (1986) *Infect. Immun.* **53,** 135–140.
44. Karch, H., Heeseman, J., Laufa, R., O'Brien, A. D., Tacket, C. O. & Levine, M. M. (1987) *Infect. Immun.* **55,** 455–461.
45. Yu, J. & Kaper, J. B. (1992) *Mol. Microbiol.* **6,** 411–417.
46. Tzipori, S., Karch, H., Wachsmuth, K. I., Robins-Browne, R. M., O'Brien, A. D., Lior, H., Cohen, M. L., Smithers, J. & Levine, M. M. (1987) *Infect. Immun.* **55,** 3117–3125.
47. Wells, J. G., Shipman, L. D., Greene, K. D., Sowers, E. G., Green, J. H., Cameron, D. N., Downes, F. P., Martin, M. L., Griffin, P. M. & Ostroff, S. M. (1990) *J. Clin. Microbiol.* **29,** 985–989.
48. Montenegro, M. A., Bulte, M., Trumpf, T., Aleksic, S., Reuter, G., Bulling, E. & Helmuth, R. (1990) *J. Clin. Microbiol.* **28,** 1417–1421.
49. Kotula, A. W., Chesnut, C. M., Emswiler, B. S. & Young, E. P. (1977) *J. Anim. Sci.* **45,** 54–58.
50. Belongia, E. A., MacDonald, K. L., Parham, G. L., White, K. E., Korlath, J. A., Lobato, M. N., Strand, S. M., Casale, K. A. & Osterholm, M. T. (1991) *J. Infect. Dis.* **164,** 338–343.
51. Taylor, J. L., Tuttle, J., Pramukul, T., O'Brien, K., Barrett, T. J., Jolbitado, B., Lim, Y. L., Vugia, D., Morris, J. G., Jr., & Tauxe, R. V. (1993) *J. Infect. Dis.* **167,** 1330–1335.
52. Weissman, J. B., Gangarosa, E. J., Schmerler, A., Marier, R. L. & Lewis, J. N. (1975) *Lancet* **i,** 88–90.
53. Black, R. E., Dykes, A. C., Sinclair, S. O. & Wells, J. G. (1977) *Pediatrics* **60,** 486–491.
54. Kostrzweski, J. & Stypulkowska-Misiurewicz, H. (1968) *Arch. Immunol. Ther. Exp.* **16,** 429–451.
55. Cohen, D., Green, M. S., Block, C., Rouach, T. & Ofek, I. (1988) *J. Infect. Dis.* **157,** 1068–1071.
56. Arai, T., Ikejima, N., Itoh, T., Sakai, S., Shimada, T. & Sakazaki, R. (1980) *J. Hyg.* **84,** 203–211.
57. Basu, S., Tharanathan, R. N. & Kontrohr, T. (1985) *FEMS Microbiol. Lett.* **28,** 7–10.
58. Levine, W. C., Smart, J. F., Bean, N. H. & Tauxe, R. V. (1991) *J. Am. Med. Assoc.* **266,** 2105–2109.
59. Carter, A. O., Borczyk, A. A., Carlson, J. A. K., Harvey, B., Hockin, J. C., Karmali, M. A., Krishnan, C., Korn, D. A. & Lior, H. (1987) *N. Engl. J. Med.* **317,** 1496–1500.
60. Black, R. E. (1986) *Rev. Infect. Dis.* **8,** Suppl., S131–S111.
61. Centers for Disease Control (1991) *Morbid. Mortal. Wkly. Rep.* **40,** 287–289.
62. Centers for Disease Control (1992) *Morbid. Mortal. Wkly. Rep.* **41,** 134.
63. Swerdlow, D. I., Mintz, E. D., Rodriguez, M., Tejada, E., Ocampo, C., Espejo, L., Greene, K. D., Saldana, W., Seminario, L. & Tauxe, R. V. (1992) *Lancet* **340,** 28–33.
64. Colwell, R. R. & Spira, W. M. (1992) in *Cholera*, eds. Barua, D. & Greenough, W. B., III (Plenum, New York), pp. 107–127.

65. McCarthy, S. A., McPhearson, R. M., Guarino, A. M.& Gaines, J. L. (1992) *Lancet* **339,** 624–625.
66. Ramamurthy, T., Garg, S., Sharma, R., Bhattacharya, S. K., Nair, G. B., Shimada, T., Takeda, T., Karasawa, T., Kurazano, H. & Pal, A. (1993) *Lancet* **341,** 703–704.
67. Cholera Working Group (1993) *Lancet* **342,** 387–390.
68. Hall, R. H., Khambaty, F. M., Kothary, M. & Keasler, S. P. (1993) *Lancet* **342,** 430.
69. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rep.* **42,** 501–503.
70. Public Health Laboratory Service (1993) *Comm. Dis. Rep. Wkly.* **3,** 173.

Dengue: The Risk to Developed and Developing Countries

THOMAS P. MONATH

In the last 20 years, dengue fever and a severe form of the disease described for the first time in the mid-1950s—dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS)—have emerged as the most important arthropod-borne viral diseases of humans (1–3). During this period the frequency of dengue fever epidemics has increased dramatically, hyperendemic transmission has been established over a geographically expanding range (Figure 1), and DHF has occurred in new areas and at higher incidence. It is estimated that up to 100 million cases of dengue fever occur annually on a worldwide basis. Approximately 250,000 cases of DHF are officially notified, and the true incidence is undoubtedly several-fold higher. Relatively few cases were recognized in the first 25 years after the initial description of this disease, while reports have risen steeply in recent years (Figure 2). The geographic expansion of epidemic dengue fever during the 1980s involved South, Central, and North America; Africa; China; and Australia, and is expected to continue in receptive regions infested by *Aedes aegypti*. Of greatest concern to the future health of the Western Hemisphere is the emergence of DHF in the Americas during the last 12 years. As illustrated below, the factors responsible for the worldwide increase in the incidence and distribution of dengue are closely linked to changes in human ecology and behavior.

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FIGURE 1 Countries with recent epidemic dengue activity and areas at risk of epidemic dengue by virtue of infestations of the principal vector, *Aedes aegypti*.

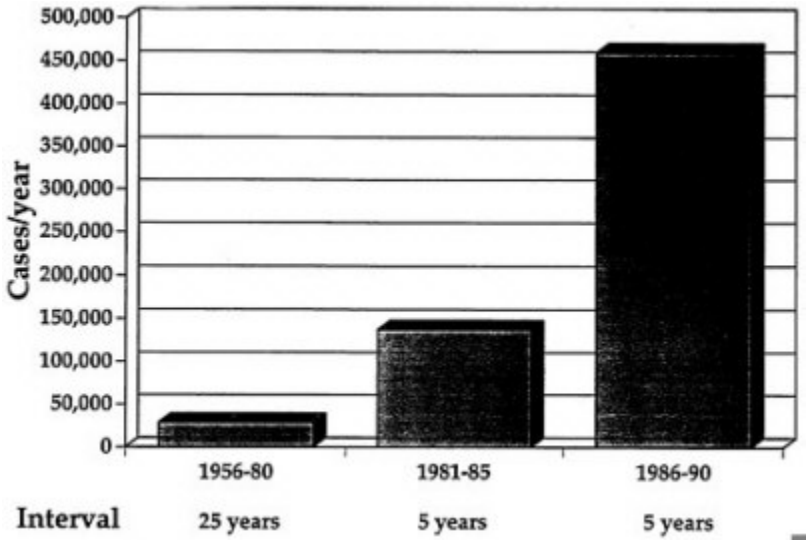

FIGURE 2 Incidence of DHF since its first description as a nosologic entity in 1954(modified from ref. 4). A dramatic increase in incidence has occurred during the 1980s, reflecting both a real increase and an apparent increase due to improved reporting.

TRANSMISSION CYCLE, DISEASE, AND PATHOGENESIS

Dengue is caused by four antigenically distinct single-strand positive-polarity RNA viruses, designated dengue types 1–4, belonging to the family Flaviviridae (5). Virus transmission in its simplest form involves the ingestion of viremic blood by mosquitoes and passage to a second susceptible human host. An extrinsic incubation period of 8–10 days is required after feeding on a viremic human for viral replication and internal dissemination in the mosquito before virus appears in the saliva and transmission on refeeding can occur. As the blood meal stimulates oviposition by the female mosquito, which undergoes at least one, and often more, reproductive cycles during the extrinsic incubation period, there is an opportunity for virus to enter the egg and be passed to the next generation of mosquitoes.

In tropical Asia and West Africa, dengue viruses are also transmitted between nonhuman primates and tree-hole-breeding mosquitoes (6), but it is uncertain what relationship, if any, exists between the forest cycle and the circulation of virus between humans and *A. aegypti*. However, the existence of a completely silent zoonotic transmission

cycle affords a potential mechanism for emergence of the disease in human populations and possibly also for selection of virus variants with altered host range and vector relationships. Virus strains representing the forest cycle have been subjected to molecular analysis and found to be distinct (see below), indicating that the forest cycle may be ecologically isolated. An important question for future research is whether the virus strains that circulate in the forest cycle are biologically distinct. Nonhuman primates challenged with strains of dengue virus isolated from humans generally develop abbreviated and significantly lower viremias than humans (7). These virus strains have not been evaluated for their capacity to induce viremic responses in their natural monkey hosts. It is possible that the apparent separation of forest and human transmission cycles reflects a reciprocal and exclusive adaptation to their hosts (or vectors).

The uncomplicated disease, classical dengue fever, is a biphasic illness beginning abruptly 3–8 days after the bite of an infected mosquito, characterized by fever, headache, severe malaise, lumbosacral aching, and generalized muscle, joint, or bone pain. Improvement after several days is followed by the reappearance of fever and development of a measles-like rash, generalized lymphadenopathy, and, sometimes, minor hemorrhagic phenomena. There are no fatalities and the disease resolves in the second week, although patients may experience prolonged convalescence, with weakness and depression. Due to the self-limited nature of the infection, little is known about the pathogenesis of classical dengue fever. High titers of virus are present in the blood during the early phase, providing the means for mosquito infection. Dengue virus is predominantly a lymphotropic agent, and the principal target cells for virus replication appear to be mononuclear phagocytes, a fact that assumes greatest relevance in the pathogenesis of DHF/DSS (8).

The onset and early phase of DHF/DSS is identical to that of dengue fever. However, shortly after onset, the patient rapidly deteriorates, developing epigastric pain, restlessness and irritability, thrombocytopenia, and signs of diffuse capillary leakage, hemoconcentration, and hypotension. Hemorrhagic manifestations of all kinds occur. In its most severe form (designated DSS, occurring in up to 1/3 of individuals with DHF), patients experience narrowing of the pulse pressure and circulatory failure. The case-fatality rate of DHF/DSS is up to 20% if untreated, but with supportive treatment consisting of fluid and electrolyte management and oxygen, fewer than 1% of such cases prove to be lethal.

The pathogenesis of DHF/DSS is only partially understood. The disease is an immunopathologic process, dependent in the vast majority

of cases on prior immune sensitization by a heterotypic dengue infection. Infection with one dengue serotype provides lifelong homologous immunity, but only transient cross-protection against other serotypes, making sequential infection possible. The relative risk of experiencing the most severe form of the disease is 100-fold higher after secondary than after a primary infection. The underlying mechanism involves enhanced infection of Fc-receptor-bearing monocyte/macrophages by dengue viruses complexed to nonneutralizing IgG antibodies (8, 9). These antibodies are the result of prior infection with a heterologous dengue virus serotype, or, in the case of infants born to immune mothers, the result of waning passive maternal antibody. The infectious immune complexes gain access to Fc-receptor-bearing monocytes more readily than dengue virus alone, with the result that the host has a larger number of infected cells containing quantitatively higher amounts of dengue virus, a phenomenon known as *immune enhancement*. A second aspect of the pathogenesis is the marked T-cell activation and induction of cross-reactive $CD4^+$ and $CD8^+$ cytotoxic T cells that recognize dengue viral antigens (principally nonstructural proteins) on infected monocytes (10). Although this process is key to the clearance of infected cells and recovery of the host from infection, the result of this interaction in a subset of patients may also have pathophysiological consequences due to release of cytokines with vasoactive or procoagulant properties (interleukins, tumor necrosis factor, platelet-activating factor, and urokinase), complement activation, and release of interferon γ. The latter molecule up-regulates expression of Fc receptors and in turn increases antibody-dependent enhancement of dengue virus replication. It is still uncertain what host- and virus-specified factors determine why one individual develops DHF/DSS and another clears secondary infection without consequence. Moreover, the precise role of different cytokine mediators in the pathogenesis of DHF/DSS remains to be defined.

HISTORY OF EPIDEMICS AND THE EMERGENCE OF DHF/DSS

Dengue fever has been recognized clinically for over 200 years. During the 18th and 19th centuries the disease occurred in intermittent pandemics affecting Asia and the Americas, occurring at intervals of up to several decades (11, 12). Spread was slow, generally by ships carrying breeding populations of A. aegypti and susceptible human hosts. In many areas, dengue was recognized only among expatriate settlers or colonial military forces, and the disease escaped attention in the indigenous populations under poor medical surveillance.

This pattern changed dramatically during and after World War II. Dengue viruses were spread by viremic military personnel to staging

areas in the Pacific. Multiple dengue serotypes were geographically shuttled by viremic troops and refugees, and the vector was spread by vehicles, water storage containers, and tires carrying along the ova and larvae of *A. aegypti*. The dissemination of virus and vector was enhanced after the war by rapid population growth and urbanization. Asian cities were characterized by poor sanitation, the necessity for domestic water storage, and crowded living conditions, creating conditions favoring breeding of *A. aegypti*. Superimposed on these phenomena was the rapid rise in air travel, providing the means for movement of viremic human beings within the region and beyond. These factors led to the establishment of hyperendemic dengue infection in Southeast Asia, a pattern of annual outbreaks caused by all four dengue serotypes, and an increasing frequency of sequential infections of children (13). It is in this setting that DHF/DSS emerged in 1954 in the Philippines. Over the next 20 years, outbreaks occurred that involved many parts of Asia and the Pacific, with a mean annual incidence of about 30,000 cases. In the 1970s and 1980s, the incidence of DHF rose dramatically, to over 250,000 cases per year (Figure 2). DHF is now the third or fourth leading cause of hospitalization of children in some areas (14).

The emergence of dengue fever and DHF in the American region provides a paradigm for the changing features of dengue epidemiology. Prior to World War II infrequent epidemics occurred at intervals of up to 37 years, probably caused by introduction of a single serotype (12). The opportunities for introduction of new viruses were limited. Outbreaks were rarely sustained for more than a few years because human populations were relatively low and isolated in island situations where immunologically susceptible hosts were rapidly exhausted. Postwar changes in dengue epidemiology in the American region occurred somewhat later than in Asia. During the 1960s, dengue virus types 2 and 3 became established in the region, and in 1977, dengue type 1 was introduced, rapidly spread, and became endemic. The pattern of intermittent epidemics at long intervals and transient circulation of one serotype changed to one of *annual* outbreaks in multiple locations and persistent cocirculation of multiple dengue serotypes. It was predictable that these events would eventually lead to the emergence of DHF in the Americas (2). In the 1970s, a few DHF cases were identified in Puerto Rico, where dengue was under intense study. The first true outbreak occurred in Cuba in 1981, with 116,000 hospitalized patients, 34,000 documented cases of DHF, and 158 deaths (15). Similar events occurred in Venezuela in 1989–1990 (16), with over 3000 cases of hemorrhagic fever, and in Rio de Janeiro in 1990. During the decade that followed the Cuban epidemic, 11 countries in the Americas have reported DHF (Figure 3).

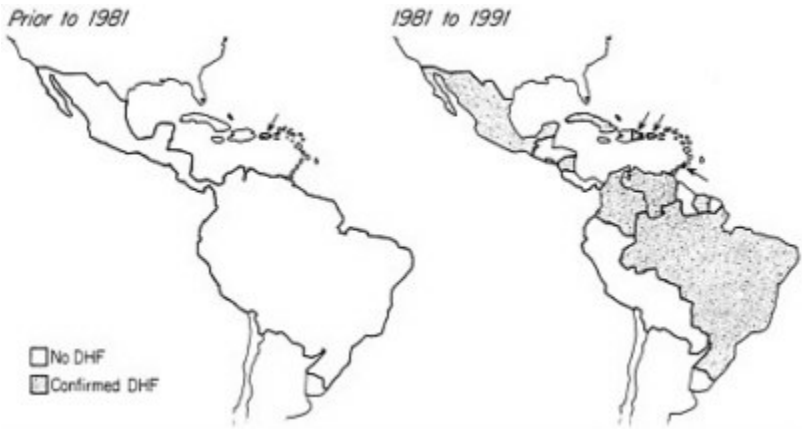

FIGURE 3 Expansion of DHF in the Western Hemisphere. Prior to 1981, only Puerto Rico reported sporadic cases of DHF. The first major outbreak occurred in Cuba in 1981. By 1991, 11 countries had been affected by the disease. Arrows indicate island nations affected in the Caribbean region.

DENGUE IN THE DEVELOPED WORLD

Every year 30–100 cases of dengue are reported in the United States in persons who have traveled to tropical countries. Many such cases undoubtedly go unreported. A similar incidence has been recorded among Swedish tourists, and it probably occurs elsewhere in Europe as well. Unlike most of Europe, parts of the United States have a resident fauna of efficient vectors (*A. aegypti* and *Aedes albopictus*; see below), and there is a risk of secondary spread. On two occasions (1980 and 1986) small outbreaks followed the introduction of dengue from Mexico into southern Texas. The introduction and spread of dengue outbreaks in the southern United States remains a potential threat, particularly in cities along the Gulf of Mexico, where *A. aegypti* and *A. albopictus* are abundant and major pest problems. As would be expected from the requirement for sequential infections, DHF has been rare in travelers and does not currently pose a threat to the developed world.

ECOLOGIC BASIS FOR THE EMERGENCE OF DENGUE

Underlying the emergence of DHF in the Western Hemisphere are changes in human and mosquito ecologies that affect the rate and geographic range of virus transmission. The principal vector, *A. aegypti*, has made extraordinary evolutionary adjustments to coexist with human

beings. This mosquito originated (and still exists) in Africa as a forest species feeding principally on rodents and other wild animals and adapted to lay eggs and undergo larval development in forest tree holes containing rainwater. However, a subspecies *A. aegypti aegypti*, evolved in Africa to become a highly domesticated animal, following humankind on its journeys and migrations to the corners of the globe, breeding in the artificial containers used for storing clean water, resting between blood meals in human habitations away from predators and harsh weather, flying rarely more than 50 yards from these convenient locations, and adopting wary biting habits around its observant and dexterous human prey. Interestingly, *A. aegypti* is not a very efficient vector of dengue viruses, and has both a low susceptibility to oral infection and low rate of transovarial infection (7). Thus, virus titers in the blood of human hosts must exceed 10^5–10^7 virus particles per ml for infection and transmission to be sustained. The vector may thus serve as an important selection mechanism or biological filter for maintaining virus virulence at a high level, since only virus strains that replicate efficiently in humans and produce high viremias are transmissible by this mosquito.

The phenomena of unchecked human population growth in the tropics and the dramatic redistribution of the human population into urban centers in search of better amenities, employment, and education have greatly influenced the epidemiology of dengue and the density and distribution of the domestic vector, *A. aegypti*. This redistribution of the human population has occurred in all countries where dengue is endemic. In the American region, for example, the urban population nearly doubled during the period (1970–1990) in which dengue emerged as a major health problem, whereas rural populations remained nearly constant. This trend will continue, with over 80% of the Latin American population predicted to inhabit cities within the next 30 years. Insufficient urban piped water supplies, necessitating the storage of water for drinking and washing, and poor sanitation, resulting in the accumulation of vast amounts of human detritus that collect rainwater, such as discarded bottles, cans, and automobile tires, have been responsible for an enormous expansion of *A. aegypti* vector populations. This environmental transformation has occurred in a setting where the supply of susceptible human hosts for dengue transmission is now virtually inexhaustible.

These changes in human and vector ecology have been accompanied by a decreased willingness of societies to undertake effective mosquito control in the context of disease prevention. In 1947, the Pan American Health Organization initiated a hemisphere-wide campaign to eradicate *A. aegypti*. Efforts were successful in a number of countries, with the

result that between 1947 and 1972, the vector had been eliminated from 19 countries, representing over 73% of the area originally infested (Figure 4) (2, 17). However, around 1972 the program ran out of steam. The United States entered the campaign late (1961) and gave up early, as entomologists and sanitarians found the job insurmountable and congressmen found the funding requirements unacceptable. The United States and other countries in the Caribbean region provided a source for exportation of *A. aegypti* to countries attempting to maintain their vector-free status. In those countries that had achieved eradication, mosquito control efforts sagged under the competition with other priorities for scarce health resources and with the increasing demands imposed by the conditions in bursting megacities such as Sao Paulo and Rio de Janeiro. Lack of funding, leadership, and morale eroded the once-proud mosquito control agencies (18). The expansion of urban human populations in Latin America and the creation of vast amounts of nonbiodegradable detritus breeding vast populations of *A. aegypti* simply outstripped the human capability to restrict this species. Within 10 years *A. aegypti* had retaken virtually all of South and Central America (2) (Figure 4). In 1986, an explosive outbreak of dengue 1 involving over 1 million cases struck Rio de Janeiro. Subsequently, dengue epidemics

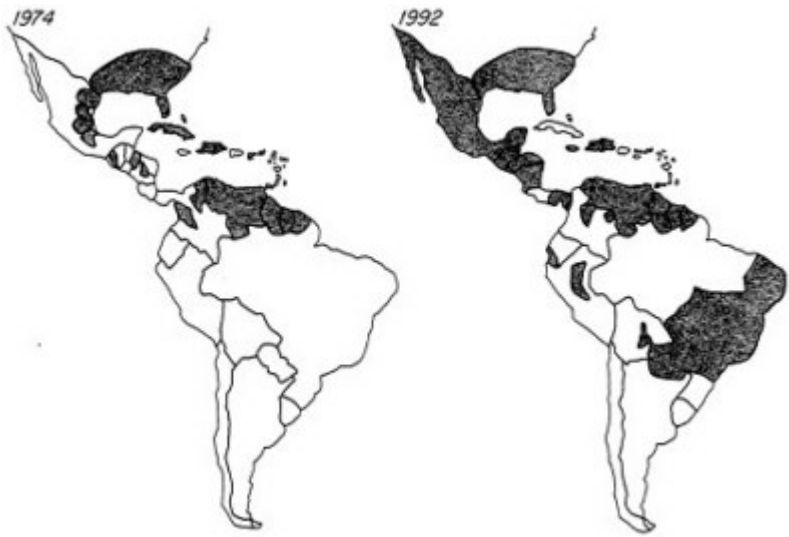

FIGURE 4 Reinfestation of Central and South America by *A. aegypti* (shaded areas) after several decades, ending in 1972, of intensive efforts to control this vector species. The reinfestation has led to explosive epidemics of dengue and DHF in South America.

have occurred in Paraguay, Bolivia, Peru, Ecuador, Colombia, and Venezuela. The sequential introduction of dengue type 1 followed by dengue 2 resulted in outbreaks of DHF in Caracas (1989) and Rio de Janeiro (1990). Three of the four dengue serotypes are currently endemic in the region, and it is probable that dengue type 3 virus will be reintroduced in the near future. It is clear that the factors that led to the emergence of DHF as a major public health problem in Asia now exist in the American region.

INTRODUCTION AND SPREAD OF A SECOND DENGUE VECTOR IN THE AMERICAS

An interesting illustration of the relationship between human and vector ecology is provided by the introduction and spread of another dengue vector, *A. albopictus*, from Asia to the Americas in the 1980s. This mosquito is responsible for endemic transmission of dengue in Asia and for epidemic spread in circumstances where *A. aegypti* is absent or in low density. In 1985, the mosquito was discovered in Houston, Texas, and a year later in Rio de Janeiro, representing separate introductions from different areas of Asia (19, 20). Human commerce in used truck tires imported for the purpose of recapping was responsible for the importation of eggs or larvae of the mosquito (21). These tires were stored in the open prior to export, where they collected rainwater and were used as oviposition sites by *A. albopictus*. Over a million tires per year were imported into the United States from Asia, and approximately 20% of these, unfit for recapping purposes, were discarded in the environment. The *A. albopictus* invasion rapidly spread, probably in large part by the tire trade, extending the range of this winter-hardy species throughout the eastern United States. A similar expansion has occurred in Brazil. Dissemination of the mosquito is continuing, and in 1993 it invaded the Dominican Republic (C. Peña, personal communication). It was predicted that this aggressive and adaptable species would become implicated in the transmission of indigenous viruses, and this has in fact occurred; five different agents, including two human pathogens (eastern equine encephalitis and dengue viruses) have now been recovered from the species (ref. 22; P. Reiter, personal communication). The full dynamics of this unique episode have yet to play out, but it will be surprising if a significant public health problem does not emerge in the future. The accumulation of vast numbers of nonbiodegradable transportable man-made mosquito-breeding devices lies at the root of the dengue vector problem. Municipal landfills reject these objects, which are thus often dumped illegally in the environment, and no recycling has proven cost effective.

MOLECULAR EPIDEMIOLOGY AND VIRUS VARIATION

Little is presently known about the role of virus-specified factors in the transmission and pathogenesis of dengue. Considerable microevolution of dengue virus strains has been found in studies employing monoclonal antibody analysis (23), RNA fingerprinting (24), or sequencing of selected regions of the genome (25). Since variants with similar genetic structure are found within a specific geographic location, such studies have elucidated movements of dengue viruses and the source of epidemics. The results also suggest that genetic variation in virus strains may determine virulence and explain the changing patterns of disease. Two examples will be given to illustrate these different points.

Use of Dengue Genetic Analysis to Explain the Emergence of Dengue in Africa

In modern times, there were virtually no reports of human dengue in Africa except for scattered cases in the 1960s in Nigeria and 1970s in Senegal. Serological surveys in 1970 in Nigeria suggested the presence of a zoonotic transmission cycle involving monkeys and a variety of tree-hole-breeding *Aedes* spp. (26), and between 1974 and 1981, French scientists verified the existence of such a cycle in Côte d'Ivoire and Senegal (27). However, no outbreaks of human disease were recorded in the region around forests supporting this cycle, and the relevance of a forest transmission cycle to human health remains obscure. Indeed, the outbreak of dengue fever closest to Africa occurred in the Indian Ocean (Seychelle Islands) in 1977.

In 1982, however, dengue appeared along the coast of Kenya, and shortly thereafter in the Ivory Coast and Burkina Faso. These were typical *A. aegypti*-borne epidemics, characterized by classical dengue fever. In 1983–1984, the disease spread up the coast of East Africa to involve Sudan and Somalia, where it has remained endemic. These events posed a number of questions: Why did these outbreaks arise? Were they interconnected? Was there a relationship between the outbreaks and the indigenous forest transmission cycle? These questions were resolved by a comparison of strains using hybridization probes (28) and by an analysis of nucleotide sequences of a 240-bp region at the junction of the E and NS1 proteins (25). Virus strains were available for study representing the forest cycle and the epidemics in Burkina Faso and the Seychelles. A close genetic relationship was found between the strains from West Africa representing the forest cycle, and these strains were quite distinct from those causing the epidemics. The virus strains from the Burkina Faso epidemic were similar to the strains responsible

for the earlier outbreak in the Seychelles, and these in turn resembled virus isolates from Sri Lanka and Indonesia. Thus, it appeared that the epidemics arose by introduction of dengue 2 virus from afar and were not the result of spill-over from the forest transmission cycle. The molecular analysis confirmed the role of humans in the dissemination of dengue viruses. The virus was apparently introduced to the Seychelles in 1977 from Sri Lanka or another location to the East, and this was also the source of introduction into East and West Africa.

Molecular and Biological Evidence for Variation in Dengue Virulence

In the Americas, dengue type 2 has been responsible for repeated outbreaks of classical dengue fever as well as for the explosive appearance of DHF in Cuba, Venezuela, and Brazil. This paradox could be explained solely on the basis of the epidemiological events leading to the establishment of hyperendemic transmission of multiple serotypes and an increased incidence of sequential infections. However, circumstantial evidence from molecular analysis suggests that the dengue 2 strain responsible for the occurrence of severe disease may represent a variant with increased virulence for humans.

A comparison of the gene sequences of dengue type 2 viruses from the Caribbean region indicated that two distinct variants were cocirculating over a period of many years (25). One genetic variant represented the Puerto Rican strain, which had been introduced into the region in 1969 and had persisted in an endemic-epidemic pattern, associated with classical dengue fever. A second variant represented a strain first isolated in Jamaica in 1981. The Jamaican genotype was responsible for the DHF epidemics in Venezuela (1989) and Brazil (1990). Since Jamaica and Cuba are geographically juxtaposed, it is probable that this variant caused the 1981 Cuban DHF outbreak (virus strains isolated in Cuba have never been available for comparative study).* Sequence homology between the Jamaican variant and virus strains from Vietnam suggested the original source of introduction—a plausible conclusion, since Cuba

* A recent report describes the genetic analysis of dengue type 2 strains recovered during the 1981 DHF epidemic in Cuba [Guzman MG, Deubel V, Pelegrino JL, et al. Partial nucleotide and amino acid sequence of the envelope and E/NS1 gene junction of four dengue 2 strains isolated during the 1981 DHF/DSS Cuban epidemic. *Am J Trop Med Hyg*, 1994, in press]. The epidemic viruses closely resemble the New Guinea C. strain, the prototype dengue 2 virus, which was isolated in 1944, and has persisted to the present day in Asia. This finding supports the conclusion that the epidemic originated by introduction of virus from Asia.

and North Vietnam had political ties at the time of the outbreak. In contrast, the Puerto Rican genotype resembled contemporary virus strains from Polynesia, where for the most part disease was mild.

Although these results imply that dengue virus strains vary in virulence and that this variation may play a role in the incidence and distribution of DHF, the molecular basis for virulence cannot be tested directly, due to the lack of an animal model of DHF. A preliminary analysis (29) demonstrating variation in the ability of dengue virus strains to replicate in human peripheral blood monocytes and higher replication of isolates from DHF patients than from dengue fever patients requires further evaluation and confirmation in prospective studies.

FLAVIVIRUS AND DENGUE EVOLUTION:A BIOLOGICAL PERSPECTIVE AND SOME SPECULATIONSON FUTURE CHANGES IN DENGUE EPIDEMIOLOGY

The 68 recognized flaviviruses are classified into serological complexes, and these in turn correspond closely to their mode of transmission. Nearly half, including the dengue viruses, are mosquito-borne, 28% are transmitted by ticks, and 20% are apparently zoonotic infections transmitted by contact between rodents and bats (30). From an evolutionary standpoint, it is of interest that the viruses belonging to the tick-borne encephalitis complex seem to have developed the capacity to infect epithelial surfaces, since members of this group are shed in milk of infected domestic livestock and can spread to humans by this means as well as by aerosols in the laboratory. This capacity for epithelial infection is apparently more highly developed in the bat- and rodent-associated agents which have no known arthropod vectors, probably representing an adaptation to transmission between hosts that are relatively abundant under circumstances of low vector density. Evolution of the flaviviruses appears to be in the direction of loss of dependence on arthropod vectors, with the most evolved forms represented by hepatitis C and simian hemorrhagic fever viruses.

The mosquito-borne flaviviruses, exemplified by St. Louis encephalitis, Japanese encephalitis, and Murray Valley encephalitis viruses, are presumed to be relatively primitive forms on the basis of their varied vector and host associations and marked radiation of closely related antigenic types. A biological common denominator of these agents is their marked neurotropism. Dengue and yellow fever viruses are distinct antigenically but share links with the more primitive mosquito-borne encephalitis viruses, as illustrated by the high nucleotide sequence homology between dengue type 2 virus and Edge Hill virus (31).

However, dengue and yellow fever viruses have evolved an important biological feature that clearly separates them from their more primitive neurotropic predecessors, namely lymphotropism, and they have subverted the primate monocyte/macrophage as principal target cell for replication (32). One result of this adaptation has been a marked restriction of host range to the primates and a parallel restriction in the virus-vector relationship to a few species of *Aedes* spp. with predilection for feeding on primate blood.

The lymphotropism of dengue viruses is consistent with the production and release of large quantities of virus into the blood stream, a feature that is essential to transmission of the virus by the relatively insusceptible *A. aegypti* vector. It is important to note that the change in tropism from nervous tissue to lymphoid cells is critical to survival of both virus and host species, since viremias of the magnitude achieved in dengue would produce a very high risk of hematogenous spread of a neurovirulent/neuroinvasive agent to the central nervous system. Loss of neurovirulence in favor of lymphotropism is thus permissive for transmission without affecting host survival.

The remarkable ability of dengue viruses to replicate to high titers in human tissues raises the possibility that, should a variant emerge with altered tropism—e.g., for epithelial tissues permitting shedding in respiratory secretions, or conversely for nervous tissues, permitting neuroinvasion—the result could fundamentally change the route of transmission or the expression of disease in the host. While this may appear to be a remote possibility, we should remember that other flaviviruses, including the bat- and rodent-associated flaviviruses and simian hemorrhagic fever virus, have evolved in these directions. It is thus not inconceivable in the context of flavivirus evolution that dengue could undergo genetic changes that would transform it into a more dangerous pathogen transmissible by the respiratory route or capable of causing encephalitis.

In general, however, dengue and other flaviviruses [e.g., yellow fever virus (33) and St. Louis encephalitis virus (34)] display a rather remarkable genetic stability, given their RNA genomes and high rates of mutation. A comparison of the genetic variability of dengue type 2 strains with that of influenza viruses, based on a comparison of nucleotide and amino acid sequence divergence, shows that dengue variation is low, similar to that of influenza C, a virus that has a single (human) host and is not subjected to the marked genetic shifts typical of influenza A virus, which undergoes recombinational events in alternative hosts and has a higher proportion of mutations that result in amino acid changes. Dengue virus strains belonging to the same serotype vary by no more than 10% at the nucleotide level and 4% at the amino acid

level. The constraints on dengue virus evolution probably reflect the need to preserve critical determinants involved in virus–cell interactions across two very diverse phyla (Arthropoda and Chordata). Nevertheless, it is clear that four dengue serotypes have evolved and that the accumulation of mutations is a continuing and directional process (24, 25). The emergence of a new serotype of dengue virus differing at one or more critical neutralization epitopes will thus undoubtedly occur at some time in the future. Dual infections of humans and vectors with different dengue serotypes may be an increasingly frequent event (35), raising the possibility that intramolecular recombination in addition to mutational change could be a mechanism for the emergence of new types. The appearance of a fifth dengue serotype would fundamentally alter the epidemiology of dengue and DHF.

PREVENTION AND CONTROL

The ability to control dengue by reduction of the vectors responsible for transmission seems increasingly remote. Efforts to develop effective live, attenuated, and genetically engineered vaccines are under way, and the impetus to move candidate vaccines into large-scale human trials will increase as DHF emerges as a major health problem in the Western Hemisphere. The immunopathogenesis of DHF demands that durable protective immunity to all four serotypes be elicited simultaneously, posing a formidable challenge to vaccine development.

SUMMARY

Dengue viruses are members of the Flaviviridae, transmitted principally in a cycle involving humans and mosquito vectors. In the last 20 years the incidence of dengue fever epidemics has increased and hyperendemic transmission has been established over a geographically expanding area. A severe form, dengue hemorrhagic fever (DHF), is an immunopathologic disease occurring in persons who experience sequential dengue infections. The risk of sequential infections, and consequently the incidence of DHF, has risen dramatically, first in Asia and now in the Americas. At the root of the emergence of dengue as a major health problem are changes in human demography and behavior, leading to unchecked populations of and increased exposure to the principal domestic mosquito vector, *Aedes aegypti*. Virus-specified factors also influence the epidemiology of dengue. Speculations on future events in the epidemiology, evolution, and biological expression of dengue were presented.

REFERENCES

1. Halstead, S. B. (1980) *Bull. W.H.O.* **58,** 1–21.
2. Gubler, D. J. (1989) *Am. J. Trop. Med. Hyg.* **40,** 571–578.
3. Pinheiro, F. P. (1989) *Epidemiol. Bull. (PAHO)* **10,** 1–8.
4. Gubler, D. J. (1991) *Virus Info. Exchange Newslett.* **8,** 2–3.
5. Chambers, T. J., Hahn, C. S. & Galler, R. (1990) *Annu. Rev. Microbiol* . **44,** 649.
6. Rudnick, A. (1978) *Asian J. Infect. Dis.* **2,** 156–168.
7. Gubler, D. J. (1988) in *The Arboviruses: Epidemiology and Ecology* , ed. Monath, T. P. (CRC, Boca Raton, FL), Vol. 2, pp. 223–260.
8. Halstead, S. B. (1989) *Rev. Infect. Dis.* **11,** S830–S839.
9. Halstead, S. B. (1988) *Science* **239,** 476–481.
10. Kurane, I. & Ennis, F. A. (1992) *Semin. Immunol.* **4,** 121–130.
11. Carey, D. E. (1971) *J. Hist. Med.* **26,** 243–262.
12. Ehrenkrantz, N. J., Ventura, A. K., Cuadrado, R. R., Pond, W. L. & Porter, J. E. (1971) *N. Engl. J. Med.* **285,** 1460–1469.
13. Gubler, D. J. (1989) in *Dengue Hemorrhagic Fever: Diagnosis, Treatment and Control*, ed. Thongcharoen, P. (W.H.O., New Delhi).
14. Halstead, S. B. (1990) *Asian J. Trop. Med. Pub. Health* **21,** 636.
15. Kouri, G. & Guzman, M. G. (1986) *Bull. PAHO* **20,** 24–26.
16. Anonymous (1990) *Epidemiol. Bull. (PAHO)* **2,** 1–2.
17. Schliesman, D. J. & Calheiros, L. B. (1974) *Mosq. News* **34,** 1–9.
18. Halstead, S. B. (1984) *Rev. Infect. Dis.* **16,** 251–264.
19. Sprenger, D. & Wuithiranyagool, T. (1986) *Am. Mosq. Control Assoc. J.* **2,** 217–221.
20. Forattini, O. P. (1986) *Rev. Saude Publica* **20,** 244–250.
21. Reiter, P. & Sprenger, D. (1987) *Am. Mosq. Control Assoc. J.* **3,** 494–500.
22. Mitchell, C. J., Niebylski, M. L., Smith, G. C., Karabatsos, N., Martin, D., Mutebi, J.-P., Craig, G. B., Jr., & Mahler, M. J. (1992) *Science* **257,** 526–527.
23. Monath, T. P., Wands, J. R., Hill, L. J., Brown, N. V., Marciniak, R. A., Wong, M. A., Gentry, M. K., Burke, D. S., Grant, J. A. & Trent, D. W. (1986) *Virology* **154,** 313–324.
24. Trent, D. W., Grant, J. A., Rosen, L. & Monath, T. P. (1983) *Virology* **128,** 271–284.
25. Rico-Hesse, R. (1990) *Virology* **174,** 479–493.
26. Monath, T. P., Lee, V. H., Wilson, D. C., Fagbami, A. & Tomori, O. (1974) *Trans. R. Soc. Trop. Med. Hyg.* **68,** 30–39.
27. Roche, J. C., Cordellier, R., Hervy, J. P., Digoutte, J. P. & Monteny, N. (1983) *Ann. Virol.* **134,** 233–239.
28. Kerschner, J. A., Vorndam, A. V., Monath, T. P. & Trent, D. W. (1986) *J. Gen. Virol.* **67,** 2645–2661.
29. Kliks, S. (1992) *AIDS Res. Hum. Retroviruses* **6,** 993–998.
30. Karabatsos, N., ed. (1985) *Catalogue of Arthropod-Borne Viruses and Certain Other Viruses of Vertebrates* (Am. Soc. Trop. Med. Hyg., San Antonio, TX).
31. Blok, J., Henchal, E. A. & Gorman, B. J. (1984) *J. Gen. Virol.* **65,** 2173–2180.
32. Monath, T. P. (1985) in *The Togaviridae and Flaviviridae*, eds. Schlesinger, S. & Schlesinger, M. J. (Plenum, New York), pp. 375–440.
33. Duebel, V., Digoutte, J. P., Monath, T. P. & Girard, M. (1986) *J. Gen. Virol.* **67,** 209–213.
34. Trent, D. W., Monath, T. P, Bowen, G. S., Vorndam, A. V., Cropp, C. B. & Kemp, G. E. (1980) *Ann. N.Y. Acad. Sci.* **354,** 219–228.
35. Gubler, D. J. (1985) *Am. J. Trop. Med. Hyg.* **34,** 170–173.

Hepatitis Viruses: Changing Patterns of Human Disease

ROBERT H. PURCELL

Viral hepatitis is caused by at least five distinct viruses. Each belongs to an entirely different family of viruses, and they have very little in common except the target organ they affect, the liver, and a certain degree of shared epidemiology. Each of the five viruses has a worldwide distribution. In general, the regional incidence rates for each virus are lowest in the Western Hemisphere and northern regions and highest in the Eastern Hemisphere and tropical regions. Thus, the incidence of infection with these five viruses is generally lowest in industrialized and developed countries and highest in less-developed regions. Two of the viruses [hepatitis A virus (HAV) and hepatitis E virus (HEV)] are spread principally by fecal–oral means and three [hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D virus (HDV)] are spread principally by exposure to blood, although HBV is frequently spread by unprotected sex. Although it has been sought, arthropod-borne or other vector-mediated transmission of the blood-borne hepatitis viruses has not been found. Other viruses, principally from the families *Arenaviridae, Bunyaviridae, Flaviviridae, Filoviridae,* and *Herpesviridae*, also cause hepatitis as part of systemic diseases, but these are generally not grouped with the hepatitis viruses.

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Changes in human ecology and behavior have had discernable effects on the epidemiology of the hepatitis viruses in different ways and to different degrees. Following is a brief summary of each virus and how it has interacted with its host. In some cases the view is but a glimpse because the existence of three of the five viruses has been recognized for less than 20 years.

HEPATITIS A VIRUS

History

HAV may be traced back to epidemics of "campaign jaundice" that afflicted the armies of the Middle Ages and that has continued to be a serious problem up to and including the Korean and Vietnamese conflicts (see ref. 1). The first civilian epidemics of hepatitis were recorded in Europe in the 17th and 18th centuries. The differentiation of hepatitis A, then called infectious hepatitis, from hepatitis B, then called serum hepatitis, came principally from studies in volunteers in Europe and the United States from the 1940s through the 1960s. It was not until the 1970s that HAV was transmitted to laboratory animals (marmoset monkeys and chimpanzees), and the virus was isolated in cell culture in 1979 (2). It remains the only one of the five hepatitis viruses that has been unequivocally isolated and serially propagated in cell culture. Effective inactivated whole virus vaccines have been developed. The first was licensed in Europe in 1991 and two inactivated hepatitis A vaccines should be licensed in the United States within the next 1 or 2 years.

Epidemiology

HAV is commonly transmitted by the fecal–oral route, either by person-to-person spread (the most common) or in common-source epidemics caused by contamination of food or water. Viremia occurs during the incubation period and the early acute phase of hepatitis A, and transmission by transfusion or recently by contaminated commercial factor VIII (3) has been reported, but such blood-borne transmissions are rare.

Virology

HAV is classified in the family *Picornaviridae*, genus *Hepatovirus*. Based on genomic sequence heterogeneity, seven genotypes of HAV have been identified, but one serotype comprises all of these (4). The

infectivity titer of HAV in the feces may be as high as 10^9 infectious doses per gram (5). Viremias lasting as long as several weeks and with titers as high as 10^5 infectious doses per ml of blood have been recorded (3, 5).

Clinical Characteristics

The incubation period of hepatitis A averages 25 days. Hepatitis A is generally mild to moderate in severity, with a mortality rate of 0.2% or less, and never becomes chronic. However, inapparent infection with shedding of virus may persist for up to 6 months in neonates.

Changing Patterns

The epidemiology of HAV is highly influenced by personal and public hygiene. Hepatitis A has diminished in importance over the past several decades in developed and industrialized countries of northern and western Europe and North America (6). A gradient from northern to southern Europe exists, in which hepatitis A is almost nonexistent in Scandinavian countries but is still present, albeit with diminishing importance, in the Mediterranean countries of Europe. A similar pattern of diminishing incidence of hepatitis A has been seen in other developed and transitional regions, including Australia, China, Japan, and Hong Kong, but HAV infection remains highly endemic in many other regions of Central and Southeast Asia, Africa, and Central and South America. As with other picornaviruses such as poliovirus, infection of the very young is usually inapparent, and disease becomes progressively more severe with increasing age. Thus, as the mean age of infection increases with improved sanitation, clinical hepatitis paradoxically becomes more apparent rather than less apparent.

In the United States, hepatitis occurred as recurring epidemics through the 1970s, with 7- to 10-year periodicity (7). When serologic tests for HAV infection became available, HAV was identified as the principal cause of these epidemics, and until the 1980s it remained the type of viral hepatitis most frequently reported to the Centers for Disease Control. Since the last epidemic in the early 1970s, HAV has diminished in importance in the United States but recently has leveled off and is still responsible for `30% of reported clinical hepatitis in this country (8). The changing epidemiology of HAV is thought to be the result of better sanitation, principally in the form of improved treatment of water and sewage and improved personal hygiene. Consequently, the epidemiology of HAV has changed from one of diffuse person-to-person spread to one of association with specific high-risk groups. Thus,

based on data from the Centers for Disease Control, international travel to areas of HAV endemicity, exposure to very young children (often in the setting of day-care facilities), drug abuse, and homosexual activity collectively accounted for `50% of cases of hepatitis A studied (7). Not specifically listed but often cited as a cause of hepatitis A is ingestion of raw or undercooked shellfish, the cause of a massive epidemic of hepatitis A in Shanghai in 1988 (9). Approximately a third of hepatitis A cases have no identifiable risk factor. Thus, hepatitis A has become a more up-scale disease in the United States, with proclivities for "yuppie" lifestyles, but it remains a serious endemic problem especially among Native Americans on or near reservations in the western United States. Hepatitis A in such settings accounts for much of the excess incidence of this disease in the western states in recent years. It also is a periodic problem among native populations in Alaska.

HEPATITIS B VIRUS

History

The first report of what must surely have been hepatitis B was that of Lürman who reported on an epidemic of hepatitis that occurred in shipyard workers in Bremen following vaccination against smallpox with glycerinated lymph of human origin in 1883 (see ref. 1). Serum hepatitis (hepatitis B) rose in importance in parallel with the increasing use of syringes and needles for the treatment of syphilis during the first half of the 20th century. Similar disease occurred in diabetics who were given insulin with improperly sterilized syringes and needles. Epidemics of hepatitis, some quite large, followed the administration of yellow fever vaccines that had been stabilized by the addition of human serum. Eventually, the association of hepatitis B specifically with blood and blood products was recognized and its distinctness from hepatitis A documented in volunteer studies. Hepatitis B was first transmitted to laboratory animals (the chimpanzee) in the 1970s. Although HBV has never been isolated and serially propagated in cell culture in any practical system, the first hepatitis B vaccine was licensed in the United States in 1981. This unique vaccine was prepared from viral envelope protein [hepatitis B surface antigen (HBsAg)] that was purified from the plasma of chronically infected individuals. Although safe and highly efficacious, the plasma-derived vaccine was replaced with a recombinant vaccine prepared in yeast, in part because the principal source of HBsAg-positive plasma for the manufacture of vaccine was from the same population that subsequently was at highest risk of contracting AIDS.

Epidemiology

Transmission of HBV is principally by exposure to blood or blood products, but transmission by unprotected sex (both homosexual and heterosexual) is also common. In Asia, perinatal transmission from the infected mother to her offspring is an important mode of spread, whereas horizontal transmission among very young children is more important in Africa.

Virology

HBV is classified in the family *Hepadnaviridae*, genus *Orthoepadnavirus*. Based upon genomic heterogeneity, five genotypes of HBV have been identified, but one serotype comprises all of these (10). The infectivity titer of HBV in blood may be 10^8 or more infectious doses per ml (11). The virus has been found in saliva and semen and this may contribute to its sexual transmission.

Clinical

The incubation period of hepatitis B averages 75 days. The disease is usually moderate but may be severe, with a mortality rate of 0.2% to 2%. Disease is less likely to be clinically severe but more likely to become chronic in the very young: >90% of infected newborns develop chronic infection, and this progressively diminishes to 6 years of age, when the adult chronicity rate of 2–7% is reached (12).

Changing Patterns

Since serologic tests for the detection of HBV infection were first applied in the mid-1960s the incidence of hepatitis B progressively increased until the mid-1980s, when it reached a plateau and began to decrease slightly, probably as a result of intensive efforts to control the spread of human immunodeficiency virus (HIV) in the same high-risk populations. Hepatitis B vaccine appears to have had little impact on the incidence of hepatitis B (13). The rise in incidence of hepatitis B during the 1960s, 1970s, and 1980s is believed to have been due principally to changing lifestyles within components of the U.S. population. Specifically, the increase in unprotected sex, both homosexual and heterosexual, and the rise in the drug culture, especially the increased use of illicit parenteral drugs, are thought to have been the most important factors in the spread of HBV. Thus, the more recent epidemic of HIV infection among these high-risk populations is an echo of the earlier epidemic of

hepatitis B. It is not surprising that many researchers with experience in studying HBV made the transition to the study of HIV and its epidemiology early in the epidemic of AIDS.

Other subsets of the population who were at high risk of acquiring hepatitis B have fared better. Indigenous populations in Alaska, in whom the incidence of hepatitis B was quite high, received universal pediatric and adult vaccination with hepatitis B vaccine. A spectacular decrease in the incidence of hepatitis B has been observed, demonstrating that control of this disease by immunoprophylaxis is possible if carried out appropriately (14). Recipients of blood and blood products were two other groups in whom hepatitis B was a serious risk. During the 1960s, up to 10% of recipients of massive blood transfusion developed transfusion-associated hepatitis B, and >80% of hemophiliacs were infected with HBV that contaminated commercial lots of pooled clotting factors. Conversion to an all-volunteer blood donor population and mandated screening of donors for serologic markers of HBV infection have virtually eradicated transfusion-associated hepatitis B. Similarly, serologic screening, coupled with incorporation of inactivation steps in the manufacture of commercial clotting factors, and vaccination of hemophiliacs with hepatitis B vaccine have virtually eliminated HBV infection among this population. However, control of hepatitis B in the general population of the United States will require universal vaccination in addition to continuing efforts to interdict transmission of HBV within the identified high-risk populations (13).

HEPATITIS C VIRUS

History

The existence of a third type of viral hepatitis was not appreciated until 1975, when the application of recently developed diagnostic tests for hepatitis A and hepatitis B to stored samples of prospectively studied cases of transfusion-associated hepatitis revealed that most of the cases were neither hepatitis A nor hepatitis B (15). The proportion of such "non-A, non-B" (NANB) cases increased from `65% before screening for HBV markers was instituted to >90% as HBV carriers were excluded from the blood donor population (16). The "new" hepatitis had an average incubation period of `7–10 weeks, which is intermediate between those of hepatitis A and hepatitis B and similar to the mean incubation period of all cases of transfusion-associated hepatitis in the 1950s and 1960s, suggesting that NANB hepatitis had also been the principal cause of transfusion-associated hepatitis in the

past (17). Although NANB hepatitis was transmitted to chimpanzees in 1978, thus establishing its infectious nature, it was not until 1989 that the virus itself was identified. Indirect evidence of the nature of the virus had been obtained by experiments in chimpanzees: it had been shown to be inactivated by lipid solvents and therefore was probably an enveloped virus, and its size had been estimated at between 30 and 60 nm in diameter. However, in 1989 a small piece of the viral RNA was reverse-transcribed, cloned, and sequenced, and this resulted in the subsequent cloning and sequencing of the entire genome (18). The virion was only recently tentatively visualized by electron microscopy (19). Nevertheless, all of the viral proteins encoded by the HCV genome have been expressed, and some of these serve as the basis for currently licensed serologic tests for antibody to HCV.

Epidemiology

HCV is commonly transmitted via blood and blood products. Its transmission by other routes, such as unprotected sex, perinatal transmission from infected mother to offspring, etc., have been proposed but remain controversial and probably of minor importance. However, >40% of cases of hepatitis C in the United States have no recognizable risk factor (20).

Virology

HCV is classified in the family *Flaviviridae*, in a separate as-yet-unnamed genus. Based on genomic sequence heterogeneity, 6 major and >12 minor genotypes of HCV have been identified (21). The major genotypes differ from one another to the same degree that other RNA viruses belonging to separate subgenera differ; minor genotypes differ to about the degree that different serotypes of other RNA viruses differ from one another. This suggests that multiple serotypes of HCV exist, but the lack of a convenient *in vitro* assay system and the failure to demonstrate lasting immunity following infection of chimpanzees, even when the virus used for rechallenge is the same as the original inoculum, suggest that serotypic variation is a prominent feature of HCV. The infectivity titer of HCV in the blood may be as high as 10^6 infectious doses per ml but is usually much lower, especially in cases of chronic infection, apparently because of complexing of virus to antibody (22, 23). Detection by polymerase chain reaction of HCV genomic RNA in other body fluids has been reported, but it is unclear whether this represents infectious virus.

Clinical Characteristics

The incubation period of hepatitis C averages 50 days. Acute hepatitis C is generally a mild disease with a mortality rate of <1%. However, >50% of acute cases progress to chronicity, and some of these will eventually progress to cirrhosis or hepatocellular carcinoma or both.

Changing Patterns

Insufficient time has passed since its discovery for us to have a clear picture of the ecology of HCV. Only `0.3% of the normal blood donor population of the United States has antibody to HCV, but the prevalence of anti-HCV in inner city populations may be >50-fold higher (24). The same inner city populations have very high prevalences of antibody to HBV and HIV, undoubtedly reflecting the high-risk lifestyles of these groups. However, since NANB hepatitis has been reported as a separate entity beginning in the early 1980s, some trends in relative importance of risk factors have emerged (25). Transfusion-associated NANB hepatitis is virtually disappearing as a result of the current comprehensive screening program that tests specifically for infections with HBV, HCV, and HIV and excludes all who identify themselves as members of high-risk populations. Intravenous drug use as a risk factor has also diminished in relative importance, probably because of efforts to control AIDS in such populations. However, control of NANB hepatitis (principally hepatitis C) in the general population will probably require the development of effective hepatitis C vaccines and their appropriate use.

The impact of hepatitis C in other countries is still being sorted out. Hepatitis C virus has a worldwide distribution, but, surprisingly, the prevalence of anti-HCV is on the order of 1% in most developed countries and <10% in most developing countries to date. An exception is Egypt, where the prevalence of anti-HCV is `10–20% (26). Much is yet to be learned about the ecology of HCV. For instance, several of the genotypes of HCV have worldwide distributions but others appear to be limited to certain regions or even countries (21). A possible connection between certain genotypes of HCV and the clinical course of infection has been reported but not confirmed. Similarly, certain genotypes of HCV appear to be more susceptible to therapy with interferon than others (27). Finally, coinfection, superinfection, and reinfection with different strains of HCV probably occur as well as the emergence of genetic variants (thought to be neutralization-escape mutants of the virus) over time in chronically infected individuals (28–32). Thus, many of the same phenomena observed in infections with HIV, another highly heterogeneous and mutable virus, have been

observed in HCV infections. This bodes ill for the rapid development of an effective hepatitis C vaccine, although the first successful steps toward vaccine development have been taken (33).

HEPATITIS D VIRUS

History

The first evidence for the existence of HDV came in 1978 when a previously unrecognized intranuclear antigen was detected by immunofluorescence in liver biopsies from Italian patients with chronic HBV infection. First thought to be another antigenic specificity of HBV, the antigen was eventually shown to be associated with the capsid protein of a previously unrecognized virus, subsequently called "hepatitis delta virus" (HDV) (34). The virus was shown to be defective, requiring a helper function from HBV, probably because HDV is enveloped with the envelope of HBV. The transmissible nature of HDV was established in 1980 by transmission of the virus to HBV-infected chimpanzees.

Epidemiology

HDV is commonly transmitted by blood and blood products. Perinatal transmission has been reported but is rare, probably because the perinatal transmission of HBV is uncommon in regions where HDV is prevalent. HDV has been found in indigenous populations of South America, Africa, and certain parts of Central and Southeast Asia, where it has caused outbreaks of severe and often fatal hepatitis. Its modes of spread in such indigenous populations are poorly understood but may include inapparent exposure to blood and, in some cases, sexual transmission. In developed countries, hepatitis D is largely restricted to certain high-risk populations, principally users of illicit parenteral drugs.

Virology

HDV is the most unusual of the hepatitis viruses. It does not resemble any other known animal virus and has been classified with the plant virus satellites, agents that are also related to viroids of plants. Based upon the genetic heterogeneity of the agent's single-stranded circular RNA genome, three genotypes have been described (35). It is not clear how many serotypes exist, since the virus is enveloped with the envelope of HBV (HBsAg), from which it probably takes its serologic specificity. HDV achieves extraordinarily high titers (10^{11} infectious doses per ml) in the blood of infected individuals (36).

Clinical Characteristics

The incubation period of HDV averages 35 days. Disease may occur as a coinfection with HBV or as a superinfection of a chronic HBV infection. In either case, hepatitis D is usually severe, being associated in coinfections with fulminant hepatitis and a high mortality and in superinfections with rapidly progressive, often fatal, subacute or chronic hepatitis. Overall, the mortality rate is 2–20%. Chronicity follows 1–3% of coinfections and 70–80% of superinfections.

Changing Patterns

As with HCV, little is known about the long-term changes in the ecology of HDV. Epidemics of severe hepatitis D in indigenous populations of Venezuela, Colombia, Brazil, and Peru are only recent manifestations of a disease that has been present for many decades as documented by retrospective analysis of stored liver tissue and the recent demonstration that South American strains of HDV are genetically different from other strains. In contrast, HDV has been introduced relatively recently into the populations of developed countries. Specifically, epidemics of hepatitis D appeared in populations of parenteral drug users in the 1970s in Norway, Sweden, Greece, and Australia and in the 1980s in Ireland and Poland (37–41). HDV infection has also been prevalent in U.S. drug abusers at least since the 1970s. However, unlike HBV and HIV, HDV has not spilled over appreciably from illicit drug-user populations to sexually promiscuous populations. Interestingly, hepatitis D has diminished in importance in the general population of southern Italy, where its medical importance as a highly endemic virus was first appreciated in the 1970s (42). Effective use of hepatitis B vaccines should eventually control hepatitis D in parallel with the control of hepatitis B, but individuals who are already chronically infected with HBV (estimated at >300 million worldwide) will continue to be at risk of contracting hepatitis D.

