Altered Immune Function Associated with Neurophysiologic Abnormalities and Executive Function Deficits in Children with Autism Spectrum Disorders

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ABSTRACT

Recent evidence suggests that deficient executive functions are fundamental to the cognitive deficits in Autism spectrum disorders (ASD). It has been suggested that individuals with ASD have disrupted neural connectivity including that in the frontal lobes that mediate executive functions. With reports of immunologic abnormalities in children with ASD, it is plausible that such abnormalities disrupt the neural connectivity in the brains of individuals with ASD. There is, however, relatively little empirical evidence to support the notion. This dissertation reports on three studies to examine whether the executive dysfunction in children with ASD is associated with their immunologic abnormalities and disordered neural connectivity.

In study one, the executive functioning of 19 high-functioning (HFA) and 19 low-functioning (LFA) children with ASD were compared to 28 children with normal development using a battery of neuropsychological tests. Results not only confirmed previous knowledge that children with ASD had significant executive dysfunctions compared with children with normal development, but also extended it to show that LFA children were significantly more impaired than HFA children. Study two built on this knowledge and examined whether immunological abnormalities are associated with the differential executive dysfunctions in 18 HFA and 19 LFA children. Results indicated that LFA children showed greater executive dysfunctions as well as higher

levels of total lymphocyte, T lymphocyte and suppressor/cytotoxic T lymphocyte levels than HFA children. In addition, executive dysfunctions were significantly associated with the three lymphocyte levels, lending support to the notion that immunological factors may play a role in the cognitive dysfunctions in individuals with ASD. Study three further examined whether the differential executive dysfunctions and immunologic levels in LFA and HFA children are associated with their neural connectivity. Results on 17 HFA and 14 LFA children showed that LFA children had significantly elevated theta coherence in the anterior network, as well as at the left *intra-hemispheric* and right-to-left *inter-hemisphere* connections than HFA children. LFA children also had significantly elevated immunologic level specifically in suppressor/cytotoxic T lymphocytes. Furthermore, the executive dysfunctions, disordered neural connectivity, and abnormal immunologic levels were found to be associated.

These findings have provided some initial evidence to support the notion that immunologic factors may play a role in causing neuronal damage in the anterior region of the brains of children with ASD, which is manifested in their disordered neural connectivity of that region, and their executive dysfunctions mediated by that same region.

摘要

有研究顯示自閉症兒童認知功能障礙源於大腦的神經連接失調，引致包括前額葉運作失衡而繼然導致行政功能欠效率。有報告顯示很多患有自閉症之人士亦同時患有免疫系統異常。所以有專家建議免疫系統異常很可能是引致自閉症兒童大腦神經連接失調的原因。但這一概念尚未有太多證據支持。此論文報告的三項研究旨在探討自閉症兒童執行功能障礙，是否與其免疫功能及神經連接異常有關。

實驗(一)，28 位發展正常及 19 位高功能和 19 位低功能自閉症的兒童參與此項研究。此實驗使用一系列神經心理學測試來評定發展正常及自閉症的兒童在行政功能的差別。結果不僅證實自閉症兒童比發展正常兒童有行政功能缺損，還發現低功能自閉症兒童比較高功能自閉症兒童在行政功能上有更大的缺損。實驗(二)探討高功能和低功能自閉症兒童在行政功能上的差異是否與免疫系統異常有關。此實驗有 18 位高功能和 19 位低功能自閉症的兒童參與。結果顯示與高功能自閉症兒童相比，低功能自閉症兒童有較多數量的淋巴細胞、T-淋巴細胞和 T-抑制性淋巴細胞。此外，自閉症兒童在行政功能上的差異亦與免疫系統功能有明顯關聯。實驗(三)進一步探討低功能自閉症兒童與高功能自閉症兒童在行政功能上的缺損及其免疫系統異常，是否與他們神經連接互相關聯。在此項研究的 17 位高功能和 14 位低功能自閉症的兒童中，結果顯示低功能自閉症兒童的θ波段腦

電同步指數比高功能自閉症兒童，在前額葉、左腦及前額葉與後方腦部位都高。而 T-抑制性淋巴細胞於低功能自閉症兒童體內亦有顯著提升。此外，自閉症兒童免疫系統功能異常，與過高的腦電同步狀況及行政功能缺損亦有明顯關係。

這些研究結果提供了一些初步的證據，以支持免疫因素可能導致自閉症兒童神經連接的缺失，及與其相關的大腦前額葉功能上異常，而導致行政功能上缺損的說法。

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PREFACE

The studies described in this dissertation examined the executive function deficits in children with autism spectrum disorders, and the contribution of abnormal immunologic function to the disordered neural connectivity and impaired executive functions in these children. This dissertation has been organized around freestanding chapters which are suitable for publications.

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PUBLICATIONS

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CONTENTS

CHAPTER ONE

GENERAL INTRODUCTION

Altered Immune Function Associated with Neurophysiologic Abnormalities and Executive Function Deficits in Children with Autistic Spectrum Disorders

Autistic spectrum disorders (ASD) is a group of complex neurodevelopmental disorder characterized by disturbances in verbal and non-verbal communication, poor social skills, and an abnormal repertoire of stereotyped behaviors (American Psychiatric Association, 2002). Autism, Asperger syndrome, and pervasive developmental disorder not otherwise specified (PDD-NOS) are the three main forms of disorders in the spectrum. Autism is the core disorder of ASD, Asperger syndrome is diagnosed when the individual does not have significant delay in cognitive and language development, and PDD-NOS is diagnosed when the individual does not meet the full criteria for a more specific disorder in the spectrum (Johnson, Myers, & Council on Children with Disabilities, 2007). Variability in the degree of language impairment, intellectual ability, and symptom severity was found among individuals with ASD (Gillberg, 1993; Wing, 1997). Some studies have estimated that about 70% of individuals with autism are mentally retarded (IQ below 70) (Fombonne, 2005; Wong, 2004). The proportion with comorbid mental retardation reduces substantially, however, when individuals with more broadly defined ASD are included (Chakrabarti & Fombonne, 2001). ASD is more common in boys, with a mean male to female ratio of 4.3:1

(Fombonne, 2003). Many individuals with ASD also have other medical conditions, with epilepsy being the most common. It was estimated that more than 17% of individuals with ASD also have epilepsy (Fombonne, 2003). Prevalence of ASD is high, with recent statistics reported by the centers for Disease Control (CDC) in U.S. at one in 150, making ASD one of the most prevalent childhood developmental disorders (Fombonne, 2005). Evidence also shows that there has been a progressive increase worldwide during recent years (Baird et al., 2000; Bertrand et al., 2001), probably as a result of increased recognition and changes in diagnostic criteria (Charman, 2002). In spite of the high prevalence rate, the causes of ASD have not been well-understood and no cure has yet been found. To date, the major form of intervention for ASD remains to be the reduction and management of symptoms. Research has unequivocally shown that early intervention is critical in improving the functioning of children with ASD (Chakrabarti & Fombonne, 2001; Chan, Sze & Cheung, 2007). To pave the way for effective intervention for ASD, it will be helpful to gain an understanding of the neural basis and the possible causes underlying the pervasive behavioral and cognitive dysfunctions such that more effective interventions can be done.

Executive Functions in ASD

Despite the fact that the causes of ASD have not been well-understood, there is evidence suggesting that deficient executive function is fundamental to the cognitive deficits in ASD (Gilotty, Kenworthy, Sirian, Black & Wagner, 2002; Ozonoff, 1997). Executive function is a broadly defined cognitive domain that includes a multidimensional set of abilities required to perform complex behaviors for the attainment of a future goal (Donders, 2002; Nyden, Gillberg, Hjelmquist, & Heiman, 1999). It is generally understood to be an umbrella term covering a number of distinct but related cognitive processes such as planning, cognitive flexibility, generativity, self-monitoring, and inhibition of inappropriate actions (Ozonoff et al., 2004; Pennington & Ozonoff, 1996). Executive dysfunction can be seen to underlie many of the key characteristics of individuals with ASD (Schmitz et al., 2006), which includes disorganized actions and strategies typified by decreased initiative, perseveration, difficulties in forming novel concepts, lack of judgment and insight, and interacting purposefully with the environment (Bennetto, Pennington & Rogers, 1996; Ozonoff et al., 2004). These individuals often appear rigid and inflexible. They are often perseverative and show a strong liking for repetitive behavior and elaborate rituals (Hill, 2004). Many children with ASD follow routines in precise details and would show great distress over trivial

changes in the environment (Ozonoff, Pennington, & Rogers, 1991). Since executive function is not a unitary construct (Bishop & Norbury, 2005) and not all of its subdomains are affected in individuals with ASD (Griffith, Pennington Wehner, & Rogers, 1999), numerous attempts have been made to delineate the specific executive deficits in ASD (Geurts, Verte, Oosterlaan, Roeyers, & Sergeant, 2004).

Measures of Executive Functioning

Neuropsychological studies of executive functions in ASD, however, have been inconclusive, with some reporting deficits in planning and cognitive flexibility but not in inhibition (Ozonoff & Jensen, 1999), while others suggested difficulties in response inhibitory control and slow information processing (Hughes, Russell, & Robbins, 1994; Nyden et al., 1999; Schmitz et al., 2006). The different findings in these studies might be due to the lack of gold standard for measurement of executive functions, and the methodological differences in the studies have made it difficult to compare the findings (Liss et al., 2001; Wong, 2004). Some researchers have further posited that low- and high-functioning individuals with ASD may have distinct primary deficits that underlie their executive dysfunctions, as indicated by the different types of repetitive, stereotyped behaviours exhibited among them (Hughes, 2001; Turner, 1997). Therefore, a comprehensive test

battery with a range of well-defined measures of executive function is necessary to assess the specificity of the executive dysfunctions in individuals with ASD. In addition, it is important for the measures to be developmentally appropriate. Therefore, tasks with a low floor and high ceiling would be most desirable.

Planning in ASD. Planning is a complex and dynamic operation, including the ability to generate alternatives, make choices, implement the plan and re-evaluate it constantly, in order to achieve a future goal (Hill, 2004). Common tasks used to assess planning include the Tower of London (Shallice, 1982) or the related Tower of Hanoi (Borys, Spitz, & Doran, 1982) tasks, in which individuals are to move discs on three different pegs to match a target arrangement with as few moves as possible, while adhering to a number of specific rules. Children and adolescents with ASD have been shown to demonstrate significantly poorer performance on the Tower tasks when compared to age-matched normal controls (Ozonoff & Jensen, 1999), as well as age-matched clinical control groups with various developmental disorders including ADHD, dyslexia, and Tourette syndrome (Bennetto et al., 1996; Ozonoff & McEvoy, 1994; Ozonoff et al., 1991).

However, the deficit in planning in individuals with ASD is not universal across all planning tasks. It has been shown that the planning impairment in individuals with ASD was seen only at the more complex levels, while they

showed intact abilities solving easy problems (Hughes et al., 1994). This may account for the observation that planning in activities of daily living appears to be especially problematic for children with ASD, as most planning in day-to-day life are complex (Hill, 2004). On the other hand, it has been reported that low-functioning (70-79 IQ points) children with ASD had poorer performance on a kinematic reach-to-grasp task compared with both high-functioning (80-109 IQ points) ASD and age-matched normal control groups, suggesting that the planning deficit might be related to IQ rather than to ASD per se (Mari, Castiello, Marks, Marraffa, & Prior, 2003). Further to the possible additive effect of learning disability on planning performance, it should also be noted that the Tower tasks are complex and involve multiple cognitive processes, such as working memory and inhibition of the prepotent moves. Hence, measurement of executive function with these tests alone should be interpreted with caution (Hill, 2004).

Set-shifting or cognitive flexibility in ASD. Set-shifting or cognitive flexibility is another executive function and refers to the ability to shift attention between different thoughts or actions in accordance with the changes in a situation (Ozonoff et al., 2004; Ozonoff & McEvoy, 1994). Individuals with poor cognitive flexibility have difficulties regulating or modulating their actions, and often exhibit perseverative and stereotyped behaviour (Hill, 2004). One typical task that

evaluates cognitive flexibility is the Wisconsin Card Sorting Task (WCST; Heaton, Chelune, Talley, Kay, & Curtiss, 1993; Nelson, 1976), in which the individual is asked to sort cards on one of three categories (color, shape, and number) according to a non-spoken rule. On this task, the examiner tells the participant if the cards have been placed correctly, but no sorting strategy is given explicitly. The sorting principle is then changed, without warning or comment from the examiner, and the participant is expected to shift set eventually to the new sorting criterion. The number of perseverative responses shown by the participant is used as a measure of cognitive flexibility. Individuals with ASD have been shown to be highly perseverative in their response to this task (Liss et al., 2001; Ozonoff & Jensen, 1999; Prior & Hoffmann, 1990). However, like the Tower tasks, the WCST also taps multiple underlying cognitive processes including attention to relevant dimensions of the stimuli, working memory to keep sorting criteria in mind, and inhibition of prepotent responses.

In addition, the WCST can be exceedingly difficult for children without a salient understanding of the category of number or with poor understanding of the verbal feedback provided by the examiner. In the present research, cognitive flexibility was assessed using the Children's Colour Trail-Making 2 Test (CCTT; Williams et al., 1995), in which duplicates of numbers from 1 to15 were embedded

in pink and yellow circles. The children are required to quickly connect the circles in ascending order, alternating between pink and yellow colors. This test is an altered version of the Trail Making Test in the Halstead-Reitan Battery (Reitan & Wolfson, 1993), designed to provide an evaluation of speeded visuo-motor tracking, sequencing and cognitive flexibility while minimizing the influence of language. The Color Trail 2 test has been shown to be particularly sensitive in discriminating children with frontal-lobe dysfunction (Williams et al., 1995). Individuals with ASD have been found to show deficit in set-shifting on the Trail-making test compared to age-matched normal controls (Rumsey & Hamburger, 1988).

Generativity in ASD. Generativity is the ability to produce novel ideas and behaviours spontaneously. Impairments in the regulation and generation of novel ideas have been suggested to be related to the lack of spontaneity and initiative that underlie the repetitive, stereotyped behaviors in individuals with ASD (Turner, 1997). Word Fluency and Design Fluency tests are two common measures of generativity, in which participants are required to produce multiple responses spontaneously following a single cue or instruction (Wong, 2004). On tests of word fluency, participants are asked to generate as many words belonging to a certain category as possible in 1 minute. On design fluency tests, participants are required

to produce as many different designs as possible within 5 minutes. Individuals with ASD have been shown to be impaired on tests of word fluency when compared to non-autistic, age- and ability-matched controls (Minshew, Goldstein, Muenz, & Payton, 1992; Rumsey & Hamburger, 1988), although some studies failed to find such evidence (Boucher, 1988; Scott & Baron-Cohen, 1996). In addition, because word fluency tasks rely heavily on vocabulary, it may not be an appropriate test to assess generativity in individuals with ASD as their verbal ability is typically impaired (Boucher, 1988; Hill, 2004; Wong, 2004). Therefore, a special emphasis was placed on design fluency in the current research, as it has been shown to be sensitive to the generativity impairment of both high- and low-functioning individuals with ASD (Turner, 1999). The Five-point Test (Regard, Strauss, & Knapp, 1982) used in the present research is a measure of design fluency, in which children are asked to create as many original and novel shapes by connecting five points with straight lines in 5 minutes. The Five-point Test is a non-verbal test that has been demonstrated to be a good measure of frontal lobe pathology (Lezak, Howieson, & Loring, 2004).

Attention and Inhibitory control in ASD. Attention and inhibitory control are important components in executive function that are essential for effective daily living (Denckla, 1996; Lezak et al., 2004; Stuss, Binns, Murphy, & Alexander,

2002). Attention is the ability to mindfully and consciously process stimuli (Robertson, Manly, Andrade, Baddeley, & Yiend, 1997), and involves alerting, orienting, and sustaining attention (Posner & DiGirolamo, 1998), as well as attention shifting (Courchesne et al., 1994; Hughes & Russell, 1993; Landry & Bryson, 2004). Inhibitory control refers to the suppression of responses to irrelevant, non-target, or distracting stimuli (Enticott, Ogloff, & Bradshaw, 2006; Friedman & Miyake, 2004; Nigg, 2000). Findings of neuropsychological studies on ASD have suggested that deficits in attention and inhibitory control may account for the executive dysfunctions that underlie many of the repetitive, stereotyped behaviors in ASD (Burack, 1994; Goldstein, Johnson, & Minshew, 2001; Nyden et al., 1999; Schmitz et al., 2006). For example, individuals with ASD were reported to have difficulties in response inhibitory control (Bishop & Norbury, 2005; Fernandez-Duque, Braid, & Posner, 2000; Hughes et al., 1994; Nyden et al., 1999; Russell & Jarrold, 1998; Russell, Jarrold, & Hood,, 1999; Schmitz et al., 2006), as well as self-monitoring impairments associated with attention and inhibitory deficits in the executive functioning tasks of memory (Hill & Russell, 2002; Russell & Jarrold, 1999), error-correction (Russell & Jarrold, 1998), and in tasks that involve suppression of a prepotent response from prior learning (Bishop & Norbury, 2005; Russell et al., 1999). Given that individuals

with ASD have deficient inhibitory control, particularly at suppressing irrelevant information and prepotent impulses, the increased number of intrusion errors can be considered a useful index of poor inhibition (Chiappe, Hasher, & Siegal, 2000). Children with ASD are expected to produce more intrusion errors than their normal counterparts in different neuropsychological measures (Chan et al., 2009).

In the current research, children with ASD were assessed on their attention and inhibitory control using the D2 Test of Concentration (D2; Brickenkamp, 1981) and Go/No-Go tests. The Total Commission Error on the Go/No-Go task has often been employed as a neuropsychological measure to assess inhibitory control (Kana, Keller, Minshew, & Just, 2007), in which the participants are required to focus on a computer monitor and make immediate response on "Go" trials and to inhibit their response on "No-Go" trials. The D2 is a letter cancellation task and the numbers of omission and wrong cancellations are recorded to give scores that measure the children's performance on inhibition, concentration and error judgment.

Neural Basis of Executive Dysfunction in ASD

Despite the fact that neurobiological determinants of the executive system have not been clearly delineated, it is widely accepted that the frontal cortex is one of the main brain regions implicated in executive function (Duncan, 1986; Schroeter, Zysset, Wahl & von Cramon, 2004). Results from behavioral and

neurobiological studies on individuals with ASD have revealed significant deficits in executive function and abnormal neurobiological processes in the frontal lobes that underlie their executive function deficits (Mundy, 2003; Ozonoff et al., 2004; Schmitz et al., 2006). Further, functional imaging studies have also found altered patterns of activation, perfusion, and glucose metabolism in various areas of the frontal lobes in individuals with ASD during neuropsychological tasks of executive function (George, Costa, Kouris, Ring & Ell, 1992; Hall, Szechtman & Nahmias, 2003; Hazlett, et al., 2004; Haznedar et al., 2000; Ohnishi et al., 2000; Pierce, Haist, Sedaghat, & Courchesne, 2004; Schmitz, et al., 2006; Wilcox, Tsuang, Ledger, Algeo & Schnurr, 2002). In addition, brain imaging studies have provided converging evidence for structural abnormalities in various subdivisions of the frontal lobes. For example, relative to a cohort of age-, race- and sex-matched controls, MRI scans assessing the brain volume of non-mentally retarded young adults with individuals with ASD have demonstrated in the latter group significant enlargement of the frontal lobes (Belmonte et al., 2004; Carper, Moses, Tigue, & Courchesne, 2002; Hardan, Minshew, Mallikarjuhn, & Keshavan, 2001).

This difference in brain size is also apparent in young children with ASD. A neuroimaging study of preschool children with ASD has also shown increased brain volume of the dorsolateral and medial frontal regions compared with normal

children (Carper & Courchesne, 2005). In addition, a recent meta-analysis of head circumference, MRI and post-mortem brain weight in individuals with ASD revealed markedly early overgrowth of the autistic brain, particularly in the frontal lobes (Redcay & Courchesne, 2005). Retrospective studies of head circumference measurements reported that much of the overgrowth occurs within 6 to 14 months of age, a critical period which coincides with exuberant synaptogensis, dendritic arborization, and ongoing myelination (Belmonte et al., 2004; Courchesne, Carper, & Akshoomoff, 2003). The researchers thus postulated that the deviant brain growth trajectory interferes with the normal developmental course of functional connectivity in the cortex, resulting in disruption to long-distance cortico-cortical connectivity between key neural networks and localized hyperconnectivity within isolated neural assemblies in the brains of individuals with ASD (Courchesne & Pierce, 2005a; Herbert, 2005; Just, Cherkassky, Keller, Kana, & Minshew, 2007; Rippon, Brock, Brown & Boucher, 2007). Indeed, diffusion tensor imaging (DTI) studies of cortico-cortical connectivity of individuals with ASD have provided evidence of reduced myelin integrity in the ventromedial prefrontal cortex and the anterior cingulate, and also at the temporoparietal junctions (Barnea-Goraly et al., 2004; Lewis & Elman, 2008). In addition to anatomical studies, evidence for disordered connectivity across neural systems in ASD is found in functional

imaging studies that demonstrate decreased correlations in cerebral metabolism and blood flow to activated brain areas on tests of sentence comprehension (Just, Cherkassky, Keller, & Minshew, 2004), social cognition (Castelli, Frith, Happe, & Frith, 2002) and working memory (Luna et al., 2002). Furthermore, consistent with the assumption that response inhibition is particularly susceptible to disruption attributed to cortical underconnectivity of the inhibition network, results from functional MRI studies on individuals with ASD performing response inhibition tasks indeed showed lower synchronization in the inhibition networks between the frontal and parietal areas of activation in the ASD group (Kana et al., 2007).

Immunological Abnormalities in ASD

Although the cause for the documented neuroanatomical abnormalities in ASD and the reported disordered neural connectivity is not well understood, increasing evidence have led to speculation that immunological factors are involved (Ashwood & Van de Water, 2004; Krause, He, Gershwin, & Shoenfeld, 2002; Pardo, Vargas, & Zimmerman, 2005). Specifically, elevated incidence of immune disorders have been reported in individuals with ASD, including abnormal cell-mediated immunity and abnormal T-cell populations and functions (Denney, Frei, & Gaffney, 1996); B-cell and natural killer cell dysfunction (Ashwood et al., 2003; Connolly et al., 1999; Gupta, 2000; Molloy et al., 2006; Pardo, et al., 2005;

Warren, Margaretten, Pace, & Foster, 1986); altered patterns of immune system activation such as abnormal CD4:CD8 ratio (Warren, Foster, & Margaretten, 1987); high blood monocyte counts and elevated percentage monocytes to total leukocytes (Denney et al., 1996; Sweeten, Posey, & McDougle, 2003); and lower Th1 and Th2-loke cytokines (Gupta, Aggarwal, Rashanravan, & Lee, 1998). Higher incidence of heightened autoimmunity and altered immune functions have also been reported in their first-degree relatives, suggesting a genetic link in the pathogenesis of ASD (Comi, Zimmerman, Frye, law, & Peeden, 1999; Singer et al., 2006).

More interestingly, some maternal viral infections are known to increase the risk for ASD; and maternal influenza infection in mice has been shown to produce profound anatomical, motor, and other behavioral defects reminiscent of autism, including anxiety in novel situations and early postnatal macrocephaly (Fatemi et al., 2002; Shi, Fatemi, Sidwell, & Patterson, 2003). Some researchers have suggested the possibility that systematic, immunologic aberrations in ASD are linked with autoimmunity, leading to the production of autoantibodies targeted against CNS proteins and resulting in destruction of neural tissue in the autistic brain (Korvatska, Van de Water, Anders, & Gershwin, 2002). Although the brain is protected and entry of potentially deleterious agents in the blood are primarily

restricted by the blood brain barrier, recent studies have demonstrated that products of immune activation such as cytokines can gain access to the brain through active transport, or impair the brain barrier function directly by binding to the receptors on brain endothelial cells (Ashwood and Van de Water, 2004; Wilson, Finch, & Cohen, 2002). Indeed, a number of studies have reported supporting evidence in detecting the presence of anti-CNS autoantibodies in children with ASD (Plioplys, Greaves, & Yoshida, 1989; Singh, Fudenberg, Emerson, & Coleman, 1988; Singh, Warren, Odell, Warren, & Cole, 1993). In addition, post-mortem studies of brain tissues in individuals with ASD demonstrated that the brains of some such individuals show clear signs of inflammation, supporting the idea that ASD may be associated with activation of the brain's immune system (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2005). Vargas et al. (2005) also found significantly elevated levels of cytokines and chemokine in innate immune responses in the cerebrospinal fluid (CSF) of children with autism. Furthermore, products of immune activation including cytokines can alter brain function and affect cognitive and emotional processing (Ashwood, Wills, & Van de Water, 2006). For example, cytokine IL-1β has been shown to influence neural plasticity that underlies learning and memory (Pugh, Fleshner, Watkins, Maier, & Rudy, 2001). Similarly, circulating cytotoxic T lymphocytes can enter the central nervous

system (CNS) when activated and can cause axonal damage of brain cells (Neumann, Medana, Bauer, & Lassmann, 2002). These findings suggest the possibility that the cognitive dysfunctions and abnormal behaviors in ASD are associated with the aberrant immune activity in the disorder (Ashwood et al., 2006; Korvatska et al., 2002).

Neurophysiological Measurements in Cognitive Neuroscience and Neuropsychology

While the argument for the role of immunologic factors disrupting the normal development in neural connectivity appears to be sound, there is relative little empirical evidence to support the notion. To study the association between the neural connectivity and immunologic functions of children with ASD, it will be helpful to explore objective methods to measure neurophysiologic activities of the brain for detecting abnormalities in brain physiology over time. A number of useful functional imaging methods have been available since the last decade, such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG). Based on different physical principles, the different brain imaging methods produce different indices of neural activities arising from cognitive operations, particularly in their relative spatial and temporal resolutions (Demonet, Thierry, & Cardebat, 2005). Whereas the high spatial resolution of the fMRI enables

localization of functions into specific brain areas, the high temporal resolution of the EEG in terms of millisecond enables sequential analyses of events and activations associated with mental processes (Minati, 2006).

Research applications of EEG. Despite the issues on source localization of the EEG, temporal and spatial changes in scalp-recorded brain activity still provide useful information on the time course of neural events involved in a cognitive process (Davidson, Jackson, & Larson, 2000; Pizzagalli, 2007). EEG oscillations that represent synchronized neuronal activity have different characteristic frequencies and spatial distributions that are closely related to different states of brain functions (Davidson et al., 2000). For example, while drowsiness has been found to be associated with low-amplitude theta (5-7 Hz) and delta (1-4 Hz) waves, deep sleep in normal adults is associated with large and slow waves in the delta frequency range (Stern, Grabner, Schumacher, Neuper, & Saalbach, 2005). Similarly, relaxed wakefulness is predominantly associated with alpha activity (8-13 Hz), and alert attentiveness during cognitive or physical activity is characterized by low-amplitude fast activity in the beta range (>13 Hz) (Pilgreen, 1995). In addition, while an increase in resting or tonic theta power is associated with memory and attention dysfunctions (Hermens et al., 2005; Klimesh, 1999), a phasic (event-related) increase in theta power but a decrease in alpha power reflect

good cognitive and memory performance (Klimesh, 1999). Still others have attempted to use the EEG as a screening tool to differentiate children with various neurological problems from those of normal children (Chan & Leung, 2006).

Empirical studies have demonstrated that patient groups with different neurologic and psychiatric disorders differed from normal individuals in their EEG characteristics, and EEG studies on children with ASD also showed abnormal quantitative EEG profiles from those of normal children (Chan et al., 2007; Chan & Leung, 2006). In particular, low-functioning children with ASD were found to have significantly higher relative slow-wave activities and greater total power than normal children (Cantor, Thatcher, Hrybyk, & Kaye, 1986). Additionally, more recent EEG studies on high-functioning individuals with ASD also showed higher theta power and increased theta coherence in the prefrontal areas of their brain (Daoust, Limoges, Bolduc, Mottron, & Godbout, 2004; Sutton et al., 2005).

Because the EEG measure is completely noninvasive, this makes it ideally suited for use in studies with infants and children, and in studies where repeated and long lasting recording are necessary (Martin, 1991). Also, the wide availability and relative low costs of the purchase and operations of EEG equipment are also notable advantages which help make the EEG the prime technique in the investigation and clinical assessment of cognitive functions, in comparison with

hemodynamic neuroimaging methods which would be prohibitively expensive (Stern at al., 2005).

EEG coherence. Coherence analysis of the EEG is a useful indicator of cortical connectivity between functional areas in the brain (Murias, Webb, Greenson, & Dawson, 2007; Rippon et al., 2007). In contrast to EEG power measurement, EEG coherence shows little maturation change in children and adolescents (Gasser, Rousson, & Gasser, 2003), thus making it well suited for neurophysiological investigation of brain connectivity in children. The EEG coherence is a measure of phase correlation which describes levels of synchronization between neuronal assemblies in terms of linearity of the relationship between two EEG signals measured at different sites of the scalp (Nunez & Srinivasan, 2006; Srinivasan, Nunez, & Silberstein, 1998). Coherence value has been shown to be independent of the amplitude of the EEG signals, due to the fact that it is normalized by the power of a given frequency band. This property makes the coherence measure suitable for comparing brain connectivity in different populations, as the coherence estimates are unaffected by the EEG power differences between the groups (Leocani et al., 2000).

In addition to the level of synchronicity, different EEG frequencies have been shown to correlate with different cognitive processes (Rippon et al., 2007). Greater

theta coherence was found in the frontal lobe as well as between the anterior and posterior brain regions during executive function tasks, including visual planning (Petsche & Etlinger, 1998), attentional and inhibitory processing (Barry, Ckarke, McCarthy, & Selikowitz, 2007; Harmony, Alba, Marroquin, & Gonzalez-Frankenberger, 2009) and memory (Chan et al., 2010; Sauseng, Klimesch, Schabus, & Doppelmayr, 2005). Higher theta coherence was also found between the frontal and temporal regions during resting condition in patients known to have executive dysfunctions (Babiloni et al., 2004; Ford, Mathalon, Whitfield, Faustman, & Roth, 2002), and in high-functioning children with ASD (Cantor et al., 1986; Murias et al., 2007).

AIMS OF STUDY

In light of the idea that successful executive control of cognitive function depends upon timely activation of cortical areas, and given the evidence suggesting abnormalities in cortical connectivity between distinct cortical systems and localized hyperconnectivity within isolated assemblies in the brains of individuals with ASD (Belmonte et al., 2004; Courchesne & Pierce, 2005a), it is postulated that children with ASD should show abnormal connectivity of the frontal cortex and its distributed network in the posterior area. This, together with findings that the immunological factors may cause direct neuronal damage in the CNS, leads to

the postulation that deficient cortical connectivity should correlate with the extent of immunologic abnormalities in children with ASD, leading to differential executive dysfunctions in these children. The purpose of the study, therefore, was to examine the relationship between immune functions, executive dysfunctions and neurophysiological activities in children with ASD. To test the hypothesis that executive functioning is differentially affected by the extent of immunologic abnormalities in ASD, high-functioning (HFA) and low-functioning (LFA) children with ASD were recruited to participate. It was hypothesized that LFA children would perform significantly poorer than HFA children in executive functions. It was also hypothesized that children with ASD would show abnormalities in their EEG coherence associated with disordered cortical connectivity. As theta coherence is critically involved in central executive processes, it was hypothesized that the executive dysfunction in children with ASD would be associated with their theta coherence in the frontal region and its distributed network. It was also hypothesized that the altered theta coherence is associated with immunologic abnormality in these children.

