



## Lymphocyte subset alterations related to executive function deficits and repetitive stereotyped behavior in autism

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### ABSTRACT

Increasing evidence suggests that immunological factors are involved in the pathogenesis of autism spectrum disorders (ASD). The present study examined whether immunological abnormalities are associated with cognitive deficits in children with ASD. Eighteen high-functioning (HFA) and 19 low-functioning (LFA) children with ASD, aged 8–17 years, were assessed on cognitive functioning using IQ tests and executive functions tests including the Five Point test, Children Color Trail-making Test, D2 Test of Concentration, Tower of California Test; Hong Kong List Learning Test, and Go/No-Go test. 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+). 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  $p < 0.05$ ). These findings support the notion that altered immune functions may act on the neural tissues of individuals with ASD, which in turn leads to their cognitive dysfunctions.

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### 1. 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).

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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 immunological 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 (Benetto, 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 immunological 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 (Han, 2010, unpublished data), 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.

## 2. Materials and methods

### 2.1. Participants

Eighteen HFA and 19 LFA children with ASD, aged 8–17 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 et al., 1994). According to the DSM-IV-TR criteria, 21 children met the diagnosis of autistic

**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 (0.83)
Characteristics	n (%)	n (%)
History of allergy	14 (77.8%)	12 (63.2%)
History of chronic GI symptoms	7 (38.9%)	7 (36.8%)

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

\*\*  $p < 0.001$ .

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 [ $t(35) = -0.74, p > 0.05$ ], 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 et al., 1994) measures of Social Interaction ( $t = 0.22, p = n.s.$ ), Communication ( $t = 0.73, p = n.s.$ ), and Repetitive/Stereotyped Behavior ( $t = -0.84, p = n.s.$ ) (Table 1).

## 2.2. Procedures and materials

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 Stanford-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 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.

## 2.3. Measures

### 2.3.1. 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.

### 2.3.2. 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.

### 2.3.3. *Stanford–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.

### 2.3.4. *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-min 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.

### 2.3.5. *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 s. The number of omission and wrong cancellations was recorded to yield performances on inhibition, concentration and error judgment.

### 2.3.6. *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 5 min. 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.

### 2.3.7. *Children's Color 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.

### 2.3.8. *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.

### 2.3.9. *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 min 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.

### 2.3.10. *Lymphocyte subsets*

Percentages (%) and absolute counts (cells/ $\mu$ l) 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 cytometer, BD Bioscience Corp., San Jose, CA, USA).

## 2.4. *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/No-Go 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. Post hoc 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 was examined using Pearson correlation. Given that planned hypotheses were tested and that the number of participants was relatively small, we did not adjust the alpha level to avoid lowering the power of the tests and inflating Type II error.

### 3. Results

#### 3.1. Executive functioning and parent behavioral observations

The LFA group had a significantly higher Executive Composite score than the HFA group ( $t = -5.21, p < 0.001$ ), suggesting that LFA children with ASD had poorer executive function than the HFA children. Post hoc results on the individual executive function measures indicated that the LFA group showed significantly lower HKLLT total Learning ( $t = 4.14, p < 0.001$ ) and discrimination ( $t = 4.55, p < 0.001$ ) scores, as well as on the D2 Concentration Performance ( $t = 5.18, p < 0.001$ ), 5-point Unique Design ( $t = 5.43, p < 0.001$ ), CCTT-2 Time ( $t = -3.22, p < 0.01$ ), and the ToC Achievement ( $t = 4.48, p < 0.001$ ) 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 ( $t = -1.89, p = 0.07$ ), 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).

#### 3.2. 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;

**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 ( $n = 18$ )	LFA ( $n = 19$ )	<i>t</i> -Value
	M (SD)	M (SD)	
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-No-Go			
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)	0.22
ADI-R Communication	12.44 (4.16)	11.32 (5.16)	0.73
ADI-R Stereotyped Behavior	4.06 (4.06)	4.89 (2.64)	-0.84

\*  $p < 0.01$ .

\*\*  $p < 0.001$ .

**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/ $\mu$ l)	2527.4 (708.1)	3136.2 (924.9)	-2.07*
T Lymphs (cells/ $\mu$ l)	1683.8 (465.9)	2138.3 (606.1)	-2.55*
Th Lymphs (cells/ $\mu$ l)	834.3 (232.4)	955.5 (225.7)	-1.74
Suppressor/cytotoxic T lymphs (cells/ $\mu$ l)	659.4 (182.7)	964.3 (393.5)	-3.05**
B lymphs (cells/ $\mu$ l)	455.6 (164.4)	578.4 (232.0)	-1.85
NK cells (cells/ $\mu$ l)	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

Notes. Standard deviations are in parentheses. Lymphs, lymphocytes.

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

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).

### 3.3. Association between intellectual functioning, executive functioning, parent behavioral observations and immunological measures

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 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 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 et al., 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 ( $r = 0.43$ ,

**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–Trail2 Time2	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

Lymphs: lymphocytes.

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

$p < 0.01$ ); 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.

#### 4. Discussion

The present study examined executive function deficits and repetitive stereotyped behavior of a group of 8–17 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 lymphocytes (Medana et al., 2000, 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.

#### 5. Conclusions

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 ASDs. This relationship may open up future directions of research and clinical trials on possible interventions for ASD.

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