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Elevated cytokine levels in children with autism spectrum disorder

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Abstract

This study compared production of IL-2, IFN-γ, IL-4, IL-13, IL-5 and IL-10 in peripheral blood mononuclear cells from 20 children with autism spectrum disorder to those from matched controls. Levels of all Th2 cytokines were significantly higher in cases after incubation in media alone, but the IFN-γ/IL-13 ratio was not significantly different between cases and controls. Cases had significantly higher IL-13/IL-10 and IFN-γ/IL-10 than controls. Conclusion: Children with ASD had increased activation of both Th2 and Th1 arms of the adaptive immune response, with a Th2 predominance, and without the compensatory increase in the regulatory cytokine IL-10.

Keywords: Autism; Cytokines; Adaptive immune responses

1. Introduction

Autism is a pervasive developmental disorder characterized by impairments in communication, language and reciprocal social interaction, and by unusual patterns of restricted and repetitive interests or behaviors, as defined by the *Diagnostic and Statistical Manual of the American Psychiatric Association* –4th ed. (DSM-IV) (American Psychiatric Association, 1994). Autism spectrum disorder (ASD) is a broader category characterized by the same set of core symptoms to varying degrees of severity, inclusive of autism as well as Pervasive Developmental Disorder–Not Otherwise Specified (PDD-NOS) and Asperger Syndrome. The prevalence of ASD is currently estimated to be 4–6 per 1000, affecting 4 times more boys than girls (Bertrand et al., 2001). Susceptibility to ASD is clearly attributable to genetic factors, with a heritability estimate >90% (Folstein and Rosen-Sheidley, 2001), but the etiology of the disorder is unknown, and no biomarkers have yet been identified as characteristic of ASD.

An association between ASD and an altered immune response was noted 30 years ago by Stubbs, who reported that 5 of 13 children with autism had no detectable Rubella titers despite previous routine vaccination (Stubbs, 1976), and that peripheral blood mononuclear cells (PBMC) from these children had a decreased responsiveness to mitogenic stimulation when compared to normal controls (Stubbs and Crawford, 1977). This finding was replicated by Warren et al., who further reported that children with ASD had a reduced number of T lymphocytes, especially $CD4^+$ cells (Warren et al., 1986). Denney et al. also found that children with ASD had a lower percentage of $CD4^+$ cells and a lower $CD4^+$:$CD8^+$ T cell ratio (Denney et al., 1996). PBMC from

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children with ASD were found to have significantly reduced cytotoxic activity (Warren et al., 1987).

Additional abnormalities related to the innate immune response have been reported to occur in children with ASD. Elevated levels of Interleukin 1 receptor antagonist (IL-1RA) have been found, indicative of an increased response by cells of the monocyte-macrophage lineage (Croonenberghs et al., 2002a). Elevated levels of the proinflammatory cytokines tumor necrosis factor (TNF)-α and IL-1β were also produced by the PBMC from children with ASD, in both media alone without stimulation and in response to lipopolysaccharide (LPS), a stimulant for cells of monocyte-macrophage lineage (Jyonouchi et al., 2002, 2001). Children with ASD also have elevated levels of urinary neopterin and bioterin, markers of cell-mediated immunity (Messahel et al., 1998).

Immune responses that are stimulated by exposure to specific antigens are considered adaptive responses and are often driven by $CD4^+$ T helper (Th) cells. Abnormalities in these adaptive responses, effected by cytokines produced by Th cell subsets, have also been reported in children with ASD. Th cell clones have been classified into distinct functional types on the basis of the cytokines they secrete (reviewed in Abbas et al., 1996). The most clearly defined of these subsets are Th1, which is characterized by the production of interferon (IFN)-γ, and Th2, which is characterized by the production of IL-4, IL-5 and IL-13. The main effector function of Th1 cytokines is phagocyte-mediated defense, especially against intracellular microbes, while Th2 cytokines function to effect IgE and eosinophil/mast cell-mediated immune responses. Cytokines of the Th1/Th2 subsets are also responsible for pathogenic immune responses. An imbalance of these cytokines, skewed toward Th2, is seen in allergic responses (Ngoc et al., 2005) and some systemic autoimmune responses such as systemic lupus erythematosus (Spadaro et al., 2003). A skewing toward Th1 cytokines is seen in some organ-specific autoimmune disorders such as insulin-dependent diabetes mellitus and multiple sclerosis (Liblau et al., 1995).