The current research consists of three studies. Study one examined the executive function profile in high- and low-functioning children with ASD. The main purposes of study one were to determine (1) whether the executive

dysfunction was differentially affected in the two groups of children with ASD, and (2) whether high- and low-functioning children with ASD would show distinct impairments of executive function. The aims and hypotheses of study one are elaborated in Chapter 2.

As there is increased evidence which suggests that immunologic factors are involved in the pathogenesis of ASD, the study two sought to extend the results of study one by examining whether immunologic abnormalities are associated with cognitive deficits in children with ASD. The aim of study two was to investigate (1) whether high- and low-functioning children with ASD showed different patterns of immunologic functions, and (2) whether there is an association between immunologic function as indexed by lymphocyte level, and cognitive function as indexed by performance on executive functions. The aims and hypotheses of this second study are addressed in Chapter 3.

Based on the documented executive dysfunction and the reported disordered neural connectivity in ASD, and that previous studies have demonstrated that immunological factors may cause direct neuronal damage in the brain, the study three attempted to confirm and extend the results of studies one and two by examining whether altered immune function was associated with neurophysiological abnormalities and executive function deficits in children with

ASD. Again, the aims and hypotheses and its extensions to previous research of this study three are discussed and elaborated in Chapter 4.

CHAPTER TWO

Executive Function Deficits in High- and Low-Functioning Children with Autism Spectrum Disorders

INTRODUCTION

Autistic spectrum disorders (ASD) consist of a spectrum of neurodevelopmental disorders that are characterized by disturbances in communication, poor social skills, and restricted / stereotyped behaviors or interest (American Psychiatric Association, 2002). Abnormalities and variation were also found in cognitive functions from severe retardation to highly superior ability (Dennis et al., 1999; Happe, 1999). While social and language impairments have been well-researched and have long been established as the defining characteristics of ASD, more recent evidence suggests that deficient executive functions are fundamental to the cognitive deficits in ASD (Gilotty, Kenworthy, Sirian, Black, & Wagner, 2002; Ozonoff, 1997). Executive functions refer to a broadly defined cognitive domain that includes a multidimensional set of abilities required to perform complex behaviors for the attainment of a future goal (Donders, 2002; Nyden, Gillber, Hjelmquist, Heiman, 1999), and are thought to be involved in cognitive processes such as planning, organization, self-monitoring, cognitive flexibility, and inhibition of inappropriate actions (Ozonoff et al., 2004). Individuals with ASD have been found to exhibit executive dysfunctions including disorganized actions and strategies typified by decreased initiative, perseveration, difficulties in forming novel concepts, and inhibition of inappropriate actions (Bennetto, Pennington & Rogers, 1996; Ozonoff & Jensen, 1999).

It is widely accepted that executive functions are mediated by the frontal cortex (Duncan, 1986; Schroeter, Zysset, Wahl & von Cramon, 2004). Given that previous studies have reported structural, physiologic, and functional abnormalities in the frontal region of individuals with ASD (Harrison, Demaree, Shenal, & Everhart, 1998; Mundy, 2003; Ozonoff et al., 2004; Rumsey & Ernst, 2000), it is

conceivable that the executive dysfunctions in these individuals are associated with their frontal abnormalities. Indeed, results from neurobiological studies have revealed abnormal neurobiological processes in the frontal lobes that underlie the deficits (Mundy, 2003; Ozonoff et al., 2004; Schmitz et al., 2006). Functional imaging studies have also found altered patterns of activation and glucose metabolism in various areas of the frontal lobes in individuals with ASD during neuropsychological tasks (Hall, Szechtman & Nahmias, 2003; Hazlett, et al., 2004; Haznedar et al., 2000; Pierce, Haist, Sedaghat, & Courchesne, 2004; Schmitz, et al., 2006). However, most of the previous studies were on high-functioning children with ASD who have normal intelligence (Dennis et al., 1999; Liss et al., 2001; Nyden et al., 1999; Ozonoff & Jensen, 1999; Ozonoff, Pennington, & Rogers, 1991). The executive dysfunction in low-functioning children with ASD is less well-understood. The present study thus aimed to extend current knowledge to the population of low-functioning children with ASD and examined their executive functioning.

Given that frontal processing might be differentially affected in low- and high-functioning individuals with ASD (Cheung, Chan, Sze, Leung, & To, 2010; Dennis et al., 1999), it is reasonable to assume that low-functioning individuals with ASD would have more severe impairments in executive functioning than their high-functioning counterparts. To test this hypothesis, the present study investigated the executive functioning of high- and low-functioning children with ASD and compared them with normally developing children in planning and organization, set-shifting, inhibitory control, and design fluency (generativity). It was anticipated that high-functioning children with ASD would demonstrate significantly poorer performance than normal children in the different measures of

executive functioning, whereas low-functioning children with ASD would show similar deficits in the different executive functions, but to a more severe degree than the high-functioning group.

METHODS

Participants

Nineteen high-functioning and 19 low-functioning children with ASD, and 28 children with normal development (NC), aged 8 to 17 years, participated voluntarily in the study. The NC children were recruited from local primary schools, and had negative history of neurological problems or abnormal developmental milestone as reported by their parents. The children with ASD were recruited from the Parents' Association of Pre-School Handicapped Children in Hong Kong or the subject pool of the Neuropsychology Laboratory of The Chinese University of Hong Kong. All children with ASD were diagnosed by pediatricians of the Child Assessment Centres of the Department of Health in Hong Kong , based on the criteria in the *Diagnostic and Statistical Manual of Mental Disorders* (4^{th} edition) (DSM-IV; American Psychiatric Association, 2002) or the Autism Diagnostic Observation Schedule (ADOS) (Lord, Rutter, DiLavore, & Risi, 2002). Diagnosis was further confirmed by a clinical psychologist through standard clinical interview and the Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, & LeCouteur, 1994). According to the DSM-IV criteria, 22 children met the diagnosis of autistic disorder, 2 met the criteria of Asperger disorder, and 14 met the criteria of pervasive developmental disorder not otherwise specified. As many of the neuropsychological measures used in the present study are paper-and-pencil tasks that require adequate motor skills, all children recruited were without

physical disabilities or reported motor dysfunction.

Table 1 shows the demographic characteristics of the children. The three groups were matched on age [$F(2, 63) = .25$, $p > .05$] and had similar gender distribution [$\chi^2 = 5.20$, $p > .05$]. Among the children with ASD, those having a general intelligence quotient (IQ) of 70 and above were classified as high-functioning (HFA), while those with IQ below 70 were classified as low-functioning (LFA). While the HFA group was matched with the NC group on IQ ($t(45) = .85$, $p > .05$), the LFA group had significantly poorer IQ [$t(36) = 9.54$, $p < .001$] ranging from borderline to mild grade mental retardation. No significant difference was found in the severity of autistic features between the HFA and LFA groups as indicated by the ADI-R (Lord et al., 1994) scores on Social Interaction ($t = .00$, $p > .05$.), Communication ($t = .29$, $p > .05$.), and Repetitive/Stereotyped Behavior ($t = -1.01$, $p > .05$).

Procedure

All children participated with informed parental consent. A neuropsychological battery consisting of the Wechsler Intelligence Scale for Children-Third Edition, short form (WISC-III short form; Kaufman, Kaufman, Balgopal, & McLean, 1996), and executive functioning tasks including the Hong Kong List Learning Test (HKLLT 2nd ed.; Chan, 2006), D2 Test of Concentration (D2; Brickenkamp, 1981), Five Point Test (5-point; Regard, Strauss, & Knapp, 1982), Children's Color Trails Test (CCTT; Williams et al., 1995), and Tower of California Test (ToC; Mattson, Goodman, Caine, Delis, & Riley, 1999) was administered to each participant individually. For non-verbal children for whom the WISC-III short form could not be used, the Standford-Binet Intelligence Scale – Fourth Edition (SB-FE) (Thorndike, Hagen, & Sattler, 1986) was

administered. The experimental procedure was approved by the NTEC-CUHK Clinical Research Ethics Committee.

Measures

Wechsler Intelligence Scale for Children-Third Edition (WISC-III) short form. The WISC-III short form (Kaufman et al., 1996) was used in the study to assess general intelligence. The test comprises the two verbal subtests of Similarities and Arithmetic; and the two performance subtests of Picture-Completion and Block Design in the original WISC-III. The short form yields an IQ score with a mean of 100 and a standard deviation of 15.

Standford-Binet Intelligence Scale – Fourth Edition (SB-FE). The. SB-FE (Thorndike et al., 1986)assesses verbal reasoning, abstract/visual reasoning, quantitative reasoning, and short-term memory, and yields an IQ score with a mean of 100 and a standard deviation of 16.

Hong Kong List Learning Test (HKLLT). The HKLLT (2nd ed.; Chan, 2006) is primarily a memory test, which also measures the frontal lobe functions of learning strategies and organization (Cheung et al., 2010). The test consists of a randomly organized list of 16 two-word Chinese characters presented once during each of three learning trials. Children in the present study were asked to recall the words immediately after each learning trial. The total number of correctly recalled words during the three learning trials gave the Total Learning score. A recognition test consisting of the 16 target words and 16 distracters was presented after a 30-minute delayed recall trial. The children were required to discriminate whether the words have been previously learnt. A discrimination score that assessed memory performance was calculated based on the number of correct hits (i.e., the correct identification of targets) and false alarms (i.e., the false positive) at the recognition

trial.

D2 Test of Concentration (D2). This test (Brickenkamp, 1981) measures inhibition, concentration and error judgment. It is a letter cancellation task, printed on a piece of paper with different letters with a different number of dashes above and below the letters. The children were asked to cancel all letter d's with 2 dashes. There were a total of 14 lines and for each line the time allowed was 20 seconds. The number of omission and wrong cancellations were recorded to give performances on inhibition, concentration and error judgment.

Five Point Test (5-point). This test (Regard et al., 1982) measures figural fluency in terms of the production of novel designs under time constraints. Children in the present study were asked to create as many original shapes by connecting five points with straight lines within five minutes. This test is a non-verbal analog to verbal fluency tasks, and was used in the present study because it is a good measure of frontal lobe pathology. Scores ranged from 0 to 40, with higher scores indicating greater cognitive fluency.

Children's Colour Trail Test (CCTT). The CCTT (Williams et al., 1995) is an altered version of the Trail Making Test in the Halstead- Reitan Battery (Reitan & Wolfson, 1993). It is specifically designed to be a culture-free test for children. The test measures the speed of attention, sequencing, mental flexibility, visual searching and motor function. This is a paper-based test, with duplicates of each number from 1 to 15 embedded within pink and yellow circles. The children were required to connect the circles in ascending order, alternating between pink and yellow colors, as quickly as possible. The completion time in seconds for the task was the score.

The Tower of California Test (ToC). The ToC (Mattson et al., 1999) is a

modification of the Tower of Hanoi (Borys, Spitz, & Dorans, 1982) and Tower of London (Morris, Ahmed, Syed, & Toone, 1993; Shallice, 1982) tests, and was administered in the present study to assess spatial planning and cognitive flexibility (Delis, Kaplan, & Kramer, 2001). It consists of nine items that involve moving discs on three colored vertical pegs to match a target arrangement while adhering to rules. The score was calculated as the number of items successfully completed.

Go/No-Go task. This is a computerized task that measured impulse control in the present study (Kana, Keller, Minshew, & Just, 2007). Children were required to press a key as quickly as possible when a black ball (Go stimulus) appeared on the computer screen, and to inhibit their responses when a red ball (No-Go stimulus) appeared. The total testing time was 6 minutes and the stimuli were displayed one at a time, in the center of the computer screen, for 500 ms in random order at a ratio of 4:1 (192 black balls: 48 red balls), followed by 1000 ms of blank intervals. The Total Commission Errors on "No-Go" trials measured inhibition.

Data Analysis

The executive functions of the HFA and LFA groups of children with ASD and NC children were compared on their seven scores from the HKLLT, D2, 5-Point, CCTT , ToC and Go/NoGo tasks using analysis of variance (ANOVA). Where significant group differences were found, posthoc comparisons were performed to identify the significant pairwise differences.

RESULTS

The two groups of children with ASD were compared with NC children on the 7 scores from the HKLLT, D2, 5-point, CCTT, ToC and Go/No-Go using analysis of variance (ANOVA), followed by post hoc *t*-tests to examine pairwise differences.

To control for inflated type 1 error as a result of multiple posthoc comparisons, Bonferroni correction with the adjusted alpha level of p=.007 was employed. Results indicated that the three groups of children showed significantly different HKLLT Total Learning [$F(2, 63) = 45.15$, p =.000] and Discrimination [$F(2, 63) = 48.08$, p = .000] scores, as well as the D2 Concentration Performance [$F(2, 63) = 38.62$, p = .000] score. The LFA group demonstrated the poorest performance, with the HFA group in between and the NC group highest (Table 2). It should be noted that the significantly lower scores on the HKLLT Discrimination and D2 Concentration Performance both groups of children with ASD were largely related to their higher false alarm rates on the HKLLT [$F(2, 63) = 13.38$, p = .000] and commission errors [$F(2, 63) = 6.53$, p = .003] on D2 . While the HFA group showed a higher false alarm rate on the HKLLT ($M = 1.94$, $SD = 3.7$) and greater commission errors on D2 ($M = 13.78$, $SD = 24.93$) than the NC group ($M = .25$, $SD = .44$; $M = 3.46$, $SD = 5.42$, respectively), the LFA group performed worst and exhibited a significantly higher false alarm rate ($M = 5.33$, $SD = 4.7$) and commission errors ($M = 107.46$, $SD = 269.43$) than both NC and HFA children. Increased commission errors and false alarm rates have been commonly observed in individuals with ASD, suggesting that they were more vulnerable to interference generated from irrelevant information. In addition, it was found that the HFA group showed comparable performance to NC children on the 5-point Unique Design ($t(45) = .28$, p =.78), CCTT-2 Time ($t(19.9) = -1.82$, p =.08), and ToC Achievement ($t(26.8) = 1.69$ p =.10) scores. Again, the LFA group performed significantly poorer than both HFA and NC children on these tasks [F values ranging from 19.66 to 28.75, p<.001]. No significant difference, however, was found among the three groups of children on the Go/No-Go Total Commission Errors [$F(2, 63) = 3.02$, p

= .06], which might be due to the large variability within the HFA and LFA groups (Table 2).

DISCUSSION

The main purpose of the present study was to examine the executive dysfunctions of a group of 8 to 17 years old high- and low-functioning children with ASD, and whether the executive function deficits were differentially affected in high- and low-functioning children with ASD. Results of the present study have extended the findings of previous studies on high-functioning children with ASD (Gilotty et al., 2002; Ozonoff, 1997) in demonstrating that high- and low-functioning children with ASD had significant differences in their degree of executive function deficits.

The present results showed that children with ASD performed significantly poorer on the D2 and HKLLT, and exhibited significantly higher false alarm rates than normal children on the HKLLT. These results suggested that children with ASD were vulnerable to interference as indicated by their increased false alarm responses, which is a function of inhibitory control mediated by the frontal lobes. This finding is consistent with those from prior studies that suggested that the cognitive profile of individuals with ASD were similar to those in patients with frontal-lobe dysfunctions (Alexander, Stuss, & Fansabedian, 2003; Baldo & Shimamura, 2002) .

The present findings also supported our hypothesis that executive functions are differentially affected among individuals with ASD, where low-functioning children performed significantly poorer than high-functioning children on different measures of executive functions. These results are consistent with findings from a

previous study which reported that individuals with ASD who have mental retardation (low-functioning) showed significantly more developmental abnormalities than those with normal intelligence (high-functioning) (Burack & Volkmar, 1992).

While the HFA children in our study performed comparably to normal children on the 5-point, CCTT, and ToC tests, both the HFA and LFA groups were relatively unimpaired in Commission errors on the Go/No-Go task, suggesting that they performed within the normal range in cognitive functions such as mental flexibility, planning and inhibition. These results are in line with some prior findings that children with frontal lobe damage performed relatively normally on some cognitive tests but showed disorganized strategies and actions when the cognitive tasks are mentally effortful, or when the information are meaningful or in vast amount (Chan et al., 2010; Mangeot, Armstrong, Colvin, Yeates & Taylor, 2002; Ozonoff & Strayer, 2001). As some researchers have pointed out, executive functions are a broadly defined cognitive domain involving "on-line" coordination of a multidimensional set of abilities to perform complex behaviors (Denckla, 2002). It is possible that standardized neuropsychological assessments measuring very specific areas of cognitive processes may not be sensitive enough to detect executive dysfunctions in everyday activities that involve a combination of different executive functions (Donders, 2002). This may explain why children with ASD in the present study were relatively unimpaired in the relatively simple commission errors on the Go/No-Go as the continuous performance tasks, but showed impaired performance in intrusion errors and false alarms on the HKLLT and D2, as list learning and visual cancellation tasks involve complex and multiple executive functions (Stuss et al., 1994). These results support the notion that a

comprehensive battery with multiple neuropsychological measures of the same construct but with varying complexity is necessary to delineate the specificity of executive dysfunctions in children with ASD (Welsh, Pennington, Ozonoff, Rouse, & McCabe, 1990).

In sum, the present findings are in line with prior studies that suggested that the overall pattern of cognitive impairment in individuals with ASD resembles those with frontal lobe damage. The results also extended those of previous studies in demonstrating that executive function deficits varied with the level of general intellectual functioning in children with ASD, where lower-functioning children showed more extensive executive function deficits and performed significantly poorer than their higher-functioning counterparts on different measures of executive functions. Future studies may explore whether the difference in severity of executive dysfunction between high- and low-functioning children with ASD are associated with the underlying neurophysiology. Such studies may help shed some light on the neural underpinnings of executive dysfunctions in individuals with ASD.

Table 1

Demographic Characteristics of the Children with Normal Development (NC), High-Functioning (HFA) and Low-Functioning (LFA) Children with Autistic Spectrum Disorders (ASD)

Variable	NC (n = 28)	HFA (n = 19)	LFA (n = 19)
Mean Age (in years)	12.0 (2.33)	11.6 (3.02)	12.1 (2.35)
Gender (male/female)	17/11	15/4	17/2
IQ	109.8 (11.59)	105.2 (21.61)	50.3 (12.73)
ADI-R Social Interaction	----	20.42 (6.25)	20.42 (6.64)
ADI-R Communication	----	11.79 (4.95)	11.32 (5.16)
ADI-R Stereotyped Behavior	----	3.84 (3.45)	4.89 (2.64)

Note. Standard deviations are in parentheses. ADI-R = Autism Diagnostic Interview- revised.

Table 2

Mean Performance and Standard Deviation on the Measures of Executive Functioning Task in NC, HFA and LFA Groups

Measures	NC (n = 28) M (SD)	HFA(n = 19) M (SD)	LFA (n = 19) M (SD)	*F*-value	Group Difference		
HKLLT-Total Learning	25.64 (4.75)	18.11 (8.18)	8.05 (5.91)	45.15**	NC>HFA**	NC>LFA**	HFA>LFA**
HKLLT-Discrimination %	93.07 (8.31)	70.56 (33.11)	21.92 (30.07)	48.08**	NC>HFA**	NC>LFA**	HFA>LFA**
D2-Concentration performance	164.8 (53.72)	118.1 (55.24)	30.26 (44.01)	38.62**	NC>HFA**	NC>LFA**	HFA>LFA**
5 Point-Unique design	23.64 (10.86)	22.74 (11.05)	5.95 (6.83)	20.62**		NC>LFA**	HFA>LFA**
CCTT-Trail2Time2	44.4 (18.86)	73.76 (68.61)	172.4 (109.0)	19.66**		NC>LFA**	HFA>LFA *
ToC-Achievement	9.57 (2.75)	7.57 (4.59)	2.37 (1.98)	28.75**		NC>LFA**	HFA>LFA**
Go/NoGo-Commission errors	9.0 (6.09)	10.05 (7.55)	15.18 (11.89)	3.02			

**p<.001, *p<.007

CHAPTER THREE

Lymphocyte Subset Alterations Related to Executive Function Deficits and Repetitive Stereotyped Behavior in Autism

INTRODUCTION

It has been documented that immune functions are related to the cognitive and emotional states in humans (Ashwood & Van de Water, 2004; McEwen, 1998). Whereas the cognitive and emotional states of an individual can compromise his/her immune function, conversely, the biochemical balances, or imbalances, of the immune system can also alter brain functions and behavior, suggesting that there is a bi-directional communication between the brain and the immune system (Ashwood, Wills, & Van de Water, 2006; Sperner-Unterweger, 2005). More specifically, cytokines of the immune system have been shown to influence brain functioning on learning and memory (Pugh, Fleshner, Watkins, Maier, & Rudy, 2001). Circulating cytotoxic T lymphocytes (CTL) have been shown to enter the central nervous system (CNS) and cause axonal damage (Boulanger & Shatz, 2004; Medana, Martinic, Wekerle, & Neumann, 2001; Neumann, Medana, Bauer, & Lassmann, 2002). Furthermore, it has been suggested that peripheral T cell deficiency is related to the cognitive dysfunction and abnormal behaviors in Schizophrenia (Kipnis, Cohen, Cardon, Ziv, & Schwartz, 2004).

Among the many neural disorders that have been suggested to be associated with immune function, increasing evidence is accumulating to suggest that immunological factors are involved in the pathogenesis of autism spectrum disorders (ASD) (Ashwood & Van de Water, 2004; Krause, He, Gershwin, & Shoenfeld, 2002; Pardo, Vargas, & Zimmerman, 2005). Although the cause of ASD is not well understood, the ASD patient population has been reported to have elevated incidence of immune disorders including heightened autoimmunity, reduced immune functions, and decreased peripheral lymphocyte numbers (Ashwood et al., 2003; Connolly et al., 1999; Molloy et al., 2006; Pardo, et al.,

2005), which was also found in their first-degree relatives (Comi, Zimmerman, Frye, law, & Peeden, 1999; Singer et al., 2006). More interestingly, it has been reported that some maternal viral infections increased the risk for ASD; and maternal influenza infection produced profound anatomical, motor and other behavioral dysfunctions similar to those of autism in mice, including anxiety in novel situations (Fatemi et al., 2002; Shi, Fatemi, Sidwell, & Patterson, 2003). Some researchers have suggested that immunologic abnormalities in ASD are linked with autoimmunity, which leads to the production of auto-antibodies targeted against CNS proteins, resulting in the destruction of neural tissues in individuals with ASD (Korvatska, Van de Water, Anders, & Gershwin, 2002). Indeed, a number of studies have reported supporting evidence in detecting the presence of anti-CNS auto-antibodies in children with ASD (Plioplys, Greaves, & Yoshida, 1989; Singh, Fudenberg, Emerson, & Coleman, 1988; Singh, Warren, Odell, Warren, & Cole, 1993). These findings raise the possibility that the cognitive and behavioral abnormalities in ASD may be associated with the abnormal immune function found in this disorder (Ashwood et al., 2006; Korvatska et al., 2002).

Behaviorally speaking, individuals with ASD are characterized by disturbances in communication, poor social skills, and an abnormal repertoire of stereotyped behaviors (American Psychiatric Association, 2002). Abnormalities were also found in the higher cognitive functions, where some individuals with ASD show severe impairments and mental retardation, while others show isolated cognitive dysfunctions such as stereotyped behavior and memory dysfunctions (Chan et al., 2010; Cheung, Chan, Sze, Leung, & To, 2010; Happe, 1999). It has been suggested that fundamental to these cognitive and behavioral deficits (Gilotty,

Kenworthy, Sirian, Black, & Wagner 2002; Ozonoff, 1997) and repetitive, stereotyped behaviors (Schmitz et al., 2006) is a deficiency in executive function. Executive functions refer to a multidimensional set of abilities required to perform complex behaviors for the attainment of a goal (Donders, 2002; Nyden, Gillberg, Hjelmquist, & Heiman, 1999). Individuals with ASD have been found to exhibit executive dysfunctions including disorganized actions and strategies characterized by reduced initiative, increased perseveration, difficulties in forming novel concepts, and inhibition of inappropriate actions (Bennetto, Pennington & Rogers, 1996).

While immunological studies suggest the involvement of immunological factors in the pathogenesis of ASD, whether the abnormalities of the immune system are associated with the cognitive and behavioral performance have not been well-studied. The present study, thus, aimed to examine whether there is an association between immune function as indexed by lymphocyte level, and cognitive function as indexed by performance on executive functions, in children with ASD. Based on the findings that immunological factors may cause neuronal damage in the CNS, it is postulated that executive dysfunctions should correlate with the extent of immunologic abnormalities in individuals with ASD. Fluorochrome-labeled antibodies and flow cytometer analysis were employed to identify and determine the levels of various mature human lymphocyte subsets in peripheral blood of children with ASD. To ensure a wide spectrum of executive functioning would be included, high-functioning (HFA) and low-functioning (LFA) children with ASD were recruited to participate. Since it has previously been demonstrated that HFA and LFA children with autism displayed significantly different executive functions (see study 1), the two groups were compared in the

present study to examine whether there are any differences in their immune function. It was hypothesized that LFA children would perform significantly poorer than HFA children in executive functions as reflected in the composite and individual scores on the executive functions, and the behaviors as observed by the parents in the ADI-R (Lord, Rutter, Le Couteur, 1994). It was also hypothesized that HFA and LFA children with ASD would show different levels of lymphocyte subsets. The executive dysfunctions, but not language or social communication dysfunctions, were further hypothesized to be associated with the immune function in these children. Findings from the present study may help shed light on the cognitive processing of children with ASD as a function of immune dysregulation, which in turn may inform future directions of research and clinical trials on possible intervention for ASD.

METHODS

Participants

Eighteen HFA and 19 LFA children with ASD, aged 8 to 17 years, participated in the study with informed consent from their parents. The study was approved by the NTEC-CUHK Clinical Research Ethics Committee. The children with ASD were recruited either from the Parents' Association of Pre-School Handicapped Children in Hong Kong, or from the database of the Neuropsychology Laboratory of The Chinese University of Hong Kong. All children were formally diagnosed by pediatricians of the Child Assessment Centres of the Department of Health in Hong Kong based on DSM-IV criteria (American Psychiatric Association, 2002). Diagnosis was further confirmed by a clinical psychologist through a standard clinical interview (4th ed., text rev.; DSM-IV-TR; American Psychiatric Association,

2002) and the Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, & LeCouteur, 1994). According to the DSM-IV-TR criteria, 21 children met the diagnosis of autistic disorder, 2 met the criteria of Asperger disorder, and 14 met the criteria of pervasive developmental disorder not otherwise specified. Children with concurrent medical problems or other forms of developmental, neurological, or psychiatric disorder, or those who were receiving psychiatric treatment for developmental problems other than ASD, were excluded from the study. High-functioning status was defined as having a general IQ of 70 or above, and low-functioning status was defined as having an IQ below 70. Children in the HFA group had IQ ranging from 70 to 143 points; children in the LFA group had IQ ranging from borderline to mild grade mental retardation (from 36 to 68 points) (Table 1). The HFA and LFA groups were matched on age [$t(35) = -.74$, $p>.05$], and had similar gender distribution. No significant difference was found in the severity of autistic features between the HFA and LFA groups on the ADI-R (Lord et al., 1994) measures of Social Interaction ($t = .22$, p=*n.s.*), Communication ($t = .73$, p=*n.s.*), and Repetitive/Stereotyped Behavior ($t = -.84$, p=*n.s.*) (Table 1).

Procedure

All children and their parents were invited to attend an individual assessment session at the authors' laboratory. During the assessment session, the parent accompanying the child was interviewed by a trained interviewer using the Autism Diagnostic Interview-Revised edition (ADI-R) (Lord et al., 1994) and a structured questionnaire to provide information on his/her child's developmental and medical history, in particular, whether the child had any allergies such as asthma, atopic dermatitis or hay fever, and chronic gastrointestinal symptoms including abdominal pain, diarrhea, constipation or bloating. All children were individually

administered five executive function tasks including the Hong Kong List Learning Test (HKLLT, 2nd ed) (Chan, 2006), D2 Test of Concentration (D2) (Brickenkamp, 1981), The Five Point Test (5-point) (Regard, Strauss, & Knapp, 1982), Children's Color Trails Test (CCTT) (Williams et al., 1995), and the Tower of California Test (ToC) (Mattson, Goodman, Caine, Delis, & Riley, 1999). To obtain estimates of general intelligence, the children were administered either the Wechsler Intelligence Scale for Children – Third Edition (WISC-III) short form (Kaufman, Kaufman, Balgopal, & McLean, 1996). For non-verbal children for whom the WISC-III short form could not be used, the Standford-Binet Intelligence Scale – Fourth Edition (SB-FE) (Thorndike, Hagen, & Sattler, 1986) was administered. In addition to the assessment and EEG recording sessions, all children had to visit a medical clinic within a week following the intelligence assessment, to have 4 ml of EDTA blood was drawn by venipuncture by a registered nurse between 1030 and 1200. The blood samples were kept in a thermally insulated bag and transported to the clinical laboratory where blood assays were performed by a laboratory technician blinded to the study.

Measures

Autism Diagnostic Interview-Revised (ADI-R). The ADI-R (Lord et al., 1994) is a rating measure completed by the parent on the child's behaviors relevant to the diagnosis of Pervasive Developmental Disorders. It consisted of three scales, Social Interaction, Communication, and Repetitive/Stereotyped Behavior, which correspond with the three diagnostic criteria of autism established in the DSM-IV-TR (American Psychiatric Association, 2002). Each scale contains detailed questions about early developmental and current functioning of the child, with higher scores indicating greater autistic features.

Wechsler Intelligence Scale for Children-Third Edition (WISC-III) short form. The WISC-III short form (Kaufman et al., 1996) was used in the study to assess general intelligence. It was individually administered to each child, and comprises the two verbal subtests of Similarities and Arithmetic; and the two performance subtests of Picture-Completion and Block Design in the original WISC-III. The short form yields the IQ score with a mean of 100 and a standard deviation of 15.

Standford-Binet Intelligence Scale – Fourth Edition (SB-FE). The. SB-FE (Thorndike et al., 1986). The test consists of 15 subtests grouped into the four areas of verbal reasoning, abstract/visual reasoning, quantitative reasoning, and short-term memory, each with a score. The test yielded an IQ score with a mean of 100 and a standard deviation of 16.

Hong Kong List Learning Test (HKLLT). The HKLLT (2nd ed.; Chan, 2006) is primarily a memory test, which also serves to measure the frontal lobe functions of learning strategies and organization (Cheung et al., 2010). The test consists of a randomly organized list of 16 two-word Chinese characters presented once during each of three learning trials. Children in the present study were asked to recall the words immediately after each learning trial. The total number of correctly recalled words during the three learning trials gave the Total Learning score. A recognition test consisting of the 16 target words and 16 distracters was presented after a 30-minute delayed recall trial. The children were required to discriminate whether the words have been previously learnt. A discrimination score that assessed memory performance was calculated based on the number of correct hits (i.e., the correct identification of targets) and false alarms (i.e., the false positive) at the recognition trial.

D2 Test of Concentration (D2). This test (Brickenkamp, 1981) measures

inhibition, concentration and error judgment. It is a letter cancellation task, consisting of a piece of paper with different letters with a different number of dashes above and below the letters. The children were asked to cancel all letter d's with 2 dashes. There were a total of 14 lines and for each line the time allowed was 20 seconds. The number of omission and wrong cancellations were recorded to yield performances on inhibition, concentration and error judgment.

