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Altered T cell responses in children with autism

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ABSTRACT

Autism spectrum disorders (ASD) are characterized by impairment in social interactions, communication deficits, and restricted repetitive interests and behaviors. A potential etiologic role for immune dysfunction in ASD has been suggested. Dynamic adaptive cellular immune function was investigated in 66 children with a confirmed diagnosis of ASD and 73 confirmed typically developing (TD) controls 2–5 years-of-age. *In vitro* stimulation of peripheral blood mononuclear cells with PHA and tetanus was used to compare group-associated cellular responses. The production of GM-CSF, TNFα, and IL-13 were significantly increased whereas IL-12p40 was decreased following PHA stimulation in ASD relative to TD controls. Induced cytokine production was associated with altered behaviors in ASD children such that increased pro-inflammatory or T_H1 cytokines were associated with greater impairments in core features of ASD as well as aberrant behaviors. In contrast, production of GM-CSF and T_H2 cytokines were associated with better cognitive and adaptive function. Following stimulation, the frequency of $CD3^+$, $CD4^+$ and $CD8^+$ T cells expressing activation markers CD134 and CD25 but not CD69, HLA-DR or CD137 were significantly reduced in ASD, and suggests an altered activation profile for T cells in ASD. Overall these data indicate significantly altered adaptive cellular immune function in children with ASD that may reflect dysfunctional immune activation, along with evidence that these perturbations may be linked to disturbances in behavior and developmental functioning. Further longitudinal analyzes of cellular immunity profiles would delineate the relationship between immune dysfunction and the progression of behavioral and developmental changes throughout the course of this disorder.

1. Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders that include autistic disorder, Asperger's disorder and pervasive developmental disorder, not otherwise specified (APA, 2000). Children with ASD have significant deficits in communication and social interactions and exhibit stereotyped or restricted behaviors and interests. Recent epidemiologic data suggest that approximately 1 in 100 children are diagnosed with an ASD (MMWR, 2009). The etiology of autism is complex and largely unknown, although it is highly heritable. While many studies yield evidence that there are genes that strongly impact the likelihood of developing autism, no definitive pattern of genes has been identified despite a multitude of different and varied candidate genes that have been implicated in ASD (Muhle et al., 2004). Moreover, replication of these data has been inconsistent; probably due in part to the heterogeneity of the phenotypes within the autism spectrum. However, among the potential genetic candidates several studies have linked ASD with immune-based genes, such as human leukocyte antigen (HLA)-DRB1*04, interleukin (IL)-4 receptor, and complement C4B null allele (Lee et al., 2006; Torres et al., 2006; Warren et al., 1992, 1995), as well as a number of other immune-related genes including macrophage migration inhibitory factor (MIF) (Grigorenko et al., 2008), MET tyrosine receptors (Campbell et al., 2006, 2007; Garbett et al., 2008), serine and threonine kinase C gene *PRKCB1* (Lintas et al., 2009), protein phosphatase and tensin homolog (PTEN) (Herman et al., 2007), and reelin (Serajee et al., 2006; Skaar et al., 2005). In addition, epidemiology studies have demonstrated associations between increased early-life infections and later ASD diagnosis (Niehus and Lord, 2006; Rosen et al., 2007; Atladóttir et al.,

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2010) supporting a potential role for abnormal immune dysfunction in ASD. Moreover, associations with maternal autoimmunity (Comi et al., 1999; Croen et al., 2005; Molloy et al., 2006; Atladóttir et al., 2009) or maternal diagnosis of allergy/asthma in the second trimester of pregnancy (Croen et al., 2005) and an ASD diagnosis in the offspring, suggests that inappropriate maternal immune responses may alter the course of immune and neurodevelopment.

Quantitative and qualitative differences in immune function between children with ASD and typically developing (TD) controls have been demonstrated, including evidence for increased neuroinflammation and cytokine production in brain specimens obtained from individuals with ASD (Vargas et al., 2005; Li et al., 2009; Garbett et al., 2008), as well as elevated levels of pro-inflammatory and decreased levels of regulatory cytokines in the CSF and peripheral blood from children with ASD (Sweeten et al., 2004; Chez et al., 2007; Vargas et al., 2005; Ashwood and Wakefield, 2006; Ashwood et al., 2008, 2010a). Alterations in both adaptive and innate immune responses have been described in ASD subjects (reviewed in Ashwood et al. (2006) and Enstrom et al. (2009a)). Abnormal innate immune responses include higher basal NK cell activation (Enstrom et al., 2009b) and increased monocyte responses to toll-like receptor ligation (Enstrom et al., 2010; Jyonouchi et al., 2008). A number of abnormal adaptive responses have been reported in ASD, including increased T lymphocyte cell production of TNFα but decreased IL-10 production (Ashwood et al., 2004; Ashwood and Wakefield, 2006; Jyonouchi et al., 2001) and, a skewing of cytokine responses towards a T_H2 cytokine profile (Gupta et al., 1998; Molloy et al., 2005). In addition, there have been reports of altered peripheral T cell activation (Plioplys et al., 1994; Warren et al., 1995), changes in lymphocyte subsets (Yonk et al., 1990; Denney et al., 1996), decreased proliferative responses to mitogen stimulation (Stubbs and Crawford, 1977), an imbalance of serum immunoglobulin levels (Enstrom et al., 2009c; Heuer et al., 2008) and the presence of antibodies reactive to brain proteins (Cabanlit et al., 2007; Connolly et al., 2006; Silva et al., 2004; Todd et al., 1988; Wills et al., 2009). Taken together, these findings support noteworthy alterations in the adaptive immune responses in a significant proportion of children with ASD.