HEPATITIS E VIRUS

History

Hepatitis E was not recognized as a unique human disease until 1980, when serologic tests for the diagnosis of hepatitis A and hepatitis B were applied to stored clinical samples collected during waterborne epidemics of viral hepatitis in India (43, 44). Among

these was the massive epidemic of hepatitis that occurred in Delhi, India, in 1955–1956 following contamination of a major water treatment plant with raw sewage. The epidemic had been cited previously as a classical example of waterborne hepatitis A, but the subsequent discovery that hepatitis A was highly endemic in Indian populations, with HAV infecting almost 100% of the population by the age of 5–10 years, made it difficult to accept that the Delhi epidemic and other waterborne epidemics that occurred principally in young adults were caused by HAV. Indeed, virtually 100% of stored serum samples from such epidemics were found to contain IgG anti-HAV but not IgM anti-HAV—strong evidence for past HAV infection with resultant immunity and therefore evidence for the existence of a previously unrecognized hepatitis agent as the cause of the epidemics. HEV was first visualized in 1983 and transmitted to a human volunteer and cynomolgus monkeys, thus establishing its etiologic role in enterically transmitted NANB (ET-NANB) hepatitis (45). Because HEV could not be grown in cell culture and the amount of virus recoverable from natural infections of man or experimental infections of primates was quite small, progress in understanding hepatitis E was slow. However, in 1990 the genome of HEV was cloned, and viral antigens encoded by this RNA genome were expressed by recombinant DNA technology (46). This resulted in the development of specific and sensitive serologic tests that have permitted rapid expansion of our knowledge of the epidemiology of this virus.

Epidemiology

HEV is transmitted by the fecal–oral route. Although it is best known from waterborne epidemics of hepatitis E, it also accounts for much sporadic disease in countries where it is endemic. Hepatitis E has a restricted distribution: disease with the epidemiologic characteristics of hepatitis E has been found in much of Central and Southeast Asia, northern and western Africa, and, to a limited extent, in Mexico. However, the application of recently developed serologic tests has revealed anti-HEV in every country in which it has been sought, including developed countries in which the disease virtually does not occur. For example, 1–5% of normal blood donors in the United States have been found to be positive for anti-HEV, a prevalence of antibody 10 times higher than that for anti-HCV (47). It is not clear whether such antibody represents missed diagnoses of hepatitis E, infection with an attenuated strain of HEV, antibody that cross-reacts with an as-yet-unrecognized agent, or some type of nonspecificity of the existing assays.

Virology

HEV is presently unclassified. Its genomic organization most closely resembles that of the caliciviruses, but it is not identical; the sequence of, for example, the putative RNA polymerase gene of HEV more closely resembles that of the togavirus-like polymerases than that of the caliciviruses and picornaviruses (48). Based on genomic sequence heterogeneity, three genotypes of HEV have been identified, but one serotype appears to comprise all of these. The infectivity titer of HEV in feces probably does not exceed 10^7 infectious doses per g, 2 orders of magnitude less than peak fecal titers of HAV. A viremia occurs during the incubation period of hepatitis E, but the titer of virus present has not been determined.

Clinical Characteristics

The incubation period of hepatitis E averages 40 days. Hepatitis E is a mild-to-moderate disease in severity (mortality rate of 0.2–1%) except in pregnancy, where the mortality rate is progressively higher in each succeeding trimester and may reach 20%. Hepatitis E appears never to become chronic.

Changing Patterns

The epidemiology and virology of hepatitis E suggest that HEV is less readily transmitted than HAV, and recent seroepidemiologic data confirm this: in populations where virtually 100% of the population was infected with HAV by the age of 5–10 years, a relatively small proportion of the population in an endemic region had anti-HEV (V. A. Arankalle, S. A. Tsarev, M. S. Chadha, D. W. Alling, S. U. Emerson, K. Banerjee, and R.H.P., unpublished data). The relatively low prevalence of anti-HEV in at-risk populations could explain the recurring epidemics among young adults. The study cited above took place in a population in which neither HAV nor HEV was diminishing in importance over time. However, it is likely that in transitional populations in which HAV is diminishing in importance, HEV, if ever present, diminished in importance at an earlier date. Some epidemiologic evidence for this comes from a review of the early literature on epidemic hepatitis during the last century (49). Such reports of epidemic hepatitis in Europe and elsewhere described a disease afflicting predominantly young adults and associated with fulminant hepatitis in pregnant women. As we have seen, age-specific antibody profiles of HAV in many countries now industrialized point to a prevalence of anti-HAV that may have been

virtually 100% during the last century. It is possible, therefore, that "infective hepatitis," occurring as sporadic and epidemic cases among adults in Europe and elsewhere before the 20th century, may have actually been hepatitis E, not hepatitis A, as has been supposed. It will be important to determine age-specific antibody profiles for populations of currently industrialized countries to determine if this can be confirmed and to determine if the anti-HEV currently detected in such populations represents the traces of a former time.

More recent interactions between changing human culture and HEV were seen in epidemics of hepatitis E that occurred among refugees living under substandard conditions in Ethiopia and Somalia in the 1980s (50). Those epidemics could be traced directly to overcrowding, substandard living conditions, and nonexistent sanitation. Such epidemics are likely to occur in the future if HEV is introduced into similar environments that are conducive to explosive spread of fecal–orally transmitted viruses. It was surprising, for example, that hepatitis E epidemics did not occur during the recent Gulf war, since the Norwalk agent, a fecal–orally transmitted calicivirus, did cause significant disease. Perhaps only the absence of HEV from the war zone prevented an epidemic.

INTERACTIONS OF THE HEPATITIS VIRUSES

Not only do changes in the culture of populations alter the epidemiology of the hepatitis viruses, but interactions among the viruses and their hosts further modify the ecology of viral hepatitis. The obligatory interaction between HDV and HBV, leading to much more severe disease, also results in suppression of replication of HBV by mechanisms that are not completely understood but may be related to the expression or action of interferon (51). Similar suppression of replication of HBV by HAV and HCV has been reported and dual infections of hepatitis viruses are thought usually to result in more severe disease than single infections. One example of this is an epidemic of severe, in some cases fatal, hepatitis B that occurred in Edinburgh, Scotland, in 1969–1970 (52). Long an enigma because other similar blood-borne epidemics of hepatitis B had not resulted in such catastrophic outcomes, the reason for the severity of this outbreak has been doggedly pursued by one of the original investigators, B. Marmion. Over the years, a variety of possible explanations, including coinfection with HDV, were ruled out. Recently, Marmion and his colleagues have provided convincing evidence that the severe hepatitis B in Edinburgh was associated with dual infection with HCV (B. Marmion, personal communication).

OTHER HEAPTITIS VIRUSES

Approximately 10% of transfusion-associated hepatitis and `4% of community-acquired hepatitis in the United States cannot be ascribed to any of the five recognized hepatitis viruses. It has been proposed that these cases may be caused by a previously unrecognized hepatitis virus, but attempts to transmit an agent from such patients to primates have yielded equivocal or negative results. Additional studies must be carried out to determine whether these cases are indeed caused by a new agent or whether their etiology may be noninfectious.

More convincing evidence for an additional, waterborne hepatitis agent has come from recent studies in Asia, where at least two waterborne epidemics of hepatitis appear not to have been caused by any of the recognized hepatitis viruses (53, 54). Thus, the epidemiology of viral hepatitis continues to evolve.

CONCLUSIONS

In summary, changes in human culture have had profound effects on the epidemiology and public health impact of the hepatitis viruses. In some cases, this has been a positive influence; in others it has been negative. For example, progressive improvement in public and personal hygiene over the last century has diminished the medical importance of hepatitis A and, possibly, at an earlier time, hepatitis E. Lapses in sanitation, through war, such as happened in Somalia, or through changing social mores, as has happened among certain groups in the United States, has resulted in increased disease caused by the fecal–orally transmitted hepatitis viruses, confirming the need for constant vigilance.

Similarly, increased sexual promiscuity, whether homosexual or heterosexual, and increasing use of illicit parenteral drugs has resulted in infection with the blood-borne hepatitis viruses in epidemic proportions. These epidemics were the forerunners of the current HIV epidemic. In most cases, these changes could have been predicted by simple epidemiology, had we the knowledge about the viruses that we now possess. However, preventing outbreaks of disease that are caused by changes in behavior patterns has never been simple or effective, and making an impact on ongoing disease by methods that rely upon changing the behavior of at-risk populations is a difficult and frustrating chore. It is one of the reasons why vaccines, although initially expensive, prove in the long run to be among the most cost-effective of all medical interventions.

SUMMARY

Viral hepatitis is a disease of antiquity, but evidence for more than one etiologic agent has been recognized only since the 1940s, when two viruses (hepatitis A virus and hepatitis B virus) were thought to account for all disease. In the past 20 years, three additional hepatitis agents (hepatitis C virus, hepatitis D virus, and hepatitis E virus) have been discovered, and there is evidence for at least one additional virus. Each of the five recognized hepatitis viruses belongs to a different virus family, and each has a unique epidemiology. The medical impact of these viruses on society has been strongly influenced by changes in human ecology. This has resulted in some cases in diminished disease and in others in increases in the incidence of disease.

REFERENCES

1. Zuckerman, A. J. (1976) *Rush Presbyt. St. Luke's Med. Bull.* **15,** 57–82.
2. Provost, P. J. & Hilleman, M. R. (1979) *Proc. Soc. Exp. Biol. Med.* **160,** 213–221.
3. Mannucci, P. M., Gdovin, S., Gringeri, A., Colombo, M., Mele, A., Schinaia, N., Ciavarella, N., Emerson, S. U., Purcell, R. H. & Italian Collaborative Group (1994) *Ann. Intern. Med.* **120,** 1–7.
4. Robertson, B. H., Jansen, R. W., Khanna, B., Totsuka, A., Nainan, O. V., Siegl, G., Widell, A., Margolis, H. S., Isomura, S., Ito, K., Ishizu, T., Moritsugu, Y. & Lemon, S. M. (1992) *J. Gen. Virol.* **73,** 1365–1377.
5. Purcell, R. H., Feinstone, S. M., Ticehurst, J. R., Daemer, R. J. & Baroudy, B. M. (1984) in *Viral Hepatitis and Liver Disease*, eds. Vyas, G. N., Dienstag, J. L. & Hoofnagle, J. H. (Grune & Stratton, New York), pp. 9–22.
6. Hadler, S. C. (1991) in *Viral Hepatitis and Liver Disease*, eds. Hollinger, F. B., Lemon, S. M. & Margolis, H. S. (Williams & Wilkins, Baltimore), pp. 14–20.
7. Shapiro, C. N., Shaw, F. E., Mandel, E. J. & Hadler, S. C. (1991) in *Viral Hepatitis and Liver Disease*, eds. Hollinger, F. B., Lemon, S. M. & Margolis, H. S. (Williams & Wilkins, Baltimore), pp. 71–76.
8. Alter, M. J., Hadler, S. C., Judson, F. N., Mares, A., Alexander, W. J., Hu, P. Y., Miller, J. K., Moyer, L. A., Fields, H. A., Bradley, D. W. & Margolis, H. S. (1990) *J. Am. Med. Assoc.* **264,** 2231–2235.
9. Yao, G. (1991) in *Viral Hepatitis and Liver Disease*, eds. Hollinger, F. B., Lemon, S. M. & Margolis, H. S. (Williams & Wilkins, Baltimore), pp. 76–78.
10. Okamoto, H., Tsuda, F., Sakugawa, J., Sastrosoewignjo, R. I., Imai, M., Miyakawa, Y. & Mayumi, M. (1988) *J. Gen. Virol.* **69,** 2575–2583.
11. Tabor, E., Purcell, R. H. & Gerety, R. J. (1983) *J. Med. Primatol.* **12,** 305–318.
12. Stevens, C. E., Beasley, R. P., Tsui, J. & Lee, W.-C. (1975) *N. Engl. J. Med.* **292,** 771–774.
13. Alter, M. J., Hadler, S. C., Margolis, H. S., Alexander, W. J., Hu, P. Y., Judson, F. N., Mares, A., Miller, J. K. & Moyer, L. A. (1990) *J. Am. Med. Assoc.* **263,** 1218–1222.
14. Carman, W. F., Zanetti, A. R., Karayiannis, P., Waters, J., Manzillo, G., Tanzi, E., Zuckerman, A. J. & Thomas, H. C. (1990) *Lancet* **336,** 325–329.

15. Feinstone, S. M., Kapikian, A. Z., Purcell, R. H., Alter, H. J. & Holland, P. V. (1975) *N. Engl. J. Med.* **292,** 767–770.
16. Dienstag, J. L. (1983) *Gastroenterology* **85,** 439–462.
17. Communicable Disease Center (1963) *Hepatitis Surveillance Report No. 16* (Communicable Disease Center, Atlanta), 1963.
18. Choo, Q.-L., Kuo, G., Weiner, A. J., Overby, L. R., Bradley, D. W. & Houghton, M. (1989) *Science* **244,** 359–362.
19. Takahashi, K., Kishimoto, S., Yoshizawa, H., Okamoto, H., Yoshikawa, A. & Mishiro, S. (1992) *Virology* **191,** 431–434.
20. Alter, M. J., Coleman, P. J., Alexander, W. J., Kramer, E., Miller, J. K., Mandel, E., Hadler, S. C. & Margolis, H. S. (1989) *J. Am. Med. Assoc.* **262,** 1201–1205.
21. Bukh, J., Purcell, R. H. & Miller, R. H. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8234–8238.
22. Feinstone, S. M., Alter, H. J., Dienes, H., Shimizu, Y. K., Popper, H., Blackmore, D., Sly, D., London, W. T. & Purcell, R. H. (1981) *J. Infect. Dis.* **144,** 588–598.
23. Hijikata, M., Shimizu, Y. K., Kato, H., Iwamoto, A., Shih, J. W., Alter, H. J., Purcell, R. H. & Yoshikura, H. (1993) *J. Virol.* **67,** 1953–1958.
24. Kelen, G. D., Green, G. B., Purcell, R. H., Chan, D. W., Qaqish, B. F., Sivertson, K. T. & Quinn, T. C. (1992) *N. Engl. J. Med.* **326,** 1399–1404.
25. Alter, M. J., Margolis, H. S., Krawczynski, K., Judson, F. N., Mares, A., Alexander, W. J., Hu, P. Y., Miller, J. K., Gerber, M. A., Sampliner, R. E., Meeks, E. L. & Beach, M. J. (1992) *N. Engl. J. Med.* **327,** 1899–1905.
26. Hibbs, R. G., Corwin, A. L., Hassan, N. F., Kamel, M., Darwish, M., Edelman, R., Constantine, N. T., Rao, M. R., Khalifa, A. S., Mokhtar, S., Fam, N. S., Ekladious, E. M. & Bassily, S. B. (1993) *J. Infect. Dis.* **168,** 789–790.
27. Purcell, R. H. (1993) *J. Prevent. Med. & Hyg.* **34,** 69–72.
28. Prince, A. M., Brotman, B., Huima, T., Pascual, D., Jaffery, M. & Inchauspé, G. (1992) *J. Infect. Dis.* **165,** 438–443.
29. Farci, P., Alter, H. J., Govindarajan, S., Wong, D. C., Engle, R., Lesniewski, R. R., Mushahwar, I. K., Desai, S. M., Miller, R. H., Ogata, N. & Purcell, R. H. (1992) *Science* **258,** 135–140.
30. Lai, M. E., Mazzoleni, A. P., Argiolu, F., De Virgilis, S., Balestrieri, A., Purcell, R. H., Cao, A. & Farci, P. (1993) *Lancet* **343,** 388-390.
31. Weiner, A. J., Geysen, H. M., Christopherson, C., Hall, J. E., Mason, T. J., Saracco, G., Bonino, F., Crawford, K., Marion, C. D., Crawford, K. A., Brunetto, M., Barr, P. J., Miyamura, T., McHutchinson, J. & Houghton, M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3468–3472.
32. Ogata, N., Alter, H. J., Miller, R. H. & Purcell, R. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3392–3396.
33. Choo, Q.-L., Kuo, G., Ralston, R., Weiner, A., Chien, D., Van Nest, G., Han, J., Berger, K., Thudium, K., Kuo, C., Kansopon, J., McFarland, J., Tabrizi, A., Ching, K., Moss, B., Cummins, L. B., Houghton, M. & Muchmore, E. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1294–1298.
34. Rizzetto, M. & Verme, G. (1985) *J. Hepatol.* **1,** 187–193.
35. Casey, J. L., Brown, T. L., Colan, E. J., Wignall, F. S. & Gerin, J. L. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9016–9020.
36. Ponzetto, A., Hoyer, B. H., Popper, H., Engle, R., Purcell, R. H. & Gerin, J. L. (1987) *J. Infect. Dis.* **155,** 72–78.
37. Hansson, B. G., Norkrans, G., Weibull, M., Weiland, O., Nielsen, J.,

Leinikki, P., Ukkonen, P., Jensson, O. & Siebke, J.-C. (1983) in *Viral Hepatitis and Delta Infection,* eds. Verme, G., Bonino, F. & Rizzetto, M. (Liss, New York), Vol. 143, pp. 155–159.
38. Hadziyannis, S. J., Papaioannou, C. & Alexopoulou, A. (1991) in *The Hepatitis Delta Virus,* eds. Gerin, J. L., Purcell, R. H. & Rizzetto, M. (Wiley–Liss, New York), Vol. 364, pp. 51–62.
39. Dimitrakakis, M. & Gust, I. (1991) in *The Hepatitis Delta Virus,* eds. Gerin, J. L., Purcell, R. H. & Rizzetto, M. (Wiley–Liss, New York), Vol. 364, pp. 89–96.
40. Shattock, A. G. (1983) in *Viral Hepatitis and Delta Infection,* eds. Verme, G., Bonino, F. & Rizzetto, M. (Liss, New York), Vol. 143, p. 281 (abstr.).
41. Laskus, T., Radkowski, M., Lupa, E., Cianciara, J., Slusarczyk, J., Halama, G. & Horban, A. (1992) *Infection* **20,** 43–44.
42. Piccinino, F. & Sagnelli, E. (1991) in *The Hepatitis Delta Virus,* eds. Gerin, J. L., Purcell, R. H. & Rizzetto, M. (Wiley–Liss, New York), Vol. 364, pp. 33–39.
43. Khuroo, M. S. (1980) *Am. J. Med.* **68,** 818–824.
44. Wong, D. C., Purcell, R. H., Sreenivasan, M. A., Prasad, S. R. & Pavri, K. M. (1980) *Lancet* **ii,** 882–885.
45. Balayan, M. S., Andzhaparidze, A. G., Savinskaya, S. S., Ketiladze, E. S., Braginskys, D. M., Savinov, A. P. & Poleschuk, V. F. (1983) *Intervirology* **20,** 23–31.
46. Reyes, G. R., Purdy, M. A., Kim, J. P., Luk, K.-C., Young, L. M., Fry, K. E. & Bradley, D. W. (1990) *Science* **247,** 1335–1339.
47. Dawson, G. J., Chau, K. H., Cabal, C. M., Yarbough, P. O., Reyes, G. R. & Mushahwar, I. K. (1992) *J. Virol. Methods* **38,** 175–186.
48. Dolja, V. V. & Carrington, J. C. (1992) *Semin. Virol.* **3,** 315–326.
49. Cockayne, E. A. (1912) *Q. J. Med.* **6,** 1–29.
50. Bradley, D. W. (1990) *Br. Med. Bull.* **46,** 442–461.
51. Shimizu, Y. & Purcell, R. H. (1989) *Hepatology* **10,** 764–768.
52. Marmion, B. P., Burrell, C. J., Tonkin, R. W. & Dickson, J. (1982) *Rev. Infect. Dis.* **4,** 619–637.
53. Doroshenko, N. V., Smolina, T. G., Zairov, G. K. & Stakhanova, V. M. (1989) *Vopr. Virusol.* **2,** 164–167.
54. Arankalle, V. A., Chadha, M. S., Tsarev, S. A., Emerson, S. U., Risbud, A. R., Banerjee, K. & Purcell, R. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3428–3432.

Population Migration and the Spread of Types 1 and 2 Human Immunodeficiency Viruses

THOMAS C. QUINN

It has been more than a decade since the recognition of the first cases of the acquired immunodeficiency syndrome (AIDS), and during this time AIDS has rapidly become a global pandemic with one million cases officially reported from 173 countries (1). Because of underreporting in many areas, the World Health Organization estimates that over 2.5 million cumulative AIDS cases and 1 million deaths have occurred to date (Figure 1). However, the impact of the AIDS epidemic is even greater when one considers the magnitude of spread of the human immunodeficiency viruses (HIV). From selected seroprevalence studies and mathematical models, it is now estimated that 16 million people are infected with HIV (Figure 1). Of these individuals, `9 million are men, 6 million are women, and 1 million are infants and children. Two-thirds of all estimated AIDS cases to date have occurred in sub-Saharan Africa, but more recently, HIV infection has increased rapidly in Southeast Asia, with over 2.5 million cumulative HIV infections estimated to have occurred within the last 5 years. It is within these developing countries that HIV will clearly have its greatest impact on morbidity and mortality, as well as having profound economic and social consequences.

In some urban centers of sub-Saharan Africa, Western Europe, and

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the Americas, AIDS has already become the leading cause of death for both men and women aged 15–49 years (2–4). It has been estimated that the HIV pandemic may have already resulted in the death of nearly 750,000 children worldwide and that, by the year 2000, 10 million children under 15 may be orphaned because of the premature death of HIV-1-infected mothers and fathers from AIDS (5). Overall infant and child mortality rates will increase as much as 30% more than previously projected as a direct consequence of perinatal HIV infection. Consequently, pediatric AIDS is now threatening much of the progress that has been made in child survival in developing countries during the past 20 years.

FIGURE 1 Estimated global distribution of HIV-1 infections by region. Data are from the World Health Organization (1).

While the origin of HIV infection remains an enigma, several factors have been hypothesized to be responsible for the eventual spread of HIV. Historically, from retrospective serologic studies it is evident that HIV existed in humans as early as the late 1950s, although it did not become epidemic until nearly 20–30 years later (6). One hypothesis is that the migration of individuals from areas of low endemicity to new uninfected areas was eventually responsible for dissemination of HIV throughout the world. This was by no means a rapid event but probably occurred over a 40-year period. Unfortunately, because of the lack of vaccine or effective antivirals, it is inevitable that further dissemination will still occur from established indigenous transmission resulting in an escalation of the epidemic over the next decade. The World Health Organization now estimates that as many as 40 million people will be HIV-infected by the year 2000 (7).

This paper will review the conceptual issues regarding the migration of populations with its associated social changes in certain regions as a major force responsible for the eventual dissemination of HIV infections worldwide. Because data are limited on the frequency and size of

population movements, and in particular in reference to HIV infection, no definitive conclusions can be made regarding the origins of HIV; rather, these concepts are presented to provide a better framework to understand the behavioral and demographic changes responsible for the AIDS epidemic.

TABLE 1 Factors contributing to population migration in sub-Saharan Africa

1.	Political instability: 75 military coups in 30 countries
2.	Environmental degradation from population pressure, bad land use, weather changes, etc.
3.	Natural disasters such as flooding and earthquakes
4.	Pursuit of productive employment
5.	Artificial boundaries established by colonists, which ignored cultural and ethnic boundaries
6.	Decline in the effect of religion on population migration

Adapted from ref. 9.

MIGRATION OF POPULATIONS: DEFINITIONS

Migration is the movement of people in space, often involving a change in the usual place of residence (8). Internal migration is such a movement within national boundaries, whereas international migration involves movement of individuals across political boundaries. Because migration is a continuous, often repeated process rather than a single event, it is often difficult to measure. Migration is further defined in terms of movement in time. The principal distinction is between circulation—i.e., involving repetitive, nonpermanent moves such as daily commuting and other short-term mobility—and definitive migration (8). Periodic movements are mostly short term, whereas seasonal movements have a regular annual rhythm. Long-term circulation involves an absence of more than 1 year but with expectation of return. Definitive migration by contrast implies a permanent movement away from one residence with little indication of return visit. Some examples of internal migration include rural–rural, rural–urban, urban–rural, and urban–urban, each with its own characteristic features and consequences (Table 1).

AFRICA

Following independence in the 1960s, many African countries experienced dramatic demographic changes, which may underlie the movement of HIV infection from potentially remote areas to more populous

areas. One of the most important types of migration during this period represented rural–urban movement, which was significant in the long-term spatial redistribution of populations. The number of cities with more than 500,000 inhabitants rapidly increased from 3 in 1960 to 28 by 1980 (10). Though the total urban population in sub-Saharan Africa increased substantially, the average growth rates did not. Between 1965 and 1985 the proportion of the total population living in urban areas in southern Africa rose from 43% to more than 50%; in middle Africa, from 21% to 35%; and in western Africa, from 17% to 29% (Figure 2) (8, 10). The least urbanized subregion, eastern Africa, has experienced increased urbanization, though the urban proportion has stayed less than 20%, rising from less than 10% in 1965. By year 2000, the projected proportion of people living in urban centers is expected to exceed one-third of the national populations in all regions except eastern Africa. As a consequence of urbanization, the crude population density increased in every country for which data are available, averaging greater than a 100% increase (8).

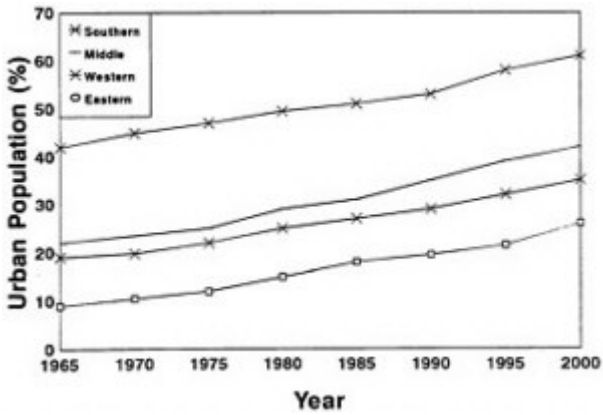

FIGURE 2 Proportion of total population living in urban areas in sub-Saharan Africa from 1965 to 2000 (9, 10).

Unlike much of the industrialized world, where urbanization followed industrialization, urbanization and industrialization have largely taken place independently in sub-Saharan Africa. As a result, employment was frequently unavailable within these centers. Demographically, the migrants tended to be young adult males who have a higher level of educational attainment than those who do not migrate. Women were not encouraged to migrate but later were forced to migrate to the urban

centers for economic reasons. Unfortunately, because of the large number of migrants to the urban centers, unemployment and social disruption became common, and many individuals reverted to commercial sex for a means of survival (9). During this period, health officials noted marked increases in sexually transmitted diseases (STDs), including HIV-1 infection, within the urban centers.

One of the first studies to suggest migration of HIV-1 from a rural setting to an urban center was based in Zaire. In 1985 Nzilambi et al. (11) tested sera for HIV-1 that had been previously collected in 1976 during an Ebola hemorrhagic fever outbreak in the remote Equateur province of Zaire. Five (0.8%) of 659 specimens were positive, and HIV-1 was isolated from one of these. Follow-up investigation 10 years later revealed that three of the five seropositive persons had died of illnesses suggestive of AIDS and that two remained healthy but seropositive. In 1986 the seroprevalence was unchanged at 0.8% among 389 randomly selected residents in the same area. However, the seroprevalence of HIV-1 was 11% in 283 prostitutes in nearby cities. Similarly, a 10-fold increase in prevalence was documented in pregnant women between 1970 and 1980 in Kinshasa. The authors concluded that HIV infection was endemic and demonstrated long-term stability in residents of rural Zaire but that urbanization with its attendant social changes may have promoted the spread of AIDS in Africa.

A cluster sampling technique was used to document the difference in rural and urban HIV infection in other countries of Central and West Africa (Figure 3) (12). The results of these studies have shown HIV prevalence rates below 1% in rural areas, with infection rates in the urban centers considerably higher. In Rwanda the HIV seroprevalence was 17.8% (14.3–21.2%) in an urban sample of 1870 and 1.3% (0.5–2.2%) in the rural sample of 742 individuals. More recent data, however, suggest that significant rates of infection are now occurring in rural areas due to return migration and other factors (14).

Other examples of population migration and spread of HIV are discussed below. Currently in Uganda there are an estimated 1 million HIV-infected individuals or 6% of the entire Uganda population. Three principal hypotheses have been proposed to explain the evolution of the AIDS epidemic and its distinct distribution throughout Uganda (15). In the "truck town" hypothesis, it has been proposed that the geographic distribution of HIV and AIDS reflects a diffusion process in which major roads act as principal corridors of virus spread between urban areas and other proximal settlements (16, 73). Truck drivers along these routes and commercial sex workers have been cited as potential vectors for the dissemination of HIV. In one study of 68 lorry drivers and their assistants, 24 (35.2%) were found to be HIV-infected (74). Epidemiologic

evidence demonstrated a wide travel history involving seven different countries served by the port of Mombasa, including Kenya, Uganda, Zaire, Burundi, and Rwanda (17, 75). Second, the "migrant labor" hypothesis proposed that HIV diffused from areas of labor demand in urban areas to areas of labor supply in rural districts through a process of return migration (18, 19). A third hypothesis suggested that the dissemination of HIV-1 in Uganda correlated with ethnic patterns of recruitment into the Ugandan National Liberation Army after the overthrow of Idi Amin some 10 years earlier in 1979 (15, 20). In fact, probably all three hypotheses together help explain the spread of HIV within Uganda.

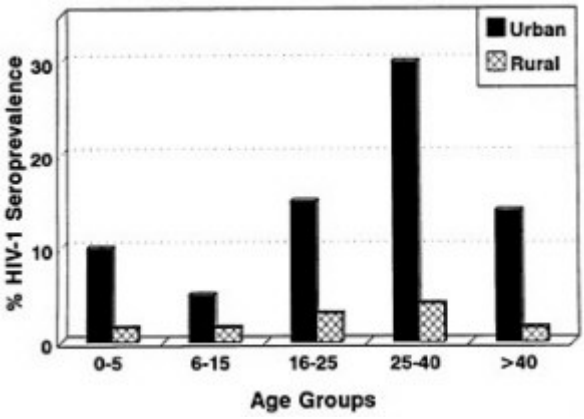

FIGURE 3 HIV-1 seroprevalence by age in urban and rural samples in Rwanda and Zaire (12, 13).

In addition to the movement of truck drivers and the military, female prostitutes, well recognized as a high-risk population for HIV infection, are also highly mobile. These women will frequently move from one locality to another due to economic pressures. For example, in South Africa, the migrant labor system created a market for prostitution in mining towns and established geographic networks of relationships within and between urban and rural communities (21). Industrialization, particularly the rapid growth of the mining industry with the migrant labor system it created, led to an epidemic of STDs among these populations (18). Gonococcal infections and syphilis have been documented in 10% and 17% of mine workers, respectively. Depending on the country of origin, HIV prevalence has varied from a low of 0.08% in residents of South Africa to as high as 17.8% in migrants from Malawi.

Women who provide migrant mine workers with sexual services also come from socially and economically marginalized groups in rural and urban areas, many of whom also have a high rate of HIV infection and STDs, further potentiating the spread of both. These data illustrate one end of the spectrum of behavior where multiple partners and frequent partner change are common. This group may represent a "core" population involved in high-risk activity that acts as a major carrier of HIV (22).

THE EFFECT ON DEMOGRAPHICS

The population pyramid in most African countries is symmetrical with a broad base and quite distinct from that observed in developed countries (23, 24). Interestingly, in urban areas one finds a prominent one-sided bulge caused by the migration of young males (age 18–35) into the cities for employment (Figure 4). The prevalence of HIV infection in urban populations is highest in the 25- to 35-year-old bracket among males and in the 15- to 25-year-old bracket for females (13). This difference is due to the fact that on the average sexual partnerships are formed between older men and younger women. The distortion of the urban population profile caused by male migration results in an overall 1:1 female-to-male prevalence ratio of infection. However, as the epidemic spreads into the larger rural population, the absolute size of the most severely affected younger female population is larger than the size of the older male population, which eventually results in a higher number of infections in women. This excess in female morbidity from HIV infection has important implications for the social and economic role of women in society (13, 23–26). It also adds fuel to an emerging epidemic of pediatric AIDS.

HIV-2 AND WEST AFRICA

By 1986, HIV-2 infection was recognized among high-risk groups in West Africa. While related to HIV-1 in terms of morphology, cell tropism, and overall genetic organization, HIV-1 and HIV-2 differed significantly in terms of nucleotide sequences, with only 42% homology (27, 28). These genomic studies further demonstrated that HIV-2 had 70% or more homology with the simian immunodeficiency virus, suggesting that HIV-2 may be evolutionarily more closely related to the simian immunodeficiency virus, a nonhuman primate retrovirus, than to its other relative, HIV-1. A common ancestor with similar properties and pathogenic potential may have existed a long time ago, and the

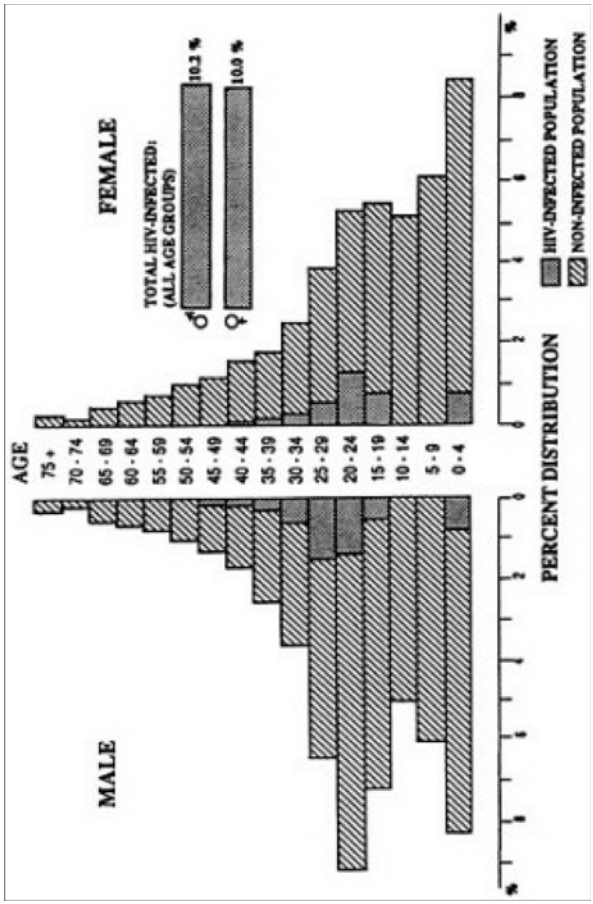

FIGURE 4 Generic urban population pyramid: HIV-infected and noninfected population (23). The prominent bulge on the male side starting at age 20 is related to increased male migration from rural to urban centers, with resultant increase in overall HIV seroprevalence. Note that HIV-1 seroprevalence is higher in the younger females and in older males, a phenomenon common to epidemiology of STDs, reflecting the cultural phenomenon of older men having sex with younger women.

emergence of the AIDS epidemic was more likely a result of simultaneous modifications of epidemiologic parameters in West and Central Africa, such as rapid urbanization, leading to the infection of larger populations with HIV-1 and HIV-2 (14, 26, 29).

International migration across national boundaries also played a role in the movement of populations and HIV-2 infection from one country to another. Of the 35 million sub-Saharan African migrants, `5.4 million are officially recognized refugees (29, 30). International migrants include those seeking employment, family members accompanying or joining those who have migrated before them, and people seeking refuge from drought, famine, political upheavals, or military conflicts. The highest concentration of migrants is found in western Africa, an area that migrants have considered as an economic unit where trading goods and services flowed freely, as did people (29). Over the past two decades, Côte d'Ivoire has become a major center for migrants from Burkina Faso, Mali, Guinea, Ghana, Niger, and elsewhere. Foreigners who were 22% of the total population in the 1975 census are now nearly 30%, giving Côte d'Ivoire by far the highest concentration of foreigners in sub-Saharan Africa (29). The second highest concentration is in Gambia, where migrants, who are about 11% of the total population, include individuals from Senegal, Guinea, Guinea-Bissau, and Mali. Interestingly, migration to Nigeria has also increased substantially during the 1980s as a combined result of Nigeria's oil boom and the protocol on freedom of movement signed in 1980. The majority of migrants were from Ghana, Togo, Benin, Niger, and Chad.

Serologic surveys for HIV-2 have demonstrated high rates of infection among several countries of West Africa. Moderate to high rates of infection have been found in urban areas of Senegal, Guinea-Bissau, Guinea, Burkina Faso, Ivory Coast, Gambia, and Cape Verde (31–36). Interestingly, significant rates of HIV-2 infection have been reported in Angola and Mozambique, two countries located in southern Africa (33). These countries were formerly Portuguese colonies and maintain ongoing relationships with countries in West Africa such as Guinea-Bissau and Cape Verde, which were also Portuguese colonies. In contrast, HIV-2 has rarely been reported from other countries of Central Africa. In Guinea-Bissau and Gambia, HIV-2 is prevalent and HIV-1 is rare. In Ivory Coast and Burkina Faso, HIV-2 and HIV-1 are both present in an appreciable proportion of the population.

Differences in these patterns of infection are likely to be consequences of different types of interrelations and contacts within West Africa, where HIV-2 predominates, and between West Africa and Central Africa, where HIV-1 predominates. In general, it seems likely that the migration of sexually active, high-risk populations played a major role in

the observed spread of these sexually transmitted viruses in these countries. One example is seasonal migration of young men and women in Senegal and Gambia. In one study by Pison *et al.* (37), of 3230 persons residing in rural Senegal, 0.8% were HIV-2 and 0.1% were HIV-1 seropositive. Seropositivity was directly associated with seasonal migration, history of blood transfusion, injections, and STDs.

In a study by Kanki *et al.* (38), of 278 female prostitutes from Ziguinchor, Senegal, HIV-2 seroprevalence was associated with women of Guinea-Bissau nationality and increased years of sexual activity. The acquisition of either HIV infection may have occurred in Senegal or in their country of origin. In addition, Ghanian and Gambian prostitutes report that they migrate and work in a number of other West African countries (39, 40), such as Burkina Faso, Ivory Coast and Mali, all countries with significant HIV-2 prevalence.

Within the last few years, investigators have noted the introduction of both HIV-1 and HIV-2 infection among certain high-risk populations in Nigeria. Although relatively spared during the early 1980s, several surveys have documented increasing rates since 1990 throughout Nigeria (41, 42). This is of critical importance since the population in Nigeria, estimated at 110 million people, represents 25% of the total population of sub-Saharan Africa. In one recent study by Dada *et al.* (42), 12.3% and 2.1% of 885 females prostitutes were infected with HIV-1 and HIV-2, respectively, a rise from a combined prevalence of only 1.7% 2 years previously. Women in the youngest age group, age 12–19, had the highest prevalence (20%). Furthermore, HIV-2 infection was significantly associated with low socioeconomic class and non-Nigerian nationality. The finding that both HIV-1 and HIV-2 are present in this population suggests that both infections are spreading within Nigeria but at differential rates. Foreign female prostitutes may represent one group responsible for the introduction of HIV-2 into Lagos. In addition, prostitutes residing in the port area of Lagos, which serves as a major convergence of overland and sea routes within and outside Nigeria, had the highest prevalence of HIV-1 infection. The federal highway region that is traversed by the overland interstate highway also had high rates. Because Lagos is the largest cosmopolitan city in Africa, there is constant migratory movement of people into and out of Lagos and a major trade center, thereby providing opportunity for further HIV dissemination.

SPREAD OF HIV-2 OUTSIDE OF WEST AFRICA

Because HIV-2 infection has been relatively confined to West Africa, its appearance in other areas generally reflects epidemiologic links to West Africa. The greatest number of cases have been reported in

Portugal, France, and Germany (31–34). The cultural and economic ties between Portugal and its former colonies may have enhanced the spread of HIV-2 to Europe and possibly to Brazil, a former Portuguese colony (43). Although most countries can link the presence of HIV-2 within their country to ties with West Africa, France and Portugal now report HIV-2 infection among the indigenous population (32, 34). In Portugal, 60% of HIV-2 cases are no longer directly linked to West African contacts. In France, 13% of cases are not directly linked to West Africa.

In the United States, the first reported case of AIDS caused by HIV-2 was reported in December 1987 in a West African residing in the United States (44). The number of HIV-2 infections documented in the United States has since risen to 32 (45). In all cases for which a history has been available, the infected individuals have previously lived in West Africa or have been sexual partners from that region. Geographically, 28 of the 32 persons with HIV-2 are from the northeastern United States. Thus far, there have been no cases of HIV-2 among i.v. drug users or homosexual men. Nevertheless, because of its presence, the Food and Drug Administration recommended in June 1992 that all blood donations should be screened for both HIV infections (46).

Reports from India now suggest the appearance of HIV-2 infection predominantly in the Maharashtra state, particularly Bombay and Pune (47, 48). Several studies have suggested that HIV-2 may represent between 3% and 7% of all HIV infections in Bombay. In other studies in Maharashtra State, HIV-2 infection, while noted, has not increased appreciably during the past 3 years. Genetic sequence analysis of these strains of HIV-2 has not been completed, and their linkage to West Africa, while suggestive, needs further evaluation.

HIV-1 IN THE AMERICAS

The United States has the highest number of reported AIDS cases worldwide, with over 339,000 cases and nearly 200,000 fatalities as of October, 1993 (49). During the 1980s HIV infection emerged as one of the leading causes of death in the U.S. In 1992, HIV infection was the leading cause of death among men aged 25–44 years and the fourth leading cause of death among women in this age group, accounting for 19% and 6% of deaths, respectively (2). In 1992, heterosexual contact accounted for the largest proportionate increase in AIDS cases compared to 1991 (17.1% in reported cases) (Figure 5). The proportionate increase in cases attributed to heterosexual contact was greater for men (26.3%) than for women (11.5%); however, women accounted for most persons infected through heterosexual contact (15.9%) (49). The second largest proportional increase was in perinatal transmission (13.4%). In 1992 the

number of AIDS cases among women infected through heterosexual contact exceeded those infected through i.v. drug use for the first time.

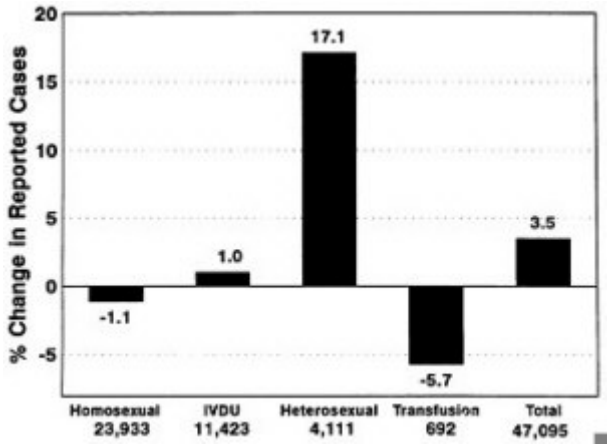

FIGURE 5 Proportional change in AIDS cases reported to the Centers for Disease Control for 1992 compared to cases reported in 1991, by HIV exposure category (49). IVDU, i.v. drug users.

While anecdotal cases of sexual clusters have been documented, rapid travel within the United States soon blurred any strong epidemiologic associations with travel. However, there are situations in the United States that are similar to the issues of migration described for Africa, which fueled the spread of HIV in the rural areas of the United States. One area that can be used as an analogy to the migrant workers in Africa is Belle Glade, Florida (50, 51). Between 1985 and 1988 alone, the prevalence of AIDS in Belle Glade quadrupled from 188 to 806 cases per 100,000 persons. Most of the spread of HIV was related to sexual intercourse, i.v. drug use, and low socioeconomic status. Socioeconomic conditions seen among the migrant population of Belle Glade date back for the past 50 years and are responsible for epidemics of STDs. Crowded living conditions, poverty, varying access to health care, and other resulting factors promote HIV transmission in Belle Glade much as they did syphilis 50 years before (50).

In a study in rural South Carolina (52), 25 (13%) of 198 migrant workers were HIV antibody positive and 32 (16%) had a reactive serologic test for syphilis. Of 166 workers who reported the frequency of condom use, 77 (46%) indicated they never use condoms. Similar to Belle Glade, rural South Carolina migrant workers also demonstrate behaviors that place them at risk for HIV infection and other STDs. In

one study in North Carolina in 1987, 2.6% of 426 migrant workers were HIV seropositive (53). Persons positive for syphilis had higher rates of HIV infection (5.6%) than those who did not (2.2%). Estimates of the prevalence of HIV infection in other migrant and seasonal farm workers are limited. The transience of this population makes it difficult for health care workers to assess the health status of these persons, who frequently do not have access to health care.

In the Americas outside of the United States, HIV-1 infection has been predominantly documented among homosexual/bisexual men who initially had sexual relations with individuals from the United States. Subsequently indigenous transmission has evolved, and there is increasing evidence for heterosexual transmission within many Latin American countries (54). One aspect of HIV transmission throughout the region has been the documented high rate of infection among female prostitutes who provide their services in different countries. In one study of 80 international prostitutes who traveled to 27 countries, 38 (49%) were HIV seropositive, which was considerably higher than that of the general population (1%), homosexual men (10%), or local prostitutes (2%) in Dominica, their country of origin (55). In view of the low prevalence of HIV in nontraveling Dominican prostitutes, these women were probably infected outside of the country. Nevertheless, many of them returned to their home, resulting in further spread within their home country.

HIV INFECTION IN SOUTHEAST ASIA

HIV was introduced relatively late into Asian countries. The first few cases of HIV infection were not documented until 1985, and it was not until 1988–1990 that the transmission of HIV escalated to epidemic proportions (56). A 1985 serosurvey of 600 individuals in Thailand including prostitutes, i.v. drug users, and STD patients revealed a very low HIV-1 seroprevalence (<0.5%) (57, 58). However, by 1988 HIV-1 began to spread rapidly among i.v. drug users to a prevalence of 40%. Seroconversion rates were as high as 3–5% per month (59). This wave of the HIV epidemic in i.v. drug users appeared to be followed by one among female prostitutes. The National Sentinel Survey conducted 1 year after the spread of HIV among i.v. drug users showed that about 44% of lower-class prostitutes in Chiang Mai were infected with HIV-1 (56). Nationally, the HIV seroprevalence among prostitutes increased steadily from 3.7% in 1989 to 15% in 1991 (Figure 6) This was followed by successive waves of transmission into male clients of the prostitutes and from them into the wives and girlfriends of these men in the general population. Based on the current available data, it is estimated that there

are 450,000 HIV-infected persons in Thailand, or 0.8% of the Thai population of 55 million (56). If this rate of HIV transmission continues, there will be 2–4 million cumulative HIV infections by the year 2000 (7).

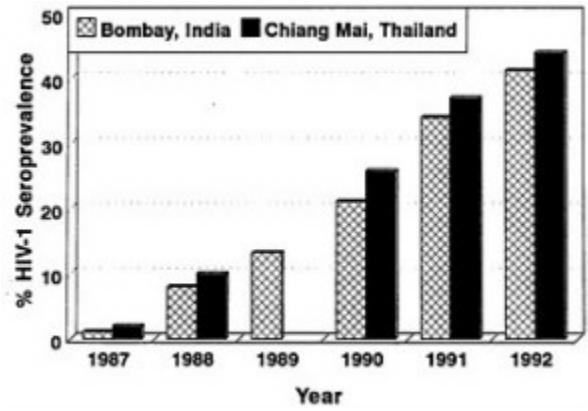

FIGURE 6 Seroprevalence rates among commercial sex workers in Thailand and India from 1986 through 1992 (data from Center for International Research, United States Bureau of the Census).

Factors that contributed to HIV transmission included Thailand's commercial sex industry, international tourism, and i.v. drug use. The majority of commercial sex workers are 16- to 24-year-old females from poor, rural areas who enter the activity to remit money back to parents and siblings. Temporary affiliation with commercial sex trade has gained legitimacy in poor regions as a means of addressing the indebtedness of rural families (60). The circulatory nature of rural–urban migration as well as the substantial patronage of the sex industry by international tourists fueled HIV transmission throughout Thailand and neighboring countries. Similar factors are involved in the spread of HIV among i.v. drug users. Poverty in the rural regions leads to dependence on the opium trade, and opium consumption became an integral part of the East Asian culture. When opium was banned in 1958, heroin injection became widespread. It is not known epidemiologically how and from where HIV originally entered the country because of the large amount of international travel by both foreigners and Thai nationals to and from all regions of the world. In 1989 alone, Thailand recorded 25,573 tourist arrivals of residents of Africa, 340,011 from the Americas, and 1.1 million from Europe (61).

Genetic analysis of strains isolated from individuals in Thailand recently enabled investigators to epidemiologically link two distinct

genotypes of HIV-1 (62, 63). Of 29 sexually infected patients, 25 (86%) had HIV-1 strains of genotype A and 4 (14%) had genotype B (62). Among 29 i.v. drug users, only 7 (24%) had genotype A and 22 (76%) had genotype B. This segregation is unlikely to have arisen by chance ($P < 0.001$). No patient was found to have dual infection. Nucleotide divergence averaged 3.4% among genotype A-infected patients and 3.5% among genotype B-infected patients, but it averaged 22% between the genotypes.

The low nucleotide divergence among people infected with the same HIV-1 genotype in Thailand is consistent with epidemiologic findings that widespread HIV transmission in Thailand has occurred only since 1988. This genetic homogeneity contrasts profoundly with the substantial diversity among HIV-1 strains from infected people in all other countries reported so far (62). The high nucleotide divergence between these two genotypes also suggests that the two variants resulted from separate, independent introductions into Thailand and that they did not evolve from a common progenitor virus already present in the country. Evolution from a common ancestor with an annual divergence of 0.5–1.0% in the C2-V_3 region of the *env* gene would have taken a minimum of 20 years (64). A continuous spectrum of intermediate strains would also be expected.

One possibility for the association of these two genotypes with behavior may be biologic—i.e., that genotype A is more efficiently sexually transmitted whereas B is more efficiently transmitted parenterally. Another possibility is that these associations are purely epidemiologically linked, demonstrating the introduction of a genotype into one high-risk group, which is distinct from another risk group in which a second genotype has been introduced. The presence of these two major distinct HIV genotypes in Thailand provides a unique opportunity to study the transmission efficiency of these strains (62, 63).

Similar increases in HIV infection have also been documented in India and Myanmar. In the northeast Indian state of Manipur, none of 2322 i.v. drug users seen from 1986 to 1989 were seropositive for HIV. However, the rate increased to 54% during the period October 1989 to June 1990 (65). In Bombay, HIV seropositivity rates among prostitutes increased from 2% in 1988–1989 to nearly 40% in 1991 (Figure 6). In Vellore, the HIV seroprevalence among prostitutes increased from 0.5% in 1986 to 34.5% in 1990 (66). These marked increases of HIV in India are of concern since India is the most densely populated country in Asia with 858 million people. an estimated 1.0 million people have already been infected in India within the past 6 years, and if unchecked this number could increase dramatically, exceeding even the number of HIV infections in Africa. As is the case in Thailand, it is not entirely clear how

HIV was introduced into India, although it is apparent that high rates among prostitutes were established rapidly in the port city of Bombay, and high rates of HIV infection among i.v. drug users were documented in Myanmar, part of the "golden triangle" for heroin exportation (65).

Genetic sequencing similar to that described for Thailand has also been carried out on infected patients from Bombay and Goa. Several isolates appear to be related to a South African "clade C" strain and others to the Thai "clade E" strain (67). This is not surprising since there are strong cultural and educational ties between South Africa and India, and workers migrate between Thailand and India. As in previous reports the sequences within each clade were found to be quite homologous to each other, suggesting a recent and fast-spreading epidemic. From these preliminary data, it is likely that HIV infections will continue to increase throughout the next decade in Asia. The annual number of HIV infections by the year 2000 will far exceed that seen in sub-Saharan Africa. Projections are that in India 3 million people may be infected by 1996 and 20 million by the year 2000 (7).

LEGAL RESTRICTIONS

Whether real or imagined, the threat of increased risk of spreading HIV infection and AIDS through international population movements has resulted in restrictive reactions by some governments (68, 69). However, mandatory screening of international travelers is unlikely to reduce the rate of spread of HIV within countries. This would be true even for a country that has no HIV infection, assuming that the nationals traveling abroad would have to be readmitted even if they tested positive. The dilemma of how to protect simultaneously the public health and the rights of individuals and social groups believed to threaten public health is not easily resolved. Policies that would restrict international movement or discriminate against travelers to prevent the spread of HIV do not address the real problems facing the control of HIV infection worldwide (69).

CONCLUSIONS

While intense educational efforts need to be focused on behavioral change as one key to preventing further spread, fundamental social change will be required if AIDS control efforts are to succeed. The migration of poor, rural, sexually active young people to urban centers in the Third World clearly played a role in the dissemination of HIV and other infectious diseases. Major causes of such population movement such as migrant work and/or political repression are social problems

that extend well beyond the behavioral factors that result in interpersonal spread of HIV (Table 2). Increasing mobility has serious implications for the control of AIDS and this phenomenon cannot be fully addressed without profound social, cultural, and economic changes that lie at the root of the problem (70, 71). Unfortunately, the situation is compounded by the economic recession and debt crisis witnessed in many African countries. Violent protests and further civil disruption have occurred in Liberia (1979), Sudan and Tunisia (1985/86), Zambia (1987, 1990), Algeria (1988), Nigeria (1988/89), Ivory Coast (1990), and Zaire (1992). Twenty-four countries in sub-Saharan Africa with a total population of over 400 million were worse off in terms of average income per capita at the end of the 1980s than at the beginning of the decade (23, 25). In summary, the economic recession has further aggravated the transmission of HIV in Africa and Asia by directly increasing the population at risk through increased urban migration, disruption of rural families, poverty, women's subordinate status in society, and prostitution and indirectly through decrease in health care provision (72). The latter entails not only reduced facilities to care for patients with AIDS but also less effective diagnosis and treatment of STDs and decreased spending on health education programs.

TABLE 2 Factors associated with HIV dissemination

1.	*Population migration*. Internal and international with >35 million people in African moving from one area to another
2.	*Urbanization*. Migration of poor, sexually active young people from rural regions to cities in search of employment
3.	*Social disruption*. Changes in social and cultural values secondary to migration from families or from political repression and civil disruption
4.	*Poor medical services*. Less effective facilities of diagnosis and treatment of increasing medical problems associated with urbanization and a failing economy
5.	*Declining economy*. Overall decrease in average income per capita, resulting in increased migration and prostitution
6.	*Low social status of women*. Difficult to obtain educational opportunities, less training for skilled labor, and consequently fewer economic possibilities, resulting in increased prostitution
7.	*STD epidemic*. Many of the factors above contributed to further spread of STDs, particularly genital ulcers, which facilitated HIV transmission

The current AIDS epidemic in developing countries is inextricably liked to socioeconomic and political factors, both current and historical. To control the AIDS epidemic, countries will need to not only promote individual behavior change but also address the related problems of increasing landlessness, mounting unemployment, accelerated urbanization, prostitution, rapid decline in health services, and drug abuse in

order to control its spread (Table 2). It is evident that the success of such AIDS control initiatives will be limited if they fail to confront such fundamental structural issues.

SUMMARY

Over 14 million people are estimated to be infected with the human immunodeficiency viruses (HIV), with nearly three-fourths of the infected persons residing in developing countries. One factor responsible for dissemination of both HIV-1 and HIV-2 worldwide was the intense migration of individuals, from rural to urban centers with subsequent return migration and internationally due to civil wars, tourism, business purposes, and the drug trade. In sub-Saharan Africa, between 1960 and 1980, urban centers with more than 500,000 inhabitants increased from 3 to 28, and more than 75 military coups occurred in 30 countries. The result was a massive migration of rural inhabitants to urban centers concomitant with the spread of HIV-1 to large population centers. With the associated demographic, economic, and social changes, an epidemic of sexually transmitted diseases and HIV-1 was ignited. Migratory patterns were also responsible for the spread of endemic HIV-2 to neighboring West African countries and eventually to Europe, the Americas, and India. Although Southeast Asia was the last region in which HIV-1 was introduced, it has the greatest potential for rapid spread due to population density and inherent risk behaviors. Thus, the migration of poor, rural, and young sexually active individuals to urban centers coupled with large international movements of HIV-infected individuals played a prominent role in the dissemination of HIV globally. The economic recession has aggravated the transmission of HIV by directly increasing the population at risk through increased urban migration, disruption of rural families and cultural values, poverty, and prostitution and indirectly through a decrease in health care provision. Consequently, social and economic reform as well as sexual behavior education need to be intensified if HIV transmission is to be controlled.

REFERENCES

1. World Health Organization (1994) *Wkly. Epidemiol. Rec.* **69,** 189–196.
2. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rep.* **42,** 869–871.
3. Selik, R. M., Chu, S. Y. & Buehler, J. W. (1993) *J. Am. Med. Assoc.* **269,** 2991–2994.
4. DeCock, K. M., Barrere, B., Diaby, L., Lafontaine, M.-F., Gnaore, E., Porter, A., Pantobe, D., Lafontant, G. C., Dago-Akribi, A., Ette, M., Odehouri, K. & Heyward, W. L. (1990) *Science* **249,** 793–796.

5. Chin, J. (1990) *Lancet* **336,** 221–224.
6. Nahmias, A. J., Weiss, J., Yao, X., Lee, F., Kodski, R., Schanfield, M., Matthews, T., Bolognesi, D., Durack, D. & Motulsky, A. (1986) *Lancet* **i,** 1279–1280.
7. World Health Organization (1993) *Wkly. Epidemiol. Rec.* **68,** 74–77.
8. Oucho, J. O. & Gould, W. T. S. (1993) in *Demographic Change in Sub-Saharan Africa*, eds. Foote, K. A., Hill, K. H. & Martin, L. G. (Natl. Acad. Press, Washington, DC), pp. 256–296.
9. Okoth-Ogendo, H. W. (1991) *People* **18,** 24–26.
10. United Nations Economic Commission for Africa (1991) *World Population Prospects 1990* (United Nations, New York).
11. Nzilambi, N., De Cock, K. M., Forthal, D. N., Francis, H., Ryder, R. W., Malebe, I., Getchell, J., Laga, M., Piot, P. & McCormick, J. B. (1988) *N. Engl. J. Med.* **318,** 276–279.
12. Rwandan HIV Seroprevalence Study Group (1989) *Lancet* **i,** 941–943.
13. Quinn, T. C., Mann, J. M., Curran, J. W. & Piot, P. (1986) *Science* **234,** 955–936.
14. Anderson, R. M., May, R. M., Boily, M. C., Garnett, G. P. & Rowley, J. T. (1991) *Nature (London)* **352,** 581–589.
15. Smallman-Raynor, M. R. & Cliff, A. D. (1991) *Epidemiol. Infect.* **107,** 69–80.
16. Wood, W. B. (1988) *Prof. Geogr.* **40,** 266–269.
17. Carswell, J. W., Lloyd, G. & Howells, J. (1989) *AIDS* **3,** 759–761.
18. Hunt, C. W. (1989) *J. Health Soc. Behav.* **30,** 353–373.
19. Larson, A. (1990) *African Affairs* **89,** 5–25.
20. Omara-Otunnu, A. (1987) *Politics and the Military in Uganda* (Macmillan, London).
21. Jochleson, K., Mothibeli, M. & Leger, J.-P. (1991) *Int. J. Health Serv.* **21,** 157–173.
22. Anderson, R. M. (1992) in *Research Issues in Human Behavior and Sexually Transmitted Diseases in the AIDS Era*, eds. Wasserheit, J. N., Aral, S. O., Holmes, K. K. & Hitchcock, P. J. (Am. Soc. Microbiol., Washington, DC), pp. 38–45.
23. Decosas, J. & Pedneault, V. (1992) *Health Policy and Planning* **7,** 227–233.
24. Bureau national du recensement du Rwanda (1978) *Recensement general de la population et de l'habitat* (Bureau national du recensement, Kigali, Rwanda).
25. Bassett, M. T. & Mhloyi, M. (1991) *Int. J. Health Serv.* **21,** 143–156.
26. Rowley, J. T., Anderson, R. M. & Wan Ng, T. (1990) *AIDS* **4,** 47–56.
27. Clavel, F., Guyader, M., Guetard, D., Salle, M., Montagnier, L. & Alizon, M. (1987) *Nature (London)* **326,** 662–669.
28. Guyader, M., Emerman, M., Sonigo, P., Calvel, F., Montagnier, L. & Alizon, M. (1987) *Nature (London)* **326,** 662–669.
29. Russell, S. S. (1993) in *Demographic Change in Sub-Saharan Africa* , eds. Foote, K. A., Hill, K. H. & Martin, L. G. (Natl. Acad. Press, Washington, DC), pp. 297–349.
30. United Nations (1989) *World Population Trends and Policies: 1989 Monitoring Report* (United Nations Population Division, New York).
31. DeCock, K. M. & Brun-Vezinet, F. (1989) *AIDS* **3** (Suppl. 1), S89–S95.
32. Horsburgh, C. R., Jr. & Holmberg, S. D. (1988) *Transfusion* **28,** 192–195.
33. Kanki, P. (1991) in *AIDS Clinical Review*, eds. Volberding, P. & Jacobson, M. A. (Marcel Dekker, New York), pp. 17–38.
34. Markovitz, D. M. (1993) *Ann. Intern. Med.* **118,** 211–218.