The Five Point Test (5-point). This test (Regard et al., 1982) measures figural fluency in terms of the production of novel designs under time constraints. Children in the present study were asked to create original and novel shapes by connecting five points with straight lines within five minutes. This test is a non-verbal analog to verbal fluency tasks, and was used in the present study because it is a good measure of frontal lobe pathology. Scores ranged from 0 to 40, with higher scores indicating greater cognitive fluency.

Children's Colour Trail Test (CCTT). The CCTT (Williams et al., 1995) is an altered version of the Trail Making Test in the Halstead- Reitan Battery (Reitan & Wolfson, 1993). It is specifically designed to be a cultural-free test for children. The test measures the speed of attention, sequencing, mental flexibility, visual searching and motor function. The test is printed on paper, with duplicates of each number from 1 to 15 embedded within pink and yellow circles. The children were required to connect the circles in ascending order, alternating between pink and yellow colors, as quickly as possible. The completion time in seconds for the task was the score.

The Tower of California Test (ToC). The ToC (Mattson, et al., 1999) is a modification of the Tower of Hanoi (Borys, Spitz, & Dorans, 1982) and Tower of London (Morris, Ahmed, Syed, & Toone, 1993; Shallice, 1982) tests, and was

administered in the present study to assess spatial planning and cognitive flexibility (Delis, Kaplan, & Kramer, 2001). It consists of nine items that involve moving dics on three colored vertical pegs to match a target arrangement while adhering to rules. The score was calculated as the number of items successfully completed.

Go/No-Go task. The computerized Go/No-Go task was used to measure impulse control in the study (Kana, Keller, Minshew, & Just, 2007). Children were required to press a key as quickly as possible when a black ball (Go stimulus) appeared on the computer screen, and to inhibit their responses when a red ball (No-Go stimulus) appeared. The total testing time was 6 minutes and the stimuli were displayed one at a time, in the center of the computer screen, for 500 ms in random order at a ratio of 4:1 (192 black balls: 48 red balls) followed by 1000 ms of blank intervals. The Total Commission Errors on "No-Go" trials measured inhibition.

Lymphocyte Subsets. Percentages (%) and absolute counts (cells/ μl) of the lymphocyte subsets in peripheral blood, including T lymphocytes (CD3+), B lymphocytes (CD19+), T helper (Th) lymphocytes (CD3+CD4+), suppressor/cytotoxic T lymphocytes (CD3+CD8+), and natural killer (NK) cells (CD3-CD16+ and/or CD56+), were measured using immunofluorescence technique with BD Multitest™ IMK kit, using flow cytometry (FACSCalibur 4 color flow cytomter, BD Bioscience Corp., San Jose, CA, USA).

Data Analyses

For the executive function measures, in order to reduce the number of statistical comparisons, the HFA and LFA groups of children with ASD were compared on their performance on the neuropsychological measures of executive function using an Executive Composite score which was derived by summing,

seven scores from the HKLLT, D2, 5-Point, CCTT , ToC and Go/NoGo tasks. The raw score for each child on each executive function measure was converted to a Z score, using the grand mean and standard deviation of the respective executive function measure derived from the normative data of measure. The Z scores from the different executive function measures were then averaged to yield the Executive Composite score. Higher scores indicated poorer executive functioning. Posthoc analyses on each executive function measure would be performed if a significant difference was found in the Executive Composite score between the LFA and HFA groups. For the parent observations on the ADI-R, the three subscale scores were compared using independent *t-tests*. For the immunological measures, the absolute counts and percentages of the lymphocyte subsets were analyzed with independent *t*-test. The relationship between executive functioning, parent behavioral observations and immune function were examined using Pearson correlation. Given that planned hypotheses were tested and that the number of participants were relatively small, we did not adjust the alpha level to avoid lowering the power of the tests and inflating Type II error.

RESULTS

Executive Functioning and Parent Behavioral Observations

The LFA group had a significantly higher Executive Composite score than the HFA group (t = -5.21, p<.001), suggesting that LFA children with ASD had poorer executive function than the HFA children. Posthoc results on the individual executive function measures indicated that the LFA group showed significantly lower HKLLT total Learning (t = 4.14, p<.001) and discrimination (t = 4.55, p<.001) scores, as well as on the D2 Concentration Performance (t = 5.18, p<.001),

5-point Unique Design (t = 5.43, p<.001), CCTT-2 Time (t = -3.22, p<.01), and the ToC Achievement (t = 4.48, p<.001) scores than the HFA group (Table 2). Independent t-test indicated no significant difference between the HFA and LFA groups on the Go/No-Go Total Commission Errors (t = -1.89, p=.07), which is possibly due to the large variability within the two groups of children.

In parent behavioral observations, the results indicated that LFA children with autism showed a higher score on ADI-R Stereotyped Behavior subscale and lower scores on the Social Interaction and Communication subscales than HFA children with autism. It was interesting to note that the differences between the two groups in Stereotyped Behavior appeared to be larger in terms of magnitude and variation than the differences in Social Interaction and Communication; and that the direction of the differences were different with LFA children with autism being observed to display more Stereotyped Behavior dysfunctions while less Social Interaction and Communication dysfunctions by their parents. It should be noted, however, that these differences did not reach statistical significance (all ps=n.s.) (Table 2).

Alterations of Immune System

Parental report on the history of allergic symptoms was collected as indicator of an autoimmune condition. Parents of both HFA and LFA children reported high incidences of clinical immune response in the two groups of children. While 78% of the HFA and 63% of the LFA children were reported to have a history of allergy such as asthma, atopic dermatitis or hay fever; about one third of the children in both HFA and LFA groups had chronic gastrointestinal symptoms including abdominal pain, bloating, constipation or diarrhea. The LFA group, however, showed significantly elevated numbers of total lymphocytes (t = -2.07, p<0.05) as

well as T lymphocytes (CD3+; t = -2.55, $p<0.05$) and suppressor/cytotoxic T lymphocytes (CD3+CD8+; t = -3.05, $p<0.01$). The percentage of suppressor/cytotoxic T lymphocytes (CD3+CD8+ /CD45+) was also found to be significantly higher in the LFA group (t = -3.17, $p<0.01$). The LFA and HFA groups showed no significant difference in the measurement of Th lymphocytes (CD3+CD4+), B lymphocytes (CD19+) and NK lymphocytes (CD3-CD16+ and/or CD56+) (all $p > 0.05$, Table 3).

Association between Intellectual Functioning, Executive Functioning, Parent Behavioral Observations and Immunological Measurements

Given that both the executive functions and some measures of the immune system were significantly different between the HFA and LFA groups, the relationship between IQ, executive functions and immunological measures were examined using Pearson correlation with the two groups of children combined (Table 4). In addition, we also examined the correlation between parent behavioral observations to examine whether immune function was related specifically to executive-function-mediated stereotyped behavior, or if it was also related to behaviors in communication and social interaction that were not mediated by executive functions. Results indicated that IQ was significantly associated with the immunological measures of Total lymphocytes (r = -0.34, $p<0.05$), T lymphocytes (r = -0.32, $p<0.05$), number of Suppressor/ Cytotoxic T lymphocytes (r = -0.38, $p<0.02$), and percentage of Suppressor/ Cytotoxic T lymphocytes (r = -0.33, $p<0.05$). The Executive Composite score was also significantly correlated with IQ (r = -0.74, $p<0.001$) and immunological measures [Total lymphocytes (r = 0.35, $p<0.05$); T lymphocytes (r = 0.36, $p<0.05$); Suppressor/ Cytotoxic T lymphocytes (r = 0.38, $p<0.05$)]. In addition, the ADI-R (Lord, Rutter, Le Couteur, 1994)

Stereotyped Behavior, but not the Social Interaction and Communication, score was significantly correlated with the immunological measures [Total lymphocytes (r =0 .45, $p<0.01$); T lymphocytes (r = 0.43, $p<0.01$); Suppressor/ Cytotoxic T lymphocytes (r = 0.44, $p<0.01$)]. Correlations between individual executive function measures, IQ, parent behavioral observations, and lymphocyte subsets are shown in Table 4.

DISCUSSION

The present study examined executive function deficits and repetitive stereotyped behavior of a group of 8 to 17 years old high- and low-functioning children with ASD, and whether these deficits were associated with their altered immune functions. The present results extended those of prior studies on executive dysfunctions in ASD (Chan et al., 2009; Cheung et al., 2010; Gilotty et al., 2002; Ozonoff, 1997) in demonstrating that among children with ASD, those who were LFA performed significantly poorer than those who were HFA on different measures of executive functions. The present results also showed that children in the LFA group showed significantly elevated levels of total lymphocytes and T lymphocytes, as well as increased number and percentage of Suppressor/cytotoxic T lymphocytes. Suppressor/cytotoxic lymphocytes (CD8+) are a subset of T lymphocytes (CD3+). Increased number and percentage of CD8+ has been reported in patients with congenital or acquired immune deficiencies as well as autoimmune diseases (Giorgi, 1993; Nicholson, 1989; Schmidt, 1989). In contrast to CD3+ and CD8+ lymphocytes, no significant difference was found in the numbers of CD3+CD4+ Th, B and NK cells between the LFA and HFA children with ASD.

Although no significant difference was found on the ADI-R (Lord et al., 1994) Repetitive/Stereotyped Behavior scores between the two groups of children, the HFA children showed higher within-group variability. More interestingly, findings in the present study showed that the poorer executive performance and repetitive stereotyped behavior, but not social interaction and communication, in these children were significantly associated with increased levels of total lymphocytes, T lymphocytes and Suppressor/cytotoxic T lymphocytes, suggesting that an altered immune system may be involved in the pathogenesis that underlies the executive processing and repetitive, stereotyped behaviors in ASD. These findings are in line with previous studies that reported abnormalities in the immune system in children with ASD (Krause et al., 2002; Molloy et al., 2006). The findings of alterations in T lymphocyte subsets are also consistent with the hypothesis that increased dysregulation of the immune system may give rise to the development of CNS-directed autoimmune responses in ASD (Hornig & Lipkin, 2001).

What appears to be an important extension of previous studies is that in the present study, it was found that executive function deficits and repetitive stereotyped behavior exacerbated as a function of increased levels of Suppressor/cytotoxic T lymphocytes in children with ASD. This provided some empirical evidence to support the notion that predominance of CD8+CTLs may underlie some of the autoimmune CNS diseases (Schirmer et al., 2001; Neumann et al., 2002). Specifically, CD8+CTLs are highly potent cells with several distinct cytotoxic functions. Recently, it has become clear that CD8+CTLs are important effectors in several autoimmune and degenerative CNS diseases and could be crucial in leading to tissue destruction. It was demonstrated that neurites of

cultured hippocampal neurons can be selectively transected by CD8+CTLs but not CD4+ Th lymphcoytes (Medana et al., 2000; Medana, Martinic, Wekerle, & Neumann, 2001). Thus, *in vitro* data provides compelling evidence that, in principle, cellular elements of the CNS can become CD8+CTL targets (Neumann et al., 2002). Since the autoreactive cytotoxic T cells can cause direct tissue damage to the CNS which may lead to neurodevelopmental damages (Krause et al., 2002), it may explain why a higher level and percentage of Suppressor/cytotoxic T lymphocytes were found in the LFA compared with the HFA children in the present study, which in turn may account for the more severe executive dysfunctions and abnormal repetitive behavior in the LFA children. However, further research is necessary to substantiate the present findings and examine the role that altered T lymphocyte subsets play in ASD.

While there are some interesting observations in the present study that suggested associations between executive dysfunction, repetitive stereotyped behavior and altered immune system in children with ASD, the following should be noted when interpreting the data. First, it should be noted that without normal control groups for comparison in the different executive functions measures, the degree of executive dysfunctions in the present sample of children with ASD was difficult to determine. The same applies to the immunological measures. Further research that includes a normal control group would allow a more confident conclusion to be drawn on whether children with ASD have executive dysfunctions and immunological aberrations. Second, IQ was observed to be significantly correlated with measures of executive functioning as well as immunological measures in the present study, and it may be argued that executive dysfunctions are mediated by the level of intelligence rather than altered immune function in the

children with ASD. Further studies to delineate the intricate relationship between IQ, executive functioning, and immune function would be useful to shed some light on the specificity of the immune function effects on general intellectual functioning and executive functioning. Finally, the generalization of the findings to individuals with ASD in general may be limited by the relatively small sample-size, and the large within-group variations in both performance and immunity measures.

In summary, the present study showed that general intelligence, executive functioning, and abnormal repetitive behavior varied as a function of the level of lymphocyte subsets in children with ASD. Low-functioning children with ASD showed more severe deficits in executive functioning and higher level of lymphocyte subsets, in particular, the Suppressor/cytotoxic T lymphocytes, compared with high-functioning children with ASD. This relationship may open up future directions of research and clinical trials on possible interventions for ASD.

Table 1

Characteristics of the High-Functioning (HFA) and Low-Functioning (LFA) Children with Autistic Spectrum Disorders (ASD).

Characteristics	HFA (n = 18)	LFA (n = 19)
Mean Age (in years)	11.4 (3.04)	12.05 (2.35)
Gender (male/female)	14/4	17/2
Intelligence Quotient	105.3 (22.24)	50.32 (12.73)**
ADI-R Social Interaction	20.89 (6.08)	20.42 (6.64)
ADI-R Communication	12.44 (4.16)	11.32 (5.16)
ADI-R Stereotyped Behavior	4.06 (4.06)	4.89 (2.64)
ADI-R Abnormal < 36 months	3.5 (1.5)	3.84 (.83)
	n (%)	n (%)
History of Allergy	14 (77.8%)	12 (63.2%)
History of chronic GI symptoms	7 (38.9%)	7 (36.8%)

Note. Standard deviations are in parentheses. ADI-R = Autism Diagnostic Interview-Revised (Lord, Rutter, Le Couteur, 1994).

** $p < .001$.

Table 2

Mean Performance and Standard Deviations on the Executive Functioning Measures and Parent Behavioral Observations of HFA and LFA Children with ASD.

Measures of Executive Functions	HFA(n = 18) M (SD)	LFA (n = 19) M (SD)	*t*-value
Executive Composite score	1.43 (1.59)	4.32 (1.82)	-5.21**
Individual test scores			
HKLLT			
Total Learning	17.8 (8.3)	8.05 (5.91)	4.14**
Discrimination	69.9 (33.9)	21.9 (30.1)	4.55**
D2			
Concentration performance	115.7 (55.9)	30.3 (44.0)	5.18**
5-Point			
Unique design	21.9 (10.7)	5.95 (6.83)	5.43**
CCTT			
Trail2 Time2	76.1 (69.8)	172.4 (109.0)	-3.22*
Tower of California			
Achievement scaled score	7.72 (4.69)	2.37 (1.98)	4.48**
Go-NoGo			
Commission errors	16.44 (33.53)	40.13 (39.49)	-1.89
Parent Behavioral Observations			
ADI-R Social Interaction	20.89 (6.08)	20.42 (6.64)	.22
ADI-R Communication	12.44 (4.16)	11.32 (5.16)	.73
ADI-R Stereotyped Behavior	4.06 (4.06)	4.89 (2.64)	-.84

Note. ** $p < .001$. *$p<.01$.

Table 3

The Absolute Numbers and Percentage Values of Lymphocyte Subsets in the Peripheral Blood of HFA and LFA Children with ASD.