Although several studies have implicated the adaptive immune responses in ASD, many have relied upon small sample sizes, varied diagnostic criteria, disparately aged case and control groups, and unevaluated siblings as controls; these have led to confusion regarding interpretation of the findings. Moreover, the previous studies have not attempted to evaluate the relationship between the adaptive cellular immune response and the core defects of ASD, impairments in associated behaviors, and/or onset patterns of ASD. To better define the adaptive immune status of children with ASD, proliferative and cytokine responses to immune challenge as well as the expression of activation markers on T cell subsets was evaluated in well-characterized children with ASD and age-matched typically developing control participants in a population-based case-control study. In addition, proliferative and cytokine production in children with ASD was investigated for possible associations with clinical behavioral and developmental function.

2. Methods

2.1. Subjects

This study examined 139 participants enrolled through the M.I.N.D. (Medical Investigations of Neurodevelopmental Disorders) Institute Clinic and the Center for Children's Environmental Health (CCEH) as part of the ongoing CHARGE (Childhood Autism Risk from Genetics and Environment) study at UC Davis (Hertz-Picciotto et al., 2006). The CHARGE study protocols, including details on recruitment and behavioral assessments have been described previously (Ashwood et al., 2008; Hertz-Picciotto et al., 2006; Enstrom et al., 2009b). After clinical evaluations using standardized assessments of behavior and developmental function at the M.I.N.D. Clinic by qualified, research-trained clinicians, participants were placed in one of two groups: (1) diagnosed with autism spectrum disorders, or (2) confirmed as typically developing controls. This study was approved by the UC Davis institutional review board and complied with all requirements regarding human subjects. Parents gave informed consent. Children were selected based on available blood draws from consecutively recruited participants and were medication free and in good health at time of blood draw. Participants included 66 children with ASD (median age 3.8 years (interquartile range 3.2–4.3), 59 males) and 73 typically developing (TD) controls median age 3.3 years (interquartile range 2.7–4.3), 51 males). Autism spectrum disorder diagnosis was based on criteria set forth in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 2000) and confirmed and further evaluated using the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) assessments. Children from the TD groups were screened for autism traits using the Social Communication Questionnaire (SCQ). For all children adaptive function was assessed by parental interview using the Vineland Adaptive Behavior Scales (VABS). Additional measures of cognitive ability were determined using the Mullen Scales of Early Learning (MSEL) and abnormal behavior profiles using the Aberrant Behavior Checklist (ABC). Patterns of development were assessed using the Early Development Questionnaire. All the specific behavioral assessments used are discussed in more detail elsewhere (Hertz-Picciotto et al., 2006).

2.2. Cell Isolation

Peripheral blood was drawn from subjects or controls into sodium citrate (ACD) treated vacutainers (BD Bioscience; San Jose, CA). Peripheral blood mononuclear cells (PBMC) were separated from the whole blood by centrifugation over Histopaque-1077 Hybri-Max lymphocyte separation medium (Sigma; St. Louis, MO) before washing twice in Hanks Balanced Salt Solution (HBSS; VWR; Brisbane, CA). The number of viable PBMC was determined by Trypan Blue exclusion (Sigma) and PBMC concentrations were adjusted to 1.5×10^6 cells/ml in a solution of 0.1% T-Stim (BD Biosciences) in X-Vivo media (Cambrex, Walkersville, MD) and were plated in 12-well flat bottom tissue culture plate (Corning, Corning, NY) for stimulation.

2.3. Cell stimulation

PBMC were either cultured in media alone, or stimulated with PHA (10 μg/mL; Sigma), or to assess the cellular recall response stimulated with tetanus toxoid (1 LF unit/ml, Sigma) for 48 h at 37 °C in 5% CO_2. Following culture, plates were centrifuged before supernatants were harvested and stored at −80 °C until cytokine analysis. Cells were collected after incubation and used for analysis of cell proliferation (see below). For the experiments involving cell proliferation and cytokine analysis, 37 children with ASD (median age 3.6 years, interquartile range 3.1–4.5, 32 males) and 35 TD controls (median age 3.3 years, interquartile range 2.5–4, 22 males) were included.

2.4. Cellular proliferation

Proliferative responses to stimulation were measured using [^{3}H]thymidine incorporation into progeny cell DNA. After cells