35. Naucler, A., Albino, P., da Silva, A. P., Andreasson, P.-A., Andersson, S. & Biberfeld, G. (1991) *AIDS* **5,** 301–304.
36. Poulsen, A.-G., Aaby, P., Gottschau, A., Kvinesdal, B. B., Dias, F., Molbak, K. & Lauritzen, E. (1993) *J. AIDS* **6,** 941–948.
37. Pison, G., Le Guenno, B., Lagarde, E., Enel, C. & Seck, C. (1993) *J. AIDS* **6,** 196–200.
38. Kanki, P., M'Boup, S., Marlink, R., Travers, K., Hsieh, C.-C., Gueye, A., Boye, C., Sankale, J.-L., Donnelly, C., Leisenring, W., Siby, T., Thior, I., Dia, M., Gueye, E.-H., N'Doye, I. & Essex, M. (1992) *Am. J. Epidemiol.* **136,** 895–907.
39. Pickering, H., Todd, J., Dunn, D., Pepin, J. & Wilkins, A. (1992) *Soc. Sci. Med.* **34,** 75–88.
40. Pepin, J., Dunn, D., Gaye, I., Alonso, P., Egboga, A., Tedder, R., Piot, P., Berry, N., Schellenberg, D., Whittle, H. & Wilkins, A. (1991) *AIDS* **5,** 69–75.
41. Olaleye, O. D., Bernstein, L., Ekweozor, C. C., Sheng, Z., Omilabu, S. A., Li, X.-Y., Sullivan-Halley, J. & Rasheed, S. (1993) *J. Infect. Dis.* **167,** 710–714.
42. Dada, A. J., Oyewole, F., Onofowokan, R., Nasidi, A., Harris, B., Levin, A., Diamondstone, L., Quinn, T. C. & Blattner, W. A. (1993) *J. AIDS* **6,** 1358–1363.
43. Cortes, E., Detels, R., Aboulafia, D., Li, X. L., Moudgil, T., Alam, M., Bonecker, C., Gonzaga, A., Oyafuso, L., Tondo, M., Boite, C., Hammershlak, N., Capitani, C., Slamon, D. J. & Ho, D. D. (1989) *N. Engl. J. Med.* **320,** 953–958.
44. Centers for Disease Control (1988) *Morbid. Mortal. Wkly. Rep.* **37,** 33–35.
45. O'Brien, T. R., George, J. R. & Holmberg, S. D. (1992) *J. Am. Med. Assoc.* **267,** 2775–2779.
46. Quinn, T. C. (1992) *Infect. Dis. Clin. Prac.* **1,** 355–356.
47. Kulkarni, S., Thakar, M., Rodrigues, J. & Banerjee, K. (1992) *Indian J. Med. Res.* **95,** 213–215.
48. Pfutzner, A., Dietrich, U., von Eichel, U., von Briesen, H., Brede, H. D., Maniar, J. K. & Rubsamen-Waigmann, H. (1992) *J. AIDS* **5,** 972–977.
49. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rep.* **42,** 547–557.
50. Grey, M. R. (1992) *Ann. Intern. Med.* **116,** 329–334.
51. Castro, K. G., Lieb, S., Jaffe, H. W., Narkunas, J. P., Calisher, C. H., Bush, T. J. & Witte, J. J. (1988) *Science* **239,** 193–197.
52. Jones, J. L., Rion, P., Hollis, S., Longshore, S., Leverette, W. B. & Ziff, L. (1991) *South Med. J.* **84,** 1088–1090.
53. Rodman, A. E. & *et al.*, (1988) *Morbid. Mortal. Wkly. Rep.* **37,** 517–519.
54. Quinn, T. C., Narain, J. P. & Zacarias, F. R. (1990) *AIDS* **4,** 709–724.
55. Koenig, E. R. (1989) *Lancet* **i,** 782–783.
56. Weniger, B. G., Limpakarnjanarat, K., Ungchusak, K., Thanprasertsuk, S., Choopanya, K., Vanichseni, S., Uneklabh, T., Thongcharoen, P. & Wasi, C. (1991) *AIDS* **5** (Suppl. 2), S71–S75.
57. Wangroonsarb, Y., Weniger, B. G., Wasi, C., Traisupa, A., Kunasol, P., Rojanapithayakorn, W. & Fucharoen, S. (1985) *Southeast Asian J. Trop. Med. Public Health* **16,** 517–520.
58. Traisupa, A., Wongba, C. & Taylor, D. N. (1987) *Genitourin. Med.* **63,** 106–108.
59. Vanichseni, S., Plangsringarm, K., Sonchai, W., Akarasewi, P., Wright, N. E. & Choopanya, K. (1990) *Thai. AIDS J.* **1,** 75–82.
60. Ford, N. & Koetsawang, S. (1991) *Soc. Sci. Med.* **33,** 405–414.

61. Tourism Authority of Thailand (1989) *Annual Statistical Report on Tourism in Thailand* (Tourism Authority of Thailand, Bangkok).
62. Ou, C.-Y., Takebe, Y., Weiniger, B. G., Luo, C.-C., Kalish, M. L., Auwanit, W., Yamazaki, S., Gayle, H. D., Young, N. L. & Schochetman, G. (1993) *Lancet* **341,** 1171–1174.
63. McCutchan, F. E., Hegerich, P. A., Brennan, T. P., Phanuphak, P., Sing-haraj, P., Jugsudee, A., Berman, P. W., Gray, A. M., Fowler, A. K. & Burke, D. S. (1992) *AIDS Res. Hum. Retroviruses* **8,** 1887–1895.
64. Myers, G., Korber, B., Berzofsky, J. A., Smith, R. F. & Pavlakis, G. N. (1992) *A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences: Human Retroviruses and AIDS 1992* (Los Alamos Natl. Lab., Los Alamos, NM).
65. Naik, T., Sarkar, S., Singh, H. L., Bhunia, S. C., Singh, Y. I., Singh, P. K. & Pal, S. C. (1991) *AIDS* **5,** 117–118.
66. Mathai, R., Prasad, P. V., Jacob, M., Babu, P. G. & John, T. J. (1990) *Indian J. Med. Res.* **91,** 239–241.
67. Dietrich, U., Grez, M., von Briesen, H., Panhans, B., Feissendorfer, M., Kuhnel, H., Maniar, J., Mahambre, G., Becker, W. B., Becker, M. L. & Rubsamen-Waigmann, H. (1993) *AIDS* **7,** 23–27.
68. Micklin, M. & Sly, D. F. (1988) in *The Global Impact of AIDS*, eds. Fleming, A. F., Carballo, M., Fitzsimons, D. W., Bailey, M. R. & Mann, J. (Liss, New York), pp. 67–77.
69. Chin, J. (1988) in *The Global Impact of AIDS*, eds. Fleming, A. F., Carballo, M., Fitzsimons, D. W., Bailey, M. R. & Mann, J. (Liss, New York), pp. 61–65.
70. Decosas, J. (1992) *Fam. Plann. World* **2,** 4.
71. Sanders, D. & Sambo, A. (1991) *Health Policy and Planning* **6**, 157–165.
72. Larson, A. (1989) *Rev. Infect. Dis.* **11**, 716-731.
73. Carswell, J. W. (1987) *AIDS* **1,** 223–227.
74. Bwayo, J. J., Omari, A. M., Mutere, A. N., Jaoko, W., Sekkade-Kigondu, C., Kreiss, J. & Plummer, F. A. (1991) *East Afr. Med. J.* **68,** 425–429.
75. Bwayo, J. J., Mutere, A. N., Omari, M. A., Kreiss, J. K., Jaoko, W., Kigondu-Sekkade, C. & Plummer, F. A. (1991) *East Afr. Med. J.* **68,** 714–719.

Impact of Malaria on Genetic Polymorphism and Genetic Diseases in Africans and African Americans

LOUIS H. MILLER

Africa is the area of the world most threatened by malaria because it has the most efficient mosquito vectors, the *Anopheles gambiae* complex. Outside the endemic areas of Africa, *An. Gambiae* has caused some of the worst epidemics of our century. One such malaria epidemic occurred when *An. gambiae* was introduced into Brazil. The effects in Africa are more insidious, in that malaria kills young children and pregnant women; adults are immune and little affected. The problem has been compounded by the spread of chloroquine resistance. This has threatened the availability of the cheapest and safest treatment to diminish the impact of malaria in these areas where mosquito control has been ineffective. The combination of sulfadoxine and pyrimethamine is a temporary stopgap. Cost is a major consideration in poor countries and will limit the availability of newer drugs. In Asia and Brazil, where the population can afford more expensive drugs, resistance to these drugs has developed. The development of malaria vaccines, which will reduce mortality and the spread of drug-resistant malaria, has not received the necessary funding or industrial interest to be successful within a reasonable period of time. The crisis in development of new modalities against malaria is made worse by the minimal industrial interest in antimalarial drug development.

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ESTIMATES OF MORTALITY FROM MALARIA

What will be the impact of parasite resistance to antimalarial drugs on death in the population? Malaria is one of the few diseases for which we can predict the impact of the loss of drugs without an alternative form of control. We obtain these estimates of past and future malarial mortality from the frequencies of certain genes observed today.

J. B. S. Haldane (1) was the first to propose that infectious diseases were the main selective force for human evolution during the past 5000 years. In a recent study of diversity at the molecular level, Murphy (2) compared sequences of genes common between rodents and humans. It was found that host defense genes were more diverse than all other classes of proteins, suggesting that selection in mice and humans resulted from exposure to different microorganisms. In Europeans, tuberculosis has been a major selective force in evolution; in Africans, malaria was one of the major selective forces in their evolution and, as a result, many genes are known to confer a survival advantage. One of the best studied is the gene for hemoglobin S. From the frequency of the gene for hemoglobin S, it is possible to estimate the mortality from malaria by using the Hardy–Weinberg equation (3). It is assumed that mortality from malaria is the sole selective force for the gene. The mortality of hemoglobin SS in West Africa is 100% in childhood. In malaria-endemic areas, SA heterozygotes have a survival advantage compared with hemoglobin AA children. This selection of a deleterious gene by survival advantage for another disease (e.g., malaria) is referred to as a balanced polymorphism. In some areas of Africa, the mortality from malaria must have been as high as 25% to account for the hemoglobin S gene frequencies that we see today.

Another example of balanced polymorphism is a mutation in erythrocyte band 3, which causes a certain form of erythrocyte membrane defect known as Melanesian ovalocytosis. This mutation is a 27-base-pair (9 amino acid) deletion in band 3 (4). It is particularly common in Papua New Guinea, the only other area of the world with transmission of malaria to equal that seen in Africa. The ovalocytic erythrocyte is partially resistant to invasion by malaria parasites. Homozygosity for this mutation is 100% lethal during fetal development, the price that is paid for a deleterious gene that helps survival in heterozygous children.

The broader implications of the above studies are as follows. (*i*) Definitive studies on the population genetics of this disease were impossible before identification of the genetic defect because there are a number of causes of abnormally shaped erythrocytes in Papua New Guinea. It only now has been possible to study its gene frequency and the impact of the mutant allele on intrauterine lethality of the homozygote.

(*ii*) The fact that the gene frequency—like that of hemoglobin S in Africa—is high argues for a high mortality from malaria in Papua New Guinea. Although high mortality is not seen today because of chloroquine, it may be a problem in the future when antimalarial drugs become ineffective. (*iii*) Why have band 3 mutations in Papua New Guinea never occurred in Africa, and why has hemoglobin S never occurred in Papua New Guinea? Other erythrocyte skeletal mutations have been identified in Africa that appear to affect parasite development (5, 6). (*iv*) Despite the slow evolution of the human genome compared to that of the parasite, the host innate resistance mechanisms can afford improved survival. It may not be in the best interest of the parasite to kill its host.

The fact that certain polymorphisms such as hemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency were selected for by malaria is well known (7, 8). I am going to explore other genetic differences that exist between Africans and Caucasians that may have been selected for by malaria and what clues they may provide for drug and vaccine development. Some of these polymorphisms are deleterious; genetic markers for these would identify individuals at risk for preventive therapy. Others are neutral as far as we know and have gone to fixation at 100%.

HYPERTENSION AND IRON OVERLOAD

Hypertension and liver disease are two major diseases that affect African Americans at a higher frequency than European Americans. Because the genetic bases are unknown, the potential that these genes confer resistance to malaria should be considered.

There are many hypotheses for the selective force that led to the increased incidence of hypertension in African Americans. Theories postulate that African Americans, who retain salt more avidly than European Americans, were selected for because of limited availability of salt in Africa, death during the transportation of slaves from Africa to America, or death during subsequent life as a slave in America. The validity of these hypotheses has been questioned by historian Philip Curtin (9). He pointed out that most slaves lived within 100 miles of the African coast where there was a readily available, inexpensive source of salt from evaporation of sea water. He also examined the causes of death in slaves and found no evidence for an excess of diseases related to salt loss.

I would like to raise the possibility that differences in salt metabolism, especially as they may be reflected in erythrocytes, may have been selected by malaria. The erythrocytes of African Americans are known

to have higher sodium, lower potassium, and altered sodium transport when compared to European Americans (10, 11). The potential connection may relate to the fact that there are no malarias of animals that have high-sodium erythrocytes such as dogs, cats, cows, and horses. There are saurine malarias, rodent malarias, avian malarias, and primate malarias, all in animals that have high-potassium erythrocytes (12). The absence of malaria in dogs and cows is not likely to have been related to an absence of opportunity for infection because of lack of receptors for cell invasion. This requirement for K^+ was consistent with the finding of Trager *et al.* (13) that asexual erythrocytic malaria parasites required a high K^+ culture medium when the parasites were grown outside of erythrocytes (13). The exact definition of how potassium affects parasite growth is complicated by the fact that the parasite grows at a normal rate in erythrocytes treated with ouabain, which increases intraerythrocytic sodium and decreases intraerythrocytic potassium (14, 15). It is possible that the shift in sodium/potassium may reduce parasite growth through a third factor such as hemoglobin S or that other ouabain-insensitive transporters may affect parasite growth. How this may affect the intraerythrocytic parasite derives in part from the work of Friedman (16) on growth of *Plasmodium falciparum* in hemoglobin SA erythrocytes (16). He found that the parasite growth in hemoglobin SA erythrocytes was normal under an atmosphere of 17% O_2, but the parasites could not grow at 3% O_2. The parasites could, however, grow normally in SA hemoglobin at 3% O_2 tension if the culture medium contained high K^+ concentrations. The parasitized erythrocytes grown in the usual culture medium at low oxygen tension sickled and lost K^+. The parasitized erythrocytes still sickled in high K^+ medium, but the erythrocyte K^+ remained normal.

The question of whether hypertension genes suppress the growth of the malaria parasite and give a survival advantage in malariaendemic areas cannot be studied until the molecular basis of hypertension and the differences in erythrocyte sodium in Africans and African Americans have been identified. Genetic markers would also identify those at risk of hypertension and may suggest drugs or dietary precautions to prevent this common disease. Mutations in the angiotensinogen gene have been associated with hypertension in Caucasians (17), but its involvement in hypertension in African Americans remains undefined.

Iron overload and concomitant liver disease in sub-Sahara Africa have only recently been shown to have a genetic basis (18). Large deposits of hemosiderin are found in the Kupffer cells and hepatic parenchymal cells and are associated with portal fibrosis and cirrhosis. Diabetes mellitus may also occur at increased frequency in these patients because

of similar hemosiderin deposits in the pancreas. Recently, Gordeuk *et al.* (18) demonstrated that the disease is genetic; however, unlike hereditary hemochromatosis of Caucasians, the disease is not linked to the *HLA* locus. In Southern Africa, the disease is associated with a high intake of beer brewed in steel drums, and it is proposed that the disease results from an interaction of genetic susceptibility and the intake of excessive iron and alcohol. Why would I speculate that the gene responsible for this disease was selected for by increased fitness in a malaria-endemic area? How could abnormalities in iron metabolism be involved in resistance to malaria when the parasite has a ready supply of iron from digestion of hemoglobin, a main source of amino acids for the parasite? Iron in the form of ferriprotoporphyrin IX is highly toxic to the parasite. The parasite detoxifies iron by forming malaria pigment, a form that is inaccessible to the parasite. Malaria pigment is β-hematin (19) that can be formed in the test tube from polymerization of ferriprotoporphyrin IX at pH 3 and high temperatures. In the cell, however, formation of β-hematin requires a polymerase, an enzymatic activity that was discovered by Slater and Cerami (20). Interestingly, polymerization is inhibited by chloroquine and other active 4-aminoquinolines (20), solving the mystery of the mode of chloroquine action. It has been further shown, through studies with iron chelation by desferroxamine, that the malaria parasite must obtain iron from the outside by unknown mechanisms (21). Iron uptake by the parasite may require a ferric reductase, a receptor, and a transporter molecule. If altered iron metabolism is proven to confer resistance to malaria, it would be an impetus to explore iron-related pathways in malaria therapy. In addition, this African disease may cause increased liver disease in African Americans. The study of these questions would require identification of the genetic basis of iron overload and the determination of whether this gene is associated with increased resistance to malaria in Africa and liver disease in African Americans. It is clear that hypertension and iron overload in Africans and African Americans may be preventable by diet, and the search for its genetic basis deserves high priority to develop molecular probes to identify those at risk.

HLA AND MALARIA

Piazza *et al.* (22) were the first to present evidence of the association between HLA and malaria from gene frequencies in Sardinia, comparing lowland areas where malaria occurred and highland areas (22). In the definitive studies by Hill *et al.* (23), a class I molecule, HLA-B53, was found in lower frequency in Gambian children with severe malaria than in the general population. Class I molecules contain a groove that

presents antigen to the T-cell receptor on $CD8^+$ T cell.s Antigens presented in this way are targets for $CD8^+$ T-cell-dependent cytotoxicity. What cells in the malaria life cycle can present antigens through the HLA class I pathway? Sporozoites injected into humans invade the liver parenchymal cells, where they multiply. Liver parenchymal cells express class I HLA antigens and, theoretically, could present malarial antigens to $CD8^+$ T cells. Merozoites released from liver cells invade erythrocytes, thereby initiating the erythrocytic stage in the life cycle that causes disease. There is no evidence in rodent malarias that $CD8^+$ T cells are critical for immunity to the erythrocytic stage. Immunity to the liver stage has been shown to be dependent in part on $CD8^+$ T cells (24, 25). For example, immunity induced by irradiated sporozoites was eliminated by $CD8^+$ T-cell depletion. Subsequently, the $CD8^+$ T-cell epitope on the CS protein was identified in *P. falciparum* (26, 27). Of great concern when this epitope was identified was the fact that it was highly variant among clones of *P. falciparum*.

The strategy used by Hill *et al*. (28) for identifying malaria epitopes presented by HLA-B53 to $CD8^+$ T cells was as follows. The peptide presented by HLA-class I molecules usually consists of eight or nine amino acids, depending on the class I molecule. The peptides eluted from the groove were sequenced, and from this information the invariant anchor amino acid, proline, at position 2 of the nonapeptides, was identified. All possible nonapeptides with a proline at position 2 from the four malaria proteins of the sporozoite and liver stages were synthesized. These were tested as targets of cytotoxicity with cells from immune, adult Gambians who were HLA-B53 (28). A peptide from one of the liver-stage antigens was identified that was a target of cytotoxic $CD8^+$ T cells. This must undergo testing as a potential antigen to induce $CD8^+$ T-cell-mediated immunity. There is one curiosity of this immunity. Although the severity of illness was less in children who expressed HLA-B53 than those who did not, the incidence of blood-stage infections was not reduced (23). How HLA-B53 could protect without reducing infection rates probably reflects our limited understanding of pathogenesis in malaria. A potential parallel may be drawn with protection against malaria by permethrin-impregnated bed nets, which reduced mortality in the age group 1–5 years (29). Curiously, the infection rate in the children protected by the impregnated bed nets was as high as in the unprotected community. One speculation is that a reduced number of clones inoculated into a child might reduce the child's risk of being exposed to a clone that is antigenically unique. Thus, either bed nets or a certain HLA type may give enough protection to decrease the number of parasite clones infecting a child and, in this way, may lead to increased survival. It is evident that such results may

change our strategy for vaccine development in endemic areas where improved survival is the goal.

TABLE 1 Blood group antigens of African origin

Blood group	African	Euro-pean	Comment	Ref(s).
Duffy negative [Fy(a− b−)]	≈100% (W. Africa)	0%	Chemokine receptor; *P. vivax* receptor	30, 31, 32, 33
Glycophorin Dantu	4%	0%	*P. falciparum* receptor; hybrid glycophorin (extracellular glycophorin B, transmembrane and cytoplasmic glycophorin A)	34
Glycophorin B negative (S− s− U−)	20% (Pygmies)	0%	*P. falciparum* receptor	35
Sl(a−)	30% (in USA)	1%	Polymorphism in complement receptor 1 (CR1)	36, 37
Rh (V+)	40%	0%	13 transmembrane loops (function unknown)	38, 39
Js^a	17% (in USA)	1%	Zinc metalloendopeptidase-like	40, 41

change our strategy for vaccine development in endemic areas where improved survival is the goal.

BLOOD GROUPS AND MALARIA

There are blood group differences between African Americans and Caucasian populations (Table 1). Sl(a-), which is found in 50% of African Americans, is rare in Caucasian populations. Sl(a-) is a polymorphism in the erythrocyte CR 1 receptor for C3b/C4b (36). It is thought that this receptor is involved in clearing immune complexes from the circulation and may be related to the 4-fold increase in systemic lupus in African Americans. We have been unable to find any reduced invasion of these erythrocytes. Although I still believe it was selected by malaria, I have been unable to find the connection.

Africans also have a high frequency of Rh and Kell antigens that differ from those found in Caucasians (38, 40). One of the genes encoding Rh has recently been cloned, and it is predicted to cross the membrane 13 times (39). Although the function of Rh remains a mystery, it is possible that it may be involved in membrane transport or membrane integrity and may affect survival of the intraerythrocytic parasite.

From the parasite's point of view, certain blood group antigens on the erythrocyte surface are receptors for the attachment and invasion of the erythrocyte. Mutations in these surface proteins in Africans were selected for by resistance to invasion of erythrocytes by malaria parasites.

Invasion consists of multiple steps that include initial recognition, reorientation to bring the apical end of the parasite in contact with the erythrocyte, junction formation, and entry into a vacuole created by the parasite (42). There are multiple receptors involved in these steps (43). This derives from the finding that Africans are unable to be infected with *Plasmodium vivax* because almost 100% lack the Duffy blood group antigens (30, 31). Knowledge of the requirements of *P. vivax* for invasion derive largely from studies on the interaction between Duffy-negative erythrocytes and *Plasmodium knowlesi*, a simian malaria that also requires the Duffy blood group system to invade human erythrocytes. The parasite attaches and apically reorients on Duffy-negative erythrocytes but cannot form a junction or invade these erythrocytes (44). Thus, the initial receptors involved in attachment are unrelated to Duffy blood group antigens. It is only at the stage of junction formation that the Duffy blood group system is involved as a receptor.

The Duffy blood group system has now been shown to be the erythrocyte chemokine receptor (32, 33). Chemokines are a family of molecules involved in chemotaxis and proinflammatory activities related to activating cells like neutrophils for chemotaxis and for binding endothelium. The erythrocyte chemokine receptor, when originally identified, was proposed to be a scavenger for inflammatory mediators (45). If this hypothesis is correct, then there should be physiologic differences between Duffy-positive and -negative individuals. It has been observed that Africans and African Americans have a lower peripheral neutrophil count (46), although this is not associated with increased susceptibility to infection. The question of whether this lower neutrophil count is causally related to Duffy negativity remains to be determined. The involvement of Duffy antigens in chemokine metabolism also raises the question of whether Duffy negativity in West Africa provides a survival advantage in terms of the ability to control *P. falciparum* infections. The absence of the receptor for *P. vivax* may have been a by-product of this other selective advantage. Alternatively, resistance to *P. vivax* may have selected for Duffy negativity.

Investigation into genetically determined resistance to *P. vivax* led to discoveries of Duffy antigens as the erythrocyte chemokine receptor. This, in turn, suggested new approaches to drug therapy and vaccine development. Chemokines block invasion by *P. knowlesi* (32) and would presumably also block *P. vivax*. The possibility now exists that chemokine analogs can be developed as antimalarial therapy through receptor blockade.

The parasite molecule that binds to Duffy antigens may provide an effective vaccine, as antibodies against the molecule may inhibit invasion. We have recently succeeded in identifying the domain of the

parasite Duffy binding ligand, which binds Duffy-positive but not Duffy-negative erythrocytes (47). Each region of the extracellular portion of the molecule was expressed in COS cells, and only region II bound. The Duffy binding molecule of *P. vivax* has structural and sequence homology with a sialic acid binding molecule of *P. falciparum* (48). The same domain in the *P. falciparum* ligand was shown to bind human erythrocytes in a sialic acid-dependent manner (49). That is, neuraminidase-treated human erythrocytes could not bind COS cells expressing *P. falciparum* region II. An erythrocyte mutant that lacks glycophorin A, En(a-) erythrocytes, also did not bind to these COS cells. This result suggests that glycophorin B, the second most common sialoglycoprotein on the erythrocyte surface, cannot act as receptor for this parasite ligand. Consistent with this finding was the fact that trypsin-treated erythrocytes that lack glycophorin A, but not glycophorin B, could not bind to the region II-expressing COS cells.

The first 25 amino acids of glycophorins A and B are identical and contain most of the sialic acid residues (50). One difference between glycophorins A and B is that glycophorin A, and not B, has one N-linked oligosaccharide. This difference in N-linked oligosaccharides is unlikely to explain the differential binding of glycophorin A, as O-Glycanase but not N-Glycanase treatment of erythrocytes rendered them refractory to binding of the *P. falciparum* sialic acid binding ligand (49). The basis of specificity of glycophorin A as a receptor for EBA-175 was tested in two ways (49). First, glycophorin A, but not glycophorin B, inhibited erythrocyte binding to COS cells expressing the binding domain of EBA-175. Second, glycophorin A or the N-terminal 64 amino acids of glycophorin A blocked binding of EBA-175 to erythrocytes; shorter N-terminal peptides that contain most O-linked oligosaccharide and the one N-linked oligosaccharide did not block attachment. These data demonstrate that EBA-175 binds specifically to glycophorin A and that the binding is dependent on sialic acid and the peptide backbone. It is unknown whether the peptide acts to present clusters of sialic acid in a specific spatial distribution or whether it functions as part of the receptor.

P. falciparum, unlike *P. vivax*, has developed alternative pathways for invasion. Thus, deletion of a receptor does not appear to make the population completely resistant to *P. falciparum*. In fact, we and others have developed data for multiple alternative pathways for invasion (51–53). One of the pathways uses glycophorin B with a different parasite ligand (54).

Although there is evidence that both glycophorin A and B are used as receptors for *P. falciparum*, the abnormalities in the population are all in glycophorin B (55–57). Glycophorin B negative erythrocytes in some pygmy populations reach frequencies of 20%. The mutation in glycophorin

B and the relative severity of *P. falciparum* in glycophorin B negative pygmies has yet to be studied. If this was selected by *P. falciparum*, which seems likely, then the parasite is unable to produce full virulence in these individuals. Multiple other mutations of glycophorin B in Africans have been observed, such as Dantu, Henshaw and U-. Dantu results from a recombination between the glycophorin A gene and the glycophorin B gene, which leads to a mutant that has part of the extracellular domain of glycophorin B and the transmembrane and cytoplasmic domains of glycophorin A. Dantu also has a normal copy of glycophorin A and lacks glycophorin B. Henshaw has point mutations in the N-terminus of glycophorin B that influences antigenicity and probably folding in other regions of the molecule. In Southeast Asia, another area of severe *P. falciparum* malaria, Miltenburger III occurs at high frequencies. Like Dantu, Miltenberger III involves a recombination between glycophorin A and B and also has a normal glycophorin A allele; the normal glycophorin B allele is missing. We can conclude that either deficiency of glycophorin A gives no selective advantage or that glycophorin A deficiency is detrimental. It is known that individuals with En(a-) erythrocytes that lack glycophorin A appear to have no associated disease. Perhaps in Africa, however, this gene deletion is more deleterious. Alternatively, in the setting of glycophorin B mutations, but not of glycophorin A mutations, the parasite is unable to express its full virulence, and consequently mutations in glycophorin B are selected. As a correlary, vaccines against the parasite ligand that binds glycophorin B may be a better target than EBA-175, the ligand for glycophorin A.

It is clear that, despite these mutations in glycophorin B, the parasite remains in the population and is able to invade erythrocytes with these mutations. When *P. vivax* lacksthis flexibility is unknown, but such flexibility is clearly an advantage for the parasite that can respond to polymorphisms in the human population.

Malaria is a major cause of mortality and, as a consequence, it has had a marked and varied impact on the genetic makeup of the human population. It should be equally clear why there is a need to invest in the discovery of alternative methods of malaria control such as vaccines and approaches to modifying the vector population. We have a few years before the antimalarial drugs become unacceptably expensive for the poor countries. It is during this precious time that we must search for alternatives to reduce mortality. Unfortunately, obtaining the resources and industrial support for vaccine development has been difficult for this disease, which little affects our lives in the wealthy countries. It is a challenge to all of us in the world community to accelerate development of methods to reduce the impact of malaria.

SUMMARY

The high mortality from malaria in sub-Sahara Africa selected multiple genes that give the population a selective advantage. Identification of the genetic basis for resistance may suggest unusual approaches to development of malarial vaccines and antimalarial drugs. Some of these genes may be deleterious, although of selective advantage within the African setting, and need to be identified for counseling for disease prevention.

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REFERENCES

1. Haldane, J. B. S. (1949) *Ric. Sci. Suppl.* **19,** 68–76.
2. Murphy, P. (1993) *Cell* **72,** 823–826.
3. Smith, S. M. (1954) *Ann. Hum. Genet.* **19,** 51–57.
4. Jarolim, P., Palek, J., Amato, D., Hassan, K., Sapak, P., Nurse, G. T., Rubin, H. L., Zhai, S., Sahr, K. E. & Liu, S.-C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11022–11026.
5. Lecomte, M.-C., Chermy, D., Solis, C., Ester, A., Féo, C., Gautero, H., Bournier, O. & Boivin, P. (1985) *Blood* **65,** 1208–1217.
6. Facer, C. A. (1986) *Int. Symp. Malaria* (Memorias do Instituto Oswaldo Cruz, Rio de Janiero, Brazil), Vol. 81, pp. 111–114.
7. Nagel, R. L. (1990) *Blood Cells* (Springer, New York), Vol. 16, pp. 321–339.
8. Yuthavong, Y. & Wilairat, P. (1993) *Parasitol. Today* **9,** 241–245.
9. Curtin, P. D. (1992) *Am. J. Public Health* **82,** 1681–1686.
10. Canessa, M., Adragna, N., Solomon, H. S., Connolly, T. M. & Tosteson, D. C. (1980) *N. Engl. J. Med.* **302,** 772–776.
11. Canessa, M., Lâski, C. & Falkner, B. (1990) *Hypertension* **16,** 508–514.
12. Garnham, P. C. C. (1966) *Malaria Parasites and Other Haemosporidia* (Blackwell, Oxford).
13. Trager, W., Langreth, S. G. & Platzer, E. G. (1972) *Proc. Helminthol. Soc. Wash.* **39,** 220–230.
14. Ginsburg, H., Handell, S., Friedman, S., Gorodetsky, R. & Krugliak, M. (1986) *Z. Parasitenkd.* **72,** 185–199.
15. Tanabe, K., Izumo, A. & Kageyama, K. (1986) *Am. J. Trop. Med. Hyg.* **35,** 476–478.
16. Friedman, M. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1994–1997.
17. Jeunemaitre, X., Sourbier, F., Kotelevtsev, Y. V., Lifton, R. P., Williams, C. S., Charru, A., Hunt, S. C., Hopkins, P. N., Williams, R. R., Lalouel, J.-M. & Corvol, P. (1992) *Cell* **71,** 169–180.
18. Gordeuk, V., Mukiibi, J., Hasstedt, S. J., Samowitz, W., Edwards, C. Q.,

West, G., Nadambier, S., Emmanual, J., Nkanza, N., Chapanduka, Z., Randall, M., Boone, P., Romano, P., Martell, R. W., Yamashita, T., Effler, P. & Brittenham, G. (1992) *N. Engl. J. Med.* **326,** 95–100.

19. Slater, A. F. G., Swiggard, W. J., Orton, B. R., Flitter, W. D., Goldberg, D. E., Cerami, A. & Henderson, G. B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 325–329.
20. Slater, A. F. G. & Cerami, A. (1992) *Nature (London)* **355,** 167–169.
21. Gordeuk, V., Thuma, P., Brittenham, G., McLaren, C., Parry, D., Backen-stose, A., Biemba, G., Msiska, R., Holmes, L., McKinley, E., Bargas, L., Gilkeson, R. & Poltera, A. A. (1992) *N. Engl. J. Med.* **327,** 1473–1477.
22. Piazza, A., Mayr, W. R., Contu, L., Amoroso, A., Borelli, I., Curtoni, E. S., Marcello, C., Moroni, A., Olivetti, E., Richiardi, P. & Ceppellini, R. (1985) *Ann. Hum. Genet.* **49,** 47–63.
23. Hill, A. V. S., Allsopp, C. E. M., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J. & Greenwood, B. M. (1991) *Nature (London)* **352,** 595–600.
24. Schofield L., Villaquiran, J., Ferreira, A., Schellekens, H., Nussenzweig, R. S. & Nussenzweig, V. (1987) *Nature (London)* **330,** 664–666.
25. Weiss, W. R., Sedegah, M., Beaudoin, R. L., Miller, L. H. & Good, M. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 573–576.
26. Kumar, S., Miller, L. H., Quakyi, I. A., Keister, D. B., Houghten, R. A., Maloy, W. L., Moss, B., Berzofsky, J. A. & Good, M. F. (1988) *Nature (London)* **334,** 258–260.
27. Malik, A., Egan, J. E., Houghten, R. A., Sadoff, J. C. & Hoffman, S. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3300–3304.
28. Hill, A. V. S., Elvin, J., Willis, A. C., Aidoo, M., Allsopp, C. E. M., Gotch, F. M., Gao, X. M., Takiguchi, M., Greenwood, B. M., Townsend, A. R. M., McMichael, A. J. & Whittle, H. C. (1992) *Nature (London)* **360,** 434–439.
29. Alonso, P. L., Lindsay, S. W., Armstrong Schellenberg, J. R. M., Keita, K., Gomez, P., Shenton, F. C., Hill, A. G., David, P. H., Fegan, G., Cham, K. & Greenwood, B. M. (1993) *Trans. R. Soc. Trop. Med. Hyg.* **87,** 37–44.
30. Miller, L. H., Mason, S. J., Dvorak, J. A., McGinniss, M. H. & Rothman, I. K. (1975) *Science* **189,** 561–563.
31. Miller, L. H., Mason, S. J., Clyde, D. J. & McGinniss, M. H. (1986) *N. Engl. J. Med.* **295,** 302–304.
32. Horuk, R., Chitnis, C. E., Darbonne, W. C., Colby, T. J., Rybicki, A., Hadley, T. J. & Miller, L. H. (1993) *Science* **261,** 1182–1184.
33. Chaudhri, A., Polyakova, J., Zbrezna, V., Williams, K., Gulati, S. & Pogo, A. O. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10793–10797.
34. Unger, P., Procter, J. L., Moulds, J. J., Moulds, M., Blanchard, D., Guizzo, M. C., McCall, L. A., Cartron, J. P. & Dahr, W. (1987) *Blut* **55,** 33–43.
35. Fraser, G. R., Giblett, E. R. & Motulsky, A. G. (1966) *Am. J. Hum. Genet.* **18,** 546–552.
36. Moulds, J. M., Nickells, M. W., Moulds, J. J., Brown, M. C. & Atkinson, J. P. (1991) *J. Exp. Med.* **173,** 1159–1163.
37. Lacey, P., Laird-Fryer, B., Block, U., Lair, J., Guilbeau, L. & Moulds, J. J. (1980) *Abstracts of Volunteer Papers,*, 33rd Annual Meeting of the Am. Assoc. of Blood Banks (Am. Assoc. Blood Banks, Arlington, VA), p. 25.
38. Denatale, A., Cahan, A., Jack, J. A., Race, R. R. & Sanger, R. V. (1955) *J. Am. Med. Assn.* **159,** 247–250.
39. Agre, P. C. & Cartron, J.-P. (1992) in *Protein Blood Group Antigens of the Human*

Red Cell: Structure, Function and Clinical Significance, eds. Agre, P. C. & Cartron, J.-P. (Johns Hopkins Univ. Press, Baltimore), pp. 20–52.
40. Giblett, E. R. & Chase, J. J. (1959) *Br. J. Haematol.* **5,** 319–326.
41. Redman, C. M. & Marsh, W. L. (1992) in *Protein Blood Group Antigens of the Human Red Cell: Structure, Function and Clinical Significance,* eds. Agre, P. C. & Cartron, J.-P. (Johns Hopkins Univ. Press, Baltimore), pp. 53–69.
42. Aikawa, M., Miller, L. H., Johnson, J. & Raggege, J. (1978) *J. Cell Biol.* **77,** 72–82.
43. Hadley, T., Klotz, F. W. & Miller, L. H. (1986) *Annu. Rev. Microbiol.* **40,** 451–477.
44. Miller, L. H., Aikawa, M., Johnson, J. G. & Shiroishi, T. (1979) *J. Exp. Med.* **149,** 172–184.
45. Darbonne, W. C., Rice, G. C., Mohler, M. A., Apple, T., Hébert, C. A., Valente, A. J. & Baker, J. B. (1991) *J. Clin. Invest.* **88,** 1362–1369.
46. Broun, G. O., Herbig, F. K. & Hamilton, J. R. (1966) *N. Engl. J. Med.* **275,** 1410–1413.
47. Chitnis, C. E., & Miller, L. H. (1994) *J. Exp. Med.* **180,** 497–506.
48. Adams, J. H., Kim, L. S. B., Dolan, S. A., Fang, Z., Kaslow, D. C. & Miller, L. H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7085–7089.
49. Sim, B. K. L., Chitnis, C. E., Wasniowska, Hadley, T. J. & Miller, L. H. (1994) *Science* **264,** 1941–1944.
50. Blanchard, D. (1990) *Transfus. Med. Rev.* **3,** 170–186.
51. Mitchell, G. H., Hadley, T. J., McGinniss, M. H., Klotz, F. W. & Miller, L. H. (1986) *Blood* **67,** 1519–1521.
52. Hadley, T. J., Klotz, G. W., Pasvol, G., Haynes, J. D., McGinniss, M. H., Okubo, Y. & Miller, L. H. (1987) *J. Clin. Invest.* **80,** 1190–1193.
53. Perkins, M. E. & Holt, E. H. (1988) *Mol. Biochem. Parasitol.* **27,** 23–34.
54. Dolan, S. A., Proctor, J. L., Alling, D. W., Okubo, Y., Wellems, T. E. & Miller, L. H. (1994) *Mol. Biochem. Parasitol.*, **64,** 55–63.
55. Fukuda, M. (1993) *Seminars in Hematology* **30,** 138–151.
56. Huang, Ch. (In press) in *Molecular Basis of Major Human Blood Group Antigens,* eds. Cartron, J.-P. & Raeger, P.
57. Holliman, S. M. (1989) in *Blood Group Systems MN and Gerbich,* eds. Unger, P. & Laird-Fryer, B. (Am. Assn. Blood Banks, Arlington, VA).

Hospital-Acquired Infections: Diseases with Increasingly Limited Therapies

M. N. SWARTZ

The `6500 acute-care hospitals in this country represent unique ecological systems and provide the settings for nosocomial (hospital-acquired) infections. The principal components making up these systems are patients, medical-care personnel, equipment and devices employed in the treatment of patients with complicated medical illnesses, and the commensal microbiota of patients and the microbial population in the hospital environment. Modern acute-care hospitals are complex institutions consisting of a variety of specialized components: burn services, oncology wards, coronary care units, intensive care units, and transplantation units. Individual units may have particular nosocomial infection problems related to the type of patient being treated or the nature of their underlying illnesses, procedures employed in individual units, and the selection pressure exerted by antimicrobial usage patterns.

Each year there are `37.7 million admissions to acute-care hospitals in the United States; among these, `2.1 million patients (5.7%) develop nosocomial infections (1). This is a sizable number and, at first glance, might seem surprising five decades after the beginning of the antibiotic era. A variety of changes account for the current significant role of nosocomial infections. These alterations include changes in the age of hospitalized patients, the nature of their underlying illnesses, the types

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of surgical and systemic therapy that are now available for treating diseases that were formerly untreatable, and the multitude of antimicrobial drugs currently available and capable of selecting a more resistant microbial flora.

CHANGING FEATURES OF THE POPULATIONOF HOSPITALIZED PATIENTS

The increase in mean life expectancy in the population as a whole is reflected in the increasing age of hospitalized patients. In an acute-care community general hospital in Madison, WI, for example, the percentage of patients 65 years of age or older rose from 13% in 1970–1973 to 24% by 1987 (2). Similarly, at the Massachusetts General Hospital, the mean age of admissions increased steadily over a 7-year period (1986–1992) from 54 to 57 years. Increasing age as a risk factor for nosocomial infections is seen in the analysis of nosocomial infections in elderly patients reported by the National Nosocomial Infections Surveillance (NNIS) system between 1986 and 1990 (3). Whereas elderly patients (>65 years of age) represented only 31% of total discharges from hospitals, 54% of nosocomial infections occurred in this group.

CHANGES IN TYPES OF PROCEDURES PERFORMED FOR COMPLEX ILLNESSES

Extensive surgical procedures are now carried out almost routinely, correcting a variety of incapacitating or debilitating illnesses and restoring patients to productive lives. Many of these procedures have been introduced and performed in large numbers only in the past several decades. Innovations in cardiac surgery or orthopedic surgery serve as examples (4, 5). In 1960 the modern era of heart valve replacement began with the first successful placement of a caged-ball valve in the subcoronary aortic valve position. Currently, an estimated 46,000-58,000 prosthetic heart valve replacement procedures are performed annually in the United States (6). The first saphenous vein aortocoronary artery bypass graft for coronary artery disease was carried out in 1967. Over the ensuing 25 years this procedure has become one of the most commonly performed operations in the United States. About 390,000 such procedures are performed each year (6).

Paralleling development of innovative and extensive operative procedures for treatment of cardiac disease have been similar advances in reconstructive orthopedic surgical procedures. The first total hip replacements in this country began in 1966, and current estimates are that `125,000 such procedures are performed annually in North America

(William Harris, personal communication). The first total knee replacements were carried out in 1970, and it is estimated that currently `125,000 such procedures are performed in North America each year.

The aforementioned surgical procedures have in common the characteristic of prolonged operative time and, in the case of valve and joint replacements, the feature of insertion of a foreign body. Both of these factors are predisposing risks for development of post-operative infections. Such infections include not only operative would infections but also lower respiratory tract infections (secondary to prolonged anesthesia, intubation, etc.), urinary tract infections (secondary to indwelling urinary catheters), and blood stream infection (complicating the use of intravenous catheters while the patient is recovering from extensive surgery).

Consequences of the benefits of modern complex surgical procedures include nosocomial infections, stemming from a variety of factors. These include the extensive nature of the surgical procedures, prolonged operative/anesthesia times, need for medical devices and invasive manipulations during the post-operative period, the often complex nature of the underlying disease or of associated illnesses, and the older ages of hospitalized patients.

Overall nosocomial infection rates vary considerably depending on the type of surgery performed. Among common surgical procedures in acute-care hospitals participating in the NNIS system, the highest rates of overall nosocomial infections occur with gastric surgery (21%), bowel surgery (19%), craniotomies (18%), coronary artery bypass graft procedures (11%), and other cardiac surgery (10%) (7). Orthopedic procedures involving introduction of metallic plates and of prosthetic joints have overall nosocomial infection rates of `8%. The fact that more major surgery is now possible than was the case three decades ago carries with it the almost inevitable consequence of a greater burden of nosocomial infections.

Individuals particularly vulnerable to nosocomial infections by virtue of the immunosuppressive treatment of their underlying conditions make up a larger component of hospitalized patients than was the case three or four decades ago. Such patients include those undergoing organ transplantation or intensive chemotherapy of leukemia, lymphoma, and other neoplastic processes. The first successful human renal transplantation was performed in 1954. By 1992, renal transplantation was performed in 10,210 patients annually (8). By 1992 multiple other types of organ transplantation have become commonplace: 3059 liver transplants and 2171 heart transplants annually. Including bone marrow transplants, of which there were `4900 performed in 1990 (9), the total number of organ transplants in 1992 numbered >21,400. Nosocomial

infections in transplant patients include those due to the common nosocomial bacterial pathogens but also some due to opportunistic viral and fungal agents. Patients undergoing induction therapy for leukemia or chemotherapy for a variety of cancers are particularly at risk for nosocomial infections by virtue of the neutropenia consequent on cytotoxic drug treatment. In a prospective study over a 42-month period at a cancer research center in New York City, an overall nosocomial infection rate of 48.3 per 100 neutropenic patients (46.3 per 1000 days at risk from neutropenia) was observed (10). The NNIS data, admittedly a different group of patients in different geographic areas, nonetheless provides a frame of reference for comparison: median overall infection rates per 100 discharges for general medical patients (3.5%), for cardiac surgical patients (9.8%), and for patients on the burn/trauma service (14.9%) (11).

CHANGING SPECTRUM OF NOSOCOMIAL PATHOGENS EARLY IN THE ANTIMICROBIAL ERA

Nosocomial infections are not unique to the past few decades but have occurred since care of patients in hospitals began. What has changed over the past half century are the properties and species of infecting microorganisms. Viral agents (herpes group viruses and hepatitis virus) and *Mycobacterium tuberculosis*, important causes of nosocomial infections, will not be considered here since they have been considered separately earlier in this colloquium.

In the preantibiotic era, the principal microbial causes of nosocomial infections were Gram-positive cocci: *Streptococcus pneumoniae*, *Streptococcus pyogenes* and other streptococcal species, and *Staphylococcus aureus*. Sir William Osler and Henry Christian recognized the role of *S. pneumoniae* as a cause of terminal pneumonia in patients dying of various other primary illnesses. "Patients with arteriosclerosis, heart disease, nephritis, etc., not infrequently are carried off by a pneumococcic pneumonia which may give few or no signs" (12).

The introduction of penicillin G into clinical medicine in the mid-1940s was viewed overly optimistically as heralding the elimination of the common bacterial infections, including those acquired within hospitals. Although highly effective initially against *S. aureus*, a major cause of human infections, subsequent developments belied the initial enthusiasm. Whereas `90% of *S. aureus* isolates at Boston City Hospital prior to 1946 were susceptible to penicillin, by 1952 `75% of isolates were resistant (13). In the early 1970s, `75% of *S. aureus* isolates at the Massachusetts General Hospital were resistant to penicillin. Within a few years, 90% were penicillin-resistant. Currently, worldwide >95% of

S. aureus isolates are resistant to penicillin G as the result of plasmid-mediated penicillinase production (14).

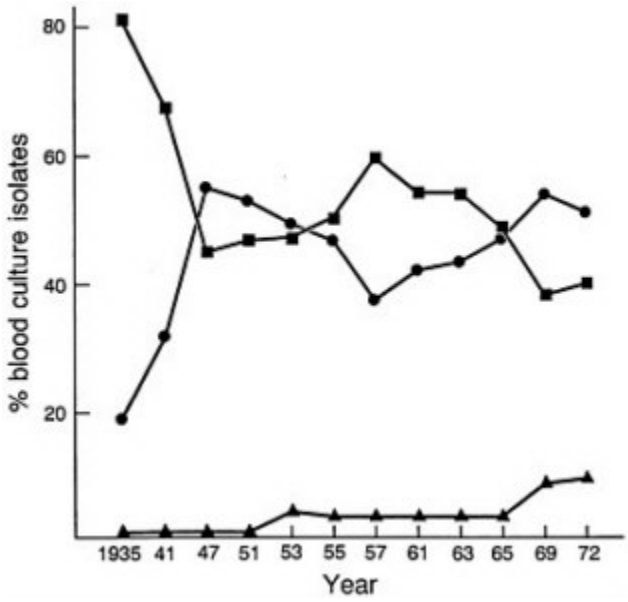

FIGURE 1 Changes in relative percentages of aerobic Gram-positive cocci (`), aerobic Gram-negative bacilli (`), and yeasts (`) as causes of nosocomial bacteremias/fungemias at Boston City Hospital between 1935 and 1972. Data are plotted from McGowan *et al*. (15).

The introduction of penicillin in treatment of human infections was followed in a few years by the introduction of streptomycin, the tetracyclines, erythromycin, and chloramphenicol. As more extensive surgery and cytotoxic drugs were used in treatment and as selection of more antibiotic-resistant microorganisms developed as a result of broad-spectrum antibiotic usage, the frequency of hospital-acquired infections increased. At Boston City Hospital, the frequency of hospital-acquired bacteremias and fungemias steadily increased (with few fluctuations) between 1935 and 1972 from a rate of 3.7 per 1000 hospital admissions to a rate of 14.7 per 1000 admissions (15). Most striking was the change that occurred in the predominant species causing the bloodstream infections. Whereas in 1935 Gram-positive aerobic cocci accounted for 80% of isolates and Gram-negative aerobic bacilli accounted for 20%, by 1972 the relative roles had undergone major changes (Figure 1). By the early 1970s, `55% of blood isolates were Gram-negative aerobic bacilli; `40%

were Gram-positive aerobic cocci, and the remainder were *Candida* or *Torulopsis* spp. Between 1941 and 1972, the percentage of *S. pneumoniae* among bacteremic Gram-positive coccal strains declined from 15 to 4% and that of enterococci increased from 3 to 19%. Among Gram-negative bacilli, the principal changes observed between 1941 and 1972 were a decline in frequency for *Escherichia coli* from 66 to 27% and an increase in *Klebsiella–Enterobacter* from 0 to 39% (15, 16).

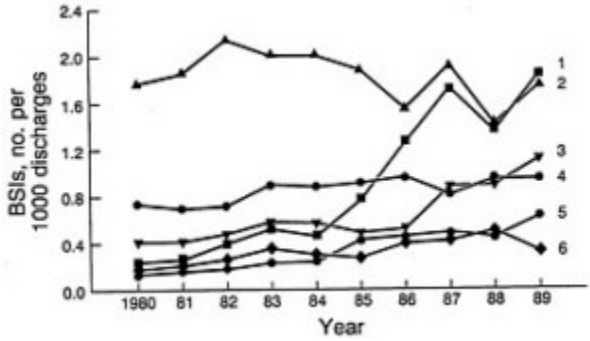

FIGURE 2 Changes in the frequencies of CNS (trace I), Gram-negative bacilli (trace 2), *S. aureus* (trace 3), others (trace 4), *Candida* spp. (trace 5), and enterococci (trace 6) as causes of nosocomial bloodstream infections (BSIs) between 1980 and 1989 in NNIS system hospitals. Modified from Banerjee *et al.* (18) and reproduced with permission (copyright Cahners).

During the decade of the 1980s, changes in the frequency of nosocomial infections and the spectrum of microorganisms involved continued. Primary bloodstream infections represent `8% of all hospital-acquired infections in the United States (3, 17). The frequency of bloodstream infection varies with the category of hospital (18). The rate for small nonteaching hospitals is the lowest (1.3 per 1000 discharges) followed by that for large nonteaching hospitals (2.5 per 1000 discharges). The highest rate (6.5 per 1000 discharges) occurs in large teaching hospitals, followed by the rate (3.8 per 1000 discharges) in small teaching hospitals. The higher rates in the large teaching hospitals presumably reflect the more seriously ill patient population within large tertiary-care hospitals. Increases in the primary bloodstream infection rates occurred between 1980 and 1989 and varied by category from 70 to 279%. The most striking changes were in the rates for individual pathogen groups (Figure 2). Four pathogen groups accounted for most of the increase in nosocomial bloodstream infections in the United States between 1980 and 1989: coagulase-negative staphylococci (CNS), *S.*

aureus, Enterococcus spp., and *Candida* spp. (18). The largest percentage increase (75.4%) in bloodstream bacterial infections occurred with CNS in large teaching hospitals. Bloodstream infections with *Candida* spp. increased in frequency by 487% in large teaching hospitals whereas those with *S. aureus* increased 176% and those with *Enterococcus* spp. increased 120%. Aerobic Gram-negative bacilli, which had achieved prominence in the previous two decades, showed no change in frequency in large teaching hospitals.

The spectrum of microbial pathogens involved overall in nosocomial infections differs somewhat from that causing only bacteremia/fungemia (19). Overall, in NNIS hospitals from 1986 to 1989, the five leading pathogens were *E. coli* (16%), *Enterococcus* spp. (12%), *Pseudomonas aeruginosa* (11%), *S. aureus* (10%), and CNS (9%). The leading role of *E. coli* reflects the frequency of urinary tract infections in hospitalized patients. Each of the other major categories of nosocomial infections has its own distinct leading pathogen: wound infection, *S. aureus;* pneumonia, *P. aeruginosa* and *S. aureus;* bloodstream infection, CNS. Over the past decade there has been a decline in the frequency of *E. coli* infections and increases in infections due to CNS (from 4% of total infections to 9%) and *Candida albicans* (from 2 to 5%). In the past decade the trend has been away from antimicrobial-susceptible species to more resistant pathogens. Even among susceptible species, there has been a shift from readily susceptible to antibiotic-resistant strains.

Overall data for a large number of hospitals are very helpful in identifying major trends. However, they may tend to obscure outbreaks that occur in individual hospitals and important shifts that occur in antimicrobial susceptibilities of pathogens that present problems in individual intensive-care units. For example, among 392 nosocomial infections occurring among 920 neutropenic cancer patients in a cancer unit, *Candida* spp. (18%) was the most common pathogen followed closely in frequency by CNS (17%) (10). In addition to common microorganisms seen in the multihospital NNIS survey, less frequently observed pathogens such as *Clostridium* spp., *Corynebacterium* spp., *Aspergillus* spp., and herpes viruses may account for 20% of the total pathogen group.

PROBLEMS OF ANTIMICROBIAL RESISTANCE IN SELECTED NOSOCOMIAL PATHOGENS

S. aureus

Methicillin-resistant *S. aureus* (MRSA) was first described in 1961 in England and over the next decade became an important nosocomial

pathogen in parts of Europe (20). MRSA is resistant to all penicillins, including semisynthetic penicillinase-resistant congeners, penems, and carbapenems. The basis for this resistance is the *mecA* gene that codes for a new penicillin-binding protein (PBP; PBP2a) with a reduced affinity for all β-lactam antibiotics (21). The *mecA* gene is chromosomal in location and not subject to dissemination among staphylococcal strains via plasmid spread. However, resistance to other antibiotics such as streptomycin, tetracyclines, trimethoprim, and erythromycin can be spread widely via plasmids and by transposition in many MRSA (21). In the early 1970s, the prevalence of MRSA temporarily receded only to reemerge in the past one and a half decades as a cause of nosocomial outbreaks of infections in Europe, Africa, Asia, Australia, and the United States (22). The prevalence of MRSA among *S. aureus* isolates reveals marked differences between countries: 0.1% in Denmark in 1988, 4% in Germany in 1989, 26% in France in 1989, and 15% in the United States in 1987–88. However, these represent mean prevalence rates for many hospitals. Although the average prevalence rate of MRSA among 24 hospitals in Italy was 26% in 1986, the survey included hospitals with prevalence rates that varied from 6 to 44% (22). In the NNIS data from U.S. hospitals evaluated over a 17-year period (1975–1991), the percentage of MRSA increased from 2.4% in 1975 to 29% (23). The prevalence of MRSA varied with the size of hospitals. In 1991 it was 14.9% for hospitals with <200 beds, 20.3% for hospitals with 200–499 beds, and 38.3% for hospitals with 500 or more beds.

Nosocomially acquired strains of MRSA do not appear to exhibit enhanced intrinsic clinical virulence in comparison with methicillin-susceptible strains. However, patients who carry MRSA may be at a higher risk of developing infection due to that organism that patients colonized with methicillin-susceptible *S. aureus* (24). Epidemiologic virulence (efficiency in colonizing patients and in spreading throughout hospitals) has been attributed to some strains (21, 23, 25). A variety of factors may contribute to the prevalence of MRSA in a given hospital: patterns of antibiotic use, biologic properties of circulating MRSA strains, the nature of the patient population, local infection control practices, and frequency of admission of colonized or infected patients from other institutions.

Careful monitoring of *S. aureus* strains from hospitalized patients in Denmark at a national laboratory has provided data on antibiotic susceptibility and phage type of 523,000 isolates over the interval from 1966 to 1988 (26). The rise in prevalence of MRSA from 3% in 1966 to `15% in the period 1967–1970 was followed by a gradual decline to a level of `0.2%. The rise in prevalence of MRSA during the late 1960s, and the subsequent decline, was paralleled by changes in phage type.

The increase in prevalence of MRSA appears to have been due to spread in hospitals of one or a few clones of *S. aureus* belonging to phage-type complex 83A and exhibiting multiple antibiotic resistance (27). In Denmark in the 1950s, the *S. aureus* strains isolated were frequently of the 52/52A/80/81 complex, but these were replaced increasingly in the early 1960s by tetracycline-resistant strains of the 83A complex; 70% of these were multiresistant (45% methicillin resistant). Subsequently, prevalence of strains of phage complex 83A steadily declined until 1980 and has remained at 6% since 1980. Currently, <2% of strains of *S. aureus* are multiresistant, and even strains of the 83A complex are no longer multiresistant.

Important factors in the control of the Danish outbreak included the extensive and continual monitoring of nosocomial *S. aureus* isolates and prompt institution of isolation precautions when MRSA was detected. The basis for the increased prevalence of the epidemic clone(s) of MRSA in the Danish outbreak and their subsequent disappearance is unclear. A role for antimicrobial use patterns in the evolution of resistance has been suggested (27). The use of tetracyclines and streptomycin in the 1960s may have provided some selective pressure for the increase in frequency of multiantibiotic-resistant MRSA. Similarly, the considerable reduction in the quantities of these two drugs used during the early 1970s may have lessened the selective pressure for streptomycin- and tetracycline-resistant subtypes of phage 83A complex MRSA.

In the mid 1980s, the newer fluoroquinolone antimicrobials offered considerable initial promise for dealing with both clinical infections due to MRSA and the carrier state (28). The minimum inhibitory concentration (MIC) for 90% of *S. aureus* isolates (both methicillin-susceptible and methicillin-resistant isolates) was 0.5 µg/ml (29). In the late 1980s and early 1990s, this promise proved disappointing as resistance rapidly developed, particularly among methicillin-resistant strains. In a large tertiary care hospital in New York City, >80% of MRSA had become resistant to the available fluoroquinolones (14).

CNS

By 1971 `65% of CNS had become resistant to penicillin at large urban teaching hospitals such as the Massachusetts General Hospital, and from 1979 on `80% of isolates have been resistant. Resistance to penicillinase-resistant penicillins, such as methicillin and oxacillin, has steadily increased. At the Massachusetts General Hospital, the percentage of methicillin-resistant isolates among CNS has risen from 8% in 1971 to 54% in 1992.

The increasing role of CNS as a pathogen in infections involving

prosthetic devices (artificial heart valves, cardiac pacemaker leads, joint replacements, nervous system ventricular shunts, peritoneal dialysis catheters, and polyethylene intravenous catheters) has occurred contemporaneously with development of increasing methicillin-resistance. *Staphylococcus epidermidis*, a common commensal on human skin, is frequently the species infecting foreign body implants or indwelling venous catheters, but other CNS species may also be involved. Binding of such organisms to foreign-body surfaces and their intercell adherence is enhanced by a biofilm matrix (extracellular "slime layer" consisting of exopolysaccharides) they produce. Colonization within a biofilm appears to protect *S. epidermidis* cells against opsonization and phagocytosis by polymorphonuclear leukocytes, and it may afford protection against the bactericidal action of antibiotics by acting as a barrier to the latter's penetration (30).

CNS (and *S. aureus*) have developed resistance mechanisms for evading the inhibitory action of many antimicrobials previously useful in treating infections due to these microorganisms (14). They have acquired plasmid genes encoding β-lactamase production that render them (>90%) resistant to penicillin G. MRSA are resistant to methicillin and other penicillinase-resistant penicillins, cephalosporins, carbapenems, and penems by virtue of a chromosomal gene (*mecA*) that encodes a new PBP (PB2a) with reduced affinity for all β-lactam antibiotics. Fluoroquinolone resistance in clinical isolates of *S. aureus* is associated with mutations in the A subunit of the DNA gyrase (31). A second form of resistance to fluoroquinolones in *S. aureus* is due to a mutation (*norA*) in the chromosomal gene that codes for a membrane transporter effecting fluoroquinolone efflux driven by the proton gradient across the cell membrane (32). Rifampin resistance in *S. aureus* is encoded on the chromosomal gene for the DNA-dependent RNA polymerase and involves an alteration in the B subunit to which rifampin binds. Rifampin is not used alone to treat staphylococcal infections because of rapid emergence of resistance.

Staphylococcal infections are not ordinarily treated with aminoglycosides, but a drug in this class is sometimes combined with a penicillinase-resistant penicillin in the treatment of life-threatening infections. Plasmid-mediated aminoglycoside-modifying enzymes (phosphotransferase, adenylylating enzyme, and acetyltransferase) alter the conformation of these antibiotics and interfere with binding of the aminoglycoside to the 30S ribosomal subunit, a necessary step in aminoglycoside action (formation of an unstable initiation complex, thus blocking translation and exerting a bactericidal effect).

Resistance in staphylococci to tetracyclines is encoded on plasmids or transposons that cause synthesis of a new membrane protein that

decreases accumulation of tetracycline by causing active efflux of the drug (33). Resistance to macrolides (erythromycin and clindamycin) is primarily plasmid-mediated and inducible, involving methylation of an adenine in the 23S ribosomal RNA of the 50S ribosomal subunit. This results in decreased binding of the macrolides to their usual targets on the ribosome (34). Trimethoprim resistance is common (`40%) among CNS and is somewhat more frequent than among *S. aureus* isolates (35). The same genetic resistance determinant is found on conjugative multiresistant plasmids and on the chromosome in both species. The principal mechanism of resistance has been production of new plasmid-mediated dihydrofolate reductases that are trimethoprim-resistant (36).

The increasing resistance of CNS to methicillin and the `38% prevalence of methicillin-resistance among *S. aureus* isolates in large U.S. hospitals (23), as well as the rapid emergence of fluoroquinolone resistance among MRSA, have required frequent use of alternative antimicrobials. MRSA are resistant as well to cephalosporins, precluding use of the latter for staphylococcal infections. While only 15% of *S. aureus* isolates are clindamycin-resistant and 25% are erythromycin-resistant, a higher percentage have inducible resistance, and the use of these drugs in severe staphylococcal infections may be limited. A more suitable antimicrobial for life-threatening staphylococcal infections is the bactericidal drug vancomycin. Thus, the continuing problems with nosocomial MRSA infections and infections due to CNS have effected a major increase in the use of vancomycin. They have also led to extensive use of vancomycin in initial treatment of nosocomial fevers and for infections where MRSA and CNS are considered among possible pathogens. In addition, vancomycin usage by the oral route has also increased in the past 15 years in the treatment for *Clostridium difficile* pseudomembranous colitis.

CHANGES IN ANTIMICROBIAL USAGE OVER THE PAST TWO DECADES

Specific antimicrobial use depends on trends in antimicrobial-resistant pathogens as causes of disease. In the 1970s, `50% of antimicrobial usage involved administration to hospitalized patients. Study of parenteral antimicrobial usage at the University of Iowa Hospital between 1978 and 1992 provides an indication of changes that have occurred in major teaching hospitals (37). Whereas only 23% of patients in 1978 received one or more antimicrobials during their hospital stay, this figure rose to 44% by 1991. The principal increases in parenteral drug use were with vancomycin (increasing from use in <1% of patients in 1978 to 10% in 1992), and third-generation cephalosporins (increasing

from use in 1% of patients to 7% of patients by 1992). The quantity of vancomycin used per 1000 patient days increased 161-fold between 1978 and 1992. This experience is typical of that at other general hospitals. Such extensive use has had the potential, and now the reality, of selecting strains of vancomycin-resistant nosocomial pathogens (*Enterococcus* spp. and *Staphylococcus hemolyticus*) for which alternative therapies are extremely limited.

THE INCREASING PROBLEM OF ANTIBIOTIC RESISTANCE IN ENTEROCOCCUS SPP.

Enterococci are intrinsically more resistant *in vitro* to penicillin (MIC, 2.5–5 μg/ml) than are other streptococci (MIC, 0.02–0.08 μg/ml). Since the reports of Hunter (38) in 1947 and of Jawetz *et al.* (39) in 1950, the capacity of penicillin and an aminoglycoside to act synergistically against enterococci has been known. The bactericidal action of this combination is due to limited penicillin inhibition of bacterial cell wall synthesis that permits enhanced uptake of aminoglycoside, the latter then acting lethally on its ribosomal target (40). Treatment of serious enterococcal infections has involved the use of combined penicillin–aminoglycoside therapy since the 1950s. Originally, the aminoglycoside was streptomycin. By the early 1970s, 25–50% of clinical isolates showed high-level (>2000 μg/ml) resistance to streptomycin and kanamycin. Accordingly, gentamicin was substituted for streptomycin. The penicillin and gentamicin combination proved to be synergistic *in vitro* against all enterococci tested during the 1970s and provided effective therapy. However, in 1979 the first enterococcus isolate with high-level (>2000 μg/ml) resistance to gentamicin was identified in Paris (41). Subsequently, enterococci with high-level gentamicin resistance and resistance to penicillin–gentamicin synergy have been observed worldwide (42). By the early 1990s, in some centers, >50% of enterococcal isolates showed resistance to penicillin–gentamicin synergy. Among *Enterococcus faecium* isolates, which prior to 1987 had been uniformly susceptible to gentamicin at 200 μg/ml, in some hospitals as many as 70% of isolates showed high-level resistance to gentamicin.

High-level resistance to streptomycin is due either to plasmid-mediated (adenylyltransferase) modification of the drug or, occasionally, can be due to a chromosomal mutation that alters ribosomal affinity for streptomycin (42). High-level gentamicin resistance is based on enzymatic modification of the aminoglycoside by a plasmid-encoded bifunctional enzyme with both 2"-phosphotransferase and 6'-acetyltransferase activities (43). Aminoglycoside modification by this bifunctional enzyme prevents penicillin (or vancomycin) synergy with all available aminoglycosides

except streptomycin. Fortunately, up to 46% of high-level gentamicin-resistant *Enterococcus faecalis* isolates are still susceptible to streptomycin. While all enterococci exhibit low-level intrinsic resistance to aminoglycosides, the MICs of certain aminoglycosides, (tobramycin, kanamycin, netilmicin, and sisomicin) are higher in *E. faecium* than *E. faecalis*. This relative resistance is based on a chromosomally mediated 6'-acetyltransferase and is of sufficient degree to prevent synergy with cell-wall-active antimicrobial agents (42).

To add to the problems produced by high-level gentamicin resistance in treatment of serious enterococcal infections, major resistance to penicillin has emerged. Although a variety of less frequently isolated *Enterococcus* species *(E. gallinarum, E. asseliflavus, E. durans, E. avium,* and *E. raffinosus*) have occasionally been responsible for infections, most clinical isolates have been *E. faecalis* (85–90%) and *E. faecium* (5–10%). Penicillin resistance in enterococci has been of two types: (*i*) higher levels of intrinsic resistance to penicillin and other β-lactams, particularly in *E. faecium,* and associated with production of greater amounts of a low-affinity PBP (PBP5) (44); (*ii*) β-lactamase production by enterococci (Bla^+) carrying plasmids and exhibiting high-level gentamicin resistance (HLGR) as well. A large outbreak of infections has been caused by a clone identified in the mid-Atlantic area (45). Such a strain (Bla^+ HLGR), once ensconced in a hospital, is difficult to eradicate, reminiscent of the problem with MRSA. In one outbreak, the β-lactamase and high-level gentamicin-resistance genes were chromosomal in location, probably on a conjugative transposon (46). Although penicillin resistance due to β-lactamase production is as yet uncommon in enterococci, it presents a major threat, particularly if conjugative spread occurs.

Vancomycin resistance among clinical enterococcal isolates, particularly *E. faecium* strains highly resistant to penicillin because of their low-affinity PBPs, was first recognized in England and France in the late 1980s, `30 years after the glycopeptide had been introduced in clinical use. Subsequent nosocomial outbreaks due to vancomycin-resistant *E. faecium* have occurred in several hospitals in the United States, including a cardiothoracic intensive care unit in one (47) and a medical-surgical intensive care unit in another (48). In the latter, nondisposable handles of electronic rectal thermometers were the means of spread of enterococci. In outbreaks some patients have developed serious infections such as bacteremia, peritonitis, and pneumonia. In a survey of 10,961 nosocomial enterococcal strains collected between 1989 and 1993 in the NNIS hospital system, 2.5% were found to be vancomycin-resistant (49). The prevalence of vancomycin resistance among nosocomial isolates rose from 0.3% in 1989 to 7.9% in 1993, and hospitals in nine states were sources of resistant isolates. The most striking increase in prevalence of

vancomycin resistance has been found in isolates from patients with nosocomial infections in intensive care units where the percentage rose from 0.4% in 1989 to 13.6% in 1993.

Vancomycin resistance in enterococci consists of several different phenotypes (50). The VanA phenotype consists of inducible high-level resistance to both vancomycin and another glycopeptide, teicoplanin, that is mediated by a transposable element (Tn1546) on a conjugative plasmid in *E. faecium, E. faecalis*, and *E. avium.* The expression of the VanB phenotype is inducible in *E. faecium* and *E. faecalis* and transferable by conjugation, but the location of the resistance genes is unknown and the resistance is to vancomycin and not to teicoplanin. The VanC phenotype is probably mediated by a chromosomal gene, present in *E. gallinarum* and *E. casseliflavus*, constitutive, and confers low-level resistance only to vancomycin.

Normal peptidoglycan synthesis involves the sequential action of D-Ala:D-Ala ligase and D-Ala-D-Ala adding enzyme to ultimately produce D-Ala-D-Ala termini on the pentapeptide side chains of the nascent bacterial cell wall (50, 51). The amide of the terminal D-Ala-D-Ala of vancomycin-susceptible bacteria appears to form a crucial hydrogen bond with a carbonyl group on the vancomycin skeleton (Figure 3) (51). This NH group appears to be essential for vancomycin binding to the cell wall and its inhibition of transglycosylation and transpeptidation of growing peptidoglycan strands.

Vancomycin-resistant bacteria, such as enterococci with the VanA phenotype, have acquired a transposable element, Tn*1546*, encoding nine genes, on a plasmid. The resistance gene products include a transmembrane sensor protein (VanS) that resembles eukaryotic transmembrane receptors. The latter senses the presence of vancomycin and transduces that information to a response regulator protein (VanR) that is a transcriptional activator of *vanH* and *vanA* genes (51). The latter two genes are critical in the subsequent expression of drug resistance (Figure 3). The *vanH* gene product is a new α-keto acid reductase that produces D-lactate or D-hydroxybutyrate from pyruvate and α-ketobutyrate, respectively. The VanA protein is a homolog of a D-Ala:D-Ala ligase with its specificity altered to preferentially utilize D-α-hydroxy acids such as D-lactate rather than D-Ala as the COOH-terminal component in ester linkage. Thus, a depsipeptide (e.g., D-Ala-D-lactate) is formed by VanA and is incorporated subsequently into the peptidoglycan termini of resistant bacteria (52). The substitution in the depsipeptide of an ester linkage for the amide linkage of the terminal D-Ala-D-Ala dipeptide appears to reduce vancomycin binding to the cell wall by `1000-fold, approximating the decreased vancomycin susceptibility of resistant strains. The novel depsipeptide peptidoglycan termini do not interfere

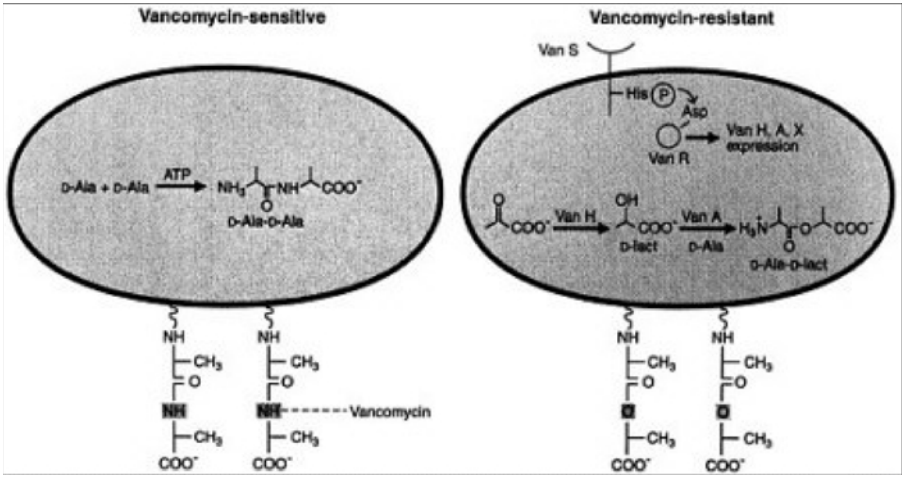

FIGURE 3 Schematic representations of vancomycin-susceptible and -resistant bacteria. Peptidoglycan strands of susceptible bacteria (*Left*) contain D-Ala-D-Ala termini to which vancomycin binds avidly. Peptidoglycan strands of resistant bacteria (*Right*) contain D-Ala-D-Lact depsipeptide termini (ester linkage) and have markedly reduced affinity for vancomycin. The plasmidborne transposable element Tn*1546* encodes the VanH and VanA proteins that are α-keto acid reductases and depsipeptide ligases (with D-lactate or D-hydroxybutyrate as the COOH-terminal partner), respectively. Reproduced from Walsh (51) with permission (copyright American Association for the Advancement of Science).

with subsequent transpeptidation and strand crosslinkage, allowing formation of structurally intact cell walls of vancomycin-resistant Gram-positive bacteria.

Gram-positive bacteria other than *Enterococcus* spp. also may be resistant to vancomycin. Some clinical isolates of *S. hemolyticus* have shown resistance to vancomycin (53). Inherent resistance to vancomycin is a feature of *Leuconostoc* spp., *Pediococcus* spp., and *Lactobacillus* spp., all three rarely causing human infections.

A threat exists of further dissemination of vancomycin resistance to other species via transposition of Tn*1546* into broad-host-range plasmids. Barriers do not exist to heterospecific expression of enterococcal resistance genes in *Listeria monocytogenes, Bacillus* spp., and *Streptococcus* spp. (50). Transfer of vancomycin resistance from *E. faecalis* to *S. aureus* via conjugation in the laboratory raises the frightening possibility of the emergence of glycopeptide resistance in MRSA (54).

RESISTANCE TO THIRD-GENERATION CEPHALOSPORINS AMONG GRAM-NEGATIVE AEROBIC BACILLI

Resistance of Gram-negative pathogens to β-lactam antibiotics is mediated by any one of three mechanisms (production of β-lactamases, reduced permeation of the drug, or alteration of the target site, i.e., PBP) or by several acting in concert. In the mid-1980s, it was noted that resistance was developing among Gram-negative bacilli possessing inducible chromosomal cephalosporinases (e.g., *Enterobacter cloacae, Proteus* spp., *Serratia* spp., *Citrobacter freundii*) when certain of the newer cephalosporins and cephamycins were used in therapy (55). Spontaneous mutation of such organisms to a stably derepressed (induced) state resulted in persistence in these mutants of high levels of β-lactamase even without the presence of an inducer. Such resistance is not transmissible but has been responsible for many instances of therapeutic failure, relapse, or nosocomial spread of such infections (56). Most outbreaks of such nosocomial infections have involved *Enterobacter* spp., and resistance has been present to virtually all β-lactam antibiotics (including newer cephalosporins and aztreonam) except imipenem.

Further problems in the treatment of infections due to Gram-negative bacilli became evident in the mid 1980s in France and Germany when *Klebsiella* isolates with plasmid-mediated resistance to broad-spectrum cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) and aztreonam appeared (57). Such resistance soon became evident worldwide (58). The basis of such resistance is the emergence of new extended-spectrum β-lactamases, observed first in *Klebsiella pneumoniae* and subsequently in *E. coli, C. freundii, Serratia marcescens,* and *Enterobacter*

cloacae. These new resistances are mediated by plasmids encoding TEM-1-, TEM-2-, and SHV-1-related (mutated) β-lactamases. These new extended-spectrum β-lactamases differ from the original plasmid-encoded TEM-1 β-lactamase (mediating ampicillin resistance in Gram-negative enteric bacteria) and plasmid-encloded SHV-1 β-lactamase in Klebsiella, by 1–4 amino acids; and, thus, they are designated (TEM-4, SHV-3, etc.) to indicate this relatedness. The prevalence of extended-spectrum β-lactamases in *Enterobacteriaceae* has risen disturbingly over the past 7 or 8 years. For example, among isolates of *K. pneumoniae* from patients in 20 French hospitals, the prevalence of such resistance increased from <1% in 1985 to 11% in 1988 (56). In 1988–1989 in one hospital in Athens, Greece, 24% of *K. pneumoniae* and 4% of *E. coli* isolates possessed extended-spectrum β-lactamases. *Enterobacteriaceae* producing extended-spectrum β-lactamases have been responsible for a number of nosocomial outbreaks, usually originating in an intensive care unit and then spreading elsewhere (56). Such dissemination of drug resistance can involve spread of a particular strain of bacteria, horizontal interspecies spread of a resistance plasmid, or spread of resistance genes. Either extensive use of an extended-spectrum cephalosporin in a hospital unit or prior use in a particular patient has usually been associated with such outbreaks. Since *K. pneumoniae* containing extended-spectrum β-lactamases also often carry genes for amikacin resistance on the same plasmids, high-level use of the aminoglycoside may serve to select for resistant strains.

The potential for selection of increasingly resistant Gram-negative bacillary pathogens in a hospital can be seen in the sequence of events in one U.S. hospital over several years. In 1988–1989, a major outbreak of infections due to *Acinetobacter* occurred, requiring extensive use of ceftazidime, and to a lesser degree, imipenem, to which the infecting strain was susceptible. Late in 1988 an outbreak of ceftazidime-resistant *K. pneumoniae* infections began and 155 patients were infected or colonized during a 2-year period (59). Predisposing factors included >2 weeks of hospitalization and >7 days of antibiotic administration. Prior to isolation of the ceftazidime-resistant *K. pneumoniae* on average almost five antibiotics per patient had been administered. These isolates were resistant to aminoglycosides and to the bactericidal action of all third generation cephalosporins, moxalactam, and cephamycins and were susceptible only to imipenem. The strains examined contained several plasmids and produced β-lactamases with enzymatic characteristics suggesting TEM-10 or TEM-26. The outbreak was finally controlled with a combination of antibiotic (ceftazidime) restriction and barrier precautions for all infected or colonized patients.

The use of imipenem to treat infected patients in the aforementioned

hospital outbreak increased 3- to 4-fold. Subsequently, an outbreak of nosocomial infections (pulmonary and bacteremic) occurred in the surgical intensive care unit of the same hospital due to a strain of multiresistant *Acinetobacter calcoaceticus* (60). The organism was resistant to all antimicrobials ordinarily employed against Gram-negative aerobic bacilli: aminoglycosides (including amikacin); ampicillin; antipseudomonal penicillins; first-, second-, and third-generation cephalosporins; cephamycins; fluoroquinolones; aztreonam; imipenem; chloramphenicol; etc. The only antimicrobials to which the *A. calcoaceticus* isolates were susceptible were ampicillin–sulbactam (sulbactam responsible for the bactericidal effect of the combination) and polymyxin.

The above-described sequential outbreaks of nosocomial infections due to increasingly resistant organisms emphasizes the potentially great selective power of extensive antimicrobial use in a given institution, particularly in intensive care units, in favoring emergence of multiresistant pathogens.

CONTROL OF NOSOCOMIAL INFECTIONS DUE TO RESISTANT BACTERIA

In the past the rate of introduction of new antimicrobial drugs has been sufficient to counter those infections caused by organisms resistant to available drugs. Although there are >155 antibiotics (14), the rate of introduction of genuinely new drugs with different modes of action or genuinely different spectra of activity into clinical usage has slackened. Therapeutic options for nosocomial infections are increasingly limited because of antimicrobial resistance.

Control of this group of infections merits consideration of changes in some current practices. (*i*) *Monitoring of nosocomial pathogens and their resistance patterns in acute-care hospitals*. Although it is common practice for hospital microbiology laboratories to collect data on the antimicrobial susceptibilities of major pathogens and to provide summary information to physicians, such data commonly is tabulated for the recent year's experience, encompasses results from all parts of the hospital (including ambulatory services), and is provided to the physicians months later. This information is helpful for guidance in antimicrobial selection when a given pathogen has been identified or is suspected but its antimicrobial susceptibilities have not yet been determined. However, such information is less helpful in identifying early phases of nosocomial outbreaks in specialized units, since the data may become available late in the outbreak and may be obscured by collating susceptibilities from all parts of a hospital. In addition, data is not readily available in the form of antimicrobial-resistance patterns. Early detection of outbreaks of nosocomial

infection may be improved by ongoing monitoring of pathogens isolated and their resistance patterns in selected intensive care units on a monthly basis. (*ii*) *Prompt institution of barrier precautions.* Early identification of the new multiresistant pathogen serves to alert intensive care unit personnel to the problem and to institute isolation precautions before the strain is widely transmitted within the unit and elsewhere in the hospital. Once the number of infected and colonized patients increases containment becomes much more problematic. (*iii*) *Judicious use of antimicrobial agents.* The use of antimicrobials by physicians in hospitals (and elsewhere) requires acute awareness of the increasing problems with resistant organisms. Thus, unnecessary use of an antimicrobial drug has public health implications. Such use may serve to select for resistant organisms that may be carried to other more vulnerable patients and produce serious difficult-to-treat infections. Antibiotic control programs can be an effective means to prevent inappropriate use of antimicrobials in hospitals. Newer antimicrobials should be included in such programs to delay the emergence of resistant strains by limiting unnecessary use of such drugs. Indeed, consideration might be given in some instances to cycling the use of certain antimicrobials in selected intensive care units.

SUMMARY

About 5% of patients admitted to acute-care hospitals acquire nosocomial infections. A variety of factors contribute: increasing age of patients; availability, for treatment of formerly untreatable diseases, of extensive surgical and intensive medical therapies; and frequent use of antimicrobial drugs capable of selecting a resistant microbial flora. Nosocomial infections due to resistant organisms have been a problem ever since infections due to penicillinase-producing *Staphylococcus aureus* were noted within a few years of the introduction of penicillin. By the 1960s aerobic Gram-negative bacilli had assumed increasing importance as nosocomial pathogens, and many strains were resistant to available antimicrobials. During the 1980s the principal organisms causing nosocomial bloodstream infections were coagulase-negative staphylococci, aerobic Gram-negative bacilli, *S. aureus*, *Candida* spp., and *Enterococcus* spp. Coagulase-negative staphylococci and *S. aureus* are often methicillin-resistant, requiring parenteral use of vancomycin. Prevalence of vancomycin resistance among enterococcal isolates from patients in intensive care units has increased, likely due to increased use of this drug. Plasmid-mediated gentamicin resistance in up to 50% of enterococcal isolates, along with enhanced penicillin resistance in some strains, leaves few therapeutic options. The emergence of *Enterobacteriaceae*

with chromosomal or plasmid-encoded extended spectrum β-lactamases presents a world-wide problem of resistance to third generation cephalosporins. Control of these infections rests on (*i*) monitoring infections with such resistant organisms in an ongoing fashion, (*ii*) prompt institution of barrier precautions when infected or colonized patients are identified, and (*iii*) appropriate use of antimicrobials through implementation of antibiotic control programs.

REFERENCES

1. Haley, R. W., Culver, D. H., White, J. W., Morgan, W. M. & Emori, T. G. (1985) *Am. J. Epidemiol.* **121,** 159–167.
2. Scheckler, W. E., Scheibel, W. & Kresge, D. (1991) *Am. J. Med.* **91,** Suppl. 3B, 90S–94S.
3. Emori, T. G., Banerjee, S. N., Culver, D. H., Gaynes, R. P., Horan, T. C., Edwards, J. R., Jarvis, W. R., Tolson, J. S., Henderson, T. S., Martone, W. J., Hughes, J. M. & the National Nosocomial Infections Surveillance System (1991) *Am. J. Med.* **91,** Suppl. 3B, 289S–293S.
4. Moss, A. J., Hamburger, S., Moore, R. M., Jr., Jeng, L. L. & Howie, L. J. (1991) *Use of Selected Medical Device Implants in the United States, 1988* (Natl. Cent. Health Statistics, Centers for Disease Control, Atlanta), No. 191.
5. Health Industry Manufacturers Association (1992) Comments to FDA on the Medical Device Tracking Regulation.
6. Survey of Society of Thoracic Surgeons (1992) *Ann. Thorac. Surg.* Brochure.
7. Horan, T. C., Culver, D. H., Gaynes, R. P., Jarvis, W. R., Edwards, J. R., Reid, C. R. and the National Nosocomial Infections Surveillance System (1993) *Infect. Control Hosp. Epidemiol.* **14,** 73–80.
8. United Network for Organ Sharing (1993) Scientific registry data.
9. Bortin, M. M., Horowitz, M. M. & Rimm, A. A. (1992) *Ann. Intern. Med.* **116,** 505–512.
10. Carlisle, P. S., Gucalp, R. & Wiernik, P. H. (1993) *Infect. Control Hosp. Epidemiol.* **14,** 320–324.
11. Martone, W. J., Gaynes, R. P., Horan, T. C., Emori, T. G. & Jarvis, W. R. (1991) *Infect. Control Hosp. Epidemiol.* **12,** 609–621.
12. Christian, H. A. (1944) *The Principles and Practice of Medicine* (Appleton–Century, New York), pp. 54–55.
13. Finland, M. (1955) *N. Engl. J. Med.* **252,** 570–580.
14. Neu, H. C. (1992) *Science* **257,** 1064–1073.
15. McGowan, J. E., Jr., Barnes, M. W. & Finland, M. (1975) *J. Infect. Dis.* **132,** 316–335.
16. McGowan, J. E., Jr. (1985) *Rev. Infect. Dis.* **7,** Suppl. 3, S357–S370.
17. Horan, T. C., White, J. W., Jarvis, W. R., Emori, T. G., Culver, D. H., Munn, V. P., Thornsberry, C., Olson, D. R. & Hughes, J. M. (1984) *Morbid. Mortal. Wkly. Rep.* **35,** 1SS, 17SS–29.
18. Banerjee, S. N., Emori, T. G., Culver, D. H., Gaynes, R. P., Jarvis, W. R., Horan, T. C., Edwards, J. R., Tolson, J. S., Henderson, T., Martone, W. J. & the National Nosocomial Infections Surveillance System (1991) *Am. J. Med.* **91,** Suppl. 3B, 86S–89S.

19. Schaberg, D. R., Culver, D. H. & Gaynes, R. P. (1991) *Am. J. Med.* **91,** Suppl. 3B, 72S–75S.
20. Kayser, F. H. (1975) *Lancet* **ii,** 650–653.
21. Lyon, B. R. & Skurray, R. (1987) *Microbiol. Rev.* **51,** 88–134.
22. Boyce, J. M. (1991) *Infect. Control Hosp. Epidemiol.* **12,** 79–82.
23. Panlilio, A. L., Culver, D. H., Gaynes, R. P., Banerjee, S., Henderson, T. S., Tolson, J. S., Martone, W. J. & the National Nosocomial Infections Surveillance System (1992) *Infect. Control Hosp. Epidemiol.* **13,** 582–586.
24. Muder, R. R., Brennen, C., Wagener, M. M., Vickers, R. M., Rihs, J. D., Hancock, G. A., Yee, Y. C., Miller, J. M. & Yu, V. I. (1991) *Ann. Intern. Med.* **114,** 107–112.
25. Cookson, B. D. & Phillips, I. (1988) *J. Antimicrob. Chemother.* **21,** Suppl. C, 57–65.
26. Rosdahl, V. T. & Knudsen, A. M. (1991) *Infect. Control Hosp. Epidemiol.* **12,** 83–88.
27. Westh, H., Jarlov, J. O., Kjersem, H. & Rosdahl, V. T. (1992) *Clin. Infect. Dis.* **14,** 1186–1194.
28. Neu, H. C. (1992) *Annu. Rev. Med.* **43,** 465–486.
29. Eliopoulos, G. M. & Eliopoulos, C. T. (1993) in *Quinolone Antimicrobial Agents*, eds. Hooper, D. C. & Wolfson, J. S. (Am. Soc. Microbiol., Washington, DC), 2nd Ed., pp. 168–169.
30. Peters, G. (1988) *J. Antimicrob. Chemother.* **21,** Suppl. C, 139–148.
31. Hooper, D. C., & Wolfson, J. S. (1993) in *Quinolone Antimicrobial Agents*, eds. Hooper, D. C. & Wolfson, J. S. (Am. Soc. Microbiol., Washington, DC), 2nd Ed., pp. 98–110.
32. Trucksis, M., Wolfson, J. S. & Hooper, D. C. (1991) *J. Bacteriol.* **173,** 5854–5860.
33. McMurry, L. M., Park, B. H., Burdette, V. & Levy, S. B. (1987) *Antimicrob. Agents Chemother.* **31,** 1648–1650.
34. Weisblum, B., Siddhikol, C., Lai, C. J. & Demohn, V. (1971) *J. Bacteriol.* **106,** 835–847.
35. Galetto, D. W., Johnston, J. L. & Archer, G. L. (1987) *Antimicrob. Agents Chemother.* **31,** 1683–1688.
36. Burchall, J. J., Pelwell, L. P. & Fling, M. E. (1982) *Rev. Infect. Dis.* **4,** 246–254.
37. Pallares, R., Dick, R., Wenzel, R. P., Adams, J. R. & Nettleman, M. D. (1993) *Infect. Control Hosp. Epidemiol.* **14,** 376–382.
38. Hunter, T. H. (1947) *Am. J. Med.* **2,** 436–442.
39. Jawetz, E., Gunnison, J. B. & Colman, V. R. (1950) *Science* **111,** 254–256.
40. Moellering, R. C., Jr., & Weinberg, A. N. (1971) *J. Clin. Invest.* **50,** 2580–2584.
41. Horodniceanu, T., Bougueleret, L., El-Solh, N., Bieth, G. & Delbos, F. (1979) *Antimicrob. Agents Chemother.* **16,** 686–689.
42. Moellering, R. C., Jr. (1991) *J. Antimicrob. Chemother.* **28,** 1–12.
43. Ferretti, J. T., Gilmore, K. S. & Courvalin, P. (1986) *J. Bacteriol.* **167,** 631–638.
44. Fontana, R., Amalfitano, G., Rossi, L. & Satta, G. (1992) *Clin. Infect. Dis.* **15,** 486–489.
45. Wells, V. D., Wong, E. S., Murray, B. E., Coudron, P. E., Williams, D. S. & Markowitz, S. M. (1992) *Ann. Intern. Med.* **116,** 285–292.
46. Rice, L. B., Eliopoulos, G. M., Wennersten, C., Goldmann, D., Jacoby, G. A. & Moellering, R. C., Jr. (1991) *Antimicrob. Agents Chemother.* **35,** 272–276.
47. Karanfil, L. V., Murphy, M., Josephson, A., Gaynes, R., Mandel, L., Hill, B. C. & Swenson, J. M. (1992) *Infect. Control Hosp. Epidemiol.* **13,** 195–200.

48. Livornese, L. L., Dias, S., Samel, C., Romanowski, B., Taylor, S., May, P., Pitsakis, P., Woods, G., Kaye, D., Levison, M. E. & Johnson, C. C. (1992) *Ann. Intern. Med.* **117,** 112–116.
49. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rep.* **42,** 597–599.
50. Arthur, M. & Courvalin, P. (1993) *Antimicrob. Agents Chemother.* **37,** 1563–1571.
51. Walsh, C. T. (1993) *Science* **261,** 308–309.
52. Handwerger, S., Pucci, M. J., Vol, K. J., Liu, J. & Lee, M. S. (1992) *J. Bacteriol.* **174,** 5982–5984.
53. Schwalbe, R. S., Stapleton, J. T. & Gilligan, P. H. (1987) *N. Engl. J. Med.* **316,** 927–931.
54. Noble, W. C., Virani, Z. & Cree, R. G. (1992) *FEMS Microbiol. Lett.* **93,** 195–198.
55. Sanders, C. C. (1987) *Annu. Rev. Microbiol.* **41,** 573–593.
56. Sanders, C. C. & Sanders, W. E., Jr. (1992) *Clin. Infect. Dis.* **15,** 824–839.
57. Philippon, A., Labia, R. & Jacoby, G. (1989) *Antimicrob. Agents Chemother.* **33,** 1131–1136.
58. Jacoby, G. A. & Medeiros, A. A. (1991) *Antimicrob. Agents Chemother.* **35,** 1697–1704.
59. Meyer, K. S., Urban, C., Eagan, J. A., Berger, B. J. & Rahal, J. J. (1993) *Ann. Intern. Med.* **119,** 353–358.
60. Urban, C., Go, E., Mariano, N., Berger, B. J., Avraham, I., Rubin, D. & Rahal, J. J. (1993) *J. Infect. Dis.* **167,** 448–451.

Evolution of Drug-Resistant Tuberculosis:A Tale of Two Species

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Charles Darwin in his treatise *The Origin of Species* delineated the requirements for "evolution" as a process to explain the multitudinous forms of life on our planet. Broadly speaking, he posited that there must be diversity among the offspring of a species. Then there should be factors in the environment that create a selective advantage for the reproduction and survival of certain of those progeny. This preferential survival for that substrain might entail either a global or a localized survival advantage within a particular niche. In a general sense, but one of which I believe Darwin might approve, the emergence of drug-resistant tuberculosis represents such a phenomenon. Unfortunately, the environmental factor that is essential for the rising prevalence of drug-resistant tuberculosis around the globe is humankind. The interplay between the two species, *Mycobacterium tuberculosis* and *Homo sapiens*, and its role in the creation of strains of tuberculosis resistant to modern medications will be described below. And the emerging influence of yet a third species, the retroviriad human immunodeficiency virus (HIV), on this ecosystem will be considered as well.

The tubercle bacillus belongs to an unusual family of bacteria that are related to and presumably developed from the microbes that constitute the "living" component of soil. On the basis of studies of genetic

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relatedness as well as circumstantial evidence, the mycobacteria probably emerged from the soil to find a niche first infesting, then infecting various mammals and birds. *M. bovis* is the most common animal pathogen, afflicting a diverse array of mammals, including ruminants and primates. Webb in his 1932 historical overview of tuberculosis speculated that the tuberculosis germ was first systematically introduced into humankind when humans domesticated cattle around 5000 B.C. (1). Indeed, modern genetic analysis indicates an extremely high degree of DNA homology between *M. bovis* and *M. tuberculosis*, indicating that they are virtually the same species (2). Thus, it is reasonable to infer that the parent strain *M. bovis*—which does have limited invasive and disease-producing capacity within humans—has undergone subtle host adaptation within the human body to become the tubercle bacillus. In this process, the microbe has developed these unique traits: (*i*) its only significant natural reservoir is humans, (*ii*) it has substantially diminished virulence for most animal species other than humans, and (*iii*) it has developed a survival-transmission strategy that is unparalleled among the mycobacteria: airborne human-to-human spread.

Skeletal artifacts indicate that tuberculosis has afflicted humankind since at least 3000–5000 B.C. From the Hippocratic writings and the work of Galen we infer that tuberculosis, referred to then as "phthisis" (translation: "I am wasting") was highly prevalent in the Greco–Roman era. For the past 500 years, tuberculosis has been pandemic in Europe and North America; at its apex in the 17th–18th centuries, the "White Plague" took the lives of 1 in 5 adults. In the 100 years from 1850–1950, it is estimated that one billion persons died of tuberculosis.

Certainly one of the most meaningful achievements of modern medicine has been the development of curative therapy for this ancient scourge. Although it is a bacterium, the tuberculosis bacillus is highly resistant to the conventional antibiotics, such as penicillin or sulfa, which were developed in the 1930s and 1940s. Selman Waksman, a specialist in soil biology at Rutgers, while screening microbes recovered from the earth, came upon a substance elaborated by one of them with substantial activity against the tubercle bacillus in 1943–1944; this compound, streptomycin, was pressed rapidly into clinical use, with initial reports of its efficacy appearing in 1945 (3). Although useful in ameliorating disease manifestations, streptomycin alone was not sufficient to cure most cases. Microbiologists soon recognized that, while most bacilli in a population of *M. tuberculosis* were susceptible to the drug (they were killed rapidly by concentrations of the medication readily achievable in tissue), some mutant offspring were present that were resistant to the drug's effects. When streptomycin was given alone, it killed the vulnerable population but left behind the resistant mutants,

a Darwinian selective process of "survival of the fittest." Without competition for the hosts' tissues, these bacilli then became the dominant subspecies.

Fortunately, two other medications were discovered shortly thereafter— *p*-aminosalicylic acid and isonicotinic acid hydrazide (isoniazid). Clinicians soon recognized that if all these drugs were given simultaneously, drug resistance did not emerge and lifetime cures of tuberculosis finally were achievable. Subsequent research showed that the explanation for this was as follows: (*i*) Random bacterial mutations that conferred resistance to individual drugs occurred infrequently during microbial replication, approximately once in 10^5–10^8 (4). (*ii*) These mutations were unlinked; therefore, the probability of a microbe spontaneously developing resistance to two drugs was the product of the individual risks or 1 in 10^5 × 1 in 10^6 = 1 in 10^{11} (3). Because the number of bacilli in a patient, even with extensive disease, rarely exceeds 10^9, it was highly improbable that multiresistant mutants would occur spontaneously (4). Thus, when isoniazid and streptomycin were given together, the isoniazid killed the mutants resistant to streptomycin and vice versa, ultimately eliminating the bacteria from the body.

In the 1950s and 1960s tuberculosis specialty hospitals (sanatoria) and clinics were widely available throughout the industrialized nations. Based on public fear of the disease and aggressive professional programs, successful treatment—despite the need for 24 months of drug therapy—was accomplished in the great majority of cases. However, as these two elements lost intensity and social disruptions became more pervasive in our society, adherence to treatment plans was eroded. Clinicians and public health authorities were hopeful that with newer, more powerful drugs the duration of treatment could be reduced sufficiently to combat noncompliance. But, despite reducing the required time from 24 to 6 months, irregular or incomplete adherence rose steadily over the past two decades (5).

As a consequence, the prevalence of drug-resistant strains of *M. tuberculosis* has risen dramatically in certain regions or populations. At the dawn of the treatment era, roughly 1–2% of strains of *M. tuberculosis* were seen to have significant drug resistance, almost universally to only one drug (6); in the 1960s and 1970s, that rate in the U.S. hovered around 3–5% (7, 8). However, over the past decade the national rate has risen steadily (9). In New York City, where a variety of elements, including poverty, substance abuse, and deteriorating public health programs, combined to confound tuberculosis control, 33% of tuberculosis strains recovered in April 1992 were resistant to at least one drug, and 19% were resistant to two or more agents (10). Tragically, in some developing nations where resources

are limited, inadequate treatment programs have resulted in drug-resistant rates in excess of 30% (11).

How has this resistance evolved? In most instances it occurs because patients either cryptically discontinue one or more of their multiple drugs or take less than the prescribed dosage (12). Alternatively, physicians—who have become generally less familiar with tuberculosis as the incidence has diminished—prescribe inappropriately (13). In either scenario, insufficient numbers or dosages of drugs are administered, creating an environment that selects for survival of the drug-resistant mutants. Note that the drugs do not induce the mutations, only tip the balance in favor of the naturally derived variants.

In this manner, a gradually increasing portion of the world's tuberculosis cases involve drug-resistant organisms. Most drug-resistant cases have historically involved failed treatment in an individual (14); however, in some instances, these strains have been transmitted to a new patient, who then develops tuberculosis with pre-formed drug resistance (15). This has occurred with relatively low frequency, presumably because the metabolic compromises made by the microbes to enable drug resistance have made them modestly less virulent (16). And, in the normal host—whose immune system has a 90% chance of containing a tuberculosis infection for a lifetime—even a small reduction in pathogenic capacity would make transmission of drug-resistant disease quite uncommon.

Enter here a third species, the HIV. Unfortunately, the HIV epidemic is afflicting persons from countries and/or socioeconomic groups in which tuberculosis latent infection and disease are highly prevalent. Because HIV infects, disables, and kills the cell that is central to tuberculosis immunity—the $CD4^+$ or helper T lymphocyte—the viral epidemic has led to a dramatic upsurge in tuberculosis in regions such as sub-Saharan Africa and cities including New York, Miami, Los Angeles, Rio de Janeiro, Brazil, and Bangkok, Thailand, where HIV and tuberculosis are coincident. A particularly alarming aspect of these coepidemics is the rising level of multidrug-resistant strains of tuberculosis (MDR-TB) in certain communities. Large-scale, highly lethal epidemics of MDR-TB among HIV-infected/AIDS patients have been reported in at least eight hospitals in New York and Florida (17). Analysis of these nosocomial outbreaks demonstrates clearly that the impaired defenses associated with HIV disease facilitate the transmission of MDR-TB (18). And, as the proportion of tuberculosis associated with HIV rises in the United States and the world over the decades to come, we may anticipate that MDR-TB strains will comprise an expanded percentage of this morbidity.

The implications are profound: (*i*) Patients will die of tuberculosis due

to inability to control the infection, (*ii*) the costs of treatment will soar, as more expensive drugs, extended therapy, and complicated surgery will be added to management, making cures unachievable for impoverished nations, and (*iii*) the highly effective prevention strategy of prophylactic treatment with isoniazid, a very inexpensive drug, to block the transition from latent infection to active disease will be rendered ineffectual.

In summary, human societal failures have potentiated the evolution of drug-resistant strains of the tubercle bacillus in the United States and around the world. Until recently, this has largely posed a threat to the health and survival of the individual in whom inadequate therapy has promoted the drug resistance. However, the HIV epidemic threatens to promote wholesale transmission of MDR-TB with the potential for immense morbidity and mortality. Reinforced treatment and control programs for TB are vital (19). Our response to this challenge will reflect on whether we deserve the appellation "sapient" or whether anthropologists will need to find another designation for our species.

SUMMARY

The history of the disease tuberculosis was briefly discussed. Now human societal failures have potentiated the evolution of drug-resistant strains of the tubercle bacillus in the United States and around the world. Until recently, this evolutionary change largely posed a threat to the health and survival of the individual in whom inadequate therapy promoted the drug resistance. However, the human immunodeficiency virus epidemic threatens to promote wholesale transmission of multidrug-resistant tuberculosis with the potential for immense morbidity and mortality. Reinforced treatment and control programs for tuberculosis are vital.

REFERENCES

1. Webb, G. B. (1936) *Tuberculosis* (Hoeber, New York).
2. Wayne, L. G. (1982) *Am. Rev. Respir. Dis.* **125,** Suppl., 31–41.
3. Feldman, W. H. & Hinshaw, H. C. (1945) *Proc. Staff Meet. Mayo Clinic* **20,** 313–315.
4. David, H. L. & Newman, C. M. (1971) *Am. Rev. Respir. Dis.* **104,** 508–515.
5. Reichman, L. B. (1987) *Tubercle* Suppl. **68,** 25–29.
6. Hobby, G. L. (1962) *Am. Rev. Respir. Dis.* **86,** 839–846.
7. Doster, B., Caras, G. J. & Snider, D. E. (1976) *Am. Rev. Respir. Dis.* **113,** 419–425.
8. Kopanoff, D. E., Kilburn, J. O., Glassroth, J. L., Snider, D. E., Farer, L. S. & Good, R. C. (1978) *Am. Rev. Respir. Dis.* **118,** 835–842.
9. Cauthen, G. M., Kilburn, J. O., Kelly, G. D. & Good, R. C. (1988) *Am. Rev. Respir. Dis.* **137,** Suppl. 4, 260 (abstr.).

10. Freiden, T. R., Sterling, T., Pablos-Mendez, A., Kilburn, J. O. , Cauthen, G. M. & Dooley, S. W. (1993) *N. Engl. J. Med.* **328,** 521–526.
11. Iseman, M. D. & Sbarbaro, J. A. (1992) *Curr. Clin. Top. Infect. Dis.* **12,** 188–207.
12. Goble, M., Iseman, M. D., Madsen, L. A., Waite, D., Ackerson, L. & Horsburgh, C. R. (1993) *N. Engl. J. Med.* **328,** 527–532.
13. Mahmoudi, A. & Iseman, M. D. (1993) *J. Am. Med. Assoc.* **270,** 65–68.
14. Costello, H. D., Caras, G. J. & Snider, D. E. (1980) *Am. Rev. Respir. Dis.* **121,** 313–316.
15. Centers for Disease Control (1990) *Morbid. Mortal. Wkly. Rep.* **39,** 369–372.
16. Middlebrook, G. & Cohn, M. L. (1953) *Science* **118,** 297–299.
17. Centers for Disease Control (1991) *Morbid. Mortal. Wkly. Rep.* **40,** 585–591.
18. Edlin, B., Tokars, J., Grieco, M., Crawford, J., Williams, J., Sordillo, E., Ong, K., Kilburn, J., Dooley, S., Castro, K., Jarvis, W. & Holmberg, S. *et al.* (1992) *N. Engl. J. Med.* **326,** 1514–1521.
19. Iseman, M. D., Cohn, D. L. & Sbarbaro, J. A. (1993) *N. Engl. J. Med.* **328,** 576–578.

Effect of Human Ecology and Behavior on Sexually Transmitted Diseases, Including HIV Infection

JUDITH N. WASSERHEIT

There are few infectious diseases that crystallize as hauntingly as sexually transmitted diseases (STDs) the effects of changes in human ecology and behavior on disease patterns. Webster's unabridged dictionary defines ecology as "a branch of science concerned with the interrelationship of organisms and their environment, especially as manifested by natural cycles and rhythms, community development and structure, interactions between different kinds of organisms, geographic distributions, and population alterations" (1). The dismaying growth in the spectrum and rates of STDs experienced over the past two decades in most parts of the world, including the United States, is, indeed, a reflection of the complex interactions of these pathogens with at least three types of environments. In this brief overview, I will summarize six changes in STD patterns that have emerged during the past 20 years and discuss their links to changes in the physiological microenvironment, the behavioral personal environment, and the sociocultural macroenvironment. Finally, I will highlight the implications of these links for effective STD prevention programs.

CHANGING PATTERNS OF SEXUALLY TRANSMITTED DISEASES

The last 20 years have witnessed six striking changes in STD patterns: emergence of new STD organisms and etiologies, reemergence of old

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STDs, shifts in the populations in which STDs are concentrated, shifts in the etiological spectra of STD syndromes, alterations in the incidence of STD complications, and increases in antimicrobial resistance (Table 1). Many STDs represent examples of more than one of these trends.

TABLE 1 Changing patterns of STDs

Pattern	Examples
Emergence of new organisms/etiologies	HIV, infertility, ectopic pregnancy, fetal death, low birth weight, prematurity, anogenital cancer, proctocolitis
Reemergence of old STDs	Syphilis
Population shifts	HIV infection, syphilis, gonorrhea
Etiology shifts	Urethritis, cervicitis, PID
Altered incidence of complications	Perinatal HIV infection, congenital syphilis, PID, dysplasia
Increased antimicrobial resistance	Gonorrhea, chancroid

PID, pelvic inflammatory disease.

Exciting insights into the pathophysiology and natural history of both bacterial and viral STDs have led to the discovery of sexually transmitted etiologies for such diverse syndromes as infertility, ectopic pregnancy, other adverse outcomes of pregnancy, anogenital cancers, and proctocolitis. However, human immunodeficiency virus (HIV) is clearly the archetype of a newly emerging STD pathogen, one that has devastated the world with a fatal pandemic involving an estimated 14 million people, of whom approximately 2.5 million have developed AIDS (2). In the United States alone, it is likely that as many as a million individuals are infected with HIV, and more than 361,000 AIDS cases have been reported since 1981, when the syndrome was first recognized (3).

Despite its recent emergence, AIDS is, in addition, a prime example of a disease that is shifting to affect new populations (Figure 1). In the United States, AIDS spread first among predominantly white homosexual and bisexual men, but AIDS incidence in this population began to plateau by the early 1990s (4, 5). The second wave of the AIDS epidemic emerged among injecting-drug users (IDUs) and was concentrated among racial and ethnic minority populations. At least since 1989, however, the greatest proportionate increase in reported AIDS cases has been due to heterosexual transmission, and the majority of these cases have occurred among women (4–7). Between 1991 and 1992, for example, a 17% increase in AIDS cases attributed to heterosexual transmission was observed, compared with a 1% decrease in cases due to homosexual or bisexual transmission, and a 1% increase in IDU-related cases among heterosexuals. In 1992, although half of all new AIDS cases

occurred among homosexual or bisexual men, almost 60% of heterosexually transmitted AIDS cases occurred in women, and, for the first time, more women were reported with AIDS as a result of heterosexual transmission than as a result of injecting-drug use. Furthermore, among women, non-Hispanic Blacks experienced the most rapid increases in heterosexually acquired AIDS (5).

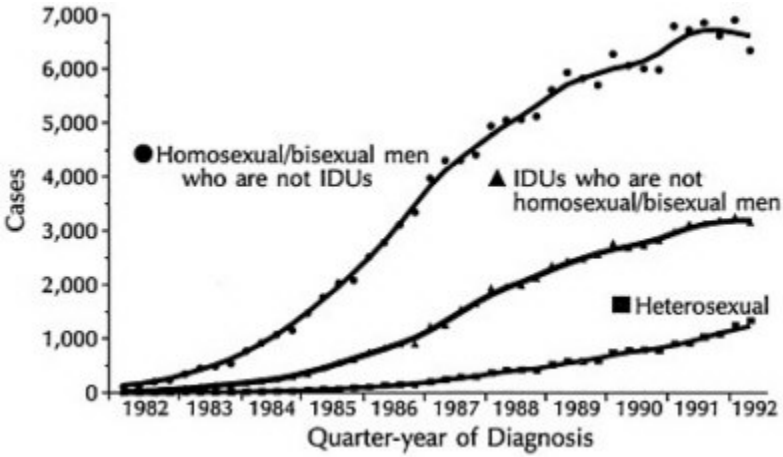

FIGURE 1 AIDS incidence by transmission category and quarter-year of diagnosis, January 1982 through June 1992, United States. Data are adjusted for delays in reporting but not for incomplete reporting.

Syphilis offers a classic example of the reemergence of an easily diagnosed and readily treatable STD. Following World War II, with the widespread availability of penicillin, infectious (primary and secondary) syphilis rates in the United States fell almost 95%, from 66 cases per 100,000 persons in 1947 to 4 cases per 100,000 persons in 1956. However, this triumph was not sustained. The incidence of syphilis rose progressively after 1956 to reach a 40-year peak of 20 cases of infectious syphilis per 100,000 persons by 1990 (8).

Echoing some aspects of the sequential AIDS epidemics discussed above, syphilis also exemplifies a STD that has shifted its principal target from one population to another. The syphilis epidemic hit the United States in two distinct waves. The first, from about 1960 to 1980, was concentrated among homosexual men, and the second, over the latter half of the subsequent decade, had its greatest impact among minority heterosexuals. As is elegantly highlighted by Rolfs and Nakashima (8), this shift was reflected in striking changes in the male/female incidence rate ratio for infectious syphilis during this 30-year period (Figure 2).

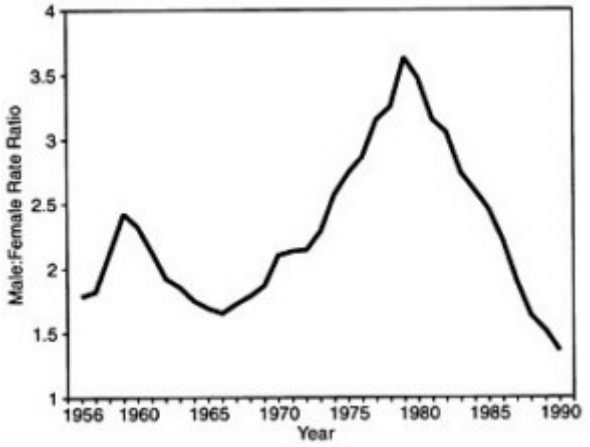

FIGURE 2 Primary and secondary syphilis in the United States, 1956–1990. Reprinted with permission from ref. 8 (copyright 1994, Blackwell Scientific Publications).

From 1966 to 1979, the ratio more than doubled, from 1.7 to 3.6, and then fell precipitously to 1.3 by 1991. Between 1985 and 1990, infectious syphilis rates also rose 165% among non-Hispanic Blacks, while they fell among persons of all other races and ethnicities. The 14-fold higher syphilis rates among Blacks than among Whites reported in 1985 exploded to a 60-fold differential by 1991 (9).

As the AIDS and syphilis epidemics penetrated heterosexual populations, the incidence of perinatal and congenital infections increased as well. AIDS cases resulting from perinatal transmission increased 13.4% between 1991 and 1992, an increase second only to that due to heterosexual transmission itself (5), and congenital syphilis rates increased at least 5-fold between 1985 and 1991, after adjustment for the new case definition that was instituted in 1989.

Gonorrhea trends in the United States present a sharp contrast to syphilis patterns and, again, highlight the protean nature of STDs. Despite the common mode of transmission shared by these STDs, gonorrhea and infectious syphilis trends have consistently diverged over the past 25 years (Figure 3). From 1965 to 1975, gonorrhea rates more than doubled, while infectious syphilis rates increased slightly. During the next 15 years, gonorrhea rates declined with increasing momentum as syphilis rates skyrocketed (10).

In addition to the population shifts characterized by sexual practices,

race, and gender observed with syphilis, age-related population shifts have been seen with gonorrhea. Over the last decade, gonorrhea rates have declined among both men and women in all age groups between 15 and 40 years of age except 15- to 19-year-olds (9). As a result, adolescents in this country now have the highest age-specific gonorrhea rates among women and the second highest age-specific rates among men. After adjustment for the proportion of the population which is sexually active, the gap between teenagers and older adults widens even more (11).

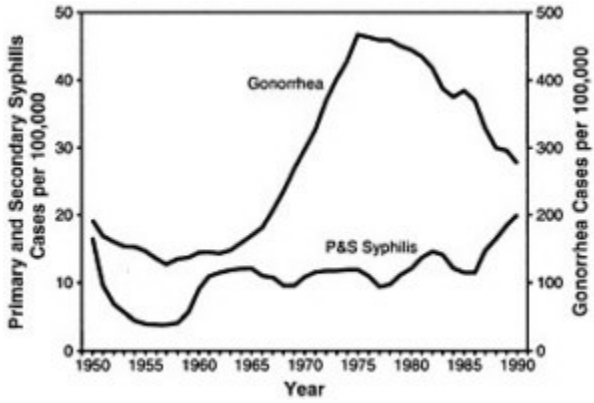

FIGURE 3 Rates of primary and secondary syphilis and gonorrhea in the United States, 1950–1990. Reprinted with permission from ref. 8 (copyright 1994, Blackwell Scientific Publications).

Gonorrhea also provides an excellent example of rapid and repeated emergence of new types of antimicrobial resistance. The recognition of penicillinase-producing *Neisseria gonorrhoeae* in 1976 has been followed by appreciation of an increasingly diverse array of resistance patterns and mechanisms, including both plasmid- and chromosomally mediated resistance to penicillins and tetracyclines, the two former mainstays of treatment (12). Between 1988 and 1991, national surveillance of gonococcal isolates conducted by the Centers for Disease Control and Prevention documented an increase in the proportion of resistant isolates from 21% to 32.4%, more than a 50% increase in only 3 years (9, 13, 14)!