Variable	HFA (n = 18) M (SD)	LFA (n = 19) M (SD)	t-value
Absolute number of Lymphocytes			
Total Lymphocytes (cells/μl)	2527.4 (708.1)	3136.2 (924.9)	-2.07*
T Lymphs (cells/μl)	1683.8 (465.9)	2138.3 (606.1)	-2.55*
Th Lymphs (cells/μl)	834.3 (232.4)	955.5 (225.7)	-1.74
Suppressor/Cytotoxic T Lymphs (cells/μl)	659.4 (182.7)	964.3 (393.5)	-3.05**
B Lymphs (cells/μl)	455.6 (164.4)	578.4 (232.0)	-1.85
NK cells (cells/μl)	388.3 (307.9)	361.4 (245.5)	1.32
Percentages within Lymphocytes			
T Lymphs (%)	65.8 (6.6)	68.5 (4.8)	-1.45
Th Lymphs (%)	33.6 (7.6)	31.4 (5.8)	0.96
Suppressor/Cytotoxic T Lymphs (%)	25.8 (3.1)	30.2 (5.0)	-3.17**
B Lymphs (%)	17.7 (5.2)	18.5 (5.5)	-0.46
NK cells (%)	14.3 (9.4)	10.9 (5.3)	1.32

Note. Standard deviations are in parentheses. ** p<.01, * p<.05. Lymphs: Lymphocytes

Table 4

Correlations between IQ, Measures of Executive Functions, Parent Behavioral Observations, and Peripheral Blood Lymphocyte Subsets in Children with ASD (N=37)

Executive Function Measures	Total Lymphocytes	T Lymphs	Suppressor/ Cytotoxic T Lymphs	Suppressor/ Cytotoxic T Lymphs %
IQ	-0.34*	-0.32*	-0.38*	-0.33*
Executive Composite score	0.35*	0.36*	0.38*	0.31
HKLLT-Total Learning	-0.40*	-0.39*	-0.42**	-0.30
HKLLT-Discrimination	-0.37*	-0.38*	-0.41**	-0.30
D2-Concentration Performance	-0.47**	-0.52**	-0.47**	-0.33*
5 Point-Unique Design	-0.34*	-0.35*	-0.40**	-0.36*
CCTT-Trail2Time2	0.17	0.21	0.21	0.22
ToC-Achievement	-0.20	-0.17	-0.20	-0.12
Go/No-Go Commission errors	0.29	0.29	0.29	0.17
ADI-R Social Interaction	0.19	0.08	0.11	-0.00
ADI-R Communication	0.02	-0.01	-0.05	-0.15
ADI-R Repetitive Behavior	0.45**	0.43**	0.44**	0.27

** p<.01, * p<.05, Lymphs: Lymphocytes

CHAPTER FOUR

Altered Immune Function Associated with Disordered Neural Connectivity and Executive Dysfunctions: A Neurophysiological Study on Children with Autism Spectrum Disorders

INTRODUCTION

Autistic spectrum disorders (ASD) is a group of lifelong developmental disorders. Individuals with ASD are characterized by impairments in communication, social interaction, and language, as well as having an abnormal repertoire of stereotyped behaviors (American Psychiatric Association, 2002). Abnormalities were also found in their higher cognitive functions such as memory (Chan et al., 2010; Cheung, Chan, Sze, Leung, & To, 2010) and executive functions (Liss et al., 2001; Ozonoff & Jensen, 1999). Some individuals with ASD may also have mental retardation, while others have normal intelligence (Happe, 1999). While the exact cognitive profile and the underlying basis of cognitive processing in ASD is not well understood, it has been suggested that deficient executive functions are fundamental to the cognitive deficits (Gilotty, Kenworthy, Sirian, Black, & Wagner 2002; Ozonoff, 1997).

Executive function consists of a multidimensional set of abilities required to perform complex behaviors for the attainment of a future goal (Donders, 2002; Nyden, Gillberg, Hjelmquist, & Heiman, 1999). Individuals with ASD have been found to have deficits in executive functions including disorganized actions and strategies, perseveration, difficulties in forming novel concepts, and inhibition of inappropriate actions (Bennetto, Pennington & Rogers, 1996). It has been widely

accepted that executive function is mediated by the frontal cortex (Duncan, 1986). Results from functional imaging studies have found altered patterns of activation, perfusion, and glucose metabolism in the frontal lobes in individuals with ASD during performance of executive function tasks (Ohnishi et al., 2000; Pierce, Haist, Sedaghat, & Courchesne, 2004; Schmitz et al., 2006).

Structural abnormalities have also been reported in individuals with ASD. Relative to a cohort of age-, race- and sex-matched controls, MRI scans assessing the brain volume of non-mentally retarded young adults with individuals with ASD have demonstrated that there is a significant enlargement of their frontal lobes (Carper, Moses, Tigue, & Courchesne, 2002; Hardan, Minshew, Mallikarjuhn, & Keshavan, 2001). Another neuroimaging study of preschool children with ASD has shown increased brain volume of the dorsolateral and medial frontal regions than normal children (Carper & Courchesne, 2005), and Redcay & Courchesne (2005) also reported converging evidence from a meta-analysis of head circumference, MRI and post-mortem brain weight in individuals with ASD revealing a marked early overgrowth of the autistic brain, particularly in the frontal lobes. These findings point to the suggestion of a transient postnatal macrocephaly (Courchesne, 2002), in which the abnormal brain growth interferes with the functional connectivity of the frontal cortex and its networks to other brain areas, resulting in

disruption to long-distance as well as localized connectivity in the brains of individuals with ASD (Belmonte et al., 2004; Courchesne & Pierce, 2005; Herbert, 2005; Just, Cherkassky, Keller, Kana, & Minshew, 2007; Rippon, Brock, Brown & Boucher, 2007).

Although the cause of the reported abnormal neural connectivity in ASD is not well understood, increasing evidence suggests that immunological factors are involved (Ashwood & Van de Water, 2004; Krause, He, Gershwin, & Shoenfeld, 2002; Pardo, Vargas, & Zimmerman, 2005). Specifically, there have been reports of elevated incidence of immune disorders, including heightened autoimmunity, reduced immune functions, and decreased peripheral lymphocyte numbers in patients with ASD (Ashwood et al., 2003; Connolly et al., 1999; Molloy et al., 2006; Pardo et al., 2005) and their first-degree relatives (Comi, Zimmerman, Frye, law, & Peeden, 1999; Singer et al., 2006).In addition, maternal viral infections have been reported to increase the risk for ASD; and maternal influenza infection in mice was associated with profound anatomical, motor, and other behavioral defects reminiscent of autism, including early macrocephaly and anxiety in novel situations (Fatemi et al., 2002; Shi, Fatemi, Sidwell, & Patterson, 2003). Some researchers have suggested that the immunologic abnormalities in ASD cause the production of autoantibodies that target central nervous system (CNS) proteins,

resulting in the destruction of neural tissues in the autistic brain (Korvatska, Van de Water, Anders, & Gershwin, 2002). Indeed, circulating cytotoxic T lymphocytes (CTL) have been shown to cause axonal damage of brain cells (Neumann, Medana, Bauer, & Lassmann, 2002). This is supported by a number of studies that reported the presence of anti-CNS auto-antibodies in children with ASD (Plioplys, Greaves, & Yoshida, 1989; Singh, Fudenberg, Emerson, & Coleman, 1988; Singh, Warren, Odell, Warren, & Cole, 1993). Together, the findings suggest that the disordered neural connectivity in ASD may be associated with abnormal immunologic activities that served to destroy neural tissues in the autistic brain (Ashwood, Wills, & Van de Water, 2006; Korvatska et al., 2002). The increased level of circulating CTL, with a report on its neural toxicity (Neumann et al., 2002), is a probable factor that should be explored.

While the argument for the role of immunologic factors disrupting the normal development in neural connectivity in ASD appears to be sound, there is relative a lack of empirical evidence to support this notion. This study, thus, aimed to examine whether there is an association between the neural connectivity and immunologic functions of young children with ASD. Given that the disordered neural connectivity involving the frontal lobes in these children has been shown to be associated with their degree of executive dysfunction (Belmonte et al., 2004;

Chan et al., 2010; Courchesne & Pierce, 2005), we also explored whether immunologic function, specifically the level of circulating CTL, would be associated with the executive dysfunction in these children.

Cortical connectivity between different functional areas in the brain can be estimated by coherence in the electroencephalography (EEG) (Murias, Webb, Greenson, & Dawson, 2007; Rippon et al., 2007). EEG coherence is a measure of phase correlation reflecting the level of synchronization between two brain areas as measured by EEG signals collected from different sites of the scalp (Nunez & Srinivasan, 2006; Srinivasan, Nunez, & Silberstein, 1998). Among different coherence measures, there is evidence to suggest that coherence in the theta band is related to the central executive processes in the frontal cortex (Mizuhara & Yamaguchi, 2007; Sauseng, Klimesch, Schabus, & Doppelmayr, 2005). Greater theta coherence was found in the frontal lobe and its posterior networks during visual planning (Petsche & Etlinger, 1998), attentional and inhibitory processing (Barry, Ckarke, McCarthy, & Selikowitz, 2007; Harmony, Alba, Marroquin, & Gonzalez- Frankenberger, 2009) and memory tasks that involved manipulation rather than simple retrieval and maintenance of information (Chan et al., 2010; Sauseng et al., 2005). Higher theta coherence was also found between the frontal and temporal regions during resting condition in patients known to have executive

dysfunctions (Babiloni et al., 2004; Ford, Mathalon, Whitfield, Faustman, & Roth, 2002), and in high-functioning children with ASD (Cantor et al., 1986; Murias et al., 2007).

Drawing together the different evidence linking ASD with executive dysfunction, disordered neural connectivity, and abnormal immunologic function, we hypothesized that children with ASD would show (1) significantly poorer executive function as measured by the Executive Composite score and scores on the different executive function tasks; (2) abnormal neural connectivity as measured by theta coherence in the frontal cortex and its network to the posterior region; and (3) a significantly higher level of circulating CD8+ T lymphocytes. Given that theta coherence is critically involved in central executive processes, we also hypothesized that (4) the executive dysfunction in individuals with ASD would be associated with theta coherence in the frontal region and its distributed network. Furthermore, given the notion that CD8+CTL might be associated with neuronal damage which leads to the disordered neural connectivity, we hypothesized that (5) the level of circulating CD8+CTL would be associated with the theta coherence in these children. Finally, given the intricate associations between neural connectivity, we hypothesized that the level of circulating CD8+CTL would be associated with the executive dysfunctions in these children.

High-functioning (HFA) and low-functioning (LFA) children with ASD, known for their significant differences in executive functioning (Han 2010, unpublished data), were recruited to participate in the present study to allow for the examination of the differences in their neural connectivity and immunologic function related to their different executive function.

METHODS

Participants

Seventeen high-functioning (HFA) and 14 low-functioning (LFA) children with ASD (18 with autistic disorder, 2 with Asperger disorder, 12 with pervasive developmental disorder not otherwise specified), aged 8 to 17 years, participated voluntarily in the study. The children with ASD were recruited either from the Parents' Association of Pre-School Handicapped Children in Hong Kong, or from the database of the Neuropsychology Laboratory of The Chinese University of Hong Kong. All children with ASD were diagnosed by pediatricians of the Child Assessment Centres of the Department of Health in Hong Kong, based on the criteria in the *Diagnostic and Statistical Manual of Mental Disorders* (4^{th} edition) (DSM-IV; American Psychiatric Association, 2002). Diagnosis was confirmed by a clinical psychologist through a standard clinical interview and the Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, & LeCouteur, 1994). Children

with a general intelligence quotient (IQ) of 70 or above belonged to the high-functioning group (HFA), and children with an IQ below 70 belonged to the low-functioning (LFA) group. Table 1 shows the demographic characteristics of the children. The HFA and LFA groups were matched on age [$t(29) = -0.42$, $p>0.05$], and had similar gender distribution. No significant difference was found in the severity of autistic features between the HFA and LFA groups on the ADI-R (Lord, Rutter, Le Couteur, 1994) in Social Interaction ($t = 0.96$, $p>0.05$), Communication ($t = 0.38$, $p>0.05$), and Repetitive/Stereotyped Behavior ($t = -0.73$, $p>0.05$).

Procedure

All children and their parents were invited to attend an individual assessment at The Chinese University of Hong Kong. The assessment consisted of a neuropsychological assessment session and an EEG recording session for the child, and an interview with one of the parents. The sequence of neuropsychological assessment and EEG recording for the children was countered-balanced to avoid order effect. In the neuropsychological assessment, each child was individually administered a battery that included the Wechsler Intelligence Scale for Children – Third Edition (WISC-III) short form (Kaufman, Kaufman, Balgopal, & McLean, 1996) for assessing their general intelligence; and measures of executive

functioning including the Hong Kong List Learning Test (HKLLT 2nd ed.; Chan, 2006), D2 Test of Concentration (D2; Brickenkamp, 1981), Five Point Test (5-point; Regard, Strauss, & Knapp, 1982), Children's Color Trails Test (CCTT; Williams et al., 1995), Tower of California Test (ToC; Mattson, Goodman, Caine, Delis, & Riley, 1999), and a discriminant Picture Completion task (PicC; Russo, Nichelli, Gibertoni, & Cornia, 1995). In the EEG recording session, resting EEG was recorded in the eyes-open condition. In addition to the neuropsychological assessment and EEG recording sessions, 4 ml of blood was drawn by venipuncture for each child within a week following the neuropsychological assessment. The EDTA blood was collected by a registered nurse in a medial clinic between 10:30 am and 12:00 noon, and kept in a 4°C cold bag for transporting to a clinical laboratory where blood assays were performed by a laboratory technician blinded to the study. All children participated with parent consent, and the procedure was approved by the NTEC-CUHK Clinical Research Ethics Committee.

Measures

Autism Diagnostic Interview-Revised (ADI-R). The ADI-R (Lord et al., 1994) is a rating measure completed by the parent on the child's behaviors relevant to the diagnosis of Pervasive Developmental Disorders. It consisted of three scales, Social Interaction, Communication, and Repetitive/Stereotyped Behavior, which

correspond to the three diagnostic criteria of autism established in the DSM-IV-TR (American Psychiatric Association, 2002). Each scale contains detailed questions about early developmental and current functioning of the child, with higher scores indicating more severe autistic features.

Wechsler Intelligence Scale for Children-Third Edition (WISC-III) short form. The WISC-III short form (Kaufman et al., 1996) was used in the study to assess general intelligence. It comprises the two verbal subtests of Similarities and Arithmetic; and the two performance subtests of Picture-Completion and Block Design from the original WISC-III. The short form yields an IQ score with a mean of 100 and a standard deviation of 15.

Hong Kong List Learning Test (HKLLT). The HKLLT (2nd ed.; Chan, 2006) is primarily a memory test, which also measures frontal lobe functions including learning strategies and organization (Cheung et al., 2010). The test consists of a randomly organized list of 16 two-word Chinese characters presented once during each of three learning trials. Children in the present study were asked to recall the words immediately after each of the three learning trials. The total number of correctly recalled words during the three learning trials gave the Total Learning score. A recognition test consisting of the 16 target words and 16 distracters was presented after a 30-minute delayed recall trial. The children were required to

discriminate whether the words have been previously learnt. A discrimination score that assessed memory performance was calculated based on the number of correct hits (i.e., the correct identification of targets) and false alarms (i.e., the false positive) at the recognition trial.

D2 Test of Concentration (D2). This test (Brickenkamp, 1981) is a letter cancellation task and measures inhibition, concentration and error judgment. It consists of a piece of paper with different letters printed on it, with different numbers of dashes above and below the letters. The children were asked to cancel all the letter d's with 2 dashes. There were a total of 14 lines and for each line the time allowed was 20 seconds. The numbers of omission and wrong cancellations were recorded to give scores indicating the children's performances on inhibition, concentration and error judgment.

The Five Point Test (5-point). This test (Regard et al., 1982) measures figural fluency through the production of novel designs under time constraints. Children in the present study were asked to create original and novel shapes by connecting five points with straight lines within five minutes. This test is a non-verbal analog to verbal fluency tasks, and was used in the present study because it is a good measure of frontal lobe pathology. Scores ranged from 0 to 40, with higher scores indicating greater cognitive fluency.

Children's Colour Trail Test (CCTT). The CCTT (Williams et al., 1995) is an altered version of the Trail Making Test of the Halstead- Reitan Battery (Reitan & Wolfson, 1993). It is specifically designed to be a cultural-free test for children. The test measures the speed of attention, sequencing, mental flexibility, visual searching and motor function. The test is printed on paper, with one set of numbers from 1 to 15 embedded within pink circles and another set within yellow circles. The children were required to connect the circles in ascending order, alternating between pink and yellow colors, as quickly as possible. The completion time in seconds for the task was the score.

