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Myeloid dendritic cells frequencies are increased in children with autism spectrum disorder and associated with amygdala volume and repetitive behaviors

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ABSTRACT

The pathophysiology of autism spectrum disorder (ASD) is not yet known; however, studies suggest that dysfunction of the immune system affects many children with ASD. Increasing evidence points to dysfunction of the innate immune system including activation of microglia and perivascular macrophages, increases in inflammatory cytokines/chemokines in brain tissue and CSF, and abnormal peripheral monocyte cell function. Dendritic cells are major players in innate immunity and have important functions in the phagocytosis of pathogens or debris, antigen presentation, activation of naïve T cells, induction of tolerance and cytokine/chemokine production. In this study, we assessed circulating frequencies of myeloid dendritic cells (defined as Lin-1$^-$BDCA1$^+$CD11c$^+$ and Lin-1$^-$BDCA3$^+$CD123$^-$) and plasmacytoid dendritic cells (Lin-1$^-$BDCA2$^+$CD123$^+$ or Lin-1$^-$BDCA4$^+$ CD11c$^-$) in 57 children with ASD, and 29 typically developing controls of the same age, all of who were enrolled as part of the Autism Phenome Project (APP). The frequencies of dendritic cells and associations with behavioral assessment and MRI measurements of amygdala volume were compared in the same participants. The frequencies of myeloid dendritic cells were significantly increased in children with ASD compared to typically developing controls ($p < 0.03$). Elevated frequencies of myeloid dendritic cells were positively associated with abnormal right and left amygdala enlargement, severity of gastrointestinal symptoms and increased repetitive behaviors. The frequencies of plasmacytoid dendritic cells were also associated with amygdala volumes as well as developmental regression in children with ASD. Dendritic cells play key roles in modulating immune responses and differences in frequencies or functions of these cells may result in immune dysfunction in children with ASD. These data further implicate innate immune cells in the complex pathophysiology of ASD.

1. Introduction

Autism spectrum disorder (ASD) appears early in childhood and is characterized by core features of impaired social interaction, deficits in communication and restricted repetitive behaviors and interests (APA, 2000). Once thought of as a rare disorder the prevalence rates for ASD are now considered to be approximately 1% of all children (MMWR, 2009). The etiology and pathophysiology of ASD largely remain a mystery but are likely to involve complex interactions between genetic, epigenetic and environmental factors. Rapidly accumulating evidence highlights a role for dysfunctional immune responses in many children with ASD (Onore et al., 2012). Many candidate genes linked with ASD have influence over immune responses while immune dysfunction in the brain and periphery have been reported (Abrahams and Geschwind, 2008; Ashwood et al., 2011a; Vargas et al., 2005). Furthermore, models of maternal immune activation cast light on how aberrant immune responses during critical periods of development can cause changes in neurodevelopment that lead to altered behaviors resembling those of core autistic features (Patterson, 2009).

Several lines of evidence point to ongoing and prominent activation of innate immune cells within the brain, such as microglia and perivascular macrophages (Vargas et al., 2005; Morgan et al., 2010; Voineagu et al., 2011). Further studies have shown increased activation of monocytes in the periphery following stimulation with Toll-like receptor ligands, including changes in gene expression, increased HLA-DR cell surface expression and the release of pro-inflammatory cytokines interleukin (IL)-1β and IL-6 (Enstrom et al., 2010; Jyonouchi et al., 2001). Moreover, circulating levels of cytokines exhibit a profile reminiscent of innate immune cell

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activation with increased IL-1β, IL-6, IL-12p40, tumour necrosis factor (TNF)-α and CCL-2 production (Ashwood et al., 2011a,b). Both the circulating levels of these pro-inflammatory cytokines and the degree of monocyte activation were associated with more impaired behaviors in children with ASD (reviewed in Onore et al., 2012). Furthermore, activation of innate immune cells leads to increases in oxygen free radicals and an increase in oxidative stress, a process that is increased in ASD (Rose et al., 2012). Together, these data point to an important role for innate immune cell dysfunction in modulating behaviors and neuroinflammation observed in ASD.

Dendritic cells serve a central role in many immune functions (Steinman, 2007). They are highly phagocytic and express many innate pattern-recognition receptors that capture pathogen-associated molecular pattern molecules (PAMPs) on microbes or damage-associated molecular pattern molecules (DAMPs) of endogenous tissues. Upon binding of these ligands/antigens, dendritic cells undergo maturation steps that increase mobility for migration, express chemokine receptors for homing to lymphoid organs, produce chemokines to recruit other immune cells, up regulate MHC class II molecules and co-stimulatory molecules for priming of naïve T cells or stimulation of effector T cells and secrete large quantities of cytokines that polarize or modulate neighboring immune cells (Banchereau and Steinman, 1998; Banchereau et al., 2000; Ueno et al., 2007). Dendritic cells also play an important role in inducing both central and peripheral tolerance. Dendritic cells in the periphery continuously capture and present low dose non-immunogenic antigens to T cells with limited or absent co-stimulation to maintain tolerance either by deletion, the induction of unresponsiveness (anergy) or generation of adaptive T regulatory cells (Steinman, 2007). The biology of dendritic cells is complex but they represent a critical link between innate and adaptive immune responses. Two dendritic cell subsets, myeloid dendritic cells and plasmacytoid dendritic cells have been described in human blood and differences in these populations have been observed in a number of autoimmune conditions (Jego et al., 2003; Pashenkov et al., 2001; Richez et al., 2009). Although, previous studies have examined a variety of innate immune cell effectors in ASD, including monocytes and NK cells, the essential role dendritic cells play in controlling many immune processes make these cells particularly interesting targets for study. To our knowledge, this is the first study to examine differences in frequencies of circulating dendritic cells in young children with ASD and typically developing controls of the same age.