In the United States, as gonorrhea rates have declined, chlamydia rates have climbed, shifting the apparent balance of etiologies of urethritis, cervicitis, and PID. Indeed, in 1991, reported chlamydia cases exceeded reported gonorrhea cases among women for the first time (9).

However, this reversal is probably an artifact of real changes in gonorrhea incidence superimposed upon improved chlamydia surveillance due to both diagnostic advances and more widespread legal requirements for chlamydia reporting. Nevertheless, a relative increase in chlamydial infection may have contributed to recent decreases in hospitalized cases of PID in the face of stable numbers of ambulatory PID cases because of the more indolent nature of chlamydial infection compared with gonococcal PID (15).

Human papillomavirus (HPV) infection provides a final example of changing patterns of STDs. HPV infection has repeatedly emerged as one of the most important risk factors for cervical dysplasia (16–18). Furthermore, several studies have documented an increased prevalence of dysplasia among HIV-infected women compared with HIV-uninfected subjects (19). In a recent New York City study, for example, HIV-infected women were almost 6 times as likely to have colposcopic evidence of cervical intraepithelial neoplasia as their HIV-uninfected counterparts, and the majority of cervical intraepithelial neoplasia was attributable to HPV infection in both groups (T. C. Wright, T. V. Ellerbrock, and M. A. Chiasson, personal communication).

ENVIRONMENTAL CHANGES AFFECTING STD PATTERNS

Why have all of these changes in STD patterns occurred? What environmental changes continue to drive the evolution of these disease patterns?

Let us consider three interrelated types of environments affecting STD patterns (Figure 4). The microbiologic, hormonal, and immunologic microenvironments most directly influence susceptibility, infectiousness, and development of sequelae. These microenvironments are shaped, in part, by the personal environments created by an individual's sexual, substance-use, and health-related behaviors.

The personal environments are also important determinants of acquisition of infection and development of sequelae but, in addition, they mediate risk of exposure to infection. These are, therefore, the environments that most directly affect changing disease patterns such as the emergence or reemergence of STDs, or the population shifts discussed above.

Finally, individuals' personal environments are, in turn, molded by powerful macroenvironmental forces, including socioeconomic, demographic, geographic, political, epidemiologic, and technological factors. Over the past 20 years, the profound changes that have occurred in many aspects of the personal environment and the macroenvironment have been reflected in new STD patterns.

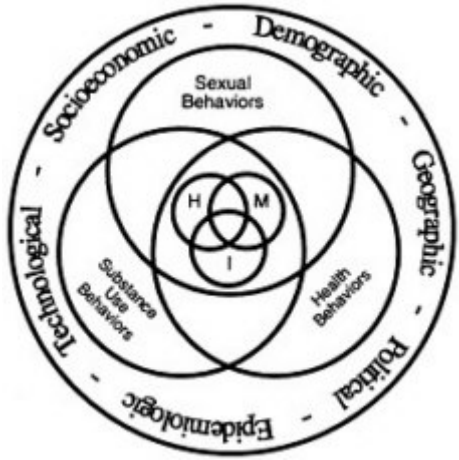

FIGURE 4 Environments affecting STD patterns. M, microbiological; H, hormonal; I, immunological.

The Physiological Microenvironment

Trend data are not available for most of the biological indices of the microenvironments affecting STD patterns (Table 2). For example, while we know that the vaginal flora and acidity (pH) of the microbiological microenvironment influence susceptibility to STDs, we do not know how these factors have changed over time among populations of women in the United States. Similarly, manifestations of the hormonal microenvironment, such as the size of the zone of cervical ectopy,* the penetrability of the cervical mucus, the patency of the cervical canal, the phase of the menstrual cycle, and possibly even the composition of seminal and prostatic fluids, may contribute to susceptibility to STDs or their sequelae (20), and we know little about population trends in these factors.

Trend data are, however, obtainable for a few of these microenvironmental parameters. By altering cervicovaginal ecology, modulating vaginal pH, transactivating other pathogens, or other mechanisms, one STD may increase susceptibility to other STDs or their complications (19, 21). Thus, recent increases in STDs may have fueled some of the

* The zone of exposed columnar epithelium that is the primary attachment site for chlamydial and gonococcal infection and that probably also plays an important role in HIV infection.

changing disease patterns biologically as well as epidemiologically. Decreases in the age of menarche, a manifestation of the hormonal microenvironment, have been documented (22, 23). Finally, competing factors that influence the immunological microenvironment, such as pregnancy, HIV infection, and prior exposure to STDs, can also be traced over time in some populations.

TABLE 2 Microenvironments affecting STD patterns

Microbiologic	Hormonal	Immunologic
Vaginal flora and acidity (pH)	Cervical ectopy	Pregnancy
STD coinfection	Cervical mucus	HIV infection
	Cervical patency	Prior STD exposure
	Menses	
	Seminal/prostatic fluid?	

The Behavioral Personal Environment

Changes in sexual behaviors have been one of the primary engines driving changing patterns of STDs, including HIV infection (Table 3). Steady decreases in age of first sexual intercourse and concomitant increases in premarital sexual activity have been documented repeatedly both in the United States and abroad (21, 24, 25). Data from the National Survey of Family Growth, for example, indicate that the percentage of 15- to 19-year-old American women reporting premarital intercourse almost doubled from 28.6% in 1970 to 51.5% in 1988 (26). Although increases were greatest among younger White adolescents, the proportion of Black women reporting premarital sexual activity was higher than the proportion of White women for each age group and year examined. This trend is particularly disturbing because early age of

TABLE 3 Personal environments affecting STD patterns

Sexual behaviors	Substance-use behaviors	Health behaviors
Age at coital debut	Intravenous drug use	Condom use
Number of sex partners	Crack cocaine	Pill and IUD use
Commercial sex	Exchange of sex for drugs	Vaginal douching
Sexual practices	Alcohol use	Circumcision
		Early health-care utilization
		Compliance with therapy
		Provider screening

IUD, intrauterine device.

coital debut appears to be associated with subsequent patterns of multiple sexual partners and sex with risky partners, such as bisexual, injecting-drug-using, or HIV-infected individuals (27). Furthermore, the hormonal microenvironment is age-dependent. Thus, for young adolescents, behavioral risk factors such as multiple, risky partners conspire with biological risk factors such as large zones of cervical ectopy to result in high STD rates.

Younger cohorts also report more sexual partners than older groups, and the proportion of young adults with multiple sexual partners appears to be increasing over time (24, 25, 28-31). In fact, between 1971 and 1988, the proportion of sexually active adolescent women reporting more than one sexual partner swelled from 39% to 62% (29). In part this may be due to the combined effect of trends toward younger age of coital debut and older age at first marriage. During the decade of the '80s, American women experienced an increase of approximately 30% in the median number of years that they spend in this relatively high risk stage of their reproductive lives (32). In addition, these increases were greatest for Black women and for women living in poverty.

Sonenstein and colleagues (33) have observed analogous trends among young men. Among 17- to 19-year-old American men, the average number of sexual partners in the previous year rose from 2.0 in 1988 to 2.6 in 1991, and the proportion reporting 5 or more partners increased from 6% to 11% over the same time period. Both this study and the 1991 National Survey of Men suggest that Black men report more sexual partners than White or Hispanic men; however, other national survey data indicate that these patterns vary substantially by gender and marital status (30-32, 34).

Both commercial sex (exchange of sex for money or drugs) and specific sexual practices such as anal intercourse, intercourse during menses, or "dry sex"* have also been linked to increased risk of STDs or their sequelae (21, 35-37). However, few trend data are available on these sexual behaviors. Cross-sectional national survey data indicate that more White men than Black men reported anal intercourse (21% vs. 13.6%) or same-gender sexual activity during the past 10 years (2.4% vs. 1.3%) (30). In contrast, anecdotal information suggests that dry sex may be practiced more frequently by Black Americans than by Whites (31).

* Sex during which efforts are made to minimize lubrication of the vagina by using intravaginal desiccants, by wiping out the vagina, or by other methods. The intent is usually to increase the pleasure of the male partner; however, this practice may increase STD or HIV transmission because it may result in vaginal inflammation, traumatic lesions, or more frequent condom breakage.

Over the past 25 years, dramatic changes in substance-use behaviors reinforced the impact of changes in sexual behaviors on STD and HIV patterns. Drug use promotes anonymous sex and exchange of sex for drugs or money. Both drug and alcohol use may also impair ability to practice safe sex. In this context, it is very disturbing that the National Institute on Drug Abuse estimates that illicit drug use increased from less than 5% of Americans ever using these substances in the early 1960s to over 10% by the early 1970s (38). By 1988, an estimated 36.6% of Americans 12 years of age or older had tried illicit drugs.

In terms of impact on STD and HIV trends, regular or recent use of illicit drugs is more important than previous experimentation. The National Household Survey of Drug Abuse indicates that use of most illicit drugs (including cocaine) at least once within the past year increased during the 1970s and peaked during the first half of the 1980s (39). Reported use of cocaine within the past year by high school seniors, for example, rose from 5.6% in 1975 to 13.1% in 1985, and subsequently declined to 3.5% by 1991.

Intravenous drug use and crack cocaine use have been particularly potent factors in the changing patterns of STDs, especially syphilis and HIV infection (21, 40). Both of these behaviors have been associated with low socioeconomic status and appear to be more common among Black and Hispanic Americans than among White Americans (31, 38).

Health-related behaviors are the third component of the personal environments affecting STD patterns (Table 3). Several of these behaviors, such as early health-care utilization, compliance with therapy, and provider screening, primarily affect the distribution of the curable STDs by reducing the duration of infectiousness of these diseases and by limiting the incidence of long-term complications.

Other health-related behaviors, such as contraceptive use, vaginal douching, and circumcision, may influence STD patterns more broadly (21). Correct and consistent condom use and male circumcision both decrease risk of STDs, including HIV infection (41). Oral contraceptive pill use, on the other hand, may augment risk of chlamydial and gonococcal cervicitis but seems to decrease risk of PID (41). Both of these effects are probably mediated by pill-induced changes in the hormonal microenvironment such as expanded zones of ectopy and decreased penetrability of cervical mucus. IUD use and vaginal douching have been linked with increased risk of PID—in the former case, principally during the first 3 months following device insertion (41, 42). These two behaviors also clearly alter the vaginal microenvironment. Finally, both pill and IUD use may increase STD risk by reducing the likelihood of condom use.

Contraceptive use trends have been a critical force in evolving STD

patterns. One of the key elements of the sexual revolution of the 1960s was the rapid diffusion of the pill, the first unobtrusive, female-controlled pregnancy prevention technology. National surveys of married women showed steady increases in pill use until 1973, a decline from 1973 to 1982, and stabilization in use rates between 1982 and 1988 (43). During the 1982-1988 period, pill use also remained unchanged among never-married and formerly married women but increased among women between the ages of 20 and 34, while IUD use declined in all groups. Condom use increased from 12% to 20% among never-married women, and from 21% to 33% among 15- to 19-year-olds during this period. Smaller gains were observed among women in their twenties. Contraceptive use at first intercourse grew from 47% of women in 1975-1979 to 66% of women in 1983-1988, with the increases entirely attributable to condom use among Whites and increases balanced between condom use and pill use among Blacks (44).

National longitudinal data on vaginal douching and on male circumcision are not available. However, national data collected in 1988 document that vaginal douching is strongly associated both with Black race and with poverty (31). Anecdotal information suggests that circumcision may be more common among White men than among other racial or ethnic groups.

Data on health-care utilization, compliance with therapy, and provider screening behaviors are also quite limited, and conflicting information on racial or ethnic differentials makes definitive interpretation difficult (31). Furthermore, a reporting bias may arise because racial/ethnic minority populations often seek care from publicly funded providers who are more likely to test for and report STDs than are private providers (21). Yet financial and social barriers to early and effective care clearly disproportionately affect adolescents and members of minority communities who live in poverty. Deterioration of the public health infrastructure in the United States during the 1980s has undoubtedly exacerbated these problems and may have contributed to the spread of HIV infection, the reemergence of adult and congenital syphilis, and the rapid evolution of gonococcal resistance.

The Sociocultural Macroenvironment

Perhaps the greatest challenge of STD and HIV prevention lies in developing strategies that acknowledge the powerful and interrelated macroenvironmental forces that shape many of the sexual, substance-use, and health-related behaviors of individuals' personal environments (Table 4). Factors such as poverty, low status of women, social up-heaval, urbanization, and migration or geographic mobility promote risk

behaviors because they destabilize societal norms by increasing inequality, anonymity, and marginalization (21, 45). These factors often cluster in and foster environments with fragile public health infrastructures, thus creating barriers to health-related behaviors among populations most in need of services. Communities of high STD and HIV prevalence result. Further social disintegration may ultimately precipitate extensive out-migration and spread of disease (46).

TABLE 4 Macroenvironments affecting STD patterns

Socioeconomic	Political	Demographic
Poverty	Public health infrastructure	Young age structures
Status of women	Social upheaveal	Sex ratio imbalance
Geographic	**Technological**	**Epidemiologic**
Urbanization	STD tests	STD prevalence
Domestic and international travel	STD therapies	
Migrant labor	Prevention technologies (e.g., female condom)	

The demographic characteristics of these populations frequently compound the problem. Young age composition and sex ratio imbalance are linked with many of the factors mentioned above, as well as with STD and HIV risk behaviors. It is noteworthy that in the United States relatively large and growing segments of minority populations are sexually active adolescents and young adults, while the proportion of the White population in these groups has been declining since the mid-1980s (21, 45).

Poverty and deterioration of the public health infrastructure have been particularly important forces in shaping STD and HIV patterns in the United States. Poverty is associated with substance abuse, exchange of sex for drugs or money, poor access to health services, and young age at first intercourse. Both the poverty rate and income inequality increased in this country during the last 20 years. The poverty rate rose to 14.2% in 1991 from 11.4% in 1978 and disproportionately affected women, young people, and racial and ethnic minority populations, particularly in urban areas (31, 47, 48). For example, the proportions of White, Hispanic, and Black Americans living in poverty in 1991 were 11.3%, 28.7%, and 32.7%, respectively. The comparable figures for 1973 (the first year for which data on Hispanic origin were recorded) were 8.4%, 21.9%, and 31.4%. These data and shifts in race/ethnicity-specific ratios of actual income to poverty level income highlight both that the extent and depth of poverty are greatest among Black Americans and

that Hispanic Americans have experienced the largest increases in poverty over the last two decades.

These socioeconomic trends have been accompanied by changes in the political environment that further limited the access to STD care for those relying on public sector providers. At the same time that chlamydia, herpes, HPV infection, and AIDS emerged, and as syphilis rates climbed and treatment of gonorrhea required increasingly expensive antibiotics, support for STD services flagged and the public health infrastructure in the United States deteriorated. Many STD clinics across the country were unable to meet the demand for services and, increasingly, were forced to turn patients away without care. Frequently, those patients who did receive care had to wait many hours to be seen. A survey of public STD clinics conducted in 1989 revealed problems of this type in 19 of 23 sites (45). It is ironic that these trends occurred in the setting of tremendous advances in diagnostic and therapeutic technologies which should have made feasible highly effective STD prevention programs. Furthermore, increased knowledge about the effectiveness of condoms and other prevention technologies was offset by the development of a more conservative political environment.

IMPLICATIONS FOR EFFECTIVE STD PREVENTION PROGRAMS

STDs offer an outstanding "case study" of the synergistic effects of changes in human ecology and behavior on patterns of infectious diseases. The last 20 years have brought explosive growth in the spectrum, complexity, range of sequelae, and, in some cases, incidence of STDs. It is likely that complete eradication of STDs, including HIV infection, must await development of vaccines that can be delivered in conjunction with antimicrobial agents and behavioral interventions. However, even in the absence of effective vaccines, enormous progress can be made in prevention and control of these infections if we broaden our focus to include all three of the environments discussed above.

Traditional, clinic-based STD programs have emphasized interventions that target the physiological microenvironments and selected health behaviors. Programs developed with an appreciation of the role of risk behaviors and macroenvironmental forces in STD and HIV epidemiology will be likely to improve the effectiveness of these clinical interventions by focusing them on populations most in need of services and by addressing barriers to care that might otherwise be ignored.

The multiple environments affecting STD patterns highlight three other important principles. First, both clinic-based and community-based behavioral interventions are essential companions to diagnosis and treatment in effective prevention programs. Second, STD/HIV

prevention, substance-abuse prevention, and pregnancy prevention efforts are likely to reinforce each other. Finally, the community can play a pivotal role in modulating the impact of macroenvironmental factors on the personal environments that shape STD and HIV patterns.

SUMMARY

The last 20 years have witnessed six striking changes in patterns of sexually transmitted diseases (STDs): emergence of new STD organisms and etiologies, reemergence of old STDs, shifts in the populations in which STDs are concentrated, shifts in the etiological spectra of STD syndromes, alternations in the incidence of STD complications, and increases in antimicrobial resistance. For example, human immunodeficiency virus (HIV) emerged to devastate the United States with a fatal pandemic involving as many as 1 million people. The incidence of syphilis rose progressively after 1956 to reach a 40-year peak by 1990. In both cases, disease patterns shifted from homosexual men to include minority heterosexuals. Over the last decade, gonorrhea became increasingly concentrated among adolescents, and several new types of antimicrobial resistance appeared. Three interrelated types of environments affect STD patterns. The microbiologic, hormonal, and immunologic microenvironments most directly influence susceptibility, infectiousness, and development of sequelae. These microenvironments are shaped, in part, by the personal environments created by an individual's sexual, substance-use, and health-related behaviors. The personal environments are also important determinants of acquisition of infection and development of sequelae but, in addition, they mediate risk of exposure to infection. These are, therefore, the environments that most directly affect changing disease patterns. Finally, individuals' personal environments are, in turn, molded by powerful macroenvironmental forces, including socioeconomic, demographic, geographic, political, epidemiologic, and technological factors. Over the past 20 years, the profound changes that have occurred in many aspects of the personal environment and the macroenvironment have been reflected in new STD patterns.

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REFERENCES

1. Grove, P. B., *et al.*, eds. (1966) *Webster's Third New International Unabridged Dictionary* (Merriam, Springfield, MA), p. 720.

2. Merson, M. H. (1993) IXth International Conference on AIDS, June 6–11, 1993, Berlin, pp. PS-01-1 (abstr.).
3. Centers for Disease Control and Prevention (1993) *HIV/AIDS Surveill. Rep.* **5**, Vol. 4, p 5.
4. Centers for Disease Control and Prevention (1992) *Morbid. Mortal. Wkly. Rep.* **41,** 463–468.
5. Centers for Disease Control and Prevention (1993) *Morbid. Mortal. Wkly. Rep.* **42,** 547–557.
6. Centers for Disease Control and Prevention (1990) *Morbid. Mortal. Wkly. Rep.* **39,** 81–86.
7. Centers for Disease Control and Prevention (1991) *Morbid. Mortal. Wkly. Rep.* **40,** 358–369.
8. Rolfs, R. T. & Nakashima, A. K. (1994) *Modern Epidemiology of Syphilis in the United States and Western Europe in Syphilis,* eds. Hook, E. W., III, & Lukehart, S. A. (Blackwell, Cambridge, MA), in press.
9. Centers for Disease Control and Prevention (1993) *Div. STD/HIV Prevent. Ann. Rep.* (Cent. Dis. Contr., Atlanta), pp. 103–174.
10. Gershman, K. A. & Rolfs, R. T. (1991) *Am. J. Public Health* **81,** 1263–1267.
11. Aral, S. O., Schaffer, J. E., Mosher, W. D. & Cates, W., Jr. (1988) *Am. J. Public Health* **78,** 702–703.
12. Whittington, W. L. & Knapp, J. S. (1988) *Sex. Transm. Dis.* **15,** 202–210.
13. Schwarcz, S. K., Zenilman, J. M., Schnell, D., Knapp, J. S., Hook, E. W., III, Thompson, S., Judson, F. N. & Holmes, K. K. (1990) *J. Am. Med. Assoc.* **264,** 1413–1417.
14. Centers for Disease Control and Prevention (1993) *Morbid. Mortal. Wkly. Rep.* **42,** 29–39.
15. Rolfs, R. T., Galaid, E. I. & Zaidi, A. A. (1992) *Am. J. Obstet. Gynecol.* **166,** 983–990.
16. Oriel, J. D. (1990) *Int. J. STD AIDS* **1,** 7–9.
17. Cancer Unit for Microbiology and Immunology, World Health Organization (1987) *Bull. World Health Org.* **65,** 817–827.
18. International Agency for Research on Cancer (WHO) (1989) (Oxford Univ. Press, New York), IARC Sci. Publ. No. 94.
19. Wasserheit, J. N. (1992) *Sex. Transm. Dis.* **19,** 61–77.
20. Ehrhardt, A. A. & Wasserheit, J. N. (1991) in *Research Issues in Human Behavior and Sexually Transmitted Diseases in the AIDS Era,* eds. Wasserheit, J. N., Aral, S. O., Holmes, K. K. & Hitchcock, P. J. (Am. Soc. Microbiol., Washington, DC), pp. 97–121.
21. Aral, S. O. & Holmes, K. K. (1990) in *Sexually Transmitted Diseases,* eds. Holmes, K. K., Mardh, P. A., Sparling, P. F., Wiesner, P. J., Cates, W., Jr., Lemon, S. M. & Stamm, W. E. (McGraw–Hill, New York), pp. 19–36.
22. Bullough, U. L. (1981) *Science* **213,** 365–366.
23. Rees, M. (1993) *Lancet* **342,** 1375–1376.
24. Biggar, R. J., Brinton, L. A. & Rosenthal, M. D. (1989) *J. Acquired Immune Defic. Synd.* **2,** 497–502.
25. ACSF investigators (1992) *Nature (London)* **360,** 407–409.
26. Centers for Disease Control and Prevention (1991) *Morbid. Mortal. Wkly. Rep.* **39,** 929–932.
27. Greenberg, J., Magder, L. & Aral, S. O. (1992) *Sex. Transm. Dis.* **19,** 331–334.
28. Johnson, A. M., Wadsworth, J., Wellings, K., Bradshaw, S. & Field, J. (1992) *Nature (London)* **360,** 410–412.

29. Kost, K. & Forrest, J. D. (1992) *Family Planning Perspectives* **24,** 244–254.
30. Billy, J. O. G., Tanfer, K., Grady, W. R. & Klepinger, D. H. (1993) *Family Planning Perspectives* **25,** 52–60.
31. Aral, S. O. & Wasserheit, J. N. (1994) in *African-Americans and Hispanics: The Role of Behavioral and Psychosocial Factors,* eds. Johnson, E. H., Amaro, H., Antoni, M., Jemmot, J. & Szapocnik, J. (Praeger, New York).
32. Forrest, J. D. (1993) *Obstet. Gynecol.* **82,** 105–111.
33. Ku, L., Sonenstein, F. & Pleck, J. (1993) *Am. J. Public Health* **83,** 1609–1614.
34. Dolicini, M. M., Catania, J. A., Coates, T. J., Stall, R., Hudes, E. S., Gagnon, J. H. & Pollack, L. (1993) *Family Planning Perspect.* **25,** 208–214.
35. Kingsley, L. A., Detels, R., Kaslow, R., Polk, B. F., Rinaldo, C. R., Chmiel, J., Detre, K., Kelsey, S. F., Odaka, N., Ostrow, D., VanRaden, M. & Visscher, B. (1987) *Lancet* **i,** 345–349.
36. European Study Group (1989) *Br. Med. J.* **298,** 411–415.
37. Sweet, R. L., Blankfort-Doyle, M., Robbie, M. O. & Schachter, J. (1986) *J. Am. Med. Assoc.* **255,** 2062–2064.
38. U.S. Department of Health and Human Services (1991) *Third Triennial Report to Congress on Drug Abuse and Drug Abuse Research* (Natl. Inst. Drug Abuse/Alcohol, Drug Abuse and Mental Health Admin., Bethesda, MD), DHHS Publ. No. (ADM)91-1704, pp. 13–29.
39. Drugs and Crime Data Center and Clearinghouse (1992) *Fact Sheet: Drug Use Trends* (U.S. Dept. of Justice, Bureau of Justice Statistics, Washington, DC), pp. 1–5.
40. Rolfs, R. T., Goldberg, M. & Sharrar, R. G. (1990) *Am. J. Public Health* **80,** 853–857.
41. Cates, W., Jr., & Stone, K. M. (1992) in *Reproductive Tract Infections,* eds. Germain, A., Holmes, K. K., Piot, P. & Wasserheit, J. N. (Plenum, New York), pp. 93–129.
42. Scholes, D., Daling, J. R., Stergachis, A., Weiss, N. S., Wang, S. & Grayston, J. T. (1993) *Obstet. Gynecol.* **81,** 601–606.
43. Mosher, W. D. (1990) *Family Planning Perspectives* **22,** 198–205.
44. Mosher, W. D. & McNally, J. W. (1991) *Family Planning Perspectives* **23,** 108–116.
45. Aral, S. O. & Holmes, K. K. (1991) *Sci. Am.* **264,** 62–69.
46. Wallace, R. (1988) *Environ. Res.* **47,** 1–33.
47. U.S. Department of Commerce (1992) *Poverty in the United States: 1991* (Bureau of the Census, Washington, DC) Current Population Rep., Series P-60, No. 181.
48. U.S. Department of Commerce (1990) *Household Wealth and Asset Ownership: 1988* (Bureau of the Census, Washington, DC) Current Population Rep., Series P-70, No. 22.

Genital Human Papillomavirus Infection

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Genital human papillomaviruses (HPVs) induce benign epithelial lesions of the internal and external genitalia and are closely associated with several anogenital malignancies, especially cervical cancer. HPVs have long been recognized as the etiologic agents of genital and nongenital warts. The involvement of these DNA tumor viruses in so-called flat condylomata of the cervix was recognized in the 1970s (1), which led to their identification in cervical cancers and other malignant genital tumors. Genital HPV infection is almost always sexually acquired, and the epidemiology of cervical cancer follows that of a sexually transmitted condition (see ref. 2 for a review).

Genital HPV infection is not a reportable condition, and the estimates of HPV prevalence vary widely depending upon the detection method used and population studied. Nevertheless, most studies indicate that genital HPV infection is even more common than genital infection with herpes simplex virus. For instance, single point detection prevalence of >40% has been found in college women in the United States (3). As with other sexually transmitted diseases, the incidence of genital HPV infection in developed countries increased in recent decades (4). An 8-fold increase in the incidence of genital warts was reported in Rochester between 1950 and 1978 (5).

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The close relationship between genital HPV infection and genital malignancies, described below, underscores the potential importance for developing more effective management of this disease. Cervical cancer is the second leading cause of cancer deaths in women worldwide (6). In many developing countries, it is the leading cause of death from cancer in women. In developed countries, the extensive use of the Pap (Papanicolaou) smear as a screening test for precursor lesions of the cervix has resulted in a substantial reduction in the incidence of cervical cancer (7). The incidence of cervical cancer may be severalfold higher in developing countries where the Pap smear has been employed more sparingly (2).

RELATIONSHIP BETWEEN HPV INFECTIONAND CERVICAL CANCER

HPVs cannot be routinely cultivated, and serotypes have not been defined. Instead, HPVs are typed by molecular hybridization of their genomes. By this analysis, >70 distinct HPV genotypes (types) have been recognized and defined since the 1970s (8). Significant clinicopathological correlations can be made for many HPV types. It is useful to divide the HPVs into three broad classes: genital-mucosal types, nongenital types, and epidermodysplasia verruciformis (EV)-specific types. EV is a rare skin condition in which patients develop widespread, chronic nongenital cutaneous HPV lesions (9). Almost one-half of the known HPV types have been identified principally in EV patients. As with other HPV types, the EV-specific types have a worldwide distribution in spite of the small number of EV patients. The nongenital HPV types infect the nongenital skin of the general population and induce common and plantar warts. These lesions have an extremely low probability of oncogenic progression. Clinical infection by the genital-mucosal HPV types (called genital types below) is found most commonly in the internal and external genitalia, although these types may also cause lesions in the upper aerodigestive tract, especially the mouth, pharynx, and larynx.

Two major classes of genital HPV types have been identified, depending upon their association with cervical cancer (reviewed in ref. 10). The "low-risk" types, especially HPV6 and HPV11, are almost never found in cervical malignancies. They are most frequently isolated from external genital warts (condylomata acuminata) or from benign cervical lesions. Viral DNAs from the "high-risk" types, by contrast, are identified in most cervical cancers, although the vast majority of lesions in which they are found are nonmalignant (reviewed in ref. 11). HPV16 and HPV18 are the types most frequently identified in cervical cancers. In

most areas, HPV16 is found in 40–60% of cervical tumors, with HPV18 being present in another 10–20% (12–15). Most of the remaining tumors contain DNA from other HPV types, such as HPV31, HPV33, and HPV45. About 10% of cervical cancers lack detectable HPV DNA.

A wealth of epidemiological and molecular data now provide important evidence for an etiologic relationship between HPV infection and cervical cancer. The molecular data followed the identification of HPV16 and HPV18 in the early 1980s (16, 17). Efforts to induce immortalization of human keratinocytes with HPV DNA revealed that the high-risk HPV types scored positive in this assay, while DNA from the low-risk types was negative (18–20). Genetic analysis indicated that efficient keratinocyte immortalization required the E6 and E7 genes from a high-risk HPV type (21–23). This result correlated with the observation that these are the two viral genes that are preferentially retained and expressed in cervical cancers and cell lines derived from these tumors (24, 25).

Both E6 and E7 encode multifunctional proteins. Among these functions, the E6 protein binds and degrades the p53 tumor suppressor protein (26, 27), while high-risk E7 protein binds the pRB tumor suppressor protein (28). These activities are greater for the proteins from high-risk HPVs than from low-risk HPVs (29–31). The apparent importance of these biochemical activities is underscored by finding that the *RB* and *p53* genes are mutationally inactivated in cervical cancer cell lines that lack HPV, while these genes are wild type in HPV-containing lines (32–34).

Much of the recent epidemiological data linking HPVs and cervical cancer comes from analysis of the relationship between HPV infection and the development of precursor lesions in the cervix. Depending upon the classification scheme, these precursor lesions may be defined as mild, moderate, or severe cervical intra-epithelial neoplasia (CIN 1, CIN 2, and CIN 3, respectively) or low-grade squamous intra-epithelial lesions (SIL), corresponding to CIN 1, and high-grade SIL, corresponding to CIN 2 and CIN 3 (35). It has been commonly thought that CIN 1, CIN 2, and CIN 3 represent a morphologic and biologic continuum. However, there is also support for the concept that CIN 1 and CIN 2–CIN 3 are distinct HPV-induced entities with only CIN 2–CIN 3 lesions being true progenitors of cervical cancer (36). Most of the cytologic abnormalities detected in Pap screening are the result of these HPV-induced lesions.

Some earlier epidemiological reports did not identify infection with high-risk HPV types as a major risk factor for cervical cancer precursor lesions. However, these studies appear to have been flawed by false-positive and/or false-negative viral DNA analysis (37). More recent evaluations, which utilize sensitive and specific polymerase chain reaction (PCR) assays that appear to have overcome earlier methodological

difficulties, demonstrate that genital infection with high-risk HPV types is a highly significant risk factor for the development of dysplastic cervical lesions (38, 39). These studies estimated that infection with high-risk HPV represents a relative risk of 30 or greater for the development of high-grade lesions.

The natural history of genital HPV infection has been incompletely analyzed. This is due in large part to the high frequency of subclinical infection and lack of a sensitive and specific serological assay for HPV infection. Molecular hybridization and PCR amplification of DNA from exfoliated cervico-vaginal cells has indicated that genital HPV infection is remarkably frequent. The highest prevalence of HPV is found among sexually active women <25 years old. Genital HPV is detected in `10–40% of these women. The majority of these infections appear to be self-limited and not to be associated with cytologic changes detectable by routine Pap screening. A minority of HPV-positive women do develop low-grade cytological changes (40–42). High-grade dysplasias, almost all of which are associated with high-risk HPV, are less common, while an even smaller number of women develop invasive cervical cancer. The highest incidence of high-grade lesions occurs in women who are >25 years old, while the highest incidence of cervical cancer occurs in women who are >35 (42). These findings suggest that high-risk HPVs normally produce a transient inapparent infection of the cervico-vaginal area. Persistent tissue infection develops in some women, perhaps as a result of defects in as yet poorly understood host defense mechanisms. Persistent lesions undergo progression to cervical cancer in a subset of these women.

Although the prevalence of HPV disease in men may be similar to that observed among females, the natural history of genital HPV infection in men is even less well understood. Many lesions are inapparent, fewer exfoliated cells are obtained from penile swabs making HPV DNA detection difficult, and biopsies are seldom obtained. Nevertheless, a strong correlation between genital HPV infection in men and CIN in their regular partners has been documented (43, 44). The incidence of penile cancer is much lower than that of cervical cancer and may not be as strongly associated with HPV infection. HPV DNA has generally been detected in 20–50% of cases (45–47). Anal cancer in both men and women, which is also relatively uncommon, is more strongly associated with HPV infection. In two recent studies using PCR-based detection, HPV DNA was found in `70% of cases (46, 48).

Patients with cellular immune deficiency have higher rates of HPV infection and tend to respond poorly to therapy (49). In this context, males and females infected with human immunodeficiency virus (HIV) appear to be at greater risk of developing persistent HPV infection, and

HIV-positive women with cervical HPV infection are more likely to have high-grade lesions (50).

MANAGEMENT OF GENITAL HPV LESIONS

The management of patients with genital HPV infections remains somewhat controversial (51). In contrast to bacterial sexually transmitted diseases, specific antimicrobial therapy for HPV infection is not yet available. Consequently, most treatment for HPV infection relies on nonspecific therapy such as laser, electrocautery, blistering agents, or agents that interfere with macromolecular synthesis (52). The approach to treatment depends, at least in part, on the goals. If the major purpose is to prevent cervical cancer, then perhaps treatment could be limited to high-grade lesions. This would seem especially true if future studies confirm the suspicion that low-grade lesions are not normally precursors of high-grade lesions. Studies are currently being conducted to determine if high-grade lesions missed by Pap screening and low-grade lesions with greater potential for progression can be effectively identified. At present, DNA typing for high-risk HPVs and cervicography, which produces high-resolution photographic images of the cervix, are the most promising ancillary tests (53). Even if these tests prove effective, some would argue it might be more cost effective to treat all cervical lesions (51).

If the goal is to prevent spread of genital infection, it might be appropriate to treat any genital HPV infection. Treatment would apply to men as well as women. Although it may seem reasonable that successful treatment of visible lesions should reduce the spread of HPV infection, there are no studies that document the efficacy of this approach. A study to evaluate the effectiveness of treating infected males found that it had no effect on recurrence rates of cervical dysplasia following treatment in their female partners (54). HPV infection may be multifocal, and some patients may have significant areas of subclinical infection. Furthermore, HPVs have been shown to establish latency in the larynx (55), and HPV DNA has been identified in normal genital epithelium (56). The high prevalence of inapparent HPV infection makes it unclear whether treatment of clinically apparent infection, in the absence of other measures, would have an impact on the rate of genital HPV infection.

PROSPECTS FOR VACCINATION

These management considerations highlight the potential utility offered by an effective vaccine against genital HPV infection. Genital

HPVs cannot be efficiently propagated. Even if they could, there might be theoretical objections to the use of an attenuated live or inactivated HPV for vaccination, since the HPV genomes contain transforming genes. Attention has therefore focused on the development of a subunit vaccine. Several lines of evidence suggest this may represent a feasible long-term goal (reviewed in ref. 57). Since E6 and E7 are selectively maintained during oncogenic progression, there is the possibility that peptides derived from these oncoproteins could serve as targets for cell-mediated immune responses to HPV-containing tumor cells. Some studies in animal models suggest that immunity against E7 (and also against L2) can induce tumor regression (58–60). However, the viral or cellular determinants that are recognized during regression of genital HPV-induced tumors in humans have not been identified. It is also unclear whether therapeutic vaccines will be able to induce an effective immune response in the persistently infected individuals (who appear to be most at risk of developing cancer), since persistence might indicate a constitutive inability to recognize critical viral determinants.

Perhaps the most encouraging experimental vaccine results come from studies of immunoprophylaxis induced by the major and minor structural viral proteins L1 and L2. Studies of the cottontail rabbit papillomavirus and bovine papillomavirus type 1 (BPV1) and type 2 (BPV2) have demonstrated that immunization with these proteins, singly or in combination, prevents experimental infection *in vivo* (60–62). These studies have used protein produced in bacteria or immunization with vaccinia vectors that express L1 and/or L2. Immunized animals were protected even when their sera demonstrated neutralizing titers of <100. The papillomaviruses in these two animal systems induce cutaneous lesions and are therefore imperfect models for cervical HPV infection. Protection from natural venereal transmission of a genital papillomavirus has not been demonstrated, but rhesus papillomavirus, which infects the genital tract of monkeys, might potentially serve as model to test this possibility.

In our laboratory, we have expressed preparative amounts of the structural viral proteins in insect cells through the use of baculovirus vectors (63). To assess the potential utility of a prophylactic vaccine based on baculovirus-derived virion proteins, we initially used BPV1, since infectious BPV1 can be readily obtained from bovine lesions and a quantitative *in vitro* BPV1 infectivity assay is available (64). Our BPV1 studies showed that expression of L1 alone in the insect cells was sufficient for self-assembly of virus-like particles that could readily be purified in preparative amounts (Figure 1). When rabbits were immunized with the L1 particles, they elicited antisera that could neutralize BPV1 infectivity at a dilution of 1:100,000. The neutralizing antibodies

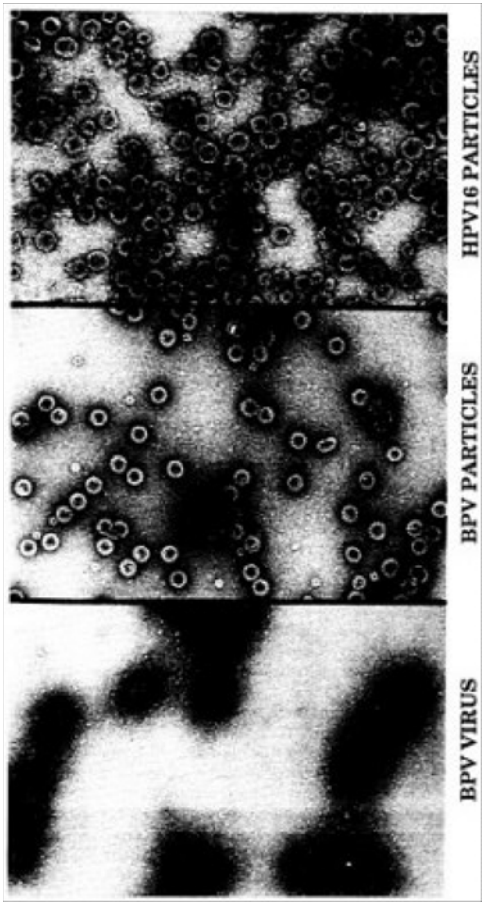

FIGURE 1 Purified BPV1 virus and BPV1 L1 and HPV16 L1 virus-like particles. The virus was purified from a bovine papilloma and the particles were purified from recombinant baculovirus-infected insect cells on preparative CsCl gradients, stained with uranyl acetate, and examined by transmission electron microscopy. (×32,400.)

were directed against conformational epitopes, in that denaturation of the particles prior to immunization abolished the ability of the preparation to induce neutralizing activity.

When efforts were made to extend this approach to the L1 of HPV16, only rare virus-like particles (three orders of magnitude less than with BPV) were detected when the source of L1 was from the widely used prototype strain of HPV16. Since reasonable self-assembly had also been observed for the low-risk HPV11 L1 (65), this observation suggested either that high-risk HPVs inefficiently self-assemble or that the L1 gene from the HPV16 prototype strain might contain one or more mutations that prevented efficient particle assembly. The latter possibility was given serious consideration, since the HPV16 genome from prototype strain had been molecularly cloned from a cervical cancer, which presumably did not make virus particles. If this speculation were correct, HPV16 L1 from a nonprogressed lesion, which presumably produced virus particles, might self-assemble with greater efficiency. We therefore tested, in collaboration with Matthias Dürst and Lutz Gissmann from the German Cancer Research Center in Heidelberg, the L1 genes from each of two HPV16 genomes that they had cloned from cases of benign condyloma accuminata. Both of these L1 genes were found to express proteins that self-assembled with an efficiency similar to that of BPV1 L1 (Figure 1) (66). Sequencing of the L1 genes from these two genomes revealed that both genes encoded aspartate at codon 202, while L1 from the prototype strain was histidine at this codon. This was the only coding difference between the prototype strain and one of the L1 genes whose protein self-assembled efficiently. These results indicate that the L1 from the HPV16 prototype strain is a mutant and that wild-type HPV16 L1 efficiently self-assembles.

When the L2s from BPV1 and HPV16 were expressed in the insect cells along with their corresponding L1s, they were found to be incorporated into the virus-like particles (66). The presence of L2 in the particles apparently increased the efficiency of particle formation, since HPV16 L1/L2 preparations contained about four times as many particles as those made with L1 only. However, the BPV L1/L2 particles did not induce higher levels of neutralizing activity than BPV L1 particles (unpublished data).

The availability of HPV16 virus-like particles has enabled us to develop, in collaboration with Cosette Wheeler and Thomas Becker from the New Mexico Tumor Registry, an ELISA that detects antivirion serum antibodies in the majority of women who test positive for genital HPV16 DNA. In an analysis of 122 women with known genital HPV DNA status, 63% (34/54) of patients who were positive for HPV16 DNA were positive in the ELISA, while 4% (2/33) of negative controls were positive

(67). A representative assay is depicted in Figure 2. Patients with a higher load of HPV16, as estimated from molecular hybridization, were more likely to be positive in the ELISA. The HPV16 ELISA does not appear to recognize antibodies in patients infected with low-risk HPV types 6 and 11. The reactivity of patients infected with low-risk HPV types 6 and 11 was similar to that of uninfected controls [9% (1/11)], which suggests that the HPV16 ELISA does not recognize antibodies directed against the virions of low-risk HPV types. Thirty-eight percent of patients who were DNA positive for high-risk HPV types 18 (5/13) and 31 (5/13) scored positive in the ELISA. The latter results, suggest either that the L1 from these types and HPV16 share some cross-reactive

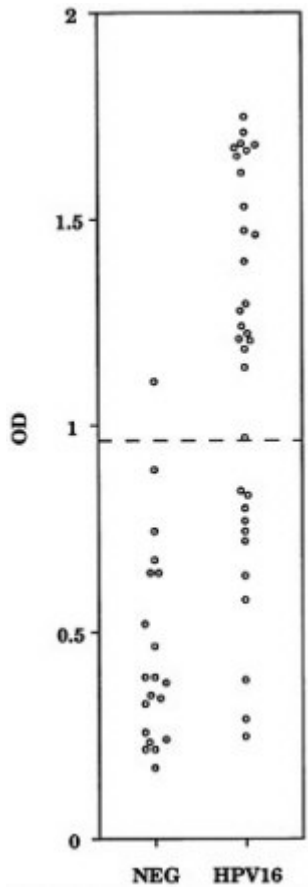

FIGURE 2 ELISA reactivity of women's sera to HPV16 L1 virus-like particles. NEG, women negative for HPV DNA; HPV16, women positive for HPV16 DNA. The dashed line indicates the mean plus two standard deviations of the values for the sera from the HPV DNA negative women.

conformational epitopes or that a subset of the women with HPV18 or HPV31 infection had been previously infected with HPV16 and remained seropositive.

The development of virus-like particle ELISAs for other HPV types should provide information on the serological relationship between the virions of different HPV types. By using particles from several HPV types, it should be possible to develop an ELISA that recognizes the majority of high-risk HPV infections. The clinical utility of this type of assay remains to be determined, especially since a substantial minority of women with HPV16 infection are negative in the assay. However, these assays should help to determine the extent of antivirion humoral immunity induced during natural genital HPV infections and if there are serological subtypes of HPV16 or other genital HPVs. This information will be important when the components for a multivalent virus-like particle vaccine to prevent genital HPV infection are considered. In addition, the ELISA will be useful to evaluate seroconversion in virus-like particle vaccine trials in animals and, perhaps ultimately, in humans.

SUMMARY

Genital human papillomavirus (HPV) infection is a common sexually transmitted disease that at the present time is not effectively controlled or treated. Many infections are inapparent and transient. However, some HPV infections result in persistent lesions that in some cases undergo carcinogenic progression. A subset of genital HPVs, designated high-risk types, are preferentially associated with high-grade dysplasias and carcinomas. About 90% of cervical cancers contain high-risk HPV DNA, most often HPV16. Development of a subunit vaccine against high-risk genital HPVs is a desirable and, it appears, an increasingly feasible long-term goal. The viral E6 and E7 oncoproteins are selectively maintained and expressed in progressed HPV tumors and could potentially be targets for therapeutic vaccines. The L1 major virion structural proteins have recently been shown to self-assemble into virus-like particles when expressed in insect cells. These particles might serve as the basis for a prophylactic vaccine to prevent genital HPV infection.

REFERENCES

1. Meisels, A. & Fortin, R. (1976) *Acta Cytol.* **20,** 505–509.
2. Brinton, L. A. (1992) in *The Epidemiology of Human Papillomavirus and Cervical Cancer*, eds. Munoz, N., Bosch, F. X., Shah, K. V. & Meheus, A. (Int. Agency of Res, on Cancer, Lyon, France), pp. 3–23.

3. Bauer, H. M., Greer, C. E., Chambers, J. C., Tashiro, C. J., Cimera, C. J., Reingold, J. & Manos, M. M. (1991) *J. Am. Med. Assoc.* **265,** 472–477.
4. States, C. A.-U. (1983) *Morbid. Mortal. Wkly. Rep.* **32,** 306.
5. Chuang, T. Y., Perry, H. O., Kurland, L. T. & Ilstrup, D. M. (1984) *Arch. Dermatol.* **120,** 469–483.
6. Parkin, D. M., Stjernsward, J. & Muir, C. S. (1984) *Bull. W.H.O.* **62,** 163–182.
7. Koss, L. G. (1989) *J. Am. Med. Assoc.* **261,** 737–743.
8. de Villiers, E.-M. (1989) *J. Virol.* **63,** 4898–4903.
9. Orth, G. (1987) in *The Papovaviridae: The Papillomaviruses*, eds. Salzman, N. P. & Howley, P. M. (Plenum, New York), Vol. 2, pp. 199–243.
10. Werness, B. A., Munger, K. & Howley, P. M. (1991) in *Important Advances in Oncology 1991*, eds. DeVita, V. T., Hellman, S. & Rosenberg, S. A. (Lippincott, Philadelphia), pp. 3–18.
11. zur Hausen, H. & Schneider, A. (1987) in *The Papovaviridae: The Papillomaviruses*, eds. Salzman, N. P. & Howley, P. M. (Plenum, New York), Vol. 2, pp. 245–263.
12. Van den Brule, A. J., Walboomers, J. M. M., du Maine, M., Kenemans, P. & Meijer, C. J. L. M. (1991) *Int. J. Cancer* **48,** 404–408.
13. Riou, G., Favre, M., Jeannel, D., Bourhis, J., Le Doussal, V. & Orth, G. (1990) *Lancet* **335,** 1171–1174.
14. Lorincz, A. T., Reid, R., Jenson, A. B., Greenberg, M. D., Lancaster, W. & Kurman, R. J. (1992) *Obstet. Gynecol.* **79,** 328–337.
15. Higgins, G. D., Davy, M., Roder, D., Uzelin, D. M., Phillips, G. E. & Burrell, C. J. (1991) *Lancet* **338,** 910–913.
16. Boshart, M., Gissmann, L., Ikenberg, H., Kleinheinz, A., Scheurlen, W. & zur Hausen, H. (1984) *EMBO J.* **3,** 1153–1157.
17. Dürst, M., Gissmann, L., Ikenberg, H. & zur Hausen, H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3812–3815.
18. Woodworth, C. D., Doniger, J. & DiPaolo, J. A. (1989) *J. Virol.* **63,** 159–164.
19. Schlegel, R., Phelps, W. C., Zhang, Y. L. & Barbosa, M. (1988) *EMBO J.* **7,** 3181–3187.
20. Pecoraro, G., Morgan, D. & Defendi, V. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 563–567.
21. Hawley-Nelson, P., Vousden, K. H., Hubbert, N. L., Lowy, D. R. & Schiller, J. T. (1989) *EMBO J.* **8,** 3905–3910.
22. Hudson, J. B., Bedell, M. A., McCance, D. J. & Laiminis, L. A. (1990) *J. Virol.* **64,** 519–526.
23. Munger, K., Phelps, W. C., Bubb, V., Howley, P. M. & Schlegel, R. (1989) *J. Virol.* **63,** 4417–4421.
24. Schneider-Gadicke, A. & Schwarz, E. (1986) *EMBO J.* **5,** 2285–2292.
25. Smotkin, D. & Wettstein, F. O. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4680–4684.
26. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J. & Howley, P. M. (1990) *Cell* **63,** 1129–1136.
27. Werness, B. A., Levine, A. J. & Howley, P. M. (1990) *Science* **248,** 76–79.
28. Dyson, N., Howley, P. M., Munger, K. & Harlow, E. (1989) *Science* **243,** 934–937.
29. Barbosa, M. S., Edmonds, C., Fisher, C., Schiller, J. T., Lowy, D. R. & Vousden, K. H. (1990) *EMBO J.* **9,** 153–160.
30. Crook, T., Tidy, J. A. & Vousden, K. H. (1991) *Cell* **67,** 547–556.

31. Munger, K., Yee, C. L., Phelps, W. C., Pietenpol, J. A., Moses, H. & Howley, P. M. (1991) *J. Virol.* **65,** 3943–3948.
32. Wrede, D., Tidy, J. A., Crook, T., Lane, D. & Vousden, K. H. (1991) *Mol. Carcinog.* **4,** 171–175.
33. Scheffner, M., Munger, K., Byrne, J. C. & Howley, P. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5523–5527.
34. Crook, T., Wrede, D. & Vousden, K. H. (1991) *Oncogene* **6,** 873–875.
35. Workshop, N. C. I. (1989) *J. Am. Med. Assoc.* **262,** 931–934.
36. Kiviat, N. B. & Koutsky, L. A. (1993) *J. Natl. Cancer Inst.* **85,** 934–935.
37. Franco, E. L. (1991) *Epidemiology* **2,** 98–106.
38. Schiffman, M., Bauer, H. M., Hoover, R. N., Glass, G. A., Cadell, D. M., Rush, B. B., Scott, D. R., Sherman, M. E., Kurman, R. J., Wacholder, S., Stanton, C. K. & Manos, M. M. (1993) *J. Natl. Cancer Inst.* **85,** 958–964.
39. Koutsky, L. A., Holmes, K. K., Critchlow, C. W., Stevens, C. E., Paavonen, J., Beckmann, A. M., ReRouen, T. A., Galloway, D. A., Vernon, D. & Kiviat, N. B. (1992) *N. Engl. J. Med.* **327,** 1272–1278.
40. Becker, T. M., Wheeler, C. M., McGough, N. S., Jordan, S. W., Dorin, M. & Miller, J. (1991) *Am. J. Public Health* **81,** 582–587.
41. de Villiers, E. M., Wagner, D., Schneider, A., Wesch, H., Munz, F., Miklaw, H. & zur Hausen, H. (1992) *Gynecol. Oncol.* **44,** 33–39.
42. Schiffman, M. H. (1992) *J. Natl. Cancer Inst.* **84,** 394–398.
43. Barrasso, R., de Brux, J., Croissant, O. & Orth, G. (1987) *N. Engl. J. Med.* **317,** 916–923.
44. Schneider, A., Sawada, E., Gissmann, L. & Shah, K. (1987) *Obstet. Gynecol.* **69,** 554–562.
45. McCance, D. J., Kalache, A. & Ashdown, K. (1986) *Int. J. Cancer* **37,** 55–59.
46. Daling, J. R., Sherman, K. J., Hislop, T. G., Maden, C., Mandelson, M. T., Beckman, A. M. & Weiss, N. S. (1992) *Am. J. Epidemiol.* **135,** 180–189.
47. Maden, C., Sherman, K. J., Beckman, A. M., Hislop, T. G., Teh, C. Z., Ashley, R. L. & Daling, J. R. (1993) *J. Natl. Cancer Inst.* **85,** 19–24.
48. Crook, T., Wrede, D., Tidy, J., Sholefield, J., Crawford, L. & Vousden, K. H. (1991) *Oncogene* **6,** 1251–1257.
49. Morison, W. L. (1975) *Br. J. Dermatol.* **92,** 625–630.
50. Judson, F. N. (1992) in *The Epidemiology of Human Papillomavirus and Cervical Cancer*, eds. Munoz, N., Bosch, F. X., Shah, K. V. & Meheus, A. (Int. Agency of Res. on Cancer, Lyon, France), pp. 199–207.
51. Richart, R. M. & Wright, T. C. (1993) *Cancer* **71,** 1413–1421.
52. Beutner, K. R. (1989) *J. Am. Acad. Dermatol.* **20,** 114–123.
53. Solomon, D. (1993) *J. Natl. Cancer Inst.* **85,** 1018–1019.
54. Krebs, H. B. & Helmkamp, B. F. (1990) *Obstet. Gynecol.* **76,** 660–663.
55. Steinberg, B. M., Topp, W. C., Schneider, P. S. & Abramson, A. L. (1983) *N. Engl. J. Med.* **308,** 1261–1264.
56. Ferenczy, A., Mitao, M., Nagai, N., Silverstein, S. J. & Crum, C. P. (1985) *N. Engl. J. Med.* **313,** 784–788.
57. Cason, J., Khan, S. A. & Best, J. M. (1993) *Vaccine* **11,** 603–611.
58. Chen, L., Thomas, E. K., Hu, S. L., Hellstrom, I. & Hellstrom, K. E. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 110–114.
59. Meneguzzi, G., Cerni, C., Kieny, M. P. & Lathes, R. (1991) *Virology* **181,** 62–69.
60. Jarrett, W. F., Smith, K. T., O'Neil, B. W., Gaukroger, J. M., Chandrachud,

L. M., Grindlay, G. J., McGarvie, G. M. & Campo, M. S. (1991) *Virology* **184,** 33–42.
61. Pilacinski, W. P., Glassman, D. L., Glassman, K. F., Reed, D. E., Lum, M. A., Marshall, R. F., Muscoplat, C. C. & Faras, A. J. (1986) in *Papillomaviruses: Ciba Foundation Symposium 120* (Wiley, Chichester, England), pp. 136–156.
62. Lin, Y.-L., Borenstein, L. A., Selvakumar, R., Ahmed, R. & Wettstein, F. O. (1992) *Virology* **187,** 612–619.
63. Kirnbauer, R., Booy, F., Cheng, N., Lowy, D. R. & Schiller, J. T. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12180–12184.
64. Dvoretzky, I., Shober, R., Chattopadhyay, S. K. & Lowy, D. R. (1980) *Virology* **103,** 369–375.
65. Rose, R. C., Bonnez, W., Reichman, R. C. & Garcea, R. L. (1993) *J. Virol.* **67,** 1936–1944.
66. Kirnbauer, R., Taub, J., Greenstone, H., Roden, R., Dürst, M., Gissmann, L., Lowy, D. R. & Schiller, J. T. (1993) *J. Virol.* **67,** 6929–6936.
67. Kirnbauer, R., Hubbert, N. L., Wheeler, C. M., Becker, T. M., Lowy, D. R. & Schiller, J. T., (1994) *J. Natl. Cancer Inst.*, **86,** 494–499.

Herpes Simplex Virus Infections of Women and Their Offspring: Implications for a Developed Society

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While herpes simplex virus (HSV) infections of humans have been recognized since ancient times (1, 2), it was not until the 18th century that Astruc, physician to the King of France, identified herpes as a cause of genital infection (3). Subsequently, in 1893, Vidal reported human-to-human transmission of HSV infections, identifying the necessity of intimate human contact for spread of infection (2). Studies of the host immune response to HSV during the early 20th century provided insight into a unique property of HSV infection—namely, that neutralizing antibodies were identified in the sera of adults who subsequently developed recurrences, a phenomenon known as reactivation of latent infection (4). Neonatal HSV infection was not described until the 1940s (5, 6); however, the association between newborn disease and genital HSV infection was not made until the late 1960s (1).

With the evolution of our society in developed countries, particularly increasing sexual freedom associated with advances in birth control, and the emergence of sexually transmitted diseases, both horizontal (sexual partners) and vertical (mother to baby) transmission of HSV infection has become prevalent. Today, genital HSV infection exists in over 60 million Americans, most of child-bearing age, and results in the majority of the 1600 cases of neonatal herpes that occur yearly in the United

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States. This article will review the changing epidemiology of genital and neonatal HSV infections with emphasis on the current status of therapy of the newborn, the cost of disease to society, and the need for the development of appropriate preventive strategies. Since the infected newborn is most likely to develop life-threatening disease and, therefore, incur the greatest costs to society, the baby becomes the starting point for our considerations.

INCIDENCE OF NEWBORN INFECTION

Although centers in the United States caring for infants with neonatal HSV infections have observed fluctuations in disease incidence, the estimated rate of occurrence is approximately 1 in 2500 to 1 in 5000 deliveries yearly (7–9). For unknown reasons, several countries worldwide, such as Africa and the United Kingdom, do not appear to recognize a significant number of cases of neonatal HSV infection in spite of the high prevalence of antibodies to HSV-2 (10, 11). In fact, the incidence of neonatal HSV infections in the United States may reflect the decreasing prevalence of HSV-1 antibodies and, therefore, the absence of transplacental humoral immunity, which might confer protection to the fetus.

PATHOGENESIS OF NEONATAL HSV INFECTIONS

At least four factors influence the incidence of newborn HSV disease. The first is the type of maternal genital infection at the time of delivery. The duration and quantity of viral excretion and the time to total healing vary with primary, initial, and recurrent maternal genital infections, such that primary, initial, and recurrent maternal genital infections, such that primary is most and recurrent is least severe (12, 13). Primary infection is associated with the excretion of 10^6–10^8 plaque-forming units of HSV for as long as 14–21 days. In contrast, recurrent infection is associated with a shorter duration of viral excretion (namely, 3–5 days) and at lower quantities (about 10^2 plaque-forming units of HSV). The incidence of neonatal herpes in babies born to women with primary or initial genital HSV infection is higher (33%) than those with recurrent infection (3%) (14). Second, the mother's HSV antibody status at delivery influences the severity of maternal infection as well as the likelihood of transmission. Transplacental maternal neutralizing and antibody-dependent cell-mediated cytotoxic (ADCC) antibodies have at least an ameliorative effect on acquisition and severity of infection for babies exposed to virus (15–18). Maternal primary infection late in gestation usually does not result in significant passage of maternal transplacental antibodies and, therefore, will increase risk to the fetus

(19). Third, the duration of ruptured membranes is an important indicator of risk for acquisition of neonatal HSV infection. Prolonged rupture of membranes (>6 hr) appears to increase the risk of fetal acquisition of infection, probably as a consequence of ascending infection from the cervix (7). Fourth, the application of fetal scalp monitors in the labor and delivery suite increases the risk of neonatal HSV infection by providing a site of inoculation of virus (20, 21). It should be remembered that between 20% and 60% of women of child-bearing age are HSV-2 seropositive, as discussed below. Thus, the probability of reactivation of latent virus, for the delivery population as a whole, increases and then provides a source of virus for scalp-electrode infection.

HSV infection of the newborn can be acquired at one of three times: *in utero*, intrapartum, or postpartum. The mother is the usual source of infection. Intrauterine infection occurs in `5% of all babies with neonatal HSV infection. These children usually have evidence of skin scarring/lesions at birth, chorioretinitis, and/or hydranencephaly. Intrapartum transmission accounts for about 85% of all cases and results from direct contact of the fetus with infected maternal genital secretions at delivery. Postnatal acquisition accounts for `;10% of cases and is the consequence of contact of the baby with an environmental source of HSV, usually a family member or care giver (22–31). Data from the National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Antiviral Study Group (CASG) indicate that the frequency of babies with neonatal HSV-1 infections is nearly 30% (16). Since HSV-1 accounts for only `15% of all genital HSV infections in the United States, concern for postnatal acquisition should be high. However, family are as likely, if not more so, to be the source of newborn infection as nurses or hospital aides on the newborn or obstetrical services.

Since the mother is the source of infection in a majority of cases, an understanding of the changing epidemiology of genital HSV infection in women of child-bearing age is essential.

EPIDEMIOLOGY OF MATERNAL HSV INFECTIONS

Type-specific reagents that allow for the unequivocal distinction between HSV-1 and HSV-2 infections have provided the opportunity to define the changing seroprevalence of HSV-2 infections worldwide. The appearance of type-specific HSV-2 antibodies positively correlates with the onset of sexual activity (32–34), although crowded living conditions may contribute to infection (35, 36). The seroprevalence of HSV-2 in healthy women ranges from 10% to 60% in Americans to 77% in Ugandans (37). As many as 50–60% of lower socioeconomic women in the United States and elsewhere develop antibodies to HSV-2 by early

adulthood (38). Antibodies to HSV-2 are virtually nonexistent in nuns (32, 39, 40). Sera collected from pregnant women in the mid-1980s from Padua, Italy, and Orebro and Stockholm, Sweden, defined seroprevalence rates varying between 8% and 28%. Over the ensuing decade, these rates have increased 2-fold. Type-specific antibodies have been found in `35% of middle class women and 10–20% of women in higher socioeconomic groups in the United States (41–45).

Overall in the United States, HSV-2 seroprevalence increases from 6.9% at 15–29 years of age to 23.4% by the age of 60. When populations are analyzed according to race, these prevalence rates become 4.6% and 19.7% for Caucasians and 21.8% and 64.7% for Blacks, respectively (46). Factors found to influence acquisition of HSV type 2 include sex (women greater than men), race (people of color more than Caucasians), martial status (divorced versus single or married), and place of residence (city greater than suburban) (47). Clearly, seropositive pregnant women have the capability of reactivating HSV-2 at the time of delivery and, therefore, transmitting infection to their child.

The most important factor that influences acquisition of infection obviously is intimate exposure to an infected individual. Thus, rates of infection are influenced by the number of sexual partners (48–50). For heterosexual women living in the United States, the probability of acquisition of HSV-2 for those having one partner was <10%. The probability increased to 40%, 62%, and >80% if the number of total lifetime sexual partners increased to 2–10, 11–50, or >50, respectively. For heterosexual men, similar data were 0% for one lifetime sexual partner and 20%, 35%, and 70% for each of the subsequent three risk groups, respectively (47). With discordant antibody status between sexual partners, a susceptible female may become infected by an infected partner, creating risk if the woman is pregnant, especially if at term (51).

From the above data, it is apparent that the incidence of HSV-2 infection is a function of exposure to infected individuals; therefore, those with the largest number of sexual partners are most likely to acquire infection within any time frame. In one prospective study of low-risk individuals—namely, college students in Columbia, South Carolina, incidence was `2% per year over 4 years (22). The rate of acquisition of HSV-2 infection during pregnancy was 0.2% in Northern California and 2.5% in Birmingham, Alabama.

MATERNAL GENITAL HERPES SIMPLEX INFECTIONS: CLINICAL AND VIROLOGIC PARAMETERS

From the seroprevalence data, genital HSV infection in the woman—pregnant or otherwise—is common. A serious, but uncommon, problem

encountered with HSV infections during pregnancy is that of widely disseminated disease whereby infection can involve multiple visceral sites, in addition to cutaneous dissemination (52–57). Before the availability of acyclovir therapy, the mortality among these pregnant women is reported to be >50%. Fetal deaths occurred in >50% of cases, although mortality did not correlate with the death of the mother.

Maternal primary infection prior to 20 weeks gestation has been associated with spontaneous abortion but not at a high incidence (45). Primary infection during gestation also has been associated with fetal disease *in utero* (58, 59). Infection that occurs later in gestation has not been associated with the termination of pregnancy (60–62), but fetal morbidity has been documented, as evidenced primarily by intrauterine growth retardation (63).

Localized genital HSV infection is the most common form of infection during pregnancy. Prospective investigations indicate that genital HSV infection occurs at a frequency of about 1% at any time during gestation, as reviewed (7, 64, 65). Most of these infections have been considered recurrent. The frequency of HSV recurrences during gestation should be of concern to women with known histories of infection. Transmission of infection to the fetus is most frequently related to shedding of virus at the time of delivery. Since HSV infection of the fetus is usually the consequence of contact with infected maternal genital secretions at the time of delivery, the determination of viral excretion at this time is of importance. The actual incidence of viral excretion at delivery has been suggested to be 0.01–0.39% for all women, irrespective of past HSV history (7, 44, 66–68). For women with a past history of genital herpes, in a predominantly white, middle-class population, documented recurrent infection occurred in 84% of pregnant women (68). Moreover, asymptomatic viral shedding occurred in at least 12% of the recurrent episodes. Viral shedding from the cervix occurred in 0.56% of symptomatic infections versus 0.66% of asymptomatic infections (44, 66, 69). The incidence of cervical shedding in pregnant women with asymptomatic HSV infection has been reported to average ` 3.0% (70). The observed rate of shedding among pregnant women with asymptomatic infection has varied more than that among nonpregnant women (from 0.2% to 7.4%), depending upon the study population and trial design (44, 62, 66, 71, 72). The frequency of recurrences has not been shown to be different from one pregnancy to the next for any given woman (72). Overall, these data indicate that the frequency of cervical shedding is low, rendering the risk of transmission of virus to the infant similarly low when the infection is recurrent in nature (7). The frequency of shedding does not appear to vary by trimester during gestation (68, 71). Given the high seroprevalence of maternal infection, protection for the

fetus must exist or the incidence of neonatal disease would be significantly higher.

Importantly, 70% of infants who develop neonatal disease are born to women who are completely asymptomatic for genital HSV infections at the time of delivery and have neither a past history of genital herpes nor a sexual partner reporting a genital vesicular rash (16). Only 20% of these mothers reported genital HSV infection in their sexual partners.

CLINICAL PRESENTATION

The clinical presentation of babies with neonatal HSV infection is a direct reflection of the site and extent of viral replication. The significance of clinical presentation in the context of developing and developed societies is of utmost relevance in that it teaches the biomedical investigator of useful approaches to decision amelioration. Neonatal HSV infection is almost invariably symptomatic and frequently lethal. Infected babies are divided into three categories—namely, those with (*i*) disease localized to the skin, eye and/or mouth; (*ii*) encephalitis with or without skin, eye and/or mouth involvement; and (*iii*) disseminated infection, which involves multiple organs, including central nervous system, lung, liver, adrenals, skin, eye, and/or mouth (7, 73). Table 1 summarizes disease classification of 291 babies with neonatal HSV infections studied by the NIAID CASG.

Disseminated Infection

Babies with the worst prognosis for both mortality and morbidity are those with disseminated infection. Disseminated disease involves multiple organs, especially the lung, liver, adrenal glands, and brain. Encephalitis appears to be a common component of this form of infection, occurring in about 60–75% of children with disseminated infection. Mortality in the absence of therapy exceeds 80%; all but a few survivors are impaired. These babies appear *not* to receive transplacental antibodies.

Encephalitis

Nearly one-third of all babies with neonatal HSV infection have encephalitis only. Babies with disseminated infection probably seed the brain by a blood-borne route, resulting in multiple areas of cortical hemorrhagic necrosis. In contrast, babies who present with only encephalitis likely have axonal transmission of virus to the central nervous system. Death occurs in 50% of babies with localized central nervous

system disease who are not treated and is usually related to brain stem involvement. With rare exceptions, survivors are left with neurologic impairment (74, 75).

TABLE 1 Demographic and clinical characteristics of infants enrolled in NIAID collaborative antiviral study

	Disease classification		
Characteristic	Disseminated	CNS	Skin, eyes, or mouth
No. of babies	93 (32)	96 (33)	102 (35)
No. of male/no. female	54/39	50/46	51/51
No. Caucasian/no. other	60/33	73/23	76/26
No. premature (<36 wk)	33 (35)	20 (21)	24 (24)
Gestational age, wk	36.5 ± 0.4	37.9 ± 0.4	37.8 ± 0.3
Enrollment age, wk	11.6 ± 0.7	17.4 ± 0.8	12.1 ± 1.1
Maternal age, yr	21.7 ± 0.5	23.1 ± 0.5	22.8 ± 0.5
Clinical findings			
Skin lesions	72 (77)	60 (63)	86 (84)
Brain involvement	69 (74)	96 (100)	0 (0)
Pneumonia	46 (49)	4 (4)	3 (3)
Mortality at 1 yr*	56 (60)	13 (14)	0 (0)
Neurologic impairment of survivors (no. affected/total no.)			
Total	15/34* (44)	45/81† (56)	10/93† (11)
Adenine arabinoside	13/25† (50)	25/51† (49)	3/34† (9)
Acyclovir	1/6† (17)	18/27† (67)	4/51† (8)
Placebo	1/2† (50)	2/3† (67)	3/8† (38)

CNS, central nervous system. Numbers in parentheses are percentages.
* Regardless of therapy.
† Denominators vary according to number with follow-up available.

Skin, Eye, and/or Mouth Infection

Infection localized to the skin, eyes and/or mouth is associated with no mortality, but it is associated with morbidity. These babies tend to receive large quantities of transplacental neutralizing and ADCC antibodies. Approximately 30% of these children eventually develop evidence of neurologic impairment (73–77). The significant neurologic findings include spastic quadriplegia, microcephaly, and blindness. Important questions regarding the pathogenesis of delayed onset neurologic debility are raised by such clinical observations. Despite normal clinical examinations in early infancy, neurologic impairment has become apparent between six months and 1 year of life. The clinical presentation is similar to that associated with congenital toxoplasmosis or syphilis.

TREATMENT OF NEONATAL HSV INFECTION

While both vidarabine and acyclovir are efficacious therapies for neonatal HSV infection, acyclovir is the treatment of choice in spite of not being licensed for this disease because of established safety for other indications (77). Acyclovir is an acyclic analog of guanosine. Virusspecified thymidine kinase phosphorylates acyclovir to its monophosphate derivative, an event that does not occur to any significant extent in uninfected cells. Acyclovir is then phosphorylated by cellular enzymes to its triphosphate derivative. Acyclovir triphosphate binds viral DNA polymerase, acting as a DNA chain terminator (78, 79). At levels 30 times higher than those used clinically, acyclovir can be teratogenic in the *in vitro* limb-bud assay, but other animal studies indicate that acyclovir is not a significant teratogen (80). Acyclovir is not a significant mutagen in the Ames test but induces chromosomal mutagenic events in a manner similar to that of caffeine (80). Because of the occasional need for acyclovir therapy during pregnancy, as well as the likelihood of frequent first trimester exposures to drug before pregnancy is recognized, an "Acyclovir in Pregnancy Registry" is established to gather data on all reported prenatal exposures to oral acyclovir. Though no significant risk to the mother or fetus has been documented, the total number of monitored pregnancies remains too small to detect any epidemiologic risk that is not overwhelming (81). The safety of acyclovir in pregnancy, therefore, has not been unequivocally established. Since acyclovir crosses the placenta and can concentrate in amniotic fluid, there is valid concern about the potential for renal toxicity in the fetus (82). Limited data suggest the safety of acyclovir administration near term for mother and fetus (83).

The efficacy of vidarabine therapy (15 mg per kg per day over 12 hr as a continuous infusion for 10–14 days) rests on the demonstration of a decrease in mortality from 75% to 40% in infants with either disseminated or isolated central nervous system disease, and `50% of survivors developed normally (76). Furthermore, therapy decreased progression of disease from localized skin, eye, and mouth involvement to either encephalitis or disseminated disease from 70% in placebo recipients to 32% in vidarabine-treated babies. A subsequent clinical trial at 30 mg per kg per day for 10–14 days decreased progression to 4%. Although there were no deaths among infants with skin, eye, and mouth infection, severe neurologic impairment was decreased from 30% to 10% with vidarabine therapy (74).

Subsequent clinical trials have compared vidarabine to acyclovir for neonatal HSV infections. The NIAID CASG compared outcome for 202 babies with neonatal HSV infection who were randomly treated with

either acyclovir or vidarabine (84). Mortality and morbidity data are summarized in Figure 1 and Table 2, respectively. Notably, there is no difference in mortality between treatment groups. Overall, the mortality was 0%, 18%, and `;55% for babies with skin, eye, or mouth disease, encephalitis, or disseminated infection, respectively. For babies with skin, eye, and mouth infection, there were no deaths; 90% and 98% of vidarabine and acyclovir recipients, respectively, were developing normally at 2 years of age. For babies surviving encephalitis, 50% of acyclovir recipients and 43% of vidarabine recipients were developing normally. For survivors of disseminated infection, 62% and 57% of vidarabine and acyclovir recipients, respectively, were normal at a 24-month follow-up. Thus, no significant differences exist between acyclovir and vidarabine therapy for any form of neonatal HSV infection.

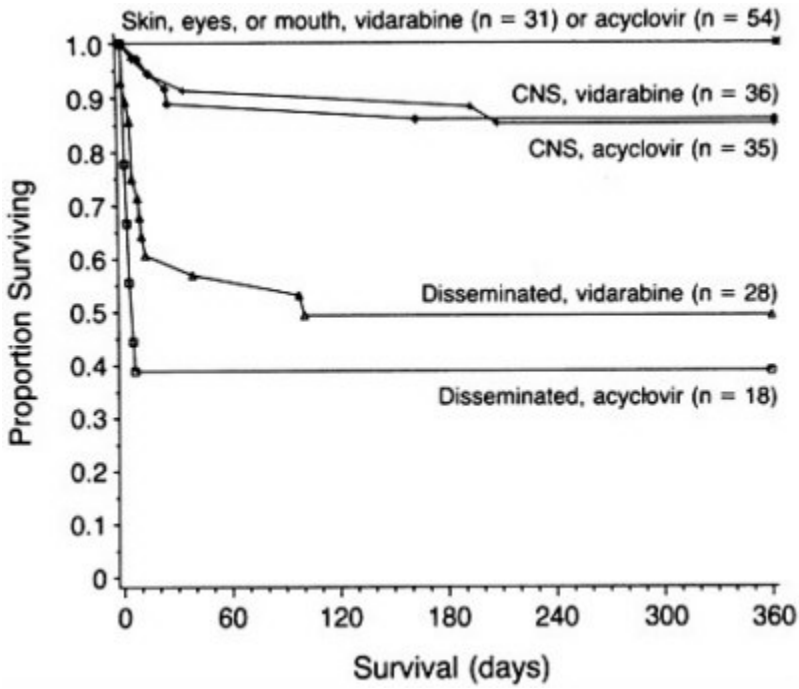

FIGURE 1 Survival of babies with neonatal HSV infection, according to treatment and the extent of disease. CNS, central nervous system. [Reproduced with permission from ref. 84 (copyright New England Journal of Medicine).]

Models of relative risk predicting patients at greatest likelihood for death or severe neurologic sequelae have been applied to the data, as

indicated in Table 3. From these data, if disease can be limited to the skin, eye, or mouth, clinical outcome is far superior to any other form of disease. It appears as though the quantity of transplacental maternal antibodies correlates positively to disease that remains limited to the skin, eye, and mouth. A confounding variable, however, is the recognition of ADCC antibodies on the sera of babies who present with encephalitis (18).

TABLE 2 Assessment of morbidity after 12 months in infants with neonatal HSV infection treated with vidarabine or acyclovir

Extent of disease	Morbidity after 12 mo					Alive after 12 mo, morbidity unknown	Dead within 12 mo	Total
	Normal	Mild	Moderate	Severe	Sub-total			
Skin, eye, or mouth infection								
Vidarabine	22	1	1	1	25	6	0	31
Acyclovir	45	0	1	0	46	8	0	54
Central nervous system infection								
Vidarabine	13	1	5	11	30	1	5	36
Acyclovir	8	5	6	9	28	2	5	35
Disseminated disease								
Vidarabine	7	1	0	4	12	2	14	28
Acyclovir	3	1	0	1	5	2	11	18
Total	98	9	13	26	146	21	35	202

The values given are the numbers of infants. [Modified and reprinted with permission from ref. 84 (copyright New England Journal of Medicine).]

To improve outcome, it will be necessary to develop strategies that prevent the development of encephalitis or disseminated disease and institute therapy before coma ensues. Furthermore, neurologic impairment of babies with disease localized to the skin, eyes, and mouth emphasizes the need to further investigate pathogenic mechanisms and treatment options.

COST-BENEFIT ANALYSES OF ANTIVIRAL THERAPY

The deployment of acyclovir in the treatment of neonatal HSV infections and recognition of enhanced survival but with persistent morbidity prompted a cost-benefit analysis of the value of antiviral therapy from a societal perspective. Defining costs as documented for

TABLE 3 Prognostic factors identified by multivariate analyses for neonates with HSV infection

Dominant factors	Relative risk	
	Mortality	Morbidity
Total group (n = 202)		
Extent of disease		
Skin, eyes, or mouth	1	1
CNS	5.8*	4.4*
Disseminated	33*	2.1*
Level of consciousness		
Alert or lethargic	1	NS
Semicomatose or comatose	5.2*	NS
Disseminated intravascular coagulopathy	3.8*	NS
Prematurity	3.7*	NS
Virus type		
1	2.3†	1
2	1	4.9*
Seizures	NS	3.0*
Infants with disseminated disease (n = 46)		
Disseminated intravascular coagulopathy	3.5*	NS
Level of consciousness		
Alert or lethargic	1	1
Semicomatose or comatose	3.9*	4.0*
Pneumonia	3.6*	NS
Infants with CNS involvement (n = 71)		
Level of consciousness		
Alert or lethargic	1	NS
Semicomatose or comatose	6.1*	NS
Prematurity	5.2*	NS
Seizures	NS	3.4*
Infants with infection of the skin, eyes, or mouth (n = 85)		
No. of skin-vesicle recurrences		
<3	NA	1
≥3	NA	21*
Virus type		
1	NA	1
2	NA	14*‡

CNS, central nervous system; NS, not statistically significant ($P > 0.05$); NA, not applicable (no baby with disease confined to the skin, eyes, or mouth died). [Modified and reprinted with permission from ref. 92 (copyright New England Journal of Medicine).]

*$P < 0.01$.

†$P < 0.05$.

‡Because of the correlation between virus type and skin-vesicle recurrence, virus type was not significant in the multivariate model; however, it was significant as a single factor.

the care of children at the University of Alabama at Birmingham and assuming an annual incidence of disease of 1600 cases per year, acyclovir treatment had no significant effect on the direct costs of health care for babies with disease, as would be anticipated. However, indirect costs, as associated with the long-term care of the child with neonatal HSV infection, are significantly decreased. The overall reduction of costs to society was from $250,000,000 per year to approximately $215,000,000 (85). In addition to providing an objective assessment of the impact of antiviral therapy on this disease, the model provides an approach to defining the potential value of improvement for subsequent clinical trials, particularly those involving therapeutic interventions.

PREVENTION OF NEONATAL HSV INFECTION

With the increased awareness of serious neonatal HSV infection occurring as a consequence of maternal genital herpes, methods of prevention have attracted attention. An unnecessarily high frequency of Cesarean sections occurs in individuals with a history of recurrent genital herpes. In large part, this increased frequency of Cesarean section is related to maternal request as well as litigious concerns. Several questions remain to be resolved regarding the value of cesarean section in the prevention of neonatal HSV infections. While surgical delivery has been associated with decreased transmission of infection when membranes are ruptured <4 hr, Cesarean section has not been proven efficacious when membranes are ruptured for longer periods of time. Nevertheless, it has been recommended that when membranes are ruptured for up to 24 hr, there is still a time frame within which Cesarean section is of value. While these recommendations seem logical, no data exist from adequately performed clinical investigations to support the recommendation.

Antiviral Prophylaxis

Some investigators have suggested that acyclovir may be useful in preventing the occurrence of neonatal HSV infections in infants who are delivered unknowingly through an infected birth canal. The prophylactic use of acyclovir has also been suggested in pregnant women who have a known history of recurrent genital lesions. No data exist that establish the value of prophylactic antiviral therapy for the newborn. Such recommendations will be of importance in considering the option of vaginal delivery for the women excreting virus at the time of onset of labor.

Suppressive therapy of genital herpes in women with a known history

of recurrent infection may pose significant but undefined risk to the fetus. Suppressive therapy in women with recurrent genital herpes may not prove efficacious. It already has been established from the trials of suppressive acyclovir administered to individuals with frequently recurrent genital herpes that reactivation of virus can occur in spite of the administration of 200 mg of acyclovir three times daily (86). It is not unreasonable to think that virus shedding could occur in women taking acyclovir for suppressive therapy for recurrent genital HSV infection during the last 4 weeks of gestation. Furthermore, the pharmacokinetics and metabolism of acyclovir in the human fetus are totally unknown, although a small study of acyclovir's tolerance and pharmacokinetics at term appears reassuring (83, 87). The possibility of acyclovir fetal nephrotoxicity introduces a potential risk of drug administration, which must be considered. In addition, it must be recognized that the women who are at greatest risk for delivering babies who develop neonatal HSV infection are those least likely to have a history of recurrent genital HSV infection. Thus, those at greatest risk remain to be identified. Perhaps, the detection of type-specific antibodies to glycoprotein (g) G-2 will be of value in identifying those women at greatest risk.

Immunoprophylaxis

Over the past several years, attention has been directed to two arenas of immunoprophylaxis: monoclonal antibody administration and immunization. The technology that has allowed for the humanization of murine monoclonal antibodies as well as the development of human monoclonal antibodies may provide compounds that could be administered to babies delivered through infected birth canals (88, 89). In so doing, the newborn child might be provided a similar level of antibodies as that encountered from transplacental maternal antibody acquisition. Studies performed in newborn mice indicate that administration of either gB- or gD-humanized monoclonal antibodies can significantly decrease acquisition of mice subsequently exposed to HSV (89, 90). It should be noted that these antibodies may have therapeutic value when administered alone or with acyclovir (ref. 91; E. Kern, P. E. Vogt, J. Palmer, M. S. Co, and R.J.W., unpublished results).

Alternatively, the administration of a vaccine—either gB or gD subunit or live-attenuated—to susceptible individuals might prevent primary infection late in gestation and, therefore, shift the risk of the child exposed to HSV at delivery to at the worst 3% for disease acquisition instead of 33%. One promising candidate subunit vaccine from Chiron is undergoing extensive field trials to prevent genital HSV infection and, possibly, to treat individuals with frequently recurrent infection. Likely,

studies with a subunit vaccine will be undertaken in the future in pregnant women or those about to conceive.

CONCLUSION

Significant progress has been made in the diagnosis and management of neonatal HSV infection over the past two decades. Nevertheless, this disease continues to increase among all socioeconomic groups of our society. As the disease has become recognized in the United States, it has become similarly an ever-increasing problem in third world countries. The lack of adequate health care delivery systems in third world countries should cause greater attention to vaccine development. Future efforts must be directed toward the prevention of this disease rather than treatment after its occurrence.

SUMMARY

Herpes simplex virus infections of humans have been known since ancient times. Contemporary society has witnessed a series of devastating manifestations of herpes simplex virus infections—namely, genital herpes simplex virus infection and neonatal herpes simplex virus infection. With the evolution of society, particularly advances in birth control and increasing promiscuity, the seroprevalence of herpes simplex virus type 2 infections has increased worldwide, however, more so in developed societies. As a consequence, individuals of child-bearing age are at risk for either reactivation of herpes simplex virus at termination of gestation or acquisition of a new primary infection at that time. The consequences of vertical transmission of herpes simplex virus from mother to child, resulting in neonatal herpes simplex virus infection, can be devastating. Current efforts, which are directed toward the treatment of neonatal herpes, have established the value of drugs such as vidarabine and acyclovir. However, the real emphasis for future programs is the prevention of herpes simplex virus infections to avoid person-to-person transmission either horizontally or vertically. The development of vaccines directed against herpes simplex virus may be of value toward this end.

REFERENCES

1. Nahmias, A. J. & Dowdle, W. R. (1968) *Prog. Med. Virol.* **10** 110–159.
2. Wildy, P. (1973) in *The Herpesviruses*, ed. Kaplan, A. S. (Academic, New York), pp. 1–25.
3. Hutfield, D. C. (1966) *Br. J. Vener. Dis.* **42.** 263–268.

4. Andrews, C. H. & Carmichael, E. A. (1930) *Lancet* **i**, 857–858.
5. Whitley, R. J. (1990) in *Virology*, eds. Fields, B. N., Knipe, D. M., Chanock, R., Hirsch, M., Melnick, J., Monath, T. & Roizman, B. (Raven, New York), pp. 1843–1887.
6. Smith, M. G., Lennette, E. H. & Reames, H. R. (1941) *Am. J. Pathol.* **17,** 55–68.
7. Nahmias, A. J., Keyserling, H. L. & Kerrick, G. M. (1983) in *Infectious Diseases of the Fetus and Newborn Infant*, eds. Remington, J. S. & Klein, J. O. (Saunders, Philadelphia), pp. 636–678.
8. Nahmias, A. J., Keyserling, H. L. & Lee, F. K. (1989) in *Viral Infections of Humans: Epidemiology and Control*, ed. Evans, A. S. (Plenum, New York), pp. 393–417.
9. Sullivan-Bolyai, J., Hull, H. F., Wilson, C. & Corey, L. (1983) *J. Am. Med. Assoc.* **250,** 3059–3062.
10. Adam, E., Sharma, S. D., Zeigler, O., Iwamoto, K., Melnick, J. L., Levy, A. H. & Rawls, W. E. (1972) *J. Natl. Cancer Inst.* **48,** 65–72.
11. Templeton, A. C. (1970) *J. Clin. Pathol.* **23,** 24–30.
12. Corey, L. (1982) *J. Am. Med. Assoc.* **248,** 1041–1049.
13. Reeves, W. C., Corey, L., Adams, H. G., Vontver, L. A. & Holmes, K. K. (1981) *N. Engl. J. Med.* **305,** 315–319.
14. Brown, Z. A., Benedetti, J., Ashley, R., Burchett, S., Selke, S. , Berry, S., Vontver, L. A. & Corey, L. (1991) *N. Engl. J. Med.* **324,** 1247–1252.
15. Yeager, A. S., Arvin, A. M., Urbani, L. J. & Kemp, J. A., III (1980) *Infect. Immun.* **29,** 532–538.
16. Whitley, R. J., Corey, L., Arvin, A., Lakeman, F. D., Sumaya, C. V., Wright, P. F., Dunkle, L. M., Steele, R. W., Soong, S. J., Nahmias, A. J., Alford, C. A. & National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (1988) *J. Infect. Dis.* **158,** 109–116.
17. Yeager, A. S. & Arvin, A. M. (1984) *Pediatrics* **73,** 188–193.
18. Kohl, S., West, M. S., Prober, C. G., Sullender, W. M., Loo, L. S. & Arvin, A. M. (1989) *J. Infect. Dis.* **160,** 770–776.
19. Boulter, E. A., Zwartouw, H. T. & Thornton, B. (1982) *Br. Med. J.* **284,** 746.
20. Kaye, E. M. & Dooling, E. C. (1981) *Neurology* **31,** 1045–1055.
21. Parvey, L. S. & Chien, L. T. (1980) *Pediatrics* **65,** 1150–1153.
22. Gibson, J. J., Homung, C. A., Alexander, G. R., Potts, W. A., Lee, F. K. & Nahmias, A. J. (1990) *J. Infect. Dis.* **162,** 306–312.
23. Douglas, J., Schmidt, O. & Corey, L. (1983) *J. Pediatr.* **103,** 908–910.
24. Francis, D. P., Hermann, K. L. & MacMahon, J. R. (1975) *Am. J. Dis. Child.* **129,** 889–893.
25. Hammerberg, O., Watts, J., Chernesky, M., Luchsinger, I. & Rawls, W. (1983) *Pediatr. Infect. Dis.* **2,** 290–294.
26. Kibrick, S. (1979) *Pediatrics* **64,** 390.
27. Light, I. J. (1979) *Pediatrics* **63,** 480–482.
28. Linnemann, C. C., Jr., Buchman, T. G., Light, I. J., Ballard, J. L. & Roizman, B. (1978) *Lancet* **i** 964–966.
29. Sullivan-Bolyai, J. Z., Fife, K. H., Jacobs, R. F., Miller, Z. & Corey, L. (1983) *Pediatrics* **71,** 455–457.
30. Sanders, D. Y. & Cramblett, H. G. (1968) *Am. J. Dis. Child.* **116,** 251–256.
31. Yeager, A. S., Ashley, R. L. & Corey, L. (1983) *J. Pediatr.* **103,** 905–907.
32. Nahmias, A. J., Josey, W. E., Naib, Z. M., Luce, C. F. & Fuest, B. (1970) *Am. J. Epidemiol.* **91,** 547–552.

33. Rawls, W. E., Tompkins, W. A. & Melnick, J. L. (1969) *Am. J. Epidemiol.* **89,** 547–554.
34. Adam, E., Kaufman, R. H., Mirkovic, R. R. & Melnick, J. L. (1979) *Obstet. Gynecol.* **54,** 171–173.
35. Wolinska, W. H. & Melamed, M. R. (1970) *Acta Cytol.* **14,** 239–242.
36. Becker, W. B. (1966) *S. Afr. Med. J.* **40,** 109–111.
37. Rawls, W. E., Adam, E. & Melnick, J. L. (1972) in *Oncogenesis and Herpesviruses*, Scientific Publication II, eds. Biggs, P. M., de The, G. & Payne, L. N. (Int. Agency Res. Cancer, Lyon, France), pp. 424–427.
38. Pagano, J. S. (1975) *J. Infect. Dis.* **132,** 209–223.
39. Evans, A. S. & Dick, E. C. (1964) *J. Am. Med. Assoc.* **190,** 699–708.
40. Rattray, M. C., Corey, L., Reeves, W. C., Vontver, L. A. & Holmes, K. K. (1978) *Br. J. Vener. Dis.* **54,** 262–265.
41. Smith, I. W., Peutherer, J. F. & MacCallum, F. O. (1967) *J. Hyg.* **65,** 395–408.
42. Arvin, A. M. & Prober, C. G. (1990) *Ped. Infect. Dis. J.* **9,** 764–767.
43. Prober, C. G., Corey, L., Brown, Z. A., Hensleigh, P. A., Frenkel, L. M., Bryson, Y. J., Whitley, R. J. & Arvin, A. M. (1992) *Clin. Infect. Dis.* **15,** 1031–1038.
44. Lee, F. K., Coleman, R. M., Pereira, L., Bailey, P. D., Tatsuno, M. & Nahmias, A. J. (1985) *J. Clin. Microbiol.* **22,** 641–644.
45. Nahmias, A. J., Josey, W. E., Naib, Z. M., Freeman, M. G., Fernandez, R. J. & Wheeler, J. H. (1971) *Am. J. Obstet. Gynecol.* **110,** 825–836.
46. Johnson, R. E., Nahmias, A. J., Magder, L. S., Lee, F. K., Brooks, C. & Snowden, C. (1989) *N. Engl. J. Med.* **321,** 7–12.
47. Nahmias, A. J., Lee, F. K. & Bechman-Nahmias, S. (1990) *Scand. J. Infect. Dis.* **69,** 19–36.
48. Rawls, W. E. & Gardner, H. L. (1972) *Clin. Obstet. Gynecol.* **15,** 913–918.
49. Rawls, W. E., Gardner, H. L., Flanders, R. W., Lowry, S. P., Kaufman, R. H. & Melnick, J. L. (1971) *Am. J. Obstet. Gynecol.* **110,** 682–689.
50. Rawls, W. E., Garfield, C. H., Seth, P. & Adam, E. (1976) *Cancer Res.* **36,** 829–835.
51. Kulhanjian, J. A., Soroush, V., Au, D. S., Bronzan, R. N., Yasukawa, L. L., Weylman, L. E., Arvin, A. M. & Prober, C. G. (1992) *N. Engl. J. Med.* **326,** 916–920.
52. Young, E. J., Killam, A. P. & Greene, J. F. (1976) *J. Am. Med. Assoc.* **235,** 2731–2733.
53. Flewett, T. H., Parker, R. G. F. & Philip, W. M. (1969) *J. Clin. Pathol.* **22,** 60–66.
54. Anderson, J. M. & Nicholls, M. W. N. (1972) *Br. Med. J.* **1,** 632.
55. Goyette, R. E., Donowho, E. M., Hieger, L. R. & Plunkett, G. D. (1974) *Obstet. Gynecol.* **43,** 191–196.
56. Hensleigh, P. A., Glover, D. B. & Cannon, M. (1979) *J. Reprod. Med.* **22,** 171–176.
57. Peacock, J. E. & Sarubbi, F. A. (1983) *Obstet. Gynecol.* **61,** 13–18.
58. Baldwin, S. & Whitley, R. J. (1989) *J. Teratol.* **39,** 1–10.
59. Hutto, C., Arvin, A., Jacobs, R., Steele, R., Stagno, S., Lyrene, R., Willett, L., Powell, D., Anderson, R., Wetherman, J., Ratliff, G., Nahmias, A. J. & Whitley, R. J. (1987) *J. Pediatr.* **110,** 97–101.
60. Thong, Y. H., Steele, R. W., Vincent, M. M., Henson, S. A. & Bellanti, J. A. (1973) *N. Engl. J. Med.* **289,** 604–606.
61. Grossman, J. H., Wallen, W. C. & Sever, J. L. (1981) *Obstet. Gynecol.* **58,** 1–4.

62. Harger, J. H., Meyer, M. P. & Amortegui, A. J. (1986) *Obstet. Gynecol.* **67,** 637–642.
63. Brown, Z. A., Vontver, L. A., Benedetti, J., Critchlow, C. W., Sells, C. J., Berry, S. & Corey, L. (1987) *N. Engl. J. Med.* **317,** 1246–1251.
64. Stagno, S. & Whitley, R. J. (1985) *N. Engl. J. Med.* **313,** 1270–1273.
65. Stagno, S. & Whitley, R. J. (1985) *N. Engl. J. Med.* **313,** 1327–1329.
66. Bolognese, R. J., Corson, S. L., Fuccillo, D. A., Traub, R., Moder, F. & Sever, J. L. (1976) *Obstet. Gynecol.* **48,** 507–510.
67. Tejani, N., Klein, S. W. & Kaplan, J. (1979) *Am. J. Obstet. Gynecol.* **135,** 547.
68. Vontver, L. A., Hickok, D. E., Brown, Z., Reid, L. & Corey, L. (1982) *Am. J. Obstet. Gynecol.* **143,** 75–84.
69. Arvin, A. M., Hensleigh, P. A., Prober, C. G., Au, D. S., Yasukawa, L. L., Wittek, A. E., Palumbro, P. E., Paryani, S. G. & Yeager, A. S. (1986) *N. Engl. J. Med.* **315,** 796–800.
70. Hatherley, L. I., Hayes, K. & Jack, I. (1980) *Med. J. Aust.* **2,** 325–329.
71. Guinan, M. E., MacCalman, J., Kern, E. R., Overall, J. C. & Spruance, S. L. (1981) *N. Engl. J. Med.* **304,** 759–763.
72. Harger, J. H., Pazin, G. J., Armstrong, J. A., Breinig, M. C. & Ho, M. (1983) *Am. J. Obstet. Gynecol.* **145,** 784–791.
73. Whitley, R. J. (1990) in *Infectious Diseases of the Fetus and Newborn Infants*, eds. Remington, J. & Klein, J. (Saunders, Philadelphia), pp. 282–305.
74. Rasmussen, L. & Merigan, T. C. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3957–3961.
75. Whitley, R. J. & Hutto, C. (1985) *Pediatr. Rev.* **7,** 119–126.
76. Whitley, R. J., Nahmias, A. J., Soong, S.-J., Galasso, G. G., Fleming, C. L., Alford, C. A., Jr., & National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (1980) *Pediatrics* **66,** 495–501.
77. Whitley, R. & Gnann, J. (1992) *N. Engl. J. Med.* **327,** 782–789.
78. Elion, G. B., Furman, P. A., Fyfe, J. A., de Miranda, P., Beauchamp, L. & Schaffer, H. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5716–5720.
79. Schaeffer, H. J., Beauchamp, L., deMiranda, P., Elion, G. B., Baver, D. J. & Collins, P. (1978) *Nature (London)* **272,** 583–585.
80. Dorsky, D. I. & Crumpacker, C. S. (1987) *Ann. Intern. Med.* **107,** 859–874.
81. Andrews, E. B., Tilson, H. H., Hurin, B. A., Path, F. R. C. & Cordero, J. F. (1988) *Am. J. Med.* **85,** 123–128.
82. Whitley, R. J. & Arvin, A. (1994) in *Seminars in Pediatric Infectious Diseases*, ed. Baker, C. J. (Saunders, Philadelphia), in press.
83. Frenkel, L. M., Brown, Z. A., Bryson, Y. J., Corey, L., Unadkat, J. D., Hensleigh, P. A., Arvin, A. M., Prober, C. G. & Connor, J. D. (1991) *Am. J. Obstet. Gynecol.* **164,** 569–576.
84. Whitley, R. J., Arvin, A., Prober, C., Burchett, S., Corey, L., Powell, D., Plotkin, S., Starr, S., Alford, C., Connor, J., Jacobs, R. F., Nahmias, A. J., Soong, S.-J. & National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (1991) *N. Engl. J. Med.* **324,** 444–449.
85. Jue, S., Koanemon, S., Rowe, M., Laughlin, C., Whitley, R. & National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (1992) *Neonatal Herpes Simplex Virus (HSV) Infections: A Cost-Benefit Analysis of Acyclovir Therapy* (ICAAC, Anaheim, CA), p. 350 (abstr. 1434).
86. Straus, S. E., Takiff, H. E., Seidlin, M., Bachrach, S., Lininger, L., DiGiovanna, J. J., Western, K. A., Smith, H. A., Lehrman, S. N., Creagl-Kirk, T. & Alling, D. W. (1984) *N. Engl. J. Med.* **310,** 1545–1550.

87. Stray-Pedersen, B. (1990) *Lancet* **336,** 756.
88. Co, M. S., Deschamps, M., Whitley, R. J. & Queen, C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2869–2873.
89. Kern, E. R., Vogt, P. E., Co, M. S., Kohl, S. & Whitley, R. J. (1992) *Treatment of Herpes Simplex Virus Type 2 Infections in Mice with Murine and Humanized Monoclonal Antibodies (MABS)* (Int. Soc. Antiviral Res., Vancouver, Canada) (abstr. 125).
90. Baron, S., Worthington, M. G., Williams, J. & Gaines, J. W. (1976) *Nature (London)* **261,** 505–506.
91. Metcalf, J. F., Koga, J., Chatterjee, S. & Whitley, R. J. (1987) *Intervirology* **185,** 1–11.
92. Whitley, R. J., Arvin, A., Prober, C., Corey, L., Burchett, S., Plotkin, S., Starr, S., Jacobs, R., Powell, D., Nahmias, A. J., Sumaya, C., Edwards, K., Alford, C., Caddell, G., Soong, S.-J. & National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (1991) *N. Engl. J. Med.* **324,** 450–454.

Human Ecology and Behavior and Sexually Transmitted Bacterial Infections

KING K. HOLMES

The four major bacterial sexually transmitted diseases (STDs), gonorrhea, chlamydial infection, syphilis, and chancroid, all rank together with human immunodeficiency virus (HIV) infection among the top 25 diseases causing loss of healthy days of life in a representative high prevalence urban area of sub-Saharan Africa (1). The prevalence of bacterial STDs in many developing countries is extremely high (2). In China and Thailand, where national STD surveillance systems do exist, major epidemics of bacterial STDs have occurred in recent years (Figures. 1 and 2). In contrast, every Western industrialized country but one has eradicated chancroid as an endemic disease and dramatically reduced the incidence of gonorrhea and syphilis during the AIDS era. Many are bringing chlamydia under control. Only the United States allowed gonorrhea, syphilis, and chancroid to go out of control during the AIDS era, as summarized elsewhere in this issue by Wasserheit (3), and the United States has failed to implement a country-wide chlamydia control program.

Beyond the extensive morbidity caused directly by these four STDs, all represent risk factors for heterosexual transmission of HIV (4–8). STD may increase heterosexual transmission of HIV by producing genital inflammation in HIV-infected persons, thereby enhancing shedding of HIV in genital secretions (9–11), or by producing genital inflammation in

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individuals who are sexually exposed to HIV, thereby enhancing susceptibility to HIV acquisition. While community randomization trials of improved STD control for HIV prevention are planned or under way in Africa, the World Health Organization (12) and U.S. Agency for International Development (13) identify treatment of bacterial STDs as one of three available public health strategies (along with condom promotion and sexual behavior change) for preventing sexual transmission of HIV. Ironically, our own country, possessing resources and technical capabilities for control of bacterial STD, has not applied the same balanced

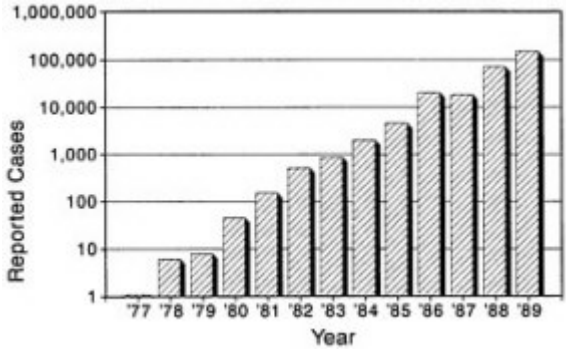

FIGURE 1 Trends in reported sexually transmitted diseases, People's Republic of China, 1977–1989.

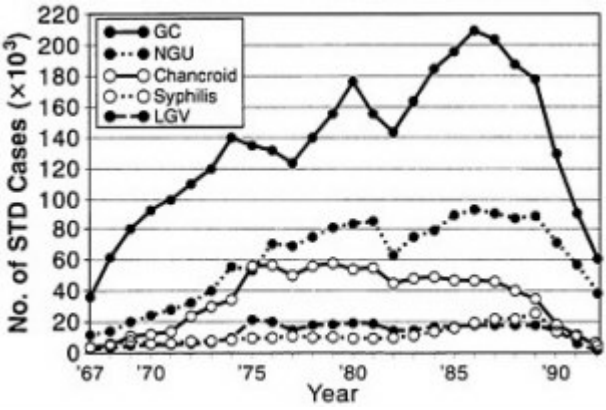

FIGURE 2 Trends in reported bacterial sexually transmitted diseases, Thailand, 1967–1992 (from the National Ministry of Health of Thailand). GC, gonorrhea; NGU, nongonococcol urethritis; LGV, lymphogranuloma venereum.

strategy within our borders. Neither promotion of condom use nor control of bacterial STDs has been aggressively pursued within the United States during the AIDS era.

Our failure to implement effective programs for preventing heterosexual HIV transmission may stem from the widespread but misguided belief that heterosexual AIDS is not a problem in the United States (14). Even as national surveillance showed that heterosexually acquired AIDS increased faster than AIDS in other risk groups every single year for the past 9 years, ultimately becoming the principal form of AIDS in women, we have allowed STD control to deteriorate in the United States. Better definition of the ecologic and behavioral determinants of the emergence of the bacterial STDs—all curable—provides the basic conceptual framework for rethinking prevention and control of STD/HIV in the 1990s.

The following discussion begins with current epidemiologic models of STD, then highlights available data on the three direct determinants of the rate of spread of STD, and finally, examines key ecologic and behavioral factors that operate through these direct determinants to explain the emergence of the four major bacterial STDs in developing countries and in subpopulations of the United States. The discussion compares causal factors in developing countries and in the United States and indicates how these factors operate at the population level and at the individual level.

EPIDEMIOLOGIC MODELS OF STD

Models of STD epidemiology draw on the concept of "core groups" of individuals who can maintain the spread of an STD within a larger population. Various authors have defined "core groups" differently, for example, referring to those with repeated STDs, those living in high-prevalence areas, those with high-risk lifestyles (e.g., sex workers and their clients), or those with prevalence rates of STDs so high as to "preempt" the introduction of new infection into their group (15, 16).

Core groups (17–19) have also been defined in terms of transmission of STDs and three principle determinants of the rate of spread of STD have been delineated. The reproductive rate, R_0, is the initial rate of secondary cases of STD arising from a new case. At $R_0 > 1$, the STD spreads; at $R_0 < 1$, it dies out. The three determinants of the rate of spread are the average risk of infection per exposure or efficiency of transmission (β); the average rate of sexual partner change within the population (c); and the average duration (D) of the infectious period for individuals with the STD. Thus $R_0 = \beta \times c \times D$.

When the population prevalence of an STD is at equilibrium, then $R_0 = 1$ and $c = 1/\beta D$. Based upon published or probable values for β and

D, Brunham and Plummer (20) have estimated values for the mean rate of partner change *(c)* required to sustain transmission of different bacterial STD. Without health care, *D* is large, and rates of partner change required for sustained transmission are highest for chancroid and syphilis, lowest for chlamydial infection, and intermediate for gonorrhea. With early diagnosis and treatment, *D* decreases, and the rate of partner change required to sustain transmission increases. In developing countries and poor urban areas with poor access to early treatment and prolonged infectiousness, lower rates of partner change sustain spread of bacterial STDs.

Subsequent work by Anderson (19) demonstrated that the greater the variance in rate of partner change within the population, the greater the calculated value of R_0. Those with highest rates of partner change (the "core group") contribute disproportionately to STD transmission. In populations where a few persons have many partners, the high variance in partner change effects a high rate of STD transmission. Thus, for example, the crack cocaine epidemic, with frequent exchange of sex for drugs by a few individuals, has a potentially big influence on STD transmission.

Patterns of partner mixing also influence R_0. At low mean rates of partner change, R_0 is highest when those with many partners tend to have sex nonrandomly with others who themselves have many partners (21). Such "assortative" patterns of sexual mixing in poor urban areas may contribute to rapid spread of STDs.

In summary, STD prevalence and incidence in a particular sociogeographic setting is determined by factors that influence β, those that influence sexual behaviors (mean rate of partner change in the population, variance in rate of change, and patterns of partner mixing), and those that influence *D* (availability and use of good STD health care). The next section elaborates on the definition and current status of ecologic factors that most closely influence each of these three direct determinants of R_0.

GLOBAL HEALTH CARE AND THE DURATION OF INFECTIOUSNESS OF BACTERIAL STDS

The quality, accessibility, and use of health care services determine the average duration of infectiousness of the bacterial STD. Early diagnosis and treatment, therefore, represent primary prevention of new infections and secondary prevention of complications.

Developing Countries

Bacterial STDs predominate among adult clinical consultations, disproportionately affecting those who cannot afford private care. Because

early diagnosis and treatment of STD have not been a high priority in the public health sector until now, many bypass the formal private and public health care systems for STD treatment. Men with urethritis often go directly to pharmacies, and often receive inappropriate, yet costly, therapy.

Women with bacterial STDs may not develop genital symptoms and would never undergo routine screening for these infections in developing countries; those who develop symptoms may not seek or find medical care; those who seek and find care seldom receive speculum or bimanual exams in the least developed countries; where such exams are done, microscopy and specific tests for gonorrhea, chlamydial infection, and *Haemophilus ducreyi* are rarely available. Although official policies require testing pregnant women for syphilis, many receive no prenatal care, and those who do often slip through without syphilis screening. Effective and relatively inexpensive STD drugs are now included on the international essential drug list but seldom reach primary care clinics that serve women. For those very select few infected women who might develop symptoms, seek care, be examined, have tests performed or receive a correct syndromic diagnosis, and receive effective therapy, treatment of the male partner is not attempted, and reinfection is likely.

Thus absence of the necessary clinical, laboratory, pharmacy, and public health infrastructure results in a very long average duration of infectiousness for bacterial STD. When combined with high-risk sexual behaviors and efficient transmission, very high equilibrium prevalence rates result.

The United States

There are several thousand public STD clinics in the United States, some free standing as categorical metropolitan STD clinics, often serving predominantly men; some integrated with family planning clinics serving predominantly women; some integrated with community or health department primary care clinics. Prior to the 1970s, STD clinics emphasized treatment and partner notification for syphilis and treatment of gonorrhea in men. Subsequently, STD clinics introduced several new services, such as gonorrhea culture testing of women, partner notification for gonorrhea, and vaginal speculum and bimanual pelvic examination. Uniform guidelines were developed by the Centers for Disease Control for treating conditions poorly managed earlier, such as nongonococcal urethritis, mucopurulent cervicitis, bacterial vaginosis, and pelvic inflammatory disease. Most recently, STD clinics have also expanded services for viral STD, including counseling and testing for HIV, and some provide cervical Pap smears.

Principal failings of our public STD clinics during the AIDS era include the inability to provide (*i*) sufficient access for all persons seeking health care (22), a problem worsened acutely by the added burden of HIV testing and counseling; (*ii*) hepatitis B vaccination of high-risk groups to reduce spread of the first vaccine-preventable STD; and (*iii*) nationwide diagnostic testing for *Chlamydia trachomatis* infection or partner notification for chlamydial infection or related syndromes.

Prior to 1992, only 1 of 10 regions in the United States, region X, received federal funds for a region-wide chlamydia screening program (23). About 150 family planning clinics in the Pacific Northwest began selective chlamydia screening in 1988. Chlamydia prevalence has since declined by >50% in women throughout the region. A few other areas, such as Wisconsin, where chlamydia control programs were also begun, replicated this experience. Screening is particularly effective in reducing transmission of chlamydial infection, because of the often silent nature and long duration of infectiousness.

In summary, inadequate health services for STD treatment provide an obvious explanation for the high rates of bacterial STD found in developing countries and in the inner cities and rural south of the United States. However, disproportionately high rates of viral STD that are little influenced by medical treatment indicate the importance of other ecologic and behavioral determinants in these same settings.

TRENDS AND PATTERNS OF SEXUAL BEHAVIORS

Developing Countries

Demographic data and ethnographic research suggest progressive liberalization of sexual behavior during the 19th and 20th centuries (24, 25), as a result of colonial and economic development, urbanization, population growth, and other factors discussed below. These changes probably best explain the subsequent epidemic spread of HIV and other STDs. Because of the HIV epidemic, qualitative behavioral research and formal sexual behavioral surveys have been undertaken recently in many countries. None approach the size of the sexual behavior surveys recently completed in the United Kingdom (26), France (27), or the United States (see below), but generalizations are possible. Invariably the rate of change of sex partners for men exceeds that for women. In six African countries surveyed in 1988–1990, the percentage of respondents who had engaged in casual or commercial sex in the last 12 months ranged from 8 to 44% for men, and 2–17% for women, with the percent of men engaging in such behavior 2–4 times higher than the percent of women in five of the countries (28). In Asia and Latin America, scattered

general population surveys indicate even higher differences in proportions of men and women engaging in casual or commercial sex. These gender differences in sexual behaviors in Asia and Latin America also exceed gender differences in the industrialized countries (26, 27).

The United States

Despite recent interference from Congress, several methodologically sound surveys of sexual behavior have been conducted in the United States. Zelnik and Kantner (29) studied adolescent sexual behavior in national probability samples in 1971, 1976, and 1979, documenting steadily increasing rates of premarital intercourse by teenaged females throughout the 1970s. The National Survey of Adolescent Males (30, 31) found that from 1988 to 1991, 17.5- to 19.0-year-old males experienced a significantly younger mean age of first intercourse and a significant increase, from 2.0 to 2.6, in the mean number of sexual partners over the past 12 months, with no increase in frequency of condom use. The 1973, 1976, 1982, and 1989 cycles of the National Survey on Family Growth (32) showed a continuing increase in the proportion of female teenagers 15–19 years old who were sexually experienced, from 28.6% in 1970 to 51.5% in 1988, including an increase among white teenagers from 44.1% to 51.5% during the AIDS era from 1985 to 1988. Similarly, the General Social Survey collected limited data annually from 1988 to 1990 on sexual behaviors of U.S. adults (33), providing perhaps the best comparison yet available of recent sexual behaviors of males and females, with data stratified by age, race, and marital status. Results help explain age and race disparities in rates of bacterial STD in the United States and suggest that >22.5 million U.S. adults had 2 or more sex partners during the preceding year, with an estimated 4.8 million having had 5 or more partners. The 1990 National AIDS Behavioral Surveys (34) assess HIV-related sexual risk behaviors. Most recently, from the landmark 1990 National Survey of Men (NSM-I), a series of five articles by Tanfer and coworkers (70–74), concerning sexual behaviors of men 20–39 years old, appeared in *Family Planning Perspectives*, March/April 1993. Age and race/ethnicity differences in numbers of sexual partners paralleled age and race/ethnicity disparities in bacterial STD rates in the United States and the General Social Survey results (33). We await results of the national survey of female sexual behavior from the same group.

These trends in heterosexual behavior over the last decade contrast with the well-documented dramatic decline in unsafe sexual behavior among homosexual/bisexual men, which strikingly cut rates of early

syphilis and rectal gonorrhea. Prior to 1982, syphilis in the United States predominantly involved homosexual or bisexual men (35). Subsequently, the incidence of syphilis and gonorrhea in gay men plummeted, although several cities now see growing numbers of young gay men with rectal gonorrhea.

Overall, sexual attitudes and behaviors became steadily more liberal in the United States throughout the 20th century. With oral contraception in the early 1960s came partial elimination of the double standard of sexual behavior of men and women. The sexual revolution extended to homosexual and bisexual men at the same time. During the 1970s and 1980s, an increasing percentage of young women had premarital intercourse, and recent birth cohorts have had more partners than earlier cohorts. During the AIDS era, gay men decreased risky sexual behaviors, but some young gay men now resume such behaviors. Surveys through 1990–1991 do not suggest dramatic reduction in multipartner, casual, or risky sex among heterosexuals, though more recent data are urgently needed. Cocaine-associated risky sexual behavior has emerged since 1985 as a major factor in the spread of bacterial STD.

DETERMINANTS OF EFFICIENCY OF TRANSMISSION OF STD

Three factors determine efficiency of STD transmission: the size of the microbial inoculum, the susceptibility of the host, and the infectious virulence of the pathogen (e.g., the ID_{50} for a given host). The stage of infection at exposure influences inoculum size, with highest inocula during early infection, with lesions or exudate. Continued sexual activity despite symptoms of STD can reflect lack of knowledge about STD, lack of access to care, dependence on income from commercial sex, or use of toxic drugs. The inoculum is reduced by use of condoms or microbicidal agents (e.g., spermicides) and, conceivably, by vaginal douching. Cultural differences in condom and spermicide use undoubtedly influence STD transmission. Finally, just as other STDs influence genital HIV shedding, genital infection or inflammation caused by one STD pathogen (e.g., gonorrhea) might also influence genital shedding of another bacterial STD pathogen (e.g., chlamydia) (36).

Host susceptibility to chancroid, and perhaps to syphilis and gonorrhea, is reduced by male circumcision (37). Traditional male circumcision practices probably protect some populations against these STDs. Male circumcision is less common in U.S. blacks (37) and Hispanics than in whites, creating an increased risk for these infections in blacks and Hispanics.

Cervical ectopy, which decreases with advancing age and increases with oral contraceptive use, increases susceptibility to chlamydial infection,

and perhaps to gonorrhea. Very young sexually active women and oral contraceptive users with ectopy are particularly susceptible to chlamydial infection. Early average onset of intercourse among females or high rates of oral contraceptive use probably foster rapid transmission of chlamydial infection.

Variations in infectious virulence of *Neisseria gonorrhoeae* may influence efficiency of transmission of gonorrhea. Certain strains of *N. gonorrhoeae* are resistant to fecal acids, and are more prevalent among homosexual men, probably because of more efficient transmission by rectal intercourse (38). Other strains that tend to cause disseminated gonococcal infection, with bacteremia and tenosynovitis, are highly susceptible to fecal acids, and uncommon in gay men, perhaps explaining why disseminated gonococcal infection has been relatively uncommon among gay men.

ECOLOGIC FACTORS INFLUENCING THE EMERGENCE OF BACTERIAL STD

Having considered the three direct determinants of the rate of spread of bacterial STD, we next consider underlying ecologic factors that have influenced the emergence of bacterial STD.

Historical Stages of Economic Development

As the economy evolved from hunting to agriculture to industry, patterns of sexual behavior, reproduction, moral codes, and religion presumably adapted. With the transition from hunting to agriculture, children became economic assets at an early age, the family became the unit of production, and land became a valuable commodity to be passed on from father to son. Early marriage, monogamy, and multiple births were reinforced by religion and moral codes (39). In sociobiological terms "greater predictability of food in space and time promotes the evolution of territoriality. When the resources are dense and easily defensible, and when food is the limiting resource, the optimum strategy is the double defense—by means of the monogamous pair bond" (40).

With the industrial revolution in Europe and North America, the family unit fragmented, as parents and children left the home and village to find work in cities, children no longer were economic assets, marriage was delayed, secular institutions began to replace religious and moral codes, and premarital and extramarital sex became more common. This scenario is replayed conspicuously today in the emerging countries throughout Asia, Latin America, and Africa.

Geography, Crowding, and Hygiene

Climate, crowding, and hygiene profoundly influence the epidemiology of the nonvenereal treponematoses of childhood (yaws, pinta, and endemic syphilis). Crowding, primitive conditions, and poor hygiene have characterized settings permitting spread of endemic syphilis, typically within family units, and yaws persists in some developing tropical countries. At one time, endemic syphilis was a problem even in Northern Europe and North America, disappearing as living conditions improved. The WHO/UNICEF-coordinated global program for eradication of the endemic treponematosis in the 1950s and 1960s greatly reduced yaws prevalence throughout the developing world. Endemic syphilis was eradicated in Bosnia, and pinta disappeared in Latin America. The next generations were rendered fully susceptible to adult syphilis, as immunity from the childhood treponematoses no longer occurred and sexual behaviors became more liberal. Many tremonematologists believe all clinical and epidemiological differences between venereal syphilis of adults and endemic syphilis in children, and perhaps even between syphilis and yaws, reflect ecologic factors and sexual behaviors, rather than essential differences in pathogenicity of *Treponema pertenue* (the cause of yaws) and the variants of *Treponema pallidum* associated with endemic syphilis of children or venereal syphilis of adults.

Similarly, poor hygiene, flies, and crowding characterize the epidemiologic niche for ocular trachoma strains of *C. trachomatis* (serovars A, B, Ba, and C) while sexually transmitted LGV strains of *C. trachomatis* now require tropical developing country settings, whereas genital serovars D-K of *C. trachomatis* cause urethral and cervical infections throughout the world.

Modern studies have not sufficiently addressed the effectiveness of hygiene or use of antiseptics in preventing transmission or acquisition of chancroid or syphilis in high-risk populations.

"Socio-Geographic Space" and Bacterial STDs

The influence of geography, crowding, and hygiene on transmission of the endemic treponematoses, chlamydia, and chancroid somewhat presages current concepts of the social and spatial concentrations of STD, and the spread of these infections along social networks and spatial contiguity or transportation links (41-44). Rothenberg (41) and others (45) documented geographic clustering of high gonorrhea incidence in a few urban census tracts. An impact on AIDS and STD of shortsighted policies for public services—not only public health services

but also fire control, housing, and others—has been inferred by Wallace (43), who decried how planned reduction in capacity to contain urban fires led to spreading urban decay, and the physical and social "ghettoization" of the Bronx. While sexual behaviors and health care most directly determine the rate of introduction and removal of bacterial STDs in the population, the patterns of introduction, spread, and removal within and between communities are determined by social networks and spatial factors, influenced by urban/community planning and services.

War, Travel, and Migration

Quinn (46) discusses these factors separately in this volume as determinants of the spread of HIV infection. They equally influence spread of bacterial STD. The possible contribution of epidemic urban decay and resulting migration described by Wallace (43) to the reemergence of gonorrhea, syphilis, and chancroid in the United States provides one example. Global examples include the epidemic increases in gonorrhea and syphilis in the industrialized countries during and after World Wars I and II and in the United States during and after the Vietnam War, and the rapid global spread of two types of β-lactamase-encoding plasmids in gonococci from separate regional foci in Asia and Africa in the 1970s. The resurgence of reported STDs in China (Figure 1) accompanied reopening of the country to foreign visitors, movement of the male work force within the country to industrial centers, and reemergence of a commercial sex industry in response. Similarly, the growth of the commercial sex industry in Thailand accompanied increased military, work-related, and tourist travel and urbanization, all of which fueled epidemic STD (Figure 2). Indeed the classic tendency of one country to attribute STD epidemics to another country (e.g., syphilis, as both "The French Disease" in Italy and "The Italian Disease" in France at the end of the 15th century) generally reflects changing behaviors in both countries during periods of war, migration, or increased travel.

The Demographic and Epidemiologic Transitions

Figure 3 contrasts the age distribution of the population of the world's developing regions with that of the industrialized countries in 1980 and shows projected growth to the year 2000. Young people predominate now in developing regions and will become even more predominant. Furthermore, the percentage of the world's population living in urban areas will grow from 37% in 1985 to 45% by the year 2000 and to 61% by

2025 (47); in developing countries, this urban migration selectively involves young adults, typically young men. This will further markedly increase the relative numbers of young adults within cities, creating even a larger predominance of young men in cities (48).

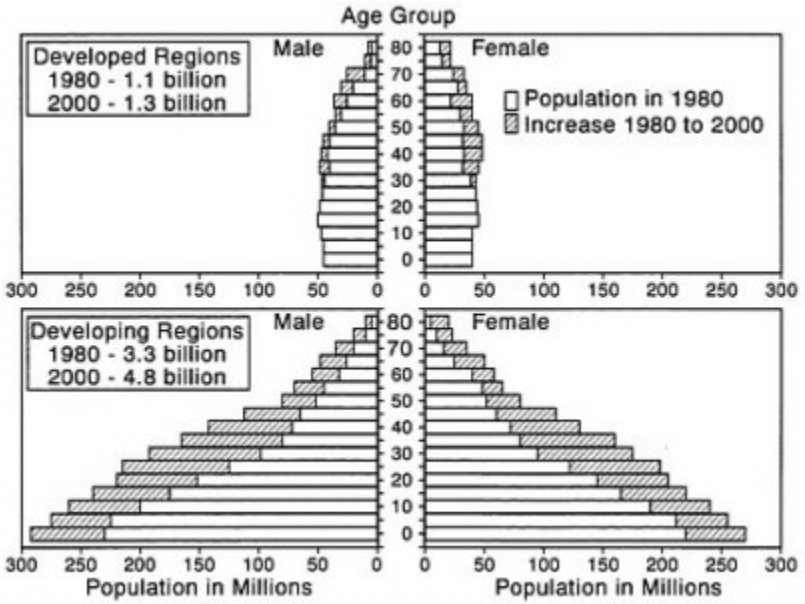

FIGURE 3 World population by age and sex for 1980 and projected for the year 2000 for developed and developing regions (United Nations).

Declining birth rates plus increasing life expectancy is producing a demographic transition to an older population in Western industrialized countries. This leads in turn to an epidemiologic transition, in which aging of the population, improved economic and health infrastructures, and declining rates of death from childhood communicable diseases result in older average age at death and growing morbidity from noncommunicable diseases of adults.

However, in developing countries, especially in Africa and South Asia, birth rates remain high, and child survival improves due to successes of the extended program on immunization, use of oral rehydration solution treatment for diarrhea, and improved care of acute respiratory infection. Rapidly growing numbers of children who survive cause a sustained and disproportionate surge in the size of the adolescent and young adult age groups, both in absolute numbers and relative

to the size of the older population. The success of child survival programs and comparative failure of family planning programs in sub-Saharan Africa and South Asia have led to an unanticipated stage in the epidemiologic transition (49), the epidemic of communicable diseases of adolescents and young adults (i.e., STDs/AIDS).

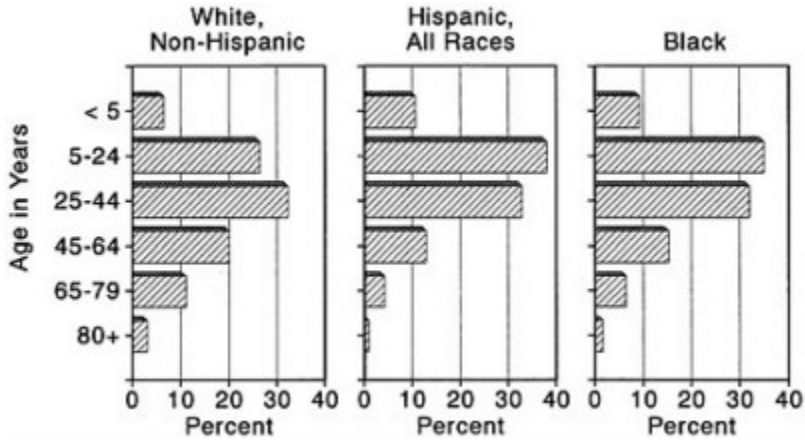

FIGURE 4 Distribution of the population of the United States by age, race, and Hispanic origin in 1990 [Bureau of the Census (1990); Census of Population; General Population Characteristics (1990); Census of Population CP-1-1;17-24].

Figure 4 shows the age distribution of the population of the United States in 1990 for blacks, Hispanics of any race, and non-Hispanic whites. The proportion of blacks and Hispanics from 5 to 24 years old substantially exceeds that of whites, so that the age pyramid for these U.S. minorities lies intermediate between that of the developing regions and that of industrialized countries.

Ages of Sexual Maturation and of Marriage

The average age of sexual maturation declined steadily during the 19th and 20th century in industrialized countries. In the Nordic countries, average age at menarche was about 16 years in the mid to late 19th century, falling steadily to 13 by the mid 20th century (50). Menarche generally occurs later in developing countries, but earlier in urban than in rural areas. This progressively earlier sexual maturation and the progressively delayed mean age of marriage greatly extends the duration of time that premarital intercourse can occur, making social, cultural, and religious proscriptions less effective than in the past.

Social and Economic Development Policies in Developing Countries

The low status of women and economic development policies that move the male work force away from families and communities into urban industrial centers contribute to the epidemics of STD/HIV in many developing countries (24, 48, 51). The relatively poor educational opportunities for women leave them ill-prepared for economic survival outside of marriage, especially after child-bearing. This problem, where coupled with the emergence of urban or periurban male slums, fosters casual and commercial sex and a new generation of urban or periurban teenagers with one available parent (who may be a sex worker) and without a stabilizing extended family or community.

In summary, interrelated factors, including separation of families with male urban migration, low status of women, increasing urban, periurban, and interurban prostitution, a demographic transition characterized by growing and destabilizing excesses of teenagers and young adults no longer under the regulatory influence of a nuclear or extended family or community, and war, migration, and travel, have fostered changes in sexual behavior and epidemics of STD in developing countries during the 20th century. Many similarities are evident in the United States.

A "Synergism of Plagues": STD/HIV, Teen Pregnancy, Violence, and Cocaine Use

In the United States, bacterial STDs, such as gonorrhea, syphilis, and chancroid, concentrate in black and Hispanic populations, particularly among teenagers. The enormous disparity between blacks and whites in annual rates of gonorrhea and syphilis has actually grown since 1985. Because this disparity largely depends upon basic structural disparities in socioeconomic attainment and in access to health care, it is not surprising that the determinants of the STD epidemic in the United States and in developing countries are similar. Race and ethnicity should be viewed not as risk factors *per se* but as risk markers for a more complex set of underlying socioeconomic, cultural, political, behavioral, and environmental risk factors.

Specifically, the nature of the demographic transition in black and Hispanic U.S. populations, a steeply rising proportion of children being born to unmarried mothers, fragmentation of the family and community, counter-productive social welfare policies, and a unique form of commercial sex related to use of crack cocaine have been the prime determinants of changing sexual behaviors; and the failure of the public

health infrastructure to cope with rising rates of STD has worsened the problem in the United States.

In 1991, 1.2 million U.S. children born to unmarried mothers represented 28% of all births. This included 68% of all births to black women, up from 26% in the 1960s; 41% of births to Hispanic mothers; and 17% of births to white women, ranging up to 44% of white women in poverty (52). The overall birth rates and pregnancy rates for U.S. teenagers in 1990 exceeded those of most developing countries (52). In 1990, 521,000 births to U.S. women 15-19 years of age represented a 20% increase from 1986 to 1990 in rate of births to teenaged women. Birthrates per 1000 women 15-19 years of age in 1990 were 42.5 for non-Hispanic white, 100.3 for black, and 116.2 for Hispanic women (53).

Trends in statistics for child neglect and abuse and in placement of children in foster home care provide further evidence of family fragmentation. Nationally, 2.2 million children were referred to child protective services in 1986, and an estimated 270,000 children nationally were in foster care (54), a 223% increase since 1976. The urban situation is worse. Reports of child abuse or neglect totaled 52,504 in New York City alone in 1992, and the number of children in foster homes in New York City increased from `18,000 in 1985 to nearly 50,000 in 1992 (data from Management and Analysis, Child Welfare Administration, Human Resources Administration, City of New York). Children commonly transfer from one family to another in the foster care system, some having been placed in 10 or more homes, precluding consistency in family values or approaches to child rearing.

In a disturbing recent article entitled "The Coming of the White Underclass," Murray (55) argues that this extraordinary increase in births to young poor single women and the resulting family fragmentation are due to social welfare policies that encourage single parenthood, policies that are the root cause of the growing problem with antisocial behavior in young people in the United States.

Not surprisingly, multiple interrelated epidemics of behavioral and emotional problems now converge in U.S. adolescents, with the epidemics of STD/HIV accompanied by concurrent epidemics of gang-related crime and violence, drug use involving teenagers, sustained high rates of teen pregnancies, and increasing rates of live births to teenage girls. In an analysis of retrospective data from successive birth cohorts of adults studied in the National Institute of Mental Health Epidemiological Catchment Area Program, Robins (56) found that the proportion meeting criteria for adolescent antisocial conduct disorder had increased from 0.5% of females and 6% of males in the oldest (65+) cohort, to 13% of females and 36% of males in the youngest (18-29) cohort. Nationally, with `1,000,000 admissions per year of adolescents

into juvenile detention facilities, the average number of adolescents in detention is ` 125,000, at an average cost of about $40,000 per detainee per year.

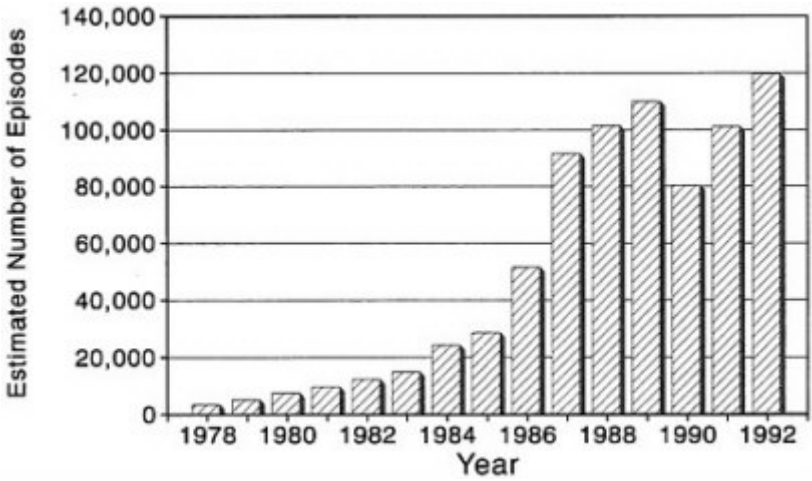

FIGURE 5 Estimated number of emergency room encounters for cocaine use in the United States from 1978 to 1992 [from the U.S. Drug Abuse Warning Network (DAWN) surveillance system] (59).

The crack epidemic contributes to the related epidemics of violence, child abuse and neglect, and the resulting placement of children in foster care. The epidemic of freebase (later crack) cocaine use appeared as early as 1982 in the Bahamas (57), where it was temporally and epidemiologically associated with major epidemics of HIV and genital ulcer disease, including chancroid, syphilis, and lymphogranuloma venereum, caused by the L2 strain of *C. trachomatis* (unpublished data). Crack use spread throughout the United States during the mid 1980s. Although the estimated number of current cocaine users dropped from a peak of 5.8 million in 1985 to 1.3 million in 1992 (58), the number of weekly users has not fallen since 1985. The estimated number of emergency room encounters for cocaine use increased rapidly from 1983 to 1989 (Figure 5). After an encouraging decline in 1990, the numbers shot up again in 1991 and 1992 (59). These encounters presumably reflect those cocaine users getting into most difficulties with the drug. Further, the percentage of cocaine users seen in emergency rooms who acknowledged use by smoking increased from 41% in 1990 to 53% in 1992, and as the "purity" of street cocaine rose from 58% in 1990 to 74% in 1992, the number of emergency room encounters for cocaine overdose rose by 47%.

The epidemics of teenage antisocial behaviors and crack use clearly promote the epidemic spread of bacterial STD. In juvenile detention populations, the combined prevalence of gonorrhea and chlamydial infection typically averages `20% in girls. Gonorrhea is now linked to membership in street gangs (60). Crack use is directly related to syphilis, gonorrhea, and chancroid, as well as HIV infection (61). Crack use leads to several STD risk behaviors, including exchange of sex for drugs or money (62).

New Technology and Product Development

Epidemic increases in gonorrhea and other STD in industrialized countries closely followed the introduction of oral contraceptives, which liberalized the sexual behavior of women, increased the efficiency of sexual transmission of chlamydial infection and perhaps of gonorrhea, and decreased condom use for contraception. On the other hand, family planning programs are now lowering fertility rates and slowing population growth, even where economic progress is slow (63), and this ultimately should slow STD spread.

Development of fluoroquinolones and new oral cepholosporins has made oral therapy for gonorrhea and chancroid more feasible, even through pharmacies, possibly contributing to the recent decline in cases reported from STD clinics in Thailand (Figure 2).

ECOLOGIC AND BEHAVIORAL DETERMINANTS OF COMPLICATIONS AND SEQUELLAE OF BACTERIAL STDS

The many complications of bacterial STD result from extension from lower to upper genital tract; bacteremia; pregnancy or puerperal infectious morbidity; congenital or perinatal transmission; or immunopathologic host responses. Good health care access, quality, and utilization provide secondary prevention of these complications. Other behaviors also influence complications. For example, vaginal douching is associated with increased risk of pelvic inflammatory disease (64, 65), is more common among black women (65, 66), and may contribute to high rates of pelvic inflammatory disease and its sequellae in black women. Conversely, oral contraception use seems to decrease the risk of pelvic inflammatory disease among women with cervical chlamydial infection (67).

HIV infection may influence the risk and severity of pelvic inflammatory disease among women with gonorrhea and may increase the risk of neurologic complications of syphilis (68). Finally, variation in the bacterial STD pathogens themselves influences disease manifestations. For

example, strains of *N. gonorrhoeae* that require arginine, hypoxanthine, and uracil for growth accounted for a high proportion of cases of gonorrhea in the United States and Europe during the 1960s and 1970s and caused most cases of gonococcal bacteremia. For unknown reasons, these strains have nearly disappeared from the United States, and disseminated gonococcal infection has become a rare disease.

POPULATIONAL VS. INDIVIDUAL DETERMINANTS OF STD RISK

In summary, at the population level, the prevalence and pattern of distribution of the direct determinants of R_0 influence the prevalence, incidence, trends, distribution, and complications of the four major bacterial STDs. These direct determinants are in turn strongly influenced by underlying ecologic factors, including structural socioeconomic factors, and development/welfare policies that are the true cause of the modern global emergence of STD and AIDS. These underlying factors must be addressed as public health programs are strengthened.

As summarized in Figure 6, for any individual, the risk of exposure to an STD depends upon the ecological (i.e., sociogeographic) setting in which partners are chosen as well as upon the individual's own sexual behaviors (such as choice of partner within that setting and frequency of partner change and sexual practices). A woman who lives and works in the Bronx (where bacterial STDs are out of control) and has only one sexual partner may have much higher risk of exposure to a bacterial STD than a woman who has 10 sex partners in Sweden (where bacterial STDs are under excellent control) (69). The risk factors influencing risk of acquiring an STD by the exposed individual, when summed across individuals, define the mean efficacy of transmission for the population. At the individual level, health care behaviors influence risk of complications, as well as risk of further transmission. Clear understanding and clear thinking about the populational and individual determinants of STD/HIV transmission and complications are required for developing, prioritizing, and implementing public health strategies for disease prevention.

Fortunately, many of these strategies are now well understood and formulated by international agencies (12). Implementation of effective programs for sexual behavior change, condom promotion, and STD treatment still represents a formidable technical and economic challenge for developing countries. However, in the United States, the technical skills are available, and only a small fraction of funds currently spent on AIDS and other complications of STD would be required for more effective control of bacterial STD. There is no longer any conceivable rationalization for not proceeding.

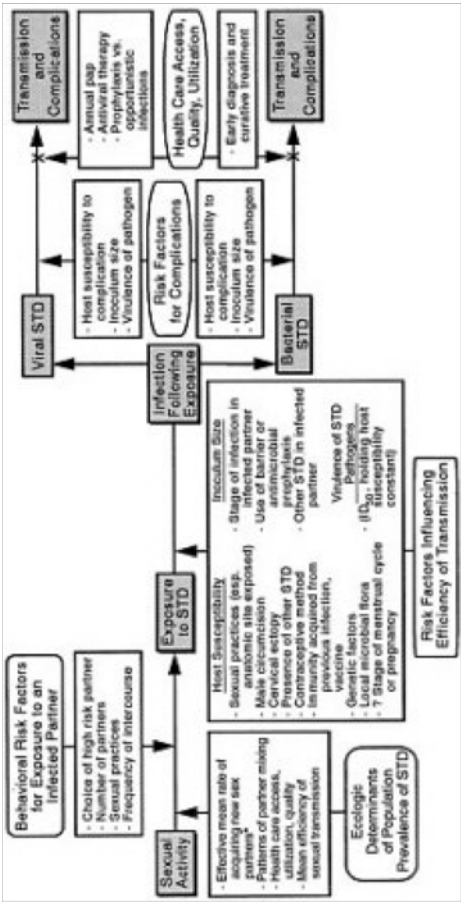

FIGURE 6 Overview of ecologic, microbial, and behavioral determinants of individual risk of acquisition and complication of STDs. * , Effective mean rate of acquiring new sex partners in the population = (mean rate of new partners + SD^2/mean rate).

SUMMARY

The three direct determinants of the rate of spread of sexually transmitted diseases (STDs) are sexual behaviors, the mean duration of infectiousness, and the mean efficiency of sexual transmission of each STD. Underlying ecological and behavioral factors that operate through one or more of these direct determinants lie on a continuum, ranging from those most proximate back to those more remote (in time or mechanism) from the direct determinants. Most remote and least modifiable are the historical stages of economic development that even today conspicuously influence patterns of sexual behavior. Next are the distribution and changing patterns of climate, hygiene, and population density; the global population explosion and stages of the demographic transition; and ongoing changes in human physiology (e.g., menarche at younger age) and culture (e.g., later marriage). More proximate on the continuum are war, migration, and travel; and current policies for economic development and social welfare. Most recent or modifiable are technologic and commercial product development (e.g., oral contraceptives); circumcision, condom, spermicide, and contraception practices; patterns of illicit drug use that influence sexual behaviors; and the accessibility, quality, and use of STD health care. These underlying factors help explain why the curable bacterial STDs are epidemic in developing countries and why the United States is the only industrialized country that has failed to control bacterial STDs during the AIDS era.

REFERENCES

1. Over, M. & Piot, P. (1993) in *Disease Control Priorities in Developing Countries*, eds. Jamison, D. T., Mosley, W. H., Measham, A. R. & Bobadilla, J. L. (Oxford Univ. Press, New York), pp. 455–527.
2. Brunham, R. C. & Embree, J. E. (1992) in *Reproductive Tract Infection: Global Impact and Priorities for Women's Health*, eds. Germaine, A., Holmes, K. K., Piot, P. & Wasserheit, J. (Plenum, New York), pp. 35–58.
3. Wasserheit, J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2401–2406.
4. Cameron, D. W., Simonsen, J. N. & D'Costa, L. J. (1989) *Lancet* **ii,** 403–407.
5. Plummer, F. A., Simonsen, J. N., Cameron, D. W., Ndinya-Achola, J. O., Kreiss, J. K., Gleinya, M. N., Waiyaki, P., Cheang, J., Piot, P., Ronald, A. R. & Ngugi, E. N. (1991) *J. Infect. Dis.* **163,** 233–239.
6. Laga, M., Manoka, A., Kivuvu, M., Malele, B., Tuliza, M., Nzila, N., Goeman, J., Behets, F., Batter, V., Alary, M., Heyward, W. L., Ryder, R. W. & Piot, P. (1993) *AIDS* **7,** 95–102.
7. Wasserheit, J. N. (1991) *Sex. Transm. Dis.* **19,** 61–77.
8. Stamm, W. E., Handsfield, H. H., Rompalo, A. M., Ashley, R. L., Roberts, P. L. & Corey, L. (1988) *J. Am. Med. Assoc.* **260,** 1429–1433.
9. Kreiss, J. K., Coombs, R., Plummer, F. A., Holmes, K. K., Nikora, B.,

Cameron, W., Ngugi, E., Ndinya-Achola, J. O. & Corey, L. (1989) *J. Infect. Dis.* **160,** 380–384.
10. Plummer, F. A., Wainsberg, M. A. & Plourde, P. (1990) *J. Infect. Dis.* **161,** 810–811.
11. Clemetson, D. A., Moss, G. B., Willerford, D. M., Hensel, M., Emonyi, W., Holmes, K. K., Plummer, F., Ndinya-Achola, J. O., Roberts, P. L., Hillier, S. & Kreiss, J. (1993) *J. Am. Med. Assoc.* **269,** 2860–2864.
12. Merson, M. H. (1993) *Science* **260,** 1266–1268.
13. Cohen, M. S., Dalabetta, G., Laga, M. & Holmes, K. K., (1994) *Ann. Int. Med.*, **120,** 340–341.
14. Fumento, M. (1993) *The Myth of Heterosexual AIDS* (Regnery Gateway, Washington, DC).
15. Yorke, J. A., Hethcoate, H. W. & Nold, A. (1978) *Sex. Transm. Dis.* **5,** 51–56.
16. Hethcoate, H. W. & Yorke, J. (1984) *Lecture Notes in Biomathematics* (Springer, New York), No. 56.
17. Anderson, R. M. & May, R. M. (1988) *Nature (London)* **333,** 514–519.
18. Anderson, R. M. & May, R. M. (1991) *Infectious Diseases of Humans: Dynamics and Control* (Oxford Univ. Press, Oxford, U.K.).
19. Anderson, R. M. (1991) in *Research Issues in Human Behavior and Sexually Transmitted Diseases in the AIDS Era*, eds. Wasserheit, J. N., Aral, S. O. & Holmes, K. K. (Am. Soc. Microbiol., Washington, DC), pp. 38–60.
20. Brunham, R. C. & Plummer, F. A. (1990) *Med. Clin. North Am.* **74,** 1339–1352.
21. Garnett, G. F. & Anderson, R. M. (1993) *Sex. Transm. Dis.* **20,** 181–191.
22. Aral, S. & Holmes, K. K. (1991) *Sci. Am.* **264,** 62–68.
23. Britton, T. F., DeLisle, S. & Fine, D. (1992) *Am. J. Gynecol. Health* **6,** 24–31.
24. Caldwell, J. & Caldwell, P. (1990) *Sci. Am.* **262,** 118–125.
25. Caldwell, J. C. & Caldwell, P. (1983) *World Health Stat. Q.* **36,** 2–34.
26. Johnson, A. M., Wadsworth, J., Wellings, K., Bradshaw, S. & Field, J. (1992) *Nature (London)* **360,** 410–412.
27. Analyse des Comportements Sexual en France (ACSF) Investigators (1992) *Nature (London)* **360,** 407–409.
28. Caraël, M., Cleland, J., Adeokun, L. & Collaborating Investigators (1991) *AIDS* **5,** 565–574.
29. Zelnik, M. & Kantner, J. F. (1980) *Fam. Plan. Perspect.* **12,** 230–231, 235–237.
30. Sonenstein, F. L., Pleck, J. H. & Ku, L. C. (1989) *Fam. Plan. Perspect.* **21,** 151–158.
31. Ku, L., Sonenstein, F. L. & Pleck, J. H. (1993) *Am. J. Publ. Health* **83,** 1609–1615.
32. Centers for Disease Control (1991) *Morbid. Mortal. Wkly. Rep.* **39,** 929–932.
33. Anderson, J. E. & Dahlberg, L. L. (1992) *Sex. Transm. Dis.* **19,** 320–325.
34. Catania, J. A., Coates, T. J., Stall, R., Turner, H., Peterson, J., Hearst, N., Dolcini, M. M., Hudes, E., Gagnon, J., Wiley, J. & Groves, R. (1992) *Science* **258,** 1101–1106.
35. Blount, J. H. & Holmes, K. K. (1976) in *The Biology of the Parasitic Spirochetes*, ed. Johnson, R. C. (Academic, New York), pp. 157–176.
36. Batteiger, B. E., Fraiz, J., Newhall, W. J., Katz, B. P. & Jones, R. B. (1989) *J. Infect. Dis.* **159,** 661–669.
37. Cook, L. S., Koutsky, L. A. & Holmes, K. K. (1993) *Genitourin. Med.* **69,** 262–264.
38. Morse, S. A., Lysko, P. G., McFarland, L., Knapp, J. S., Sandström, E. G., Critchlow, C. W. & Holmes, K. K. (1982) *Infect. Immun.* **37,** 432–438.

39. Durant, W. & Durant, A. (1968) *The Lessons of History* (Simon & Schuster, New York).
40. Wilson, E. O. (1980) *Sociobiology: The Abridged Edition* (Belknap Press, Cambridge, MA), p. 256.
41. Rothenberg, R. B. (1983) *Am. J. Epidemiol.* **117,** 688–694.
42. Potterat, J. J., Rothenberg, R. B., Woodhouse, D. E., Muth, J. B., Pratts, C. I. & Fogle, J. S. (1985) *Sex. Transm. Dis.* **12,** 25–32.
43. Wallace, R. (1990) *Soc. Sci. Med.* **31,** 801–813.
44. Wallace, R. (1991) *Soc. Sci. Med.* **33,** 1155–1162.
45. Rice, R. J., Roberts, P. L., Handsfield, H. H. & Holmes, K. K. (1991) *Am. J. Public Health* **81,** 1252–1258.
46. Quinn, T. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2407–2414.
47. Rossi-Espagnet, A., Goldstein, G. B. & Tabibzadeh, I. (1991) *World Health Stat. Q.* **44,** 185–244.
48. Decosas, J. & Pedneault, V. (1992) *Health Policy Planning* **7,** 227–233.
49. Rogers, R. G. & Hackenberg, R. (1987) *Soc. Biol.* **34,** 234–243.
50. Bell, T. A. & Hein, K. (1984) in *Sexually Transmitted Diseases* , ed. Holmes, K. K. (McGraw-Hill, New York), pp. 73–84.
51. Larson, A. (1989) *Rev. Infect. Dis.* **11,** 716–731.
52. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rept.* **42,** 733–737.
53. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rept.* **42,** 398–403.
54. Trupin, E. W., Tarico, V. S., Low, B. P., Jemelka, R. & McClellan, J. (1993) *Child Abuse Neglect* **17,** 345–355.
55. Murray, C. (1992) *The Wall Street Journal*, Oct. 29.
56. Robins, L. N. (1988) in *Risk in Intellectual and Psychosocial Development*, eds. Farran, D. C. & McKinney, J. D. (Academic, New York), pp. 261–269.
57. Jekel, J. F., Allen, D. F. & Podlewski, H. (1986) *Lancet* **i,** 459–462.
58. Substance Abuse and Mental Health Services Administration (1993) *Preliminary National Household Survey on Drug Abuse: Advance Report No. 3* (Dept. of Health and Human Services, Washington, DC).
59. Substance Abuse and Mental Health Services Administration (1993) *Estimate from the Drug Abuse Warning Network 1992* (Dept. of Health and Human Services, Washington, DC).
60. Centers for Disease Control (1993) *Morbid. Mortal. Wkly. Rept.* **42,** 25–28.
61. Marx, R., Aral, S. O., Rolfs, R. T., Sterk, C. E. & Kahn, J. G. (1991) *Sex. Transm. Dis.* **18,** 92–101.
62. Greenberg, J., Schnell, D. & Conlon, R. (1992) *Sex. Transm. Dis.* **19,** 346–350.
63. Speidell, J. J. (1993) *Physician Soc. Responsibility Q.* **3,** 155–165.
64. Wølner-Hanssen, P., Eschenbach, D. A., Paavonen, J., Stevens, C. E., Critchlow, C., DeRouen, T. & Holmes, K. K. (1990) *J. Am. Med. Assoc.* **263,** 1936–1941.
65. Chow, W.-H., Daling, J. R., Weiss, N. S., Moore, D. E. & Soderstrom, R. (1985) *Am. J. Obstet. Gynecol.* **153,** 727–729.
66. Aral, S. O., Mosher, W. D. & Cates, W., Jr. (1992) *Am. J. Public Health* **82,** 210–214.
67. Wølner-Hanssen, P., Eschenbach, D. R., Paavonen, J., Kiviat, N., Stevens, C., Critchlow, C., DeRouen, T. & Holmes, K. K. (1990) *J. Am. Med. Assoc.* **263,** 54–59.
68. Musher, D. M. (1991) *J. Infect. Dis.* **163,** 1201–1206.
69. Cronberg, S. (1993) *Genioturin. Med.* **69,** 184–186.

70. Billy, J. O. G., Tanfer, K., Grady, W. R. & Klepinger, D. H. (1993) *Fam. Plan. Perspect.* **25,** 52–60.
71. Tanfer, K., Grady, W. R., Klepinger, D. H. & Billy, J. O. G. (1993) *Fam. Plan. Perspect.* **25,** 61–66.
72. Grady, W. R., Klepinger, D. H., Billy, J. O. G. & Tanfer, K. (1993) *Fam. Plan. Perspect.* **25,** 67–73.
73. Klepinger, D. H., Billy, J. O. G., Tanfer, K. & Grady, W. R. (1993) *Fam. Plan. Perspect.* **25,** 74–82.
74. Tanfer, K. (1993) *Fam. Plan. Perspect.* **25,** 83–86.

Vaccines for Bacterial Sexually Transmitted Infections: A Realistic Goal?

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The majority of the world research effort on sexually transmitted infections is now focused on human immunodeficiency virus (HIV) infection, for the understandable reasons that HIV infections are spreading dramatically around the globe, are increasingly spread by heterosexual behaviors, and are ultimately lethal. Current treatment for HIV is only of modest benefit, and intense efforts to create an HIV vaccine have been thwarted to date by the antigenic variability of the virus.

The curable sexually transmitted diseases (STDs) caused by bacterial pathogens (*Neisseria gonorrhoeae, Treponema pallidum, Haemophilus ducreyi, and Chlamydia trachomatis*) have received relatively less research and clinical attention compared with HIV. Use of a combination of approaches, including public education and accessible treatment clinics, in many nations in the industrialized West has resulted in dramatic declines in incidence of most bacterial STDs in the last 10–14 years, particularly gonorrhea (1, 2). The situation in the United States is less optimistic. Whereas gonorrhea has declined by 40%, heterosexually transmitted syphilis, congenital syphilis, chancroid, and chlamydia have increased (3). Problems with these infections are even worse in many developing and underdeveloped nations in Africa, Asia, and

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elsewhere. Increasing antibiotic resistance in gonococci (3) and *H. ducreyi* (4) has necessitated use of newer, more expensive, antimicrobials, a circumstance that can prohibit effective therapy in developing nations.

The importance of the bacterial STDs rests in part on their adverse effects on reproductive health. Both gonorrhea and genital chlamydia are major causes of salpingitis, ectopic pregnancy, and infertility. A sense of urgency for the control of these diseases has emerged recently because of evidence that they significantly increase the rate of HIV transmission between sexual partners (5–9). Genital ulcer disease is recognized as an important cofactor for HIV transmission (7–9) and *H. ducreyi* and *T. pallidum* are prominent causes of the genital ulcer syndrome. The effects of gonorrhea and genital chlamydia on HIV transmission (5, 6) may be due to microulceration (10) and increased local accumulation of activated lymphocytes and macrophages, with a corresponding increase in release of HIV into genital secretions (11).

Because of the apparent roles of bacterial STDs in HIV transmission, the World Health Organization has concluded that strategies to control HIV should include development of effective programs for control of bacterial STDs (12). Although experience in several nations shows this can be accomplished (at least temporarily) without vaccines (1, 2), experience in other nations, including the United States, suggests that this is a difficult task. Vaccines against bacterial STDs would improve the public health and would offer long-lasting and cost-effective solutions to common and expensive problems. The question is, can such vaccines be developed? This paper briefly reviews relevant progress in research towards this goal.

CHANCROID

Chancroid is an ulcerating cutaneous infection caused by *H. ducreyi*. Infection may persist for months without effective antibiotic therapy. In earlier times, clinicians inoculated normal skin with pus taken from genital ulcers of the patient, and development of typical ulcerating lesions after such inoculation was used to confirm the diagnosis of chancroid (13). Indeed, Ducrey (13) was able to serially propagate the infectious agent at least 15 times in the same individual, indicating that little or no immunity occurs in naturally acquired infection. Today, diagnosis is made by *in vitro* culture. A renewed surge of research interest occurred after recognition that *H. ducreyi* is a cofactor for HIV transmission (7–9), and factors involved in pathogenesis are beginning to be delineated (14).

The cardinal feature of chancroid is the development of ulcers on stratified squamous epithelium, which suggests that *H. ducreyi* might

produce cytotoxins or other tissue-destructive extracellular products. Recent evidence shows that many *H. ducreyi* isolates apparently do produce cytotoxins (15). Lagergard and Purven (16) found that injection of live *H. ducreyi* into rabbits resulted in low-titer anti-cytotoxin antibodies; a subsequent injection of live organisms resulted in neutralizing antibodies. Immunization of rabbits with crude preparations of cytotoxin (cell sonicates) resulted in neutralizing anti-cytotoxin antibodies that cross-reacted in similar titers with each of 12 heterologous cytotoxin-producing strains (16). Immunization with non-cytotoxin-producing *H. ducreyi* or other Gram-negative bacteria failed to produce cytotoxin-neutralizing antibodies (16). However, natural infection of humans apparently results in anti-cytotoxin antibodies, without evident protection from disease (16). The role of cytotoxin in the pathogenesis of human chancroid is unclear at present, and much remains to be learned about the genetics, biochemistry, and immunobiology of the putative cytotoxins. Interest in the cytotoxins is prompted, of course, by evidence that many other cytotoxins can be used to create effective vaccines, including diphtheria toxin.

A variety of other *H. ducreyi* proteins also have been identified, including outer membrane proteins and pili (14). Some of these proteins appear to be antigenically variable during infection of a subcutaneous chamber, a property that could explain the ability of *H. ducreyi* to persist *in vivo* (17). Little is known about the nature of the immune response in human chancroid, although there is evidence for both a T-cell (18) and a B-cell response.

SYPHILIS

The question of immunity in syphilis has long been the subject of investigation. Some level of protective immunity was suggested by early observations that only one-third of untreated cases of human syphilis developed late complications of disease (19). During early stages of active untreated infection, immunity to reinfection seemed to be present, the so-called "chancre immunity"; studies of experimental syphilis in rabbits confirmed this phenomenon (20). Development of chancre immunity is correlated with a vigorous humoral and cellular immune response to multiple antigens (21). After several weeks of untreated early syphilis, treponemes are cleared from the primary and secondary lesions. However, viable treponemes persist in untreated experimental animals for years in sites such as lymph nodes (22), suggesting that *T. pallidum* is capable of evading the immune response. Consistent with this is the protracted course of untreated disease in humans, with its characteristic waxing and waning in the first 1-2 years, followed by long intervals that

ultimately (in one-third of cases) lead to late destructive lesions of the heart, central nervous system, or other organs.

Support for the possibility that an effective syphilis vaccine can be developed rests in large part on studies of experimental syphilis in human prison volunteers, conducted nearly 40 years ago at Sing Sing Prison in New York State by Magnuson *et al*. (23). They injected 10^5 (>100 times the ID_{50}) live *T. pallidum* harvested from rabbits into the skin of 62 adult males, 60 of whom were followed either until they developed lesions or until 4 months had elapsed. All were treated with penicillin at the end of the study. Each of 8 controls (no prior syphilis) developed dark-field-positive (spirochetes visible in dark-field microscopy) papules or ulcers at the inoculation site, whereas no lesions developed in each of 5 men with previously untreated latent syphilis. Among 11 men previously treated for early syphilis, 9 developed dark-field-positive lesions, and the other 2 developed dark-field-negative lesions. Among 31 men previously treated for late or congenital syphilis, only 2 developed dark-field-positive lesions, 15 developed dark-field-negative lesions (limited immunity?), and 14 appeared to be totally immune. Thus, durable immunity developed in many, but not all, men treated previously for natural infection, although at least several years of untreated infection were required to provide immunity.

Studies of immunity to *T. pallidum* in rabbits or hamsters confirmed the existence of slowly developed immunity. Both humoral and cellular arms of the immune system are important to immunity in animals (24-28). Antibodies with both neutralizing (27) and opsonic (28) activities, either of which could contribute to immunity, develop during infection.

Mechanisms by which treponemes persist in the presence of vigorous IgG, IgM, and cellular immune responses to multiple antigens (reviewed in ref. 29) are unknown. Studies of the physiology, biochemistry, and genetics of *T. pallidum* have been limited because the organisms still cannot be grown outside of animals, and because of lack of a genetic system for constructing mutants of *T. pallidum*. Antigenic structures seem stable during animal passage, and there is no evidence of the type of phase and antigenic variation or blocking antibodies that have confounded the search for a vaccine against gonorrhea. Persistence in the face of a vigorous antibody response could be due to intracellular localization, although the pathogenesis of *T. pallidum* infections seems to involve penetration between cells (30) rather than survival within epithelial cells or phagocytes.

One factor that may contribute to the persistence of *T. pallidum* is the apparent paucity of proteins in the outer membranes or envelope of the organism, which has been demonstrated by freeze-fracture techniques by two groups (31, 32). When compared with *Escherichia coli, T. pallidum*

has only about 1% the density of integral outer membrane proteins. Proteins in the outer membrane of *T. pallidum* thus are termed TROMPs—treponemal rare outer membrane proteins. *T. pallidum* also lacks lipopolysaccharide in its outer membrane. Identities of TROMPs have been elusive to date, although searching recombinant *T. pallidum* libraries for *phoA* (alkaline phosphatase) fusions has identified *T. pallidum* genes that encode membrane-spanning proteins containing N-terminal signal sequences (33). Progress in the molecular characterization of *T. pallidum* genes and their predicted proteins by use of recombinant DNA technology is impressive (29).

Initial attempts to develop vaccines for syphilis employed the Nichols strain of *T. pallidum*, which fully retained its virulence for humans after decades of serial passage in rabbit testicles (23). Vaccination of rabbits with *T. pallidum* killed by either irradiation (34) or prolonged cold storage (35) resulted in immunity, but multiple large doses were required over considerable periods of time, limiting enthusiasm for analogous approaches in humans. Subsequently, Fitzgerald induced considerable immunity in rabbits with a single injection of heat-killed *T. pallidum*, when the animals were pretreated with cyclophosphamide and given various adjuvants (36). Fitzgerald concluded that whole-cell vaccines caused immunosuppression, which could be overcome by the sequential use of cyclophosphamide and adjuvants. No confirmatory results have yet been reported.

Current efforts to develop a syphilis vaccine focus on recombinant *T. pallidum* proteins. Three of these show limited efficacy in animals. An antigen designated TpN19 (or previously 4D) elicited partial protection in rabbits (37). TpN19 is interesting because in its native form it assembles into an oligomeric ringlike structure (38), although its function in pathogenesis or in the physiology of *T. pallidum* is unknown. Vaccination with recombinant endoflagellar protein (39) or another protein designated either TpN36 or TmpB (40) also resulted in partial protection in animals.

These results do not permit conclusions about development of vaccines for syphilis, other than that some progress is being made. Difficulties in working with the organism are considerable, but application of modern biotechnology can be expected to hasten progress if funding for the few groups working on syphilis is stable.

GONORRHEA

Gonorrhea is a common disease and was known to ancient physicians. The quest for a vaccine is not new, and crude whole-cell vaccines were actually used as immunotherapy in the preantibiotic era (41). It is

not possible to judge the efficacy of the old gonococcal vaccines because of insufficient description of the experiments and lack of controls, yet anecdotal experience suggested that modest doses of the homologous strain were useful (41). In more recent times, a variety of crude or partially purified antigens were used to vaccinate experimental animals against gonorrhea, with modestly encouraging results. For instance, immunization of chimpanzees with a whole-cell killed vaccine made from a chimpanzee virulent isolate conferred partial resistance to urethral challenge (42). Most current studies, however, are focused on purified and well-characterized antigens.

There are lessons to be derived from study of the course of untreated natural gonococcal infection. Symptomatic urogenital gonorrhea eventually subsided into a nonsymptomatic state in the preantibiotic era, and many patients became culture-negative and noninfectious for their partners (41, 43). By inference, the immune response was effective in controlling infection. However, immunity apparently was strain specific, since patients commonly reacquired gonorrhea many times. James Boswell's superb description of his 29 separate bouts of urogenital "gonorrhea" (some of which undoubtedly were due to *C. trachomatis*) illustrates the point (reviewed in ref. 44). Strain-specific immunity to genital gonorrhea was claimed in a recent study of recurrent gonorrhea in female prostitutes in Nairobi, most of whom were immunosuppressed because of infection by HIV. Reinfection was common, but was less likely to be due to strains exhibiting the same serovar of principal outer membrane protein, porin (Por) than by other Por serovars, suggesting partial immunity based on Por-specific epitopes (45). Subsequent work in the same population showed that presence of serum antibodies to Por was not correlated with protection (46). No attempt was made in the latter study to determine whether serovar-specific serum or genital Por antibodies to surface-exposed epitopes were correlated with protection from reinfection, which are the type of data one would desire, based on the earlier work.

Por as a Vaccine Candidate

Por is one of the antigens currently being studied as a possible vaccine. Por is the most abundant membrane protein, is expressed constitutively, and does not undergo high-frequency phase or antigenic variation *in vitro* or *in vivo*. Clinical isolates, however, do exhibit antigenic differences in some surface-exposed domains, presumably as a result of low-frequency mutations of por that allow escape from anti-Por antibodies (47). Some evidence suggests that Por participates in pathogenesis by translocating into eukaryotic cells (48), which might promote

invasion, or in the case of neutrophils, apparently impairs neutrophil function (49).

Prior to the epidemiological studies of Nairobi prostitutes (45, 46), the possibility of a Por vaccine was suggested by Heckels and colleagues (50, 51), who showed that certain monoclonal antibodies (mAbs) against Por were bactericidal, stimulated chemiluminescence of neutrophils, and reduced toxicity to cultured epithelial cells. Moreover, some of the potentially protective mAbs reacted widely with either one or the other of the two main Por-based serogroups of gonococci—i.e., with strains of either the PorA or PorB serogroups (50, 51).

The *por* structural genes from several isolates have been cloned and sequenced (52, 53), allowing comparisons of the amino acid sequences of different Por molecules. PorA and PorB are closely related to each other and are products of variants of a single *por* gene (52, 53). There are multiple predicted membrane-spanning domains and models predict eight surface-exposed loops (54). By use of synthetic peptide technology, the common PorB epitope that reacts with the broadly protective PorB-specific mAb SM24 was defined as YSIP (55). The common PorA epitope that reacts with the broadly protective PorA-specific mAb SM101 (51) appears to be conformation dependent and cannot be localized to a single linear Por domain (53).

Elkins *et al*. (56) studied the immunobiology of polyclonal rabbit antisera raised against six synthetic Por peptides, four for PorA strain FA19 and two for PorB strain MS11. Results were encouraging in that polyclonal IgG purified from serum raised against the most N-terminal surface-exposed loop (loop 1) were bactericidal not only for the homologous strain but also for many other serological variants (serovars) of the same serogroup and to a limited extent for serovars of the other serogroup.

Purified Por also has been evaluated as a vaccine in animals, using *in vitro* bactericidal and opsonic assays as surrogates for possible protection of humans. Wetzler *et al*. (57) purified Por from gonococci containing a mutation in *rmp*, which encodes the reduction-modifiable protein (Rmp) formerly designated PIII; the importance of using an *rmp* mutant was in removal of the highly immunogenic Rmp antigen, which stimulates production of complement-fixing, but generally nonbactericidal, antibodies that block the bactericidal effects of anti-lipooligosaccharide (LOS) or anti-Por antibodies (58). Since mAbs against Rmp, Por, or LOS immunoprecipitate all three molecules, they apparently are tightly associated in clumps or patches in the outer membrane, helping to explain why antibodies against Rmp block bactericidal effects of anti-Por or anti-LOS. Using purified Por free of Rmp contamination, Wetzler *et al*. (59, 60) showed that Por liposomes were highly immunogenic and

resulted in bactericidal and opsonic antibodies that recognized surface-exposed epitopes.

Elkins *et al*. (unpublished data) purified recombinant Por (rPor) expressed from an inducible promoter in *E. coli*. This approach has the potential advantages of permitting easy growth of large batches of cells, allowing use of novel hybrid PorA/PorB molecules (53), and also avoiding Rmp contamination. *E. coli* and other Gram-negative bacteria contain an outer membrane protein, OmpA, that exhibits significant C-terminal homology with Rmp (61), but rPor can be purified with <0.5% OmpA contamination, and anti-OmpA polyclonal sera do not react with Rmp (unpublished data). Immunization of rabbits with liposomal rPor purified with decyl β-D-maltoside so as to retain some conformational epitopes resulted in immune sera that bound well to Por on whole gonococci and exhibited opsonic, but not bactericidal, activity (unpublished data). Lack of bactericidal activity was not due to blocking antibodies, since gonococcal *rmp* mutants also were resistant to killing by anti-rPor rabbit sera.

A problem for all Por vaccines concerns the effects of sialylation of LOS. Sialylation of LOS, which lies physically proximate to Por in the outer membrane, abolished the bactericidal effect of anti-loop 1 peptide antisera by partially masking surface exposure of Por epitopes on whole bacteria and also by inhibition of complement activation (56). Sialylation of LOS also abrogated bactericidal and opsonophagocytic effects of anti-Por sera (60). Since gonococcal LOS is sialylated *in vivo* (62), vaccines based on these Por peptides (and possibly any Por antigens) might be ineffective. Blocking of certain bactericidal anti-Por mAbs by sialylation was incomplete, however, and could be overcome by use of more mAb (C.E., unpublished data). This suggests that killing might be possible with polyclonal anti-Por sera, if sufficiently high titers or high-affinity antibodies could be raised.

Recently, it has been possible to construct recombinant *Salmonella typhimurium* strains that express gonococcal Por constitutively from the por promoter without evident toxicity to the host strain (63). This should allow studies of the vaccine potential of Por delivered to the gut immune system and of the ability of Por to protect against homologous and heterologous infection in various (imperfect) mouse models of gonococcal infection (64). The advantage of this approach is that it should stimulate both humoral and mucosal immunity, both of which presumably are required for an effective gonococcal vaccine. It presumably will be possible to construct analogous strains in attenuated hosts suitable for use in humans, such as *Salmonella typhi*.

Ultimately, the utility of Por vaccines will have to be tested in humans. Recent studies of experimental urethral gonorrhea in male

volunteers (65) show that such experiments can be conducted ethically and safely. Sialylation of LOS *in vivo* may effectively block any Por vaccine, but it should be possible to test this hypothesis directly in humans soon. If parenteral or oral vaccination with Por or peptides derived from Por results in protection against an ID_{80} intraurethral (male) challenge dose of the homologous strain, further consideration of Por vaccines will be warranted.

Pili as a Vaccine Candidate

Pili (Pil) are filamentous appendages composed of multiple pilin subunits, which function as adherence ligands. They appear to be necessary for infection, since Pil^- variants are essentially noninfectious in experimental infection of male volunteers, whereas Pil^+ gonococci are highly infectious under the same conditions (66). It was soon recognized that Pil undergo high-frequency phase and antigenic variation (67–70) and that Pil antigenic variants also exhibit different adherence properties (71, 72). The mechanism of many Pil variations involves recombination (69) between the *pilE* (structural gene) and one of multiple incomplete ("silent") variable copies of *pil* DNA present in several chromosomal loci (67–70). The rapidity and extent of Pil antigenic variation might help to explain how gonococci escape a vigorous immune response, and/or how gonococci rapidly adapt to adhere to quite different cells in diverse ecological niches (cervix, urethra). Sequencing of expressed *pil* genes from urethral isolates from previously uninfected males with experimental gonorrhea shows extensive variations, occurring so rapidly (first few days after inoculation) that immune pressure is an unlikely explanation (73) (H. S. Seifert, C. A. Wright, A. E. Jerse, M.S.C., and J. G. Cannon, unpublished data). Extensive antigenic variation obviously might make it difficult to produce an effective vaccine based on Pil, yet enthusiasm for a Pil-based vaccine once was high because of evidence that anti-Pil antibodies block adherence and are opsonic (72, 74, 75). Moreover, certain regions of the pilin subunit are relatively conserved (67, 75), and polyclonal rabbit sera against such partially conserved pilin peptides reportedly blocked *in vitro* adherence of heterologous gonococci (75). Enthusiasm lessened, however, when subsequent studies failed to confirm surface exposure of the conserved regions on intact pili expressed on whole gonococci (76). Moreover, extensive work in Heckel's laboratory showed that protective pilin mAbs all were directed at highly variable pilin epitopes (72, 74).

While these elegant studies of Pil variations and immunobiology were being conducted, several laboratories worked in a very directed fashion to implement a Pil vaccine for human use. Intramuscular injection of

purified Pil resulted in serum (77) and genital IgG and IgA anti-Pil antibodies that blocked adherence (78) and, in a historical study, partially protected male volunteers from experimental urethral infection by the homologous strain (79). There was some evidence of development of cross-reactive anti-Pil antibodies (78, 79). A field trial of a vaccine composed of a single antigenic type of gonococcal Pil was conducted in Korea, and as might have been anticipated because of extensive Pil antigenic variation, there was no evidence of efficacy (80). Most investigators have concluded that Pil are subject to such a high degree of antigenic variation that a Pil-based vaccine is unlikely to succeed. It is conceivable that relatively conserved domains from a modest number of Pil variants might be useful in a vaccine, and limited research continues with the aim of exploring this possibility.

Other Possible Gonococcal Vaccines

Opacity proteins (Opa) are a family of up to 11 or 12 related outer membrane proteins that, like pili, also serve as adherence ligands, undergo high-frequency phase and antigenic variations, and appear to be necessary to establish genital infection (81). One or a few Opa proteins appear to promote invasion of eukaryotic cells (82). The molecular mechanisms for Opa variation are quite different from Pil variations. Opa expression is regulated by translational frameshifting, which is the consequence of high-frequency spontaneous variations in the number of a pentameric CTCTT oligonucleotide repeat present in the *opa* region encoding the signal sequence (83). Each of the approximately 11 *opa* genes (84) is constitutively transcribed, but most are not translated; at any time, a single cell expresses zero to four or rarely five Opas. Each Opa contains two regions of highly variable protein sequence (HV1 and HV2), and expression of different *opa* genes results in antigenic variation (83–85). Although mAbs against particular Opas decrease adherence (86, 87), no cross-reacting Opa antibodies have been described that either block adherence or have other potentially protective effects. Relatively little work has been done on possible Opa vaccines, influenced no doubt by considerations of difficulties encountered with the highly antigenically variable Pil vaccine. If subsequent work were to demonstrate that only one or at most a few Opas were required to initiate or maintain infection (which has yet to be proven), or if a common essential Opa domain were discovered, this situation could change.

LOS is another potentially useful immunogen. Gonococcal LOS has relatively typical core sugars, but no O antigen. The terminal core sugar is a close molecular mimic of certain host glycolipids (88). Antibodies

against terminal core sugars are bactericidal, and many persons who have never been exposed to gonococci have serum IgM anti-LOS bactericidal antibodies against many gonococci (89). Since gonococci do not have a carbohydrate capsule (unlike closely related meningococci), LOS core sugars are the only carbohydrate antigens that might be considered for a vaccine. Unfortunately, evolution has enabled gonococci to escape attack on LOS, by at least three mechanisms. (*i*) Mimicry of host antigens reduces immune response to certain epitopes (88). (*ii*) Phase and antigenic variations affect the length and composition of core sugars and loss of terminal epitopes helps to evade antibodies against the core, although gonococci with a truncated core are complement sensitive. These variations occur at a frequency (`1 × 10-3) similar to Pil and Opa variations (90). The genetics of LOS core variations are not well understood. (*iii*) Sialylation of the terminal core sugar occurs *in vitro* and *in vivo* (62, 91), when bacterial sialyltransferase and host-derived CMP-N-acetylneuraminic acid (CMP-NANA) as a substrate are used. Sialylation of the terminal LOS core sugar results in partial masking of both LOS and the physically proximate Por (56), inhibiting antibody attack on both LOS and Por. Sialylation also interferes with effective formation of the terminal complement complex, thereby inhibiting bactericidal activity of antibodies against other nonmasked antigens such as Opa (56). Phase variation of core sugar composition results in transient loss of the terminal CMP-NANA acceptor site, and therefore, inability to undergo sialylation. This in turn seems to result in phase variation between a nonsialylated, invasive, but serum-susceptible, phenotype and a sialylated noninvasive serum-resistant phenotype (92).

On the basis of knowledge gained from studies of Por, Pil, Opa, and LOS, an ideal vaccine candidate might be one that does not undergo high-frequency antigenic variations, that is not protected by anti-Rmp blocking antibodies, and that is not masked by sialylation. It also should contain one or a few epitopes that are conserved and that are targets for protective antibodies. LOS seems a very unlikely candidate because of mimicry, phase and antigenic variation, protection by blocking anti-Rmp antibodies, and masking by sialylation. Pili seem to be too antigenically variable. Por remains a possible candidate because of relatively stable expression of cross-reactive epitopes for protective polyclonal and monoclonal antibodies and rather weak epidemiologic evidence for partial Por-based immunity, although partial protection by blocking anti-Rmp antibodies and LOS sialylation are major problems. Another candidate is one or both of the proteins that constitute the transferrin receptor. Transferrin-binding protein 1 (Tbp1) is a 100-kDa protein (93), and transferrin-binding protein 2 (Tbp2) is an unrelated 85-kDa protein. Together, these proteins constitute a specific and

perhaps essential receptor for binding transferrin and removing iron from it (94). Expression of Tbp1 and Tbp2 is repressed by iron, and the structural genes *tbp*A and *tbp*B for Tbp1 and Tbp2, respectively, appear to be part of an iron-regulated polycistronic operon (J. Anderson, C. Cornelissen, and P.F.S., unpublished data). Comparison of the predicted protein sequences for Tbp1 in gonococci (93) and meningococci (95), and for Tbp2 in gonococci (C. Cornelissen, J. Anderson, and P.F.S., unpublished data) and meningococci (95) shows that these proteins are closely related. Antibodies against meningococcal Tbp1 and Tbp2 block meningococcal transferrin binding (96), cross-react rather broadly among meningococci (97), and are bactericidal[*]. Similar studies have yet to be conducted with gonococcal Tbp1 and Tbp2, but it is known that sialylation of LOS does not mask the gonococcal transferrin receptor (unpublished data of C. Cornelissen and P.F.S.). It is premature to do more than speculate about use of transferrin receptor proteins in a gonococcal vaccine, but the limited data are encouraging. IgA1 protease might also be considered on the basis of evidence that antibodies against meningococcal IgA protease cross-react broadly and block proteolytic activity (98).

CHLAMYDIA

C. trachomatis is an obligate intracellular bacterial pathogen that causes trachoma and genital tract infections. There are 15 principal serovars arranged into three serogroups. Serovars A, B, and C are responsible for most trachoma, and serovars D through K cause most genital infections (urethritis, cervicitis, salpingitis). Infection results in both protective immunity and tissue-damaging hypersensitivity responses, which has complicated efforts to develop vaccines. There has been remarkable progress in understanding the immunopathogenesis of chlamydia infections in the past decade, and a vaccine for genital chlamydia is now a realistic hope.