The Tower of California Test (ToC). The ToC (Mattson et al., 1999) is a modification of the Tower of Hanoi (Borys, Spitz, & Dorans, 1982) and Tower of London (Morris, Ahmed, Syed, & Toone, 1993; Shallice, 1982) tests, and was administered in the present study to assess spatial planning and cognitive flexibility (Delis, Kaplan, & Kramer, 2001). It consists of nine items that involve moving discs on three colored vertical pegs to match the target arrangements while adhering to rules. The score was calculated as the number of items successfully completed.

Go/No-Go task. The computerized Go/No-Go task was used to measure impulse control in the study (Kana, Keller, Minshew, & Just, 2007). The task was

administered on computer. Children were required to press a key as quickly as possible when a black ball (Go stimulus) appeared on the screen, and to inhibit their responses when a red ball (No-Go stimulus) appeared. The total testing time was 6 minutes and the stimuli were displayed one at a time, in the center of the computer screen, for 500 ms in random order at a ratio of 4:1 (192 black balls: 48 red balls) followed by 1000 ms of blank intervals. The Total Commission Errors on "No-Go" trials measured inhibition.

Picture Completion Task (PicC). The PicC is an implicit memory task adapted from the picture completion paradigm developed by Russo *et al.* (1995). The line drawings were taken from the Snodgrass and Vanderwart's object database (1980). The task is designed to assess a relatively nonstrategic, automatic perceptual implicit learning, and was used in the present study as a discriminant non-executive function task. This task consists of a study phase and a test phase. In the study phase, 16 pictures were presented, and the children were asked to name each picture. The test phase follows and the children were shown 8 targets and 8 new pictures and their fragmented sequences. The most fragmented versions of the16 randomly intermixed pictures were presented first, followed by the next levels of fragmentation. Scoring was based on the level of fragmentation at which identification occurred. A Repetition Priming score that assessed perceptual

implicit learning was computed based on the identification scores for studied target pictures and new non-studied pictures.

Immunologic Measures. Given our hypothesis that cytotoxic T lymphocytes (CD8+CTLs) can cause direct tissue damage to the CNS which may lead to neuronal damage (Krause et al., 2002; Schirmer et al., 2001; Neumann et al., 2002), percentage and absolute count of CD8+ in the peripheral blood of the children with ASD were measured using immunofluorescence technique with BD Multitest™ IMK kit and 4 color flow cytometer (FACSCalibur, BD Bioscience Corp., San Jose, CA, USA).

EEG Recording. All parents and children were briefed on the procedure, and their written informed consents were obtained before EEG recording was taken. Each child was tested individually in a sound- and light-attenuated room using the DEYMED Diagnostic TruScan 32 Biofeedback Device. An electrode cap with 19 electrodes, based on the International 10-20 System referenced to linked ears, was used to collect EEG data at a sampling rate of 256 Hz with a low pass filter of 30 Hz and high pass filter of 1 Hz. Impedance at each electrode site was kept below 10 kΩ. Resting EEG was collected in the eyes-open condition. The children were asked to focus on a figure (e.g., a car) displayed on a computer screen, and body movements were time-marked by a research assistant for off-line analyses. EEG

data was stored and later displayed on computer, and visually examined for eye movements and muscle artifacts. A minimum of one minute of artifact-free data were selected (John et al., 1988 for discussion of qEEG method) and spectrally processed to the different frequency domain using the fast Fourier Transformation (FFT) with the Neuroguide (version 2.1.8) software to compute coherence values. Theta coherence measures (4 – 7.5 Hz) were used in the present study.

Coherence measures. Coherence, defined as the cross-spectral power between an electrode pair normalized by their power spectra (Sauseng et al., 2005), is an index that measures temporal synchronization of EEG activity between two brain regions underneath the electrodes and reflects the functional connectivity between the two regions. 171 coherence values were computed among the 19 electrode positions (Fp1, Fp2, F3, F4, F7, F8, Fz, T3, T4, T5, T6, C3, C4, Cz, P3, P4, Pz, O1 and O2). Two short-range and four long-range coherence measures of the anterior and posterior cortical regions were computed to examine the frontal and posterior cortical connections. Short-range coherence measures of the anterior region was computed by averaging the signals from electrode sites FP1, FP2, F3, Fz and F4, and short-range coherence measures of the posterior region was computed by averaging the signals from electrode sites P3, Pz, P4, O1 and O2. Fronto-posterior long-range coherence measures were computed to give two mean

intra-hemispheric (Left: FP1-T5, FP1-P3, FP1-O1, F7-T5, F7-P3, F7-O1, F3-T5, F3-P3, F3-O1; Right: FP2-T6, FP2-P4, FP2-O2, F8-T6, F8-P4, F8-O2, F4-T6, F4-P4, F4-O2), and two mean *inter-hemispheric* (Left to right: FP1-T6, FP1-P4, FP1-O2, F7-T6, F7-P4, F7-O2, F3-T6, F3-P4, F3-O2; Right to left: FP2-T5, FP2-P3, FP2-O1, F8-T5, F8-P3, F8-O1, F4-T5, F4-P3 and F4-O1) long-range coherence measures. Long-range coherence was defined as any electrode pairs that were separated by at least one electrode in between (i.e., the distance between the electrodes must be greater than 10 cm). Each coherence value was transformed using the Fisher's Z-transform.

Data Analyses

Executive functioning of HFA and LFA children with ASD were compared and examined for differences. To reduce the number of statistical comparisons, one Executive Composite score was computed from the seven neuropsychological measures including the HKLLT, D2, 5-Point, CCTT, ToC and Go/NoGo tasks. This was done by converting the raw scores on the different executive function measures to Z scores, using the grand mean and standard deviation of the executive measures derived from the normative data. The Z scores from the different executive function measures were then averaged to yield the Executive Composite score. Higher scores indicated poorer executive functioning. The Executive

Composite score and the priming index of the discriminant implicit memory task were compared using independent student's *t*-tests. Posthoc analyses on the individual executive function measures were performed using independent *t*-tests. For the immunological measures, the absolute count and percentage of CD8+ were compared between the HFA and LFA groups using independent student's *t*-tests. EEG theta coherence measures of the two groups were compared on the two short-range (anterior, posterior), two *intra-hemispheric* long-range (left to left, right to right), and two *inter-hemispheric* long-range (left to right, right to left) coherence measures also using independent *t*-tests. The relationship between executive functioning, EEG coherence and immune function were examined using Pearson correlation. Given that specific hypotheses were tested and that the number of participants were relatively small, we did not adjust the alpha level to maintain a reasonable balance between the risks of Type I and Type II errors.

RESULTS

Neuropsychological Measures on Executive Functioning

Results on the executive function measures confirmed our hypothesis that LFA children showed a significantly higher Executive Composite score than HFA children (t = -4.42, $p<0.01$), suggesting that LFA children had poorer executive

functioning compared with their HFA counterparts. Post hoc results on the individual executive function measures indicated that as predicted, the LFA group showed significantly lower HKLLT total Learning ($t = 3.51$, $p<0.01$) and discrimination ($t = 3.51$, $p<0.01$) scores, as well as on the D2 Concentration Performance ($t = 4.12$, $p<0.001$), 5-point Unique Design ($t = 4.37$, $p<0.001$), CCTT-2 Time ($t = -2.27$, $p<0.05$), and the ToC Achievement ($t = 3.86$, $p<0.001$) scores than the HFA group (Table 2). Independent *t*-test indicated no significant difference between the HFA and LFA groups on the Go/No-Go Total Commission Errors ($t = -1.29$, $p>0.05$). In contrast, there was no significant difference between the LFA and HFA groups on the Repetition Priming score of the discriminant PicC task ($t = .31$, $p>0.05$).

EEG Coherence Measures

Figure 1a shows the differences of Z-transformed coherence values between the HFA and LFA groups. Visual examination showed that as predicted, there were significant differences between HFA and LFA children in their theta coherence. Results indicated that LFA children showed sharply elevated theta coherence across multiple electrode sites during eye-open resting condition compared with HFA children. Specifically, LFA children demonstrated significant increases in the short-range coherence in the anterior region ($t=-2.06$, $p<0.05$), and also long-range

coherences in the left *intra-hemispheric* (left anterior–left posterior) (t = -3.18, p< 0.01), and right-to-left *inter-hemisphere* (right anterior—left posterior) (t = -2.69, p<0.01) connections (Figure 1b). The LFA group also showed a trend towards elevated long-range left-to-right *inter-hemisphere* (left anterior–left posterior) (t = -1.93, p=0.07) coherence compared with HFA children. No significant difference was found between the two groups in the short-range coherence in the posterior region (t = 0.29, p>0.05).

Immunologic Measures

Consistent with our argument that executive function deficits would be associated with immunologic abnormalities that causes neuronal damage which led to our hypothesis the children with poorer executive function, i.e. LFA children, would have a higher level of circulating CD3+CD8+ T lymphocytes, the results confirmed our hypothesis and indicated that LFA children showed significantly higher number (t = -3.13, p<0.01) and percentage (t = -4.10, p<0.001) of CD3+CD8+ T lymphocytes than HFA children (Table 4).

Association between Executive Function, EEG Coherence and Immunologic Measure

Given that LFA children were found to differ from their HFA counterparts in measures of executive function, EEG coherence as well as immunologic function,

we examined the relationship between the three using Pearson correlation (Table 5). Results showed that for the combined group (LFA and HFA) of children with ASD, executive function was associated with both EEG theta coherence and measures of immunologic CD3+CD8+ T lymphocytes. No significant correlation was found between PicC Repetition Priming score with any of the neuropsychological, neurophysiologic and immunologic measures (all $p > 0.05$).

Association between executive function and EEG coherence. Specifically, the Executive Composite score was significantly associated with the short-range coherence of the anterior region ($r = .40$, $p<0.05$), and fronto-posterior long-range *intra-hemisphere* (left anterior – left posterior) ($r = 0.36$, $p<0.05$) and *inter-hemisphere* (right anterior – left posterior) ($r = 0.38$, $p<0.05$) coherences in children with ASD.

Association between executive function and immunologic function. The Executive Composite score was also found to be significantly associated with the immunological measure of the number of Suppressor/Cytotoxic T lymphocytes (CD3+CD8+; $r = 0.51$, $p<0.01$). In addition, the percentage of Suppressor/Cytotoxic T lymphocytes (CD3+CD8+/CD45+) was most strongly and significantly associated with the measures of executive functioning ($r = 0.56$, $p<0.01$)

Association between EEG coherence and immunologic function. Results also indicated that as predicted, the percentage of Suppressor/Cytotoxic T lymphocytes (CD3+CD8+/CD45+) was strongly and significantly associated with different EEG coherences [anterior short-range coherence ($r = 0.47$, $p<0.01$); left *intra-hemisphere* coherence ($r = 0.43$, $p<0.05$) and right-to-left *inter-hemisphere* coherence ($r = 0.40$, $p<0.05$)].

DISCUSSION

The present study examined the executive function, neural connectivity, and immunologic functions in children with ASD, and whether these abnormalities were associated. Results showed that in executive function, LFA children performed significantly poorer than HFA children as shown on their lower Executive Composite as well as individual executive function scores. Results on neural connectivity showed that LFA children demonstrated a different pattern of EEG coherence from HFA children as shown in the significantly elevated theta coherence in the anterior network, as well as at the left *intra-hemispheric* (LA-LP) and right-to-left *inter-hemisphere* (RA-LP) connections of LFA children. In immunologic function, results showed that LFA children had significantly elevated level of Suppressor/cytotoxic T lymphocytes (CD8+). In addition, the executive

dysfunction, disordered neural connectivity, and abnormal immunologic function were found to be associated. These results provided some initial evidence to support that notion that immunologic factors causes neuronal damage, measureable by EEG coherence and manifested as executive dysfunctions. No significant correlation was found between PicC Repetition Priming score and any of the executive and neurophysiological measures.

The significantly elevated theta coherence in LFA children, during eyes-open resting condition compared with HFA children, is in line with prior findings that reported increased coherence of the slow bands in patients with severe cognitive impairments (Comi et al., 1998; Mann, Maier, Franke, Roschke, & Gansicke, 1997; Newton et al., 1994). Our findings that elevated short-range theta coherence in the anterior network was significantly associated with poorer executive functioning is consistent with previous studies reporting structural, physiologic, and functional abnormalities in the frontal region of individuals with ASD (Mundy, 2003; Rumsey & Ernst, 2000). Our results on the association between the elevated long-range (inter-hemisphere anterior-posterior regions) theta coherence and poorer executive functioning also provided support for the contribution of the anterior-posterior brain-circuit in the executive control of cognitive processing as shown in other EEG studies (Belmonte et al., 2004; Courchesne & Pierce, 2005; Rippon et al.,

2007). In addition, the present study also showed that the significantly elevated theta coherence in children with ASD was more pronounced in the left hemisphere. This finding is consistent with previous neuroimaging and neurophysiological studies of ASD reporting greater abnormality found in the left side of the brain, as well as ASD patients' behavioral manifestation similar to those with left hemisphere disorders (Chan et al., 2010).

Our EEG results indicating elevated intra-hemispheric coherence in the left hemisphere in children with ASD are in contrast to a previous coherence study on normal individuals which concluded that higher intra-hemispheric coherence of the right, rather than the left, hemisphere is reflective of poorer cortical differentiation (Thatcher, Walker, & Giudice, 1987). On the other hand, this finding is consistent with the notion of a functional disconnection between the two hemispheres in individuals with ASD (Nyden, Carlsson, Carlsson, & Gillberg, 2004), with the significantly elevated theta coherence of the left hemisphere being a possible compensatory mechanism by the left hemisphere to compensate for the lower cortical differentiation in individuals with ASD (Koeda et al., 1995; Leocani & Comi, 1999). This compensatory notion is also supported by some neuroimaging and neuropsychological studies that reported a reduced size of the corpus callosum (Egaas, Courchesne, & Saitoh, 1995; Piven, Bailey, Ranson, & Arndt, 1997) and

aberrant inter-hemispheric transfer in patients with ASD (Nyden et al., 2004). Given that decreased connectivity through the corpus callosum diminishes the internal communication of the brain, and that executive control of cognitive processing relies on the integration of multiple brain regions (Osaka et al., 2004; Sauseng et al., 2005), this may explain why individuals with ASD showed impaired performance on tasks that involve complex, inter-hemispheric integration of information while performing normally on relatively simple cognitive tasks that require less neural integration (Nyden et al., 2004).

Our findings on the immunologic function showed that LFA children had significantly elevated level, in terms of the actual number and the percentage, of Suppressor/cytotoxic T lymphocytes (CD8+), are in line with previous studies that reported abnormalities in the immune system in children with ASD (Krause et al., 2002; Molloy et al., 2006). In addition, we also showed that there is a strong association between the severity of executive dysfunction and increased CD8+ level, providing evidence to support the hypothesis that predominance of cytotoxic T lymphocytes (CD8+CTLs) may be involved in the pathogenesis that underlies the deficient executive processing in ASD. Apart from showing that there is an association between executive dysfunction and elevated CD8+ level, the present study has extended previous knowledge in demonstrating an association between

immunologic abnormalities and EEG coherence in these children. We found a strong association between EEG coherence and the percentage of CD3+CD8+ / CD45+ in children with ASD, with higher ratio of CD3+CD8+ / CD45+ level associated with more elevated theta coherence in the frontal and long-range anterior-posterior connections. These results support the notion that the auto-reactive cytotoxic T cells can cause direct neuronal damage (Bilzer, & Stitz, 1994; Boulanger & Shatz, 2004) which may lead to the neuro-developmental damages in ASD (Krause et al., 2002). Specifically, CD8+CTLs have highly potent cytotoxic functions. It has been reported that CD8+CTLs are important effectors in several autoimmune and degenerative CNS diseases that can lead to neuronal tissue destruction, for example, neurites of cultured hippocampal neurons were found to be selectively destroyed by CD8+CTLs but not CD4+ T cells (Medana et al., 2000; Medana, Martinic, Wekerle, & Neumann, 2001). These *in vitro* result provides compelling evidence that tissues of the CNS can become CD8+CTL targets (Neumann et al., 2002). In view of that, it is conceivable that the higher ratio of CD3+CD8+ / CD45+ cells might cause neuronal damage that leads to the observed disordered neural connectivity as indicated by EEG coherence, which might manifest as executive dysfunction as shown in children with ASD. It is worth noting, however, that in contrast to the relative percentage of CD8+

lymphocytes, the absolute count of CD8+ T lymphocytes did not show any significant association with the EEG coherence measures. This may be due to the fact that we included both suppressor T cells as well as the cytotoxic T cells in our blood test analysis, which might have been a possible source of the confounded results. Future research is necessary to further differentiate the CD8+CTLs from the CD8+ suppressor T cells and substantiate the role CD8+CTLs plays in ASD.