Through the UC Davis M.I.N.D. Institute Autism Phenome Project, we evaluated frequencies of circulating dendritic cells in very young children with ASD and age-matched typically developing controls. We also evaluated whether levels of circulating dendritic cells are associated with brain volume measurements and/or the core symptoms of ASD. Specifically, we evaluated the association between dendritic cells and amygdala volume. Abnormal amygdala structure and function has been widely reported in neuropathological and imaging studies of individuals with ASD (Schumann and Nordahl, 2011). However, the mechanism underlying abnormal amygdala enlargement in ASD remains unknown. As association with circulating dendritic cells may provide a clue to the pathophysiology of abnormal amygdala growth in ASD.

2. Methods

2.1. Participants and behavioral assessments

Eighty-six study participants aged between 2–3 years of age were recruited through the UC Davis M.I.N.D. Institute as part of the Autism Phenome Project (APP). Participants consisted of 57 children with ASD (mean age 2.92 ± 0.05 years; minimum–maximum range 2.08–3.75 years; 48 males) and 29 typically developing (TD) controls children (mean age 2.96 ± 0.06 years; range 2.17–3.58; 19 males). The study protocol was approved by the Institutional Review Board for the UC Davis School of Medicine, and parents or guardians of each subject provided written informed consent for their child to participate.

All diagnostic assessments were conducted or directly observed by trained, licensed clinical psychologists who specialize in ASD and had been trained according to research standards for these tools. Inclusion criteria for a diagnosis of ASD were based on the NIH Collaborative Programs of Excellence in Autism network. These involved: (1) meeting either the Autism Diagnostic Observation Schedule-Generic (ADOS-G) cut-off score for autistic disorder or PDD, (2) or meeting the Autism Diagnostic Interview-Revised (ADI-R) cutoff score for autistic disorder and scoring within two points of this cutoff on the other measure i.e. within 2 points on ADI-R or 2 points on ADOS, (3) combined with clinical judgment. Of the 57 children with ASD, 49 met the cut-offs for autistic disorder and 8 met the cut-offs for PDD-NOS. The ADI-R also collects information about the onset of symptoms. 26 participants demonstrated an early onset pattern, in which signs of autism were present in the first year of life, while 31 participants were reported by parents to have a regressive pattern of onset, in which early development appeared typical, followed by a loss of social-communication skills in the second year of life. All participants were administered the Mullen Scales of Early Development (MSEL) and a developmental quotient (DQ) was calculated based on of the ratio between the average of mental age equivalent scores and chronological age multiplied by 100. Three quotients were calculated: verbal, nonverbal, and overall. The TD children were screened and included after assessment with the Social Communication Questionnaire (excluded if scores >11) (SCQ – Lifetime Edition) ruled out ASD risk and the MSEL revealed developmental scores within two standard deviations of the mean for performance quotient and verbal quotient subscales. Exclusion criteria for TD controls included a diagnosis of mental retardation or specific language impairment, or any known developmental, neurological, or behavioral problems. Further exclusion criteria for all subjects consisted of the presence of Fragile X or other serious neurological, psychiatric or known medical conditions including autoimmune disease and inflammatory bowel diseases/celiac disease. Further inclusion criteria for all children, both TD controls and children with ASD, included being native English speakers, ambulatory, and with no suspected vision or hearing problems.

All subjects were screened via parental interview for current and past physical illness. The ASD and TD children had similar vaccination histories. No differences were noted for time from last vaccine in the two groups. Children with known endocrine, cardiovascular, pulmonary, liver, kidney disease, or current fever were excluded from enrollment in the study. Additional exclusionary criteria were limited to those with physical contraindications to MRI.

In addition to the diagnostic measures, all children were assessed using the Vineland Adaptive Behavior Scales (VABS), a parent report measure for adaptive behaviors and the Repetitive Behavior Scale-Revised (RBSR), a parent report questionnaire for measuring repetitive behavior in children. Parent report of frequent or always gastrointestinal symptoms of irregular bowel habits (i.e. constipation and/or diarrhea) was also collected to determine if there were associations with dendritic cell frequencies.

2.2. Flow cytometry

Peripheral blood was drawn from the participants into sodium citrate (ACD) treated vacutainers (BD Bioscience; San Jose, CA) on

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