The effects of cytokines produced by Th1 and Th2 cells are mutually antagonistic (Abbas et al., 1996; Mosmann and Coffman, 1989). This finding initially led to the hypothesis that allergy, a response dominated by cytokines of the Th2 subset, would be protective against autoimmune responses that were dominated by cytokines of the Th1 subset. However, there are recent reports that Th1 type autoimmune disorders and allergic diseases are positively associated (Dales et al., 2005; Karsh et al., 2005; Kero et al., 2001), and that infectious disease may be protective against both (Bach, 2002). This supports the hypothesis that these disorders share similar risk factors that cause the immune system to generate both Th1 and Th2 responses (Simpson et al., 2002), and, while it is a useful paradigm, the separation of T helper cells into polarizing subsets is likely an oversimplification of these complex processes (Kelso, 1995).

Elevated levels of both Th1 and Th2 cytokines have been reported in children with ASD. Singh reported increased plasma levels of the Th1 cytokines IL-12 and IFN-γ (Singh, 1996), and Croonenberghs et al. found an increase in the level of IFN-γ in the supernatant of whole blood cultures from children with ASD (Croonenberghs et al., 2002a). In contrast, using monoclonal antibodies, Gupta et al. reported increased proportions of IL-4 containing $CD4^+$ and $CD8^+$ T cells, and decreased proportions of IFN-γ^+CD4^+, IL-2^+CD4^+, IFN-γ^+CD8^+, and IL-2^+CD8^+ T cell subsets in children with ASD compared to controls (Gupta et al., 1998). Elevated levels of serum IgE, further evidence of an allergic response, have also been reported in children with ASD (Gupta et al., 1996).

An elevated level of serum IgG is associated with ASD (Croonenberghs et al., 2002b), as well as increased autoantibodies to neuronal elements that have been interpreted as evidence of an autoimmune response (Connolly et al., 1999; Singh et al., 1997). Vargas et al. reported an increased inflammatory response in postmortem brain tissue specimens from individuals with ASD, as evidenced by microglial and astroglial activation, and increased proinflammatory cytokines in the cerebral spinal fluid of patients with autism compared to controls (Vargas et al., 2005). In addition, there have been reports of an association between ASD and familial immune disorders, including an increased prevalence of autoimmune disorders among first degree relatives of children with ASD (Comi et al., 1999; Sweeten et al., 2003). Croen et al. reported that a diagnosis of maternal asthma or allergy recorded during the second trimester of pregnancy was associated with a two-fold increased risk for ASD, while there was no difference in the proportion of ASD case and control mothers with the diagnosis of an autoimmune disorder in the 4-year period surrounding the pregnancy (Croen et al., 2005).

These preliminary studies have provided a mounting body of evidence for an association between ASD and immune dysfunction, but the nature of this relationship remains unclear. Differences in T cell function or number have not been reproducible. This may be secondary to methodologic differences between studies. Cases have not always been rigorously defined in terms of their ASD classification or well characterized in terms of their immunologic symptoms or use of psychotropic medications. Controls were sometimes recruited at a different time and place or were of a markedly different age range, and immunization status has not always been reported. The aim of the present study was to compare the adaptive immune responses in the PBMC of children with well-characterized ASD to the responses of neurotypical controls matched for age, race, gender and date of study visit. The specific immune responses measured were the absolute eosinophil count in a peripheral blood smear and the levels of six cytokines produced by the PBMC: IL-2, necessary for T cell growth (Th0), the representative Th1 cytokine, IFN-γ, the Th2 cytokines IL-4, IL-5 and IL-13 and the regulatory