While some very interesting associations have been observed in the present study that suggestive of the role immunologic factor plays in the development of ASD, the following should be noted when interpreting the data. First, the establishment of impairments and abnormalities in executive function, neural connectivity, and immunologic function in our sample of children with ASD was not absolute as there was no norm group for comparison. Further research that includes a normal control group would allow a more confident conclusion to be drawn on the degrees of impairments in the different aspects in these children. Second, IQ was observed to be significantly correlated with executive function as well as immunologic measures in the present study, lending itself to the argument that executive dysfunctions are due to intelligence rather than altered immune function. Further studies to delineate the intricate relationship between IQ, executive function, cortical connectivity and immunologic function would be

useful to shed some light on the specificity of the immunologic effect. Finally, the generalization of the findings to individuals with ASD in general may be limited by the relatively small sample size, and the large within-group variations in both executive function and immunologic measures. In spite of these limitations, the present study provided evidence to show that general intelligence, executive function, and neural connectivity varied as a function of the level of CD8+ T lymphocytes in children with ASD. This relationship may open up future directions of research on the role of CD8+ T lymphocytes on the development of ASD, and related clinical studies on possible interventions for ASD.

Table 1

Characteristics of the High-Functioning autistic (HFA) and Low-Functioning autistic (LFA) Children with Autistic Spectrum Disorders (ASD)

Variable	HFA (n = 17)	LFA (n = 14)
Mean Age (in years)	11.71 (3.13)	12.15 (2.12)
Gender (male/female)	14/3	11/1
Intelligence Quotient	106.18 (20.62)	56.08 (11.83)**
ADI-R Social Interaction	20.47 (6.14)	18.08 (7.28)
ADI-R Communication	11.65 (5.22)	10.92 (4.87)
ADI-R Stereotyped Behavior	3.76 (3.29)	4.58 (2.47)

Note. Standard deviations are in parentheses. ADI-R = Autism Diagnostic Interview-Revised. ** $p < .001$.

Table 2

Mean Performance and Standard Deviation on the Executive Functioning and Discriminant Picture Completion Task of Children in the High-Functioning (HFA) and Low-Functioning (LFA) Group

Measures	HFA(n = 17) M (SD)	LFA (n = 14) M (SD)	*t*-value
Executive Function Composite	1.21 (1.23)	3.62 (1.72)	-4.42**
HKLLT-Total Learning	19.24 (7.31)	10.33 (5.76)	3.51**
HKLLT-Discrimination	73.48 (29.76)	31.94 (33.67)	3.51**
D2-Concentration performance	122.7 (49.44)	47.67 (46.52)	4.12***
5 Point-Unique design	23.29 (10.48)	7.83 (7.52)	4.37***
CCTT-Trail2Time2	62.15 (43.62)	135.26 (105.5)	-2.27*
ToC-Achievement	7.82 (4.54)	2.92 (2.19)	3.86***
Go/NoGo-Commission errors	9.71 (7.93)	14.33 (11.37)	-1.29
PicC-Repetition Priming	0.57 (0.11)	0.55 (0.20)	0.32

***p<0.001, **p<0.01, *p<0.05.

Table 3

Mean (SD) of Short- and Long-range Fisher-Z Transformed Coherence of HFA and LFA Children with Autistic Spectrum Disorder (ASD) during Eyes-open Resting Condition

Measures	HFA (n = 17) M (SD)	LFAD (n = 14) M (SD)	*t*-value
Short-Range			
Anterior	1.16 (0.09)	1.24 (0.13)	-2.06*
Posterior	1.13 (0.10)	1.12 (0.10)	0.29
Long-Range			
Intra-hemisphere			
Left	0.29 (0.07)	0.37 (0.08)	-3.18**
Right	0.42 (0.09)	0.46 (0.11)	-1.09
Inter-hemisphere			
Left to Right	0.25 (0.04)	0.30 (0.08)	-1.93
Right to Left	0.27 (0.06)	0.34 (0.08)	-2.69**

** $p < 0.01$. *$p < 0.05$.

Table 4

The Absolute Count and Percentage of Suppressor/cytotoxic T Lymphocytes in the Peripheral Blood of HFA and LFA Children.

Variable	HFA (n = 17) Mean (SD)	LFA (n = 14) Mean (SD)	t-value
Absolute count (cells/μL)			
Total T Lymphocytes (CD3+)	2512.6 (681.4)	3260.0 (991.2)	-2.41*
Suppressor/Cytotoxic T Lymphocytes (CD3+CD8+)	642.4 (172.9)	1045.4 (421.4)	-3.13**
Percentage (%)			
Suppressor/Cytotoxic T Lymphocytes (CD3+CD8+ / CD 45+)	25.8 (3.2)	31.8 (4.7)	-4.10***

Note. Standard deviations (SD) are in parentheses. ***p<0.001, ** p<0.01, * p<0.05.

Table 5

Correlations between IQ, Executive Function, EEG Coherence and Peripheral Blood Lymphocyte Subsets in Children with ASD (n =31).

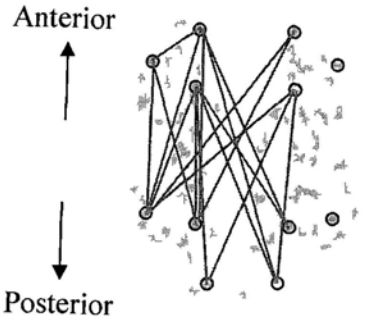
	IQ	EF	PiC	Ant. Coherence	Intra-hemi Left	Inter-hemi Rt to Left	CD3+CD8+ T lymphocytes (absolute count)	CD3+CD8+/ CD45+ (%)
IQ	1.00							
EF Composite score	-0.68***	1.00						
PicC - Repetition Priming	0.14	-0.24	1.00					
EEG Coherence								
Anterior	-0.35	0.40*	-0.23	1.00				
Intra-hemisphere - Left	-0.43*	0.36*	0.02	0.35	1.00			
Inter-hemisphere - Right to Left	-0.44*	0.38*	0.01	0.35	0.64***	1.00		
Immunologic Measures								
CD3+CD8+ T lymphocytes (absolute count)	-0.48**	0.51**	0.13	0.21	0.09	0.08	1.00	
CD3+CD8+ / CD45+ (%)	-0.48**	0.56**	-0.02	0.47**	0.43*	0.40*	0.77***	1.00

***p<0.001, **p<0.01, * p<0.05

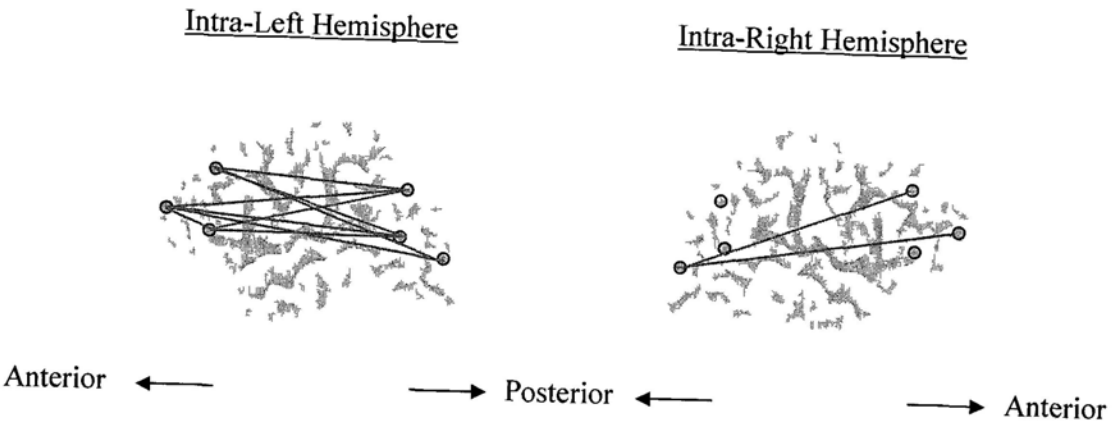
Figure Legend

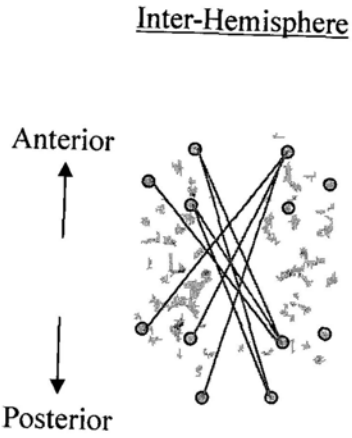
Figure 1 Maps showing the difference in theta coherence between LFA and HFA children. (a) Black lines represent significantly higher Fisher-Z transformed coherence values in the LFA group than the HFA group during eye-open resting condition. (b) Topographic maps demonstrating the between-subject differences in intra- and inter-hemispheric theta coherence.

(a)


Anterior
Posterior

(b)


Intra-Left Hemisphere
Intra-Right Hemisphere
Anterior
Posterior
Anterior


Inter-Hemisphere
Anterior
Posterior

CHAPTER FIVE

SUMMARY and CONCLUSIONS

Autism spectrum disorders (ASD) is a group of lifelong developmental disorders characterized by poor social interaction with others, language delay or impairment, and repetitive and stereotyped behavior (American Psychiatric Association, 2002). Recent evidence suggests that deficient executive functions are fundamental to the cognitive deficits in ASD (Gilotty, Kenworthy, Sirian, Black, & Wagner, 2002; Ozonoff, 1997). Executive functions have been widely accepted to be mediated by the frontal cortex (Duncan, 1986; Schroeter, Zysset, Wahl & von Cramon, 2004). It has been suggested that deviant brain growth in individuals with ASD interferes with the normal developmental course, resulting in disruption to the neural connectivity between key networks as well as localized neural assemblies (Belmonte et al., 2004; Courchesne & Pierce, 2005b; Herbert, 2005; Just, Cherkassky, Keller, Kana, & Minshew, 2007; Rippon, Brock, Brown & Boucher, 2007), which may include the frontal lobes. While the cause of ASD is not well-understood, immunologic abnormalities have been reported in children with ASD (Plioplys, Greaves, & Yoshida, 1989; Singh, Fudenberg, Emerson, & Coleman, 1988; Singh, Warren, Odell, Warren, & Cole, 1993). In parallel, cytokines have been shown to influence learning and memory (Pugh, Fleshner, Watkins, Maier, & Rudy, 2001), and circulating cytotoxic T lymphocytes have been shown to cause axonal damage (Boulanger & Shatz, 2004; Medana, Martinic,

Wekerle, & Neumann, 2001; Neumann, Medana, Bauer, & Lassmann, 2002). While the argument for immunologic factors disrupting the neural connectivity in the brains of individuals with ASD appears to be sound, there is relatively little empirical evidence to support the notion.

This dissertation reports on a series of three studies to examine whether the executive dysfunctions in children with ASD are associated with their immunologic abnormalities and disordered neural connectivity.

Study one compared high-functionting (HFA) and low-functioning (LFA) children with ASD with normally developing children (NC) on different executive functions using a neuropsychological test battery which included the Hong Kong List Learning Test (HKLLT), D2 Test of Concentration (D2), Five Point Test (5-point), Children's Colour Trail Test (CCTT), the Tower of California Test (ToC), and the Go/No-Go task (GNG). Nineteen high-functioning and 19 low-functioning children with ASD, and 28 NC children, aged 8 to 17 years, participated voluntarily in the study. Results showed that the three groups of children showed significantly different scores on the HKLLT Total Learning and Discrimination scores, as well as the D2 Concentration Performance score. The LFA group demonstrated the poorest performance, with the HFA group in between and the NC group highest. These results have extended previous knowledge in demonstrating

that LFA and HFA children had significant differences in their degree of executive function deficits.

Study two examined whether the executive dysfunctions in LFA and HFA children are associated with their immunological abnormalities. Eighteen HFA and 19 LFA children with ASD, aged 8 to 17 years, were assessed on executive functions using the same neuropsychological test battery in study one. They were also assessed on autoimmune symptoms reported by their parents; and immunological measures including T lymphocytes (CD3+), B lymphocytes (CD19+), T helper lymphocytes (CD3+CD4+), suppressor/cytotoxic T lymphocytes (CD3+CD8+), and natural killer (NK) cells (CD3-CD16+ and/or CD56+). Results indicated that LFA children showed greater deficits in executive functions as well as higher levels of total lymphocyte, T lymphocyte and suppressor/cytotoxic T lymphocyte levels than HFA children (all $p< 0.05$). Their executive functions were also significantly associated with the three lymphocyte levels (all $p < 0.05$), providing initial evidence to support the notion that altered immune functions have a role to play in the executive dysfunctions in these children.

Study three further examined whether the executive dysfunctions and abnormal immunologic functions in LFA and HFA children are associated with

their disordered neural connectivity. Seventeen HFA and 14 LFA children, aged 8 to 17 years, participated voluntarily in the study. The two groups of children were compared on their executive functions using the neuropsychological test battery in study one and two; a non-executive cognitive task as measured by the Picture Completion Task; neural connectivity as measured by theta coherence in the anterior and posterior regions; and immunologic function as measured by the level of circulating CD3+CD8+ suppressor/cytotoxic T lymphocytes in a blood sample. Results on executive functions showed that LFA children performed significantly poorer than HFA children as shown on their lower Executive Composite as well as individual executive function scores. However, there was no group difference on the Picture Completion Task. Results on neural connectivity showed that LFA children demonstrated a different pattern of electroencephalography (EEG) coherence from HFA children as shown in the significantly elevated theta coherence in the anterior network, as well as at the left *intra-hemispheric* and right-to-left *inter-hemisphere* connections of LFA children. In immunologic function, results showed that LFA children had significantly elevated level of suppressor/cytotoxic T lymphocytes (CD3+CD8+) ($p < 0.05$). In addition, the executive dysfunction, disordered neural connectivity, and abnormal immunologic function were found to be associated.

The results from the three studies have extended previous knowledge in showing that LFA children had a significantly greater degree of executive dysfunctions than their HFA counterparts. This greater degree of dysfunctions was also associated with the significantly higher levels of total lymphocyte, T lymphocyte and suppressor/cytotoxic T lymphocyte levels in LFA children. Furthermore, the significantly elevated level, specifically in suppressor/cytotoxic T lymphocytes, was found to be associated with the disordered neural connectivity in the anterior region and its related network, as shown in the significantly elevated theta coherence in LFA children.

While these studies are limited in their generalization of the findings to individuals with ASD in general by the relatively small sample-size, the large within-group variations in both performance and immunity measures, and the restricted age-range, the findings have provided some important initial evidence to support the notion that immunologic factors may play a role in causing neuronal damage in the anterior region of the brains of children with ASD, which is manifested in their disordered neural connectivity of that region, and their executive dysfunctions mediated by that same region. This has opened up a new direction of research in which behavioral and cognitive dysfunctions may be studied from the perspective of immunologic functions. It would be a new

direction of neuropsychological intervention if future research would indicate whether improvements in immunologic functions are associated with corresponding improvements in behavioral and cognitive performance.

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