Heat Shock Proteins and Hypersensitivity

Important lessons were learned from attempts to develop a vaccine against trachoma (reviewed in ref. 99 and 100). Ocular scarring and blindness result from chronic and repeated infection. Serovar-specific

[*] Irwin, S., Jacobs, E., Danve, B., Quentin-Millet, M. J. & Schryvers, A. B., Eighth International Pathogenic Neisseria Conference, October 4–9, 1992, Cuernavaca, Mexico, p. 50 (abstr.).

immunity develops over time, but other serovars remain infectious and trigger an apparent hypersensitivity response. Attempts to prevent infection with a crude whole-cell vaccine may actually have potentiated the hypersensitivity response after reexposure to infection. Protection of experimental animals from ocular infection was achieved with mAbs against the major outer membrane protein (MOMP), the serovar-specific typing antigen. An ocular hypersensitivity response was elicited in guinea pigs with the *Chlamydia psittaci* guinea-pig inclusion conjunctivitis agent (101), and topical application of one protein, a 57-kDa heat shock protein closely related to the *groEL* product (102), triggered a delayed hypersensitivity response in previously infected animals (103). Thus, the concept emerged that certain antigens such as MOMP may be useful in a vaccine, whereas others such as the 57-kDa GroEL homologue could be harmful in a vaccine.

Genital chlamydia infections also tend to be chronic and recurring and associated with scarring complications. Considerable evidence links chlamydia (as well as gonococci) to salpingitis, tubal infertility, and ectopic pregnancy. Tubal infertility may occur even in the absence of acutely symptomatic pelvic inflammatory disease, apparently due to smoldering and persistent chlamydia infection. Hypersensitivity to the 57-kDa GroEL homologue may contribute to complications of infections, since there is a strong correlation between presence of antibodies to this heat-shock protein and salpingitis (104), ectopic pregnancy (104), and tubal infertility (105). Indeed, antibodies to this protein may be predictive of women with chlamydia infection who will develop complications of infection (105). Women with salpingitis apparently also are more likely to have a delayed hypersensitivity response to the 57-kDa protein than other women with chlamydia infection (106). In infected primates, the pathology of diseased fallopian tubes is consistent with a cellular hypersensitivity response (107).

Both B-cell and T-cell responses probably are important to the protective immune response (99, 100, 108, 109). Interferon γ (IFN-γ) plays a prominent role in protection against genital chlamydia (110), and exciting recent evidence suggests that IFN-γ may result in a persistent, quiescent infection in which chlamydia express normal amounts of 57-kDa GroEL homologue but markedly decreased amounts of MOMP and other outer membrane antigens (111). If confirmed, this would help to explain how the immune response leads to chronic infection, and subsequently to a tissue-damaging hypersensitivity response to cross-reacting host heat shock protein antigens. Similar mechanisms may be involved in the arthritis–dermatitis–mucositis syndrome (Reiter syndrome) that often complicates genital chlamydia infection (112). It is unclear why the response is directed particularly to fallopian tubes,

joints, and certain other tissues. Murine immune responses to chlamydial heat shock proteins are H-2 linked (113) which could help to explain why only a proportion of persons develop immune-related complications, assuming similar genetic control of anti-chlamydia responses in humans.

Momp as a Protective Antigen

There is at least partial immunity to infection by the same strain after genital chlamydia infection in women (114), monkeys (115), and guinea pigs (116). Recovery of chlamydiae from the cervix is inversely correlated with presence of IgA antibodies reactive with *C. trachomatis* in cervical secretions (114), suggesting IgA may be protective for genital chlamydia. Antibody to several antigens is correlated with apparent protection against postabortal chlamydia salpingitis (117), but the best evidence for antigen-specific protection concerns MOMP. Anti-MOMP polyclonal and monoclonal antibodies are neutralizing and protective against tissue culture infection by elementary bodies, the infectious extracellular form of chlamydia, and against toxic death in mice (118–121). Some MOMP mAbs neutralize more than a single serovar. Protection extends in some instances to subspecies specific epitopes but not to all serogroups of chlamydia.

These results led to the cloning and sequencing of the structural gene for MOMP from all serovars of *C. trachomatis* (122–124), with several important findings. MOMP is a transmembrane protein, with four surface-exposed and variable domains (VDI–VDIV) (125). Non-surface-exposed portions of the expressed protein are highly conserved. Proteolytic cleavage of VDII or VDIV reduces infectivity (126), as do mAbs specific for variable domain epitopes (127–129). By use of synthetic peptides, epitopes specific for neutralizing MOMP mAbs have been mapped to short linear peptides on the surface-exposed loops (100, 130). T-help (T_H) epitopes are located within conserved as well as variable domains of MOMP (131, 132), as well as on other chlamydia proteins (133).

A neutralizing epitope composed of seven amino acids located in MOMP VDIV, when coupled to a common T_H epitope composed of MOMP amino acids 106–130, elicited polyclonal antibodies in mice and cynomolgus monkeys that bound to the surface of *C. trachomatis* serovars D, E, F, and G, which cause about 80% of genital chlamydia infections, and neutralized tissue culture infectivity (129). Six of eight congenic mice that differed in major histocompatibility complex class II haplotype developed anti-VDIV antibodies after immunization, showing that there was not severe T-cell restriction in immune response to

this peptide (129). These encouraging results suggest that a synthetic peptide vaccine against genital chlamydia is possible. An analogous peptide containing a T_H epitope and a VDI neutralizing epitope shows promise as a trachoma vaccine (134). The utility of purified recombinant MOMP as a vaccine also is being explored (135, 136).

MOMP does not appear to be protected by blocking antibodies or by strategies analogous to sialylation of LOS, both of which at least partially protect Por in gonococci. This suggests that it may prove to be easier to develop chlamydial MOMP vaccines than gonococcal Por vaccines. Both MOMP (124, 137) and Por appear to be subject to genetic drift, although how serious a problem this may be for vaccines is unclear.

Recently, a chlamydial 75-kDa outer membrane protein has been identified as a member of the heat shock protein 70 family (138) and more specifically as a DnaK homologue (139). Antibodies against this protein might be protective, since polyclonal antisera react with surface-exposed epitopes and are neutralizing *in vitro* (138). Moreover, there is a correlation between presence of antibodies to this antigen (as well as several others) and apparent protection from salpingitis (117). Unlike the 57-kDa GroEL homologue, the 75-kDa protein does not trigger an ocular hypersensitivity response in previously infected animals (140). Caution is warranted, however, because it shares regions of similarity with a sperm cell receptor (139).

PROSPECTS FOR THE FUTURE

It is much too early to predict future success for vaccines against any bacterial STD. Prospects appear best for a MOMP-based chlamydial vaccine, followed perhaps by a gonococcal vaccine based on Por and/or other antigens. It may be necessary to develop polyvalent vaccines, containing multiple epitopes of a single molecule such as MOMP or Por. This might be accomplished by synthetic peptide technology, or by use of recombinant DNA methods to construct novel chimeric proteins. It also may be necessary to combine different antigens, although this would greatly increase the number of possible vaccine candidate combinations to be tested. Lack of really good animal models limits development of vaccines for most of these infections, although there are several good models in which to study chlamydia vaccines. Delivery of a vaccine is another issue that will need to be addressed, and it could be crucial to initial efforts to determine whether a vaccine candidate holds real promise and is worth further development. Here, too, there is promise; a MOMP VDI neutralizing peptide for trachoma serovar A has been expressed in poliovirus, with evidence for strong immunogenicity in rabbits and production of high-titered neutralizing antibodies (141).

The ideal vaccine would decrease transmission of the infection between partners and would prevent complications of disease.

Bacterial STDs are common infections and they cause important morbidity as well as potentiation of HIV transmission. The effort to develop vaccines to prevent these infections is challenging and is a very important goal.

SUMMARY

Bacterial infections of the genital tract (gonorrhea, chlamydia, chancroid, syphilis) are common and cause significant morbidity. Their importance is heightened by recent appreciation of their roles in facilitation of transmission of the human immunodeficiency virus (HIV). Each is capable of causing repeated infections, suggesting lack of permanent broadly effective immunity. An effective vaccine has yet to be developed for any of these diseases. Rapid progress in understanding the molecular basis for pathogenesis of infection, including mechanisms for escape from otherwise effective immune surveillance and mechanisms for causing injury to host cells, has stimulated renewed efforts to make vaccines for some of these infections. Progress has been greatest for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Present emphasis is on the major or principal outer membrane proteins of *N. gonorrhoeae* and *C. trachomatis,* based on evidence for neutralizing antibodies directed against surface-exposed variable domains of each of these proteins. Other surface-exposed proteins, including the iron-repressible transferrin receptor in gonococci and certain heat-shock proteins in chlamydia, also may be targets for vaccines. Although much remains to be learned, cautious optimism is warranted.

REFERENCES

1. Danielsson, D. (1990) *Scand. J. Infect. Dis. Suppl.* **69,** 69–76.
2. Torvald, R. (1990) *Scand. J. Infect. Dis. Suppl.* **69,** 157–167.
3. Division of STD/HIV Prevention, U.S. Department of Health and Human Services, Public Health Service (1992) *Sexually Transmitted Disease Surveillance, 1991* (Cent. Dis. Control, Atlanta), Nov/Dec 1992.
4. Sturm, A. W. (1987) *J. Antimicrob. Chemother.* **19,** 187–191.
5. Plummer, F. A., Simonsen, J. N., Cameron, D. W., Ndinya-Achola, J. O., Kreiss, J. K., Gakinya, M. N., Waiyaki, P., Cheang, M., Piot, P., Ronald, A. R. & Ngugi, E. N. (1991) *J. Infect. Dis.* **163,** 233–239.
6. Laga, M., Manoka, A., Kivuvu, M., Malele, B., Tuliza, M., Nzila, N., Goeman, J., Behets, F., Batter, V., Alary, M., Heyward, W. L., Ryder, R. W. & Piot, P. (1993) *AIDS* **7,** 95–102.
7. Cameron, D. W., D'Costa, L. J., Maitha, G. M., Cheang, M., Piot, P.,

Simonsen, J. N., Ronald, A. R., Gakinya, M. N., Ndinya-Achola, J. O. , Brunham, R. C. & Plummer, F. A. (1989) *Lancet* **ii,** 403–407.
8. Simonsen, J. N., Cameron, D. W., Gakinya, M. N., Ndinya-Achola, J. O., D'Costa, L. J., Karasira, P., Cheang, M., Ronald, A. R., Piot, P. & Plummer, F. A. (1988) *N. Engl. J. Med.* **319,** 274–278.
9. Stamm, W. E., Handsfield, H. H., Rompalo, A. M., Ashley, R. L., Roberts, P. L. & Corey, L. (1988) *J. Am. Med. Assoc.* **260,** 1429–1433.
10. Kiviat, N. B., Paavonen, J. A., Wolner-Hanssen, P., Critchlow, C. W., Stamm, W. E., Douglas, J., Eschenbach, D. A., Corey, L. A. & Holmes, K. K. (1990) *Hum. Pathol.* **21,** 831–837.
11. Clemetson, D. B. A., Moss, G. B., Willerford, D. M., Hensel, M., Emonyi, W., Holmes, K. K., Plummer, F., Ndinya-Achola, J., Roberts, P. L., Hillier, S. & Kreiss, J. K. (1993) *J. Am. Med. Assoc.* **269,** 2860–2864.
12. World Health Organization (1992) *The Global AIDS Strategy* (WHO, Geneva), WHO AIDS Series, 11.
13. Ducrey, A. (1889) *Monatsh. Prakt. Dermatol.* **9,** 387–405.
14. Morse, S. A. (1989) *Clin. Microbiol. Rev.* **2,** 137–157.
15. Purven, M. & Lagergard, T. (1992) *Infect. Immun.* **60,** 1156–1162.
16. Lagergard, T. & Purven, M. (1993) *Infect. Immun.* **61,** 1589–1592.
17. Abeck, D. & Johnson, A. P. (1992) *Int. J. STD. AIDS* **3,** 319–323.
18. Abeck, D., Korting, H. C., Zaba, R., Dangor, Y., Fehler, G. & Ballard, R. C. (1990) *Int. J. STD. AIDS* **1,** 282–284.
19. Gjestland, T. (1955) *Acta Dermatol. Venereol.* **35,** Suppl. 32, 1, 11–365.
20. Turner, T. B. & Hollander, D. H. (1957) *Biology of the Treponematoses* (World Health Org., Geneva).
21. Wicher, V., Zabek, J. & Wicher, K. (1991) *J. Infect. Dis.* **163,** 830–836.
22. Collart, P., Borel, L.-J., Durel, P., Dunoyer, M. F., Ferrandez, D. & Marien, J. (1964) *Br. J. Vener. Dis.* **40,** 81–89.
23. Magnuson, H. J., Thomas, E. W., Olansky, S., Kaplan, B. I., de Mello, L. & Cutler, J. C. (1956) *Medicine* **35,** 33–82.
24. Schell, R. F., Chan, J. K. & Le Frock, J. L. (1979) *J. Infect. Dis.* **140,** 378–383.
25. Liu, H., Alder, J. D., Steiner, B. M., Stein-Streilein, J., Lim, L. & Schell, R. F. (1991) *Infect. Immun.* **59,** 529–536.
26. Liu, H., Steiner, B. M., Alder, J. D., Baertschy, D. K. & Schell, R. F. (1990) *Infect. Immun.* **58,** 1685–1690.
27. Bishop, N. H. & Miller, J. N. (1976) *J. Immunol.* **117,** 197–207.
28. Baker-Zander, S. A., Shaffer, J. M. & Lukehart, S. A. (1993) *J. Infect. Dis.* **167,** 1100–1105.
29. Norris, S. J. & *T. pallidum* Polypeptide Research Group (1993) *Microbiol. Rev.* **57,** 750–779.
30. Thomas, D. D., Navab, M., Haake, D. A., Fogelman, A. M., Miller, J. N. & Lovett, M. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3608–3612.
31. Radolf, J. D., Norgard, M. V. & Shulz, W. W. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2051–2055.
32. Walker, E. M., Zamphigi, G. A., Blanco, D. R., Miller, J. N. & Lovett, M. A. (1989) *J. Bacteriol.* **171,** 5005–5011.
33. Bianco, D. R., Giladi, M., Champion, C. I., Haake, D. A., Chikami, G. K., Miller, J. N. & Lovett, M. A. (1991) *Mol. Microbiol.* **5,** 2405–2415.
34. Miller, J. N. (1973) *J. Immunol.* **110,** 1206–1215.
35. Metger, M. & Smogor, W. (1969) *Br. J. Vener. Dis.* **45,** 308–312.
36. Fitzgerald, T. J. (1991) *Vaccine* **9,** 266–272.

37. Borenstein, L. A., Radolf, J. D., Fehniger, T. E., Blanco, D. R., Miller, J. N. & Lovett, M. A. (1988) *J. Immunol.* **140,** 2415–2421.
38. Fehniger, T. E., Radolf, J. D. & Lovett, M. A. (1986) *J. Bacteriol.* **165,** 732–739.
39. Champion, C. I., Miller, J. N., Borenstein, L. A., Lovett, M. A. & Blanco, D. R. (1990) *Infect. Immun.* **58,** 3158–3161.
40. Wicher, K., Schouls, L. M., Wicher, V., van Embden, J. D. & Nakeeb, S. S. (1991) *Infect. Immun.* **59,** 4343–4348.
41. Eyre, J. W. H. & Stewart, B. H. (1909) *Lancet* **177,** 76–81.
42. Arko, R., Duncan, W., Brown, W., Peacock, W. & Tomizawa, T. (1976) *J. Infect. Dis.* **133,** 441–447.
43. Wharton, L. R. (1937) *Am. J. Syph. Gonorrhea Vener. Dis.* **21,** 593–608.
44. Ober, W. B. (1969) *N. Y. Acad. Med.* **45,** 587–636.
45. Plummer, F. A., Simonsen, J. N., Chubb, H., Slaney, L., Kimata, J., Bosire, M., Ndinya-Achola, J. O. & Ngugi, E. N. (1989) *J. Clin. Invest.* **83,** 1472–1476.
46. Plummer, F. A., Chubb, H., Simonsen, J. N., Bosire, M., Slaney, L., MacLean, I., Ndinya-Achola, J. O., Waiyaki, P. & Brunham, R. C. (1993) *J. Clin. Invest.* **91,** 339–343.
47. Knapp, J. S., Tam, M. R., Nowinski, R. C., Holmes, K. K. & Sandström, E. G. (1984) *J. Infect. Dis.* **150,** 44–48.
48. Lynch, E. C., Blake, M. S., Mauro, A. & Cohen, Z. (1984) *Biophys. J.* **45,** 104–107.
49. Haines, K. A., Yeh, L., Blake, M. S., Cristello, P., Korchak, H. & Weissmann, G. (1988) *J. Biol. Chem.* **263,** 945–951.
50. Virji, M., Zak, K. & Heckels, J. E. (1986) *J. Gen. Microbiol.* **132,** 1621–1629.
51. Virji, M., Fletcher, J. N., Zak, K. & Heckels, J. E. (1987) *J. Gen. Microbiol.* **133,** 2639–2646.
52. Gotschlich, E. C., Seiff, M. E., Blake, M. S. & Koomey, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8135–8139.
53. Carbonetti, N. H., Simnad, V. I., Seifert, H. S., So, M. & Sparling, P. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6841–6845.
54. van der Ley, P., Heckels, J. E., Virji, M., Hoogerhout, P. & Poolman, J. T. (1991) *Infect. Immun.* **59,** 2963-2971.
55. Butt, N. J., Virji, M., Vayreda, F., Lambden, P. R. & Heckels, J. E. (1990) *J. Gen. Microbiol.* **136,** 2165–2172.
56. Elkins, C., Carbonetti, N. H., Varela, V. A., Stirewalt, D., Klapper, D. G. & Sparling, P. F. (1992) *Mol. Microbiol.* **6,** 2617–2628.
57. Wetzler, L. M., Blake, M. S. & Gotschlich, E. C. (1988) *J. Exp. Med.* **168,** 1883–1897.
58. Rice, P. A., Vayo, H. E., Tam, M. R. & Blake, M. S. (1986) *J. Exp. Med.* **164,** 1735–1748.
59. Wetzler, L. M., Blake, M. S., Barry, K. & Gotschlich, E. C. (1992) *J. Infect. Dis.* **166,** 551–555.
60. Wetzler, L. M., Barry, K., Blake, M. S. & Gotschlich, E. C. (1992) *Infect. Immun.* **60,** 39–43.
61. Gotschlich, E. C., Blake, M. S. & Seiff, M. (1987) *J. Exp. Med.* **165,** 471–482.
62. Smith, H. (1991) *Proc. R. Soc. London Biol.* **246,** 97–105.
63. Elkins, C., Coimbre, A. J., Carbonetti, N. H. & Sparling, P. F. (1994) *Gene* **138,** 43–50.
64. Arko, R. J. (1989) *Clin. Microbiol. Rev.* **2,** S56–S59.

65. Cohen, M. S., Cannon, J. G., Jerse, A. H., Charniga, L., Isbey, S. & Whicker, L. (1994) *J. Infect. Dis.* **169,** 532–537.
66. Kellogg, D. S., Peacock, W. L., Deacon, W. E., Brown, L. & Pirkle, C. I. (1963) *J. Bacteriol.* **85,** 1274–1279.
67. Hagblom, P., Segal, E., Billyard, E. & So, M. (1985) *Nature (London)* **315,** 156–158.
68. Segal, E., Hagblom, P., Seifert, H. S. & So, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2177–2181.
69. Koomey, M., Gotschlich, E. C., Robbins, K., Bergstrom, S. & Swanson, J. (1987) *Genetics* **117,** 391–398.
70. Haas, R. & Meyer, T. F. (1987) *Antonie van Leeuwenhoek* **53,** 431–434.
71. Virji, M. & Heckels, J. E. (1984). *J. Gen. Microbiol.* **130,** 1089–1096.
72. Heckels, J. E. & Virji, M. (1985) in *Monoclonal Antibodies Against Bacteria*, eds. Macario, A. J. L. & Macario, E. C. (Academic, New York), pp. 1–35.
73. Swanson, J., Robbins, K., Barrera, O., Corwin, D., Boslego, J., Ciak, J., Blake, M. & Koomey, J. M. (1987) *J. Exp. Med.* **165,** 1344–1357.
74. Virji, M. & Heckels, J. E. (1985) *Infect. Immun.* **49,** 621–628.
75. Rothbard, J. B., Fernandez, R., Wang, L., Teng, N. N. H. & Schoolnik, G. K. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 915–919.
76. Robinson, E. J., Clemens, C. M., Schoolnik, G. K. & McGee, Z. A. (1989) *Mol. Microbiol.* **3,** 57–64.
77. Seigel, M., Olsen, D., Critchlow, C. & Buchanan, T. M. (1982) *J. Infect. Dis.* **145,** 300–310.
78. McChesney, D., Tramont, E. C., Boslego, J. W., Ciak, J., Sadoff, J. & Brinton, C. C. (1982) *Infect. Immun.* **36,** 1006–1012.
79. Brinton, C. C., Jr., Wood, S. W., Brown, A., Labik, A. M., Bryan, J. R., Lee, S. W., Polen, S. E., Tramont, E. C., Sadoff, J. & Zollinger, W. (1982) in *Seminars in Infectious Disease*, eds. Weinstein, L. & Fields, B. N. (Thieme-Stratton, New York), Vol. 4, pp. 140–159.
80. Boslego, J. W., Tramont, E. C., Chung, R. C., McChesney, D. G., Ciaks, J., Sadoff, J. C., Piziak, M. V., Brown, J. D., Brinton, C. C., Jr., Wood, S. W. & Bryan, J. W. (1991) *Vaccine* **9,** 154–162.
81. Swanson, J., Barrera, O., Sola, J. & Boslego, J. (1988) *J. Exp. Med.* **168,** 2121–2129.
82. Makino, S., van Putten, J. P. M. & Meyer, T. F. (1991) *EMBO J.* **10,** 1307–1315.
83. Stern, A., Brown, M., Nickel, P. & Meyer, T. F. (1986) *Cell* **47,** 61–71.
84. Bhat, K. S., Gibbs, C. P., Barrera, O., Morrison, S. G., Jähnig, F., Stern, A., Kupsch, E-M., Myer, T. F. & Swanson, J. (1991) *Mol. Microbiol.* **5,** 1889–1901.
85. Connell, T. D., Shaffer, D. & Cannon, J. G. (1990) *Mol. Microbiol.* **4,** 439–449.
86. Elkins, C. & Rest, R. F. (1990) *Infect. Immun.* **58,** 1078–1084.
87. Sugasawara, R. J., Cannon, J. G., Black, W. J., Nachamkin, I., Sweet, R. L. & Brooks, G. F. (1983) *Infect. Immun.* **42,** 980–985.
88. Mandrell, R. E., Griffiss, J. M. & Macher, B. A. (1988) *J. Exp. Med.* **168,** 107–126.
89. Rice, P. A. (1989) *Clin. Microbiol. Rev.* **2,** 112–117.
90. Apicella, M. A., Shero, M., Jarvis, G. A., Griffiss, J. M. L., Mandrell, R. E. & Schneider, H. (1987) *Infect. Immun.* **55,** 1755–1761.
91. Mandrell, R. E., Lesse, A. J., Sugai, J. V., Shero, M., Griffis, J. M., Cole, J. A., Parson, N. J., Smith, H., Morse, S. A. & Apicella, M. A. (1990) *J. Exp. Med.* **171,** 1649–1664.

92. van Putten, J. P. M. (1993) *EMBO J.* **12,** 4043–4051.
93. Cornelissen, C. N., Biswas, G. D., Tsai, J., Paruchuri, D., Thompson, S. A. & Sparling, P. F. (1992) *J. Bacteriol.* **174,** 5788–5797.
94. Blanton, K., Biswas, G. D., Tsai, J., Adams, J., Dyer, D., Davis, S., Koch, G., Sen, P. & Sparling, P. F. (1990) *J. Bacteriol.* **172,** 5225–5235.
95. Legraine, M., Mazarin, V., Irwin, S. W., Bouchon, B., Quentin-Miller, M-J., Jacobs, E. & Schryvers, A. B. (1993) *Gene* **130,** 73–80.
96. Ala'Aldeen, D. A., Powell, N. B., Wall, R. A. & Borriello, S. P. (1993) *Infect. Immun.* **61,** 751–759.
97. Stevenson, P., Williams, P. & Griffiths, E. (1992) *Infect. Immun.* **60,** 2391–2396.
98. Lomholt, H., Poulsen, K., Caugant, D. A. & Kilian, M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2120–2124.
99. Morrison, R. P. (1990) in *Chlamydial Infections*, eds. Bowie, W. R., Caldwell, H. D., Jones, R. P., Mardh, P.-A., Ridgway, F. L., Schachter, J., Stamm, W. E. & Ward, M. E. (Cambridge Univ. Press, New York), pp. 163–172.
100. Ward, M. E. (1992) *J. Infect. Dis.* **25,** Suppl. 1, 11–26.
101. Watkins, N. G., Hadlow, W. J., Moos, A. B. & Caldwell, H. D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7480–7484.
102. Morrison, R. P., Belland, R. J., Lyng, K. & Caldwell, H. D. (1989) *J. Exp. Med.* **170,** 1271–1283.
103. Morrison, R. P., Lyng, K. & Caldwell, H. D. (1989). *J. Exp. Med.* **169,** 663–675.
104. Wagar, E. A., Schachter, J., Bavoil, P. & Stephens, R. S. (1990) *J. Infect. Dis.* **162,** 922–927.
105. Toye, B., Laferrière, C., Claman, P., Jessamine, P. & Peeling, R. (1993) *J. Infect. Dis.* **168,** 1236–1240.
106. Toth, M., Jeremias, J., Ledger, W. J. & Witkin, S. S. (1992) *Surg. Gynecol. Obstet.* **174,** 359–362.
107. Patton, D. L. (1985) *Rev. Infect. Dis.* **7,** 746–753.
108. Rank, R. G., Soderberg, L. S. F., Sanders, M. M. & Batteiger, B. E. (1989) *Infect. Immun.* **57,** 706–710.
109. Igietseme, J. U. & Rank, R. G. (1991) *Infect. Immun.* **59,** 1346–1351.
110. Byrne, G. I., Lehmann, L. K. & Landry, G. J. (1986) *Infect. Immun.* **53,** 347–351.
111. Beatty, W. L., Byrne, G. I. & Morrison, R. P. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3998–4002.
112. Martin, D. H., Pollock, S., Kuo, C.-C., Wang, S.-P., Brunham, R. C. & Holmes, K. K. (1984) *Ann. Intern. Med.* **100,** 207–213.
113. Zhong, G. & Brunham, R. C. (1992) *Infect. Immun.* **60,** 3143–3149.
114. Brunham, R. C., Kuo, C.-C., Cles, L. & Holmes, K. K. (1983) *Infect. Immun.* **39,** 1491–1494.
115. Wolner-Hanssen, P., Patton, D. L. & Holmes, K. K. (1991) *Sex. Transm. Dis.* **18,** 21–25.
116. Lamont, H. C., Semine, D. Z., Leveille, C. & Nichols, R. L. (1978) *Infect. Immun.* **19,** 807–813.
117. Brunham, R. C., Peeling, R., Maclean, I., McDowell, J., Persson, K. & Osser, S. (1987) *J. Infect. Dis.* **155,** 749–755.
118. Caldwell, H. D. & Perry, L. J. (1982) *Infect. Immun.* **38,** 745–754.
119. Zhang, Y.-X., Stewart, S., Joseph, T., Taylor, H. R. & Caldwell, H. D. (1987) *J. Immunol.* **138,** 575–581.

120. Zhang, Y.-X., Stewart, S. J. & Caldwell, H. D. (1989) *Infect. Immun.* **57,** 636–638.
121. Qu, Z., Cheng, X., de la Maza, L. M. & Peterson, E. M. (1993) *Infect. Immun.* **61,** 1365–1370.
122. Stephens, R. S., Sanchez-Pescador, R., Wagar, E. A., Inouye, C. & Urdea, M. S. (1987) *J. Bacteriol.* **169,** 3879–3885.
123. Yuan, Y., Zhang, Y.-X., Watkins, N. G. & Caldwell, H. D. (1989) *Infect. Immun.* **57,** 1040–1049.
124. Dean, D., Patton, M. & Stephens, R. S. (1991) *Infect. Immun.* **59,** 1579–1582.
125. Baehr, W., Zhang, Y.-X., Joseph, T., Su, H., Nano, F. E., Everett, K. D. E. & Caldwell, H. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4000–4004.
126. Su, H., Zhang, Y.-X., Barrera, O., Watkins, N. G. & Caldwell, H. D. (1988) *Infect. Immun.* **56,** 2094–2100.
127. Toye, B., Zhong, G., Peeling, R. & Brunham, R. C. (1990) *Infect. Immun.* **58,** 3909–3913.
128. Cheng, X., Pal, S., de la Maza, L. M. & Peterson, E. M. (1992) *Infect. Immun.* **60,** 3428–3432.
129. Su, H. & Caldwell, H. D. (1993) *Vaccine* **11,** 159–166.
130. Stephens, R. S., Wagar, E. A. & Schoolnik, G. K. (1988) *J. Exp. Med.* **167,** 817–831.
131. Su, H., Morrison, R. P., Watkins, N. G. & Caldwell, H. D. (1990) *J. Exp. Med.* **172,** 203–212.
132. Allen, J. E., Locksley, R. M. & Stephens, R. S. (1991) *J. Immunol.* **147,** 674–679.
133. Allen, J. E. & Stephens, R. S. (1993) *Eur. J. Immunol.* **23,** 1169–1172.
134. Su, H. & Caldwell, H. D. (1992) *J. Exp. Med.* **175,** 227–235.
135. Koehler, J. E., Birkelund, S. & Stephens, R. S. (1992) *Mol. Microbiol.* **6,** 1087–1094.
136. Tuffrey, M., Alexander, F., Conland, W., Woods, C. & Ward, M. (1992) *J. Gen. Microbiol.* **138,** 1707–1715.
137. Dean, D., Schachter, J., Dawson, C. R. & Stephens, R. S. (1992) *J. Infect. Dis.* **166,** 383–392.
138. Danilition, S. L., MacLean, I. W., Peeling, R., Winston, S. & Brunham, R. C. (1990) *Infect. Immun.* **58,** 189–196.
139. Raulston, J. E., Davis, C. H., Schmiel, D. H., Morgan, M. W. & Wyrick, P. B. (1993) *J. Biol. Chem.* **268,** 1–9.
140. Taylor, H. R., MacLean, I. W., Brunham, R. C., Pal, S. & Whittum-Hudson, J. (1990) *Infect. Immun.* **58,** 3061–3063.
141. Murdin, A. D., Su, H., Manning, D. S., Klein, M. H., Parnell, M. J. & Caldwell, H. D. (1993) *Infect. Immun.* **61,** 4406–4414.

Quest for Life-Long Protection by Vaccination

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Vaccines represent one of our most powerful and cost-effective prevention tools. Successful vaccination programs have led to marked reductions in disease, disability, and death (1). The successes of our current vaccination efforts raise the question of how we can eliminate the disease burdens that remain.

THE IDEAL VACCINE

The ideal vaccine is one that is stable (i.e., will maintain potency under most environmental conditions), safe, administered orally in a single dose at birth, 100% effective, reasonably priced, and protective for a lifetime. Unfortunately, there is no such vaccine. Some of the vaccines currently available have one or more of these ideal characteristics, but no single vaccine has all of them. The goal of future research is to obtain vaccines as close to these ideal characteristics as possible. Such research should be targeted toward a better understanding of the host and pathogen interactions as well as the immunology of candidate vaccines.

Successful use of a vaccine requires widespread acceptance by the

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public, the health-care providers, and the public-health community. Such acceptance is enhanced the closer the vaccine comes to meeting the ideal set of characteristics previously mentioned. Much discussion about vaccines is devoted to assuring efficacy, but safety is equally as important. Widespread acceptance of vaccines can only be assured if the public believes vaccines will not harm them. Concerns about vaccine safety led to marked drops in pertussis vaccine coverage in Japan and the United Kingdom in the 1970s, with resultant major epidemics of whooping cough (2, 3). The quest for life-long protection needs to be accompanied by a quest for completely safe products.

Even though no vaccine today is perfectly safe or perfectly effective, with the right strategy less-than-perfect vaccines can be powerful prevention tools. Diseases can be eradicated with a strategy for vaccine use tailored to both the characteristics of the vaccine and the epidemiology of the disease.

PROTECTION THROUGH VACCINATION

Life-long protection from disease through vaccination can be accomplished in two ways: (*i*) individual protection—assuring a life-long immune response capable of repelling challenges individuals may receive at any time in their lives, and (*ii*) community protection—reducing or even eliminating the possibility that nonimmune individuals will be exposed to the infectious agent.

All vaccines are given to protect individuals. Individual protection is the only way to assure life-long protection against certain diseases with inanimate or animal reservoirs for infectious agents, such as tetanus and rabies (4, 5). For these diseases, vaccinated humans do not help to protect unvaccinated humans. All susceptible persons are at risk, and that risk is not modified by reducing the number of susceptibles through vaccination. Individual protection is also the only protection available when vaccines are recommended for selected populations whose behaviors place them at increased risk of disease exposure. These are vaccines needed for international travel, special life-styles, selected occupations, and other special uses. Included among these are Japanese encephalitis (6), yellow fever (7), typhoid (8), adenoviruses 4 and 7 (9), anthrax (9), cholera (10), rabies (11), and meningococcal (12) vaccines.

While influenza and pneumococcal vaccines are recommended for all adults ≥65 years of age and selected others with high-risk medical conditions, these vaccines do not substantially reduce population exposure to the organism (13, 14).

For each of these diseases, temporary or life-long protection can only be obtained through vaccination of each individual at risk. The ability to

induce lasting protection varies widely for available vaccines. Live attenuated viral vaccines are generally believed to induce long-term, probably life-long, protection among the great majority of individuals (9). Measles, mumps, rubella, and oral polio vaccines are in this category. Inactivated vaccines and toxoids usually induce shorter-term protection and require periodic boosters. Experience with a number of the newer inactivated vaccines, such as hepatitis B vaccine and enhanced-potency inactivated polio vaccine, is too limited to know the precise duration of immunity (15, 16). Immunologic memory, even in the absence of detectable antibody, may confer life-long protection.

Research is proceeding into new ways of developing vaccines that will lead to longer-lasting immunity. An example includes the incorporation of genes coding for key antigens of pathogens in live vectors that do not cause disease (such as vaccinia) (17). Microencapsulation of antigens in polymers, which can lead to either sustained release or pulsed release of those antigens over prolonged periods, also offers promise (18).

Most discussions of life-long protection focus on individual protection. This is an important consideration for any vaccine. However, we as a society derive far more benefit by seeking protection for all by eliminating exposure to infectious diseases.

For diseases transmitted solely from human to human, prevention of exposure can be accomplished by creating high levels of population "herd" immunity (19). Transmission of diseases such as measles is sustained when a transmitting case comes in contact with a susceptible person. After an incubation period, the susceptible person becomes contagious and the chain of transmission is maintained. The chain of transmission is broken if the transmitting case comes in contact only with immune individuals.

When there are high levels of immunity in the population, the likelihood that a transmitting case will come in contact with a susceptible person is reduced, thereby resulting in indirect protection of the few remaining susceptibles, including those too young for vaccination, those with legitimate contraindications to vaccination, and those whose vaccination fails to protect them. While there is no absolute level of population immunity short of 100% that will guarantee elimination of disease transmission, it is clear that the higher the level of immunity, the lower the probability of significant transmission (19).

For example, use of a cellular pertussis vaccines in Japan among 2-year-old children led to marked reductions of pertussis among younger children who were not targets for vaccination (20). Similar effects have been seen in the United States with *Haemophilus influenzae* type b and measles (21, 22). Any effort to induce life-long immunity requires that vaccines be used and used widely among targeted groups.

RESULTS WITH CURRENT VACCINES

It would be useful to review how well we have protected both the individual and the community with the currently available vaccines.

In the United States, there are nine vaccines routinely recommended for all children: diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, measles, mumps, rubella, hepatitis B, and polio (15, 23–28). Of these, all but tetanus toxoid can lead to community protection from widespread use, reducing disease transmission through herd immunity.

Vaccination of children with these vaccines has yielded spectacular results. Shortly after the measles vaccine was introduced in 1963, measles decreased from an annual average of >500,000 cases reported in the decade preceding licensure to <25,000 cases in 1968. Since 1968, there has been a further decrease in incidence, broken by three periods of increased incidence. Although small by comparison to the incidence before the introduction of the vaccine, they were, nevertheless, serious. During the last prolonged epidemic, >55,000 cases, 11,000 hospitalizations, and 130 deaths were recorded (CDC, unpublished data).

Rubella has shown an equally steep decline, from nearly 60,000 cases reported at the time the vaccine was licensed in 1969 to only a few hundred in recent years. As with measles, there are very few periods of increases in cases of rubella, the most recent coming during 1990 and 1991.

Mumps has shown a similar decline from 1968 to 1992.

There have been no cases of paralytic polio caused by indigenously acquired wild viruses since 1979 in the United States. There has been an annual average of approximately 8 vaccine-associated polio cases (29).

The figures for the first half of 1993 show considerable improvement over the same period for 1992, with decreases in reported measles cases of >90%. With the exception of pertussis, tetanus, and rubella, all other reported cases are going in the right direction—down. The increases in tetanus and rubella are small, following record lows in 1992. We would like to think that these decreases reflect the major vaccination effort between 1989 and 1991, particularly for measles, but it is unlikely that we can take full credit. Epidemics run their course, often without the help of vaccines.

Clearly, vaccination of children has been an enormously successful public health strategy. Young parents today do not have the memories of childhood infectious diseases that parents of 40 years ago have. By all criteria, as a nation, we have accomplished even more than we dared dream when many of these vaccines were first introduced. Much of our population has never experienced these childhood infectious diseases for which vaccines are available. However, the elimination of these childhood diseases is still an elusive goal. Our job is incomplete.

Nearly all children have received the appropriate vaccines prior to entry into school or other organized settings, primarily as a result of state laws. Of children at school entry, >95% have received appropriate vaccines. Vaccination levels for children enrolled in licensed day-care or Head Start exceed 90%. These findings do not apply to children under age 2.

The most recent data from the National Center for Health Statistics in 1991 reveal that nationally, among children 19–35 months of age, coverage rates for the recommended vaccines were as follows: polio (three or more doses), 53%; DTP (three or more doses), 69%; and MMR (one dose), 82%. (DTP is diphtheria/tetanus toxoid/pertussis vaccine; MMR is measles/mumps/rubella vaccine.) Just before the measles outbreak in 1989, full vaccination coverage was <50% in some urban areas—a percentage far below that of many developing countries. Children in Cairo, Bangkok, and Bombay have better vaccination rates today than those in the inner cities of Houston or New York. Although none of the childhood vaccines have 100% efficacy, a vaccine not given has a 0% efficacy.

This low vaccination coverage rate for preschool children in the United States is not new (30). Increasing the vaccine usage in this country has been a constant challenge. At the time of the passage of the first Federal Vaccination Assistance Act in 1962, only about two-thirds of the children under 5 years of age had received each of the recommended series of vaccines.

How have we, certainly one of the most affluent countries in the world, allowed this to happen? There are no simple answers, but three points should be made. First, vaccination of the approximately 4 million children added to the United States population each year is no simple task. Full protection before 2 years of age requires that each child receive a complicated series of 14–15 doses of vaccines in four to five visits to a health care provider (15, 23–28). Second, unlike school entry, where there are laws in each state requiring proof of appropriate vaccination, there are no laws requiring vaccination of all infants. Aside from children entering licensed day-care or Head Start, vaccination is voluntary. Third, unlike most other developed and many developing countries, the United States does not have a structured system of health care that assures parents access to providers (31). Achieving high levels of coverage is further hampered by the lack of a system of record keeping that would allow providers knowledge about the immunization status of a new patient.

In regard to the first point, combining vaccines and simplifying the dosage schedule for children <2 years of age would help considerably. Not only does the 15-dose, four- to five-visit schedule impose a major economic burden on many parents, the three to four injections per visit poses pain for the child, and anxiety for the parent and the health care

provider. The complexity of the schedule leads to parental misunderstanding and confusion, with high dropout rates, missed vaccination opportunities, and higher societal costs.

The probability is high that still more vaccines will be licensed for children. The number of vaccines has become a limiting factor.

Multinational organizations have recognized the need for more efficient delivery of new and better vaccines for developing countries and in 1990 made a commitment to the production and delivery of easily administered and affordable children's vaccines. The result of that commitment is the Children's Vaccine Initiative (CVI) established by the United Nations Children's Fund (UNICEF), The United Nations Development Program (UNDP), The Rockefeller Foundation, The World Bank, and The World Health Organization (WHO). The CVI concept is largely directed toward developing countries, with special needs for an expanding number of combined, low-cost, and heat-stable vaccines.

The recent Institute of Medicine report *The Children's Vaccine Initiative 1993* describes the advantages that would accrue to the United States through support of The Children's Vaccine Initiative (32).

Children from developed, as well as developing, countries can greatly benefit from fewer and less expensive injections. It is heartening to learn that one United States manufacturer is now marketing a combined DTP and Hib (*Haemophilus influenza* b) vaccine and that several manufacturers are developing other combinations that will reduce the number of injections.

Even without such combinations, the United States has demonstrated that it can vaccinate the vast majority of its children when immunization is linked to something more valuable—i.e., requirement for entry to school (33). In those settings, barriers to immunization are overcome. The need for school laws to assure immunization of schoolchildren has led some to feel that we will reach our goals for 2-year-old children only if there are also laws requiring the vaccination of preschool children. It is not clear how such laws would be written or how they might be enforced if passed. In any event, there seems to be no visible legislative support for such laws at present.

As to the final point, the absence of a single health infrastructure to assure vaccination of those <2 years of age means that the full responsibility for vaccination rests with the parents, who are frequently poorly informed about the needs for early vaccination. In contrast to the United Kingdom, where providers are held accountable for immunizing their patients, most providers in the United States do not receive rewards or suffer consequences depending upon the immunization status of their patients (34).

Congress and the current administration are determined to close the

vaccination gap. The President's budget for 1994 contains funds for increased purchasing of vaccines, building state and local infrastructure for vaccine delivery, and conducting public education and outreach. The goal for 1996 is 90% vaccine coverage for most of the recommended vaccinees and zero incidence of most of the vaccine-preventable childhood diseases.

DISEASE ERADICATION THROUGH VACCINATION

Thus, in our quest for life-long protection by vaccination, our first responsibility is to a better use of the vaccines that we already have.

The ultimate goal of community protection is disease eradication. The most effective means of providing life-long protection through vaccination is elimination of the infectious agent. Several factors related to disease and vaccines make the eradication of an infectious disease possible. Diseases that should be the easiest to eradicate with effective vaccines are characterized by person-to-person transmission, humans as the only natural host, absence of an inanimate reservoir, limited duration of shedding of the organism when infected, no long-term carrier state, and a distinctive, clinically apparent syndrome to facilitate case detection and response. Appropriate vaccines should be characterized by high efficacy at preventing not only disease but also infection and transmission. Such vaccines should be easy to use and stable and should provide long-term duration of protection with a limited number of doses. Finally, and critically, there must be commitment to eradication at both the international and national levels. Some of the viral diseases for which vaccines are available have many of the biological characteristics desirable for eradication, although the commitment of the international community remains a challenge.

Smallpox vaccine represents an ideal example of life-long protection. The story of the eradication of smallpox is well known (35). The World Health Organization (WHO) Smallpox Eradication Program ran from 1966 to 1980. The last case of smallpox occurred in 1977 in Somalia.

When the WHO eradication program began in 1967, smallpox was endemic in 33 countries, with an estimated 10–15 million cases and 2 million deaths. Perhaps less well known was the high human toll associated with routine vaccination against smallpox. In the United States in 1968 alone, 9 people died from complications of primary vaccination; >8000 had complications requiring medical attention, and >200 were hospitalized, 4 being permanently disabled (36). Untold numbers of other vaccinees had adverse reactions not requiring medical attention.

The smallpox vaccine was not ideal. Adverse reactions were common, and the duration of protection was limited. Eradication was achieved

despite these shortcomings through a strategy tailored specifically to the epidemiology of smallpox—targeted vaccination in the immediate vicinity of cases otherwise known as "ring vaccination" or "search and containment" (35). Eradication eliminated not only a serious infectious disease but also eliminated the need to use a vaccine that had accompanying adverse reactions.

The total U.S. contribution to the smallpox eradication campaign was $32 million, an investment that has been returned every 2.5 months since 1971 (37). The discontinuation of smallpox vaccination in the United States resulted in a direct savings since 1983 of >3 billion dollars (J. D. Sencer and N. W. Axnick, cited in ref. 35, p. 1365). These savings continue to accrue day after day and year after year, thus making these funds available for other pressing public health needs.

Today the debate focuses on the final destruction of smallpox virus stocks held by the Centers for Disease Control and Prevention (CDC) in Atlanta and the Institute of Viral Preparations in Moscow.

Ten years after the last smallpox case, the World Health Assembly called for eradication of another virus disease, polio (39, 40). The target dates were for the American region by 1990, the western Pacific and European regions by 1995, and worldwide by 2000.

Through the efforts of WHO's Expanded Programme on Immunization, donor agencies, multinational organizations, and individual countries, an estimated 84% of the infants born in 1990 were immunized against polio. The decreases in the number of cases of polio reported to WHO, although considered a fraction of the total cases, have been encouraging.

The WHO estimate of the number of cases of paralytic polio has been reduced from >400,000 annually in 1980 to 127,000 annually today. China, for example, reported a 58% decrease in cases between 1989 and 1991. Polio-free zones are beginning to emerge in North Africa, southern and eastern Africa, the Middle East, Europe, and the Pacific Rim. In the Western Hemisphere, the last case of paralytic polio caused by a wild virus was identified in 1991 (41, 42).

There are still enormous challenges ahead. India, Pakistan, and Bangladesh account for two-thirds of the cases reported to WHO. The Indian subcontinent will remain a reservoir of polio, reinfecting other countries, unless major progress is made over the next few years. There are also the ever-present wars and civil disruptions that interfere with the expanded immunization programs.

Much more help is needed from the developed world if the eradication effort is to succeed. Specific polio eradication efforts, including vaccine production and distribution, disease surveillance, and laboratory networks cost money. However, full investment in the effort to eradicate polio could pay enormous dividends. In the United States, eradication would

mean discontinuing the use of the vaccine, resulting in savings of approximately $110 million per year in vaccine costs alone—independent of administration costs. Eliminating the use of oral vaccine would also eliminate the annual average of eight or so vaccine-associated cases of poliomyelitis that occur in the United States each year (29).

In fact, with time, there is no good reason why any of the current childhood viral diseases for which vaccines are available could not be eradicated. Measles, while potentially most difficult to eradicate as a result of its high communicability, deserves highest priority because of its substantial health consequences (43, 44). Before vaccination, WHO estimated measles caused 2.5 million deaths annually, most of which were in the developing world.

Measles decreases with high levels of vaccination. An example includes the apparent termination of measles transmission recently in much of the United States, where only 167 cases were reported during the first 26 weeks of 1993—the lowest total ever reported (45). Innovative strategies may be needed, but there is promise. For example, mass campaigns vaccinating all children between 9 months and 14 years of age (regardless of prior vaccination history) in the English-speaking Caribbean and multiple Latin American countries have led to virtual elimination in many areas (46).

Hepatitis B must also be considered a potentially eradicable disease, despite the high carrier rates in many developing countries. In the United States, there are an estimated 1 to 1.25 million chronic hepatitis B carriers (15). Eradication will require a lifetime, or maybe two, but it, too, is doable.

Experience today with hepatitis B virus vaccine has been encouraging. Universal immunization of an Alaskan (47) population with a virus carrier rate in some groups of >30% reduced the number of clinical hepatitis B cases to zero in <5 years.

Results of childhood hepatitis B virus vaccination programs in several countries where hepatitis B virus infection is highly epidemic are very encouraging. In Taiwan (48), perinatal and routine infant hepatitis B vaccination have resulted in a decline of chronic hepatitis B infection identified among children from 10% to 2.2% after 5 years. In Gambia (49), the overall prevalence of hepatitis B infection among children declined from 53% to 9%, and the prevalence of chronic hepatitis B declined from 12% to 0.5% within 6 years after the introduction of routine infant hepatitis B vaccination. In American Samoa (38), prevalence of hepatitis B infection among children declined from 23% to 11.5%, and prevalence of chronic hepatitis B declined from 7% to 1.5% within 4 years after the implementation of perinatal, routine infant, and catch-up hepatitis B vaccination programs.

In conclusion, the ultimate goal for vaccination is not simply long-term protection of the individual but also long-term protection of all individuals. The epidemiology of many infectious diseases allows for societal protection when high levels of immunity in the population are achieved, even if that immunity level is not 100%. But those high levels of immunity must be sustained with high dollar costs to society. Any reduction in effort or funding can lead to return of disease. Instead, our objective should be eradication of the infectious agent whenever feasible. The quest is not simply for better vaccines. The quest that will lead us to our goal will be through a better understanding of the epidemiology of vaccine-preventable diseases and an elucidation of the strategies for vaccine use. Any disease of sufficient public health importance to warrant routine vaccination is also of sufficient importance to warrant reasonable attempts at eradication.

SUMMARY

Life-long protection from disease through immunization can be accomplished through individual or community protection. Individual protection is the goal for vaccination against diseases that have inanimate or animal reservoirs or that pose risks for certain populations. Community protection is the goal for vaccination against diseases that are transmitted only from human to human. Community protection afforded by childhood vaccines has been highly successful against measles, rubella, mumps, and polio. However, outbreaks of measles, rubella, and mumps continue to occur, primarily because of inadequate immunization of children under age 2. Simplification of vaccination regimens, provision of incentives to care providers and parents, and increased access to care should improve vaccination rates in the United States. Better protection requires better use of available vaccines. Eradication of disease through vaccination is the ultimate goal of community protection. Elimination of the infectious agent is the most effective means of achieving life-long protection. The World Health Organization's (WHO) smallpox eradication campaign eliminated a serious disease as well as the need for a vaccine with frequent and severe adverse reactions. The discontinuation of smallpox vaccination in the United States has produced a savings of over $3 billion. Polio has been targeted by WHO for eradication by the year 2000. The eradication of polio and the elimination of the need for polio vaccination in the United States should result in a savings of $110 million per year in vaccine costs alone. Strong United States support is crucial for WHO to reach its goal. Any of the vaccine-preventable childhood virus diseases could be eradicated with sufficient national and international will. Measles and hepatitis B

should be high priorities. The ultimate goal of vaccination is life-long protection of all individuals. Any disease of sufficient public health importance to warrant routine vaccination is of sufficient importance to warrant eradication wherever judged to be possible.

REFERENCES

1. Orenstein, W. A. (1993) *Future Directions*, 26th National Immunization Conference Proceedings, June 1–5, 1992, St. Louis (Centers for Disease Control and Prevention, Atlanta), pp. 13–22.
2. Cherry, J. D. (1984) *Curr. Probl. Pediatr.* **14**, 1–78.
3. Kimura, M., Kuno-Sakai, H. (1990) *Lancet* **336**, 30–32.
4. Smith, L. D. (1978) *Health Lab. Sci.* **15**, 74–80.
5. Anderson, L. J., Nicholson, R. G., Tauxe, R. V. & Winkler, W. G. (1984) *Ann. Intern. Med.* **100**, 728–735.
6. Centers for Disease Control and Prevention (1993) *Morbid. Mortal. Wkly. Rep.* **42** (RR-1), 1–15.
7. Centers for Disease Control and Prevention (1990) *Morbid. Mortal. Wkly. Rep.* **39** (RR-6), 1–6.
8. Centers for Disease Control and Prevention (1990) *Morbid. Mortal. Wkly. Rep.* **39** (RR-10), 1–5.
9. Hinman, A. R., Orenstein, W. A., Bart, K. J. & Preblud, S. R. (1990) in *Principles and Practice of Infectious Diseases Immunization* , eds. Mandell, G. L., Douglas, R. G., Jr., & Bennett, J. E. (Churchill Livingstone, New York), 3rd Ed., pp. 2320–2334.
10. Centers for Disease Control and Prevention (1988) *Morbid. Mortal. Wkly. Rep.* **37**, 617–624.
11. Centers for Disease Control and Prevention (1991) *Morbid. Mortal. Wkly. Rep.* **40** (RR-3), 1–19.
12. Centers for Disease Control and Prevention (1985) *Morbid. Mortal. Wkly. Rep.* **34**, 255–259.
13. Centers for Disease Control and Prevention (1993) *Morbid. Mortal. Wkly. Rep.* **42** (RR-6), 1–14.
14. Centers for Disease Control and Prevention (1989) *Morbid. Mortal. Wkly. Rep.* **38**, 64–68, 73–76.
15. Centers for Disease Control and Prevention (1991) *Morbid. Mortal. Wkly. Rep.* **40** (RR-13), 1–25.
16. Centers for Disease Control and Prevention (1987) *Morbid. Mortal. Wkly. Rep.* **36**, 795–798.
17. Anonymous (1985) *Bull. WHO* **63**, 471–477.
18. Eldridge, J. H., Hammond, C. J., Meulbroek, J. A., Staas, J. K., Gilley, R. M. & Tice, T. R. (1990) *J. Controlled Release* **11**, 205–214.
19. Anderson, R. M. & May, R. M. (1982) *Science* **215**, 1053–1060.
20. Noble, G. R., Bernier, R. H., Esber, E. C., Hardegree, M. C., Hinman, A. R., Klein, D. & Saah, A. J. (1987) *J. Am. Med. Assoc.* **257**, 1351–1356.
21. Adams, W. G., Deaver, K. A., Cochi, S. L., Plikaytis, B. D., Zell, E. R., Broome, C. V. & Wenger, J. D. (1993) *J. Am. Med. Assoc.* **269**, 221–226.
22. Schlenker, T. L., Bain, C., Baughman, A. L. & Hadler, S. C. (1992) *J. Am. Med. Assoc.* **267**, 823–826.

23. Centers for Disease Control and Prevention (1991) *Morbid. Mortal. Wkly. Rep.* **40** (RR-10), 1–28.
24. Centers for Disease Control and Prevention (1991) *Morbid. Mortal. Wkly. Rep.* **40** (RR-1), 1–7.
25. Centers for Disease Control and Prevention (1989) *Morbid. Mortal. Wkly. Rep.* **38** (S-9), 1–13.
26. Centers for Disease Control and Prevention (1989) *Morbid. Mortal. Wkly. Rep.* **38**, 388–392, 397–400.
27. Centers for Disease Control and Prevention (1982) *Morbid. Mortal. Wkly. Rep.* **31**, 22–26, 31–34.
28. Centers for Disease Control and Prevention (1990) *Morbid. Mortal. Wkly. Rep.* **39** (RR-15), 1–18.
29. Strebel, P. M., Sutter, R. W., Cochi, S. L., Biellik, R. J., Brink, E. W., Kew, O. M., Pallansch, M. A., Orenstein, W. A. & Hinman, A. R. (1992) *Clin. Infect. Dis.* **14**, 568–579.
30. Stehr-Green, P. A., Zell, E. R. & Eddins, D. L. (1993) *J. Am. Med. Assoc.* **270**, 1932.
31. The National Vaccine Advisory Committee (1991) *J. Am. Med. Assoc.* **266**, 1547–1552.
32. Mitchell, V. S., Philipose, N. M. & Sanford, J. P. (1993) *The Children's Vaccine Initiative* (Inst. of Medicine, Natl. Acad. Press, Washington, DC), pp. 1–221.
33. Orenstein, W. A., Hinman, A. R. & Williams, W. W. (1992) in *The Impact of Legislation on Immunisation in the United States*, Proceedings of the Second National Immunisation Conference, Canberra, 27–29 May 1991, Canberra, Australia (Public Health Assoc. of Australia, Canberra), pp. 58–62.
34. Salisbury, D. (1992) in *The Immunization Program in England*, 25th National Immunization Conference Proceedings, June 10–13, 1991, Washington, DC (Centers for Disease Control and Prevention, Atlanta), pp. 49–52.
35. Fenner, F., Henderson, D. A., Arita, I., Jazek, Z. & Ladnyi, I. D. (1988) *Smallpox and Its Eradication* (World Health Org., Geneva).
36. Sencer, J. D. & Axnick, N. W. (1973) in *International Symposium on Vaccination Against Communicable Diseases, Monaco, March 14–17, 1973*, Immunobiological Standardization Symposia Series, ed. Perkins, F. T. (Karger, Basel), Vol. 22, pp. 37–46.
37. Ogden, H. G. (1987) in *CDC and the Smallpox Crusade*, DHHS Publ. No. (CDC) 87-8400 (Centers for Disease Control, Atlanta), pp. 1–141.
38. Mahoney, F. J., Woodruff, B. A., Erben, J. J., Coleman, P. J., Reid, E. C., Schatz, G. C. & Kane, M. A. (1993) *J. Infect. Dis.* **167** , 203–207.
39. Wright, P. F., Kim-Farley, R. J., DeQuadros, C. A., Robertson, S. E., Scott, R. McN., Ward, N. A. & Henderson, R. H. (1991) *N. Engl. J. Med.* **325**, 1774–1779.
40. Hinman, A. R., Foege, W. H., DeQuadros, C. A., Patriarca, P. A., Orenstein, W. A. & Brink, E. W. (1987) *Bull. WHO* **65**, 835–840.
41. DeQuadros, C. A., Andrus, J. K., Olive, J. M. & deMacedo, C. G. (1992) *Annu. Rev. Public Health* **13**, 239–252.
42. DeQuadros, C. A., Andrus, J. K., Olive, J. M., Da Silveira, C. M., Eikhof, R. M., Carrasco, P., Fitzsimmons, J. W. & Pinheiro, F. P. (1991) *Pediatr. Infect. Dis.* **10**, 222–229.
43. Hopkins, D. R., Hinman, A. R., Koplan, J. P. & Lane, M. J. (1982) *Lancet* **i**, 1396–1398.
44. Hinman, A. R. (1982) *Rev. Infect. Dis.* **4**, 933–936.

45. Centers for Disease Control and Prevention (1993) *Morbid. Mortal. Wkly. Rep.* **42**, 813–816.
46. Pan American Health Organization (1990) *Bull. Pan Am. Health Org.* **24**, 240–246.
47. Wainwright, R. B., McMahon, B. J., Bulkow, L. R., Parkinson, A. J., Harpster, A. P. & Hadler, S. C. (1991) in *Proceedings of the 1990 International Symposium on Viral Hepatitis and Liver Disease: Contemporary Issues and Future Prospects*, eds. Hollinger, F. B., Lemon, S. M. & Margolis, H. (Williams & Wilkins, Baltimore), pp. 762–765.
48. Tsen, Y. J., Chang, M. H., Hsu, H. Y., Lee, C. Y., Sung, J. L. & Chen, D. S. (1991) *J. Med. Virol.* **34**, 96–99.
49. Whittle, H. C., Inskip, H., Hall, A. J., Mendy, M., Downes, R. & Hoare, S. (1991) *Lancet* **337**, 747–750.

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