Short communication

Th1- and Th2-like cytokines in CD4\(^+\) and CD8\(^+\) T cells in autism

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Abstract

Th1-like (IL-2, IFN-\(\gamma\)) and Th2-like (IL-4, IL-6, and IL-10) cytokines were examined in CD4\(^+\) and CD8\(^+\) T cells in children with autism. Intracellular cytokines were measured using specific antibodies to various cytokines and anti-CD4 or anti-CD8 monoclonal antibodies by FACScan. Proportions of IFN-\(\gamma\) CD4\(^+\) T cells and IL-2 CD4\(^+\) T cells (Th1), and IFN-\(\gamma\) CD8\(^+\) and IL-2 CD8\(^+\) T cells (TC1) were significantly lower in autistic children as compared to healthy controls. In contrast, IL-4 CD4\(^+\) T cells (Th2) and IL-4 CD8\(^+\) T cells (TC2) were significantly increased in autism. The proportions of IL-6 CD4\(^+\), IL-6 CD8\(^+\) and IL-10 CD4\(^+\), IL-10 CD8\(^+\) T cells were comparable in autism and control group. These data suggest that an imbalance of Th1- and Th2-like cytokines in autism may play a role in the pathogenesis of autism. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Autism is a syndrome of developmental disorder whose etiology and pathogenesis remain unclear. A number of factors, including genetic, environmental, and immunological factors, have been implicated. There is evidence to suggest that the immune system plays an important role in the pathogenesis of autism. This includes: decreased T-cell subsets and cell-mediated immunity (Stubbs et al., 1977; Warren et al., 1986; Gupta et al., 1996a) increased levels of serum IgM and IgE (Gupta et al., 1996a); presence of autoantibodies to neural tissues (Todd et al., 1988; Singh et al., 1993); and positive response to transfer factor (Fudenberg, 1997) and pentoxifyllin, a modulator of cytokines (Gupta et al., 1996b). CD4\(^+\) T cells, based upon the profile of cytokine secretion, have been divided into two major subpopulations: the Th1 that produce IL-2 and IFN-\(\gamma\), and Th2 that produce IL-4, IL-5, IL-6, IL-10 (Romagnani, 1995). Th1 cytokines are responsible for cell-mediated immunity, whereas Th2 type cytokines are responsible for providing help to B cells to produce immunoglobulins, including IgE. More recently, CD8\(^+\) T cells have also been shown to produce Th1-like (TC1 cells) and Th2-like (TC2 cells) cytokines (Sad et al., 1995). Because of depressed cell-mediated immunity and increased serum IgE levels in children with autism, we examined Th1-like and Th2-like CD4\(^+\) and CD8\(^+\) T cells in autism and controls. Our data show that the proportions of both CD4\(^+\) and CD8\(^+\) T cells that produce IFN-\(\gamma\) and IL2 (Th1 and TC1 respectively) are significantly reduced, whereas those CD4\(^+\) and CD8\(^+\) T cells that produce IL-4 (Th2 and TC2 respectively) were significantly elevated in children with autism as compared to controls.

2. Materials and methods

Twenty children with autism (range 3–7 years; 16 male and 4 female) and 20 age-matched controls (range 4–8 years; 17 males and 3 females) were examined. Patients with autism were diagnosed according to DSM IV criteria. None of the patients or controls were on any medications. The protocol was approved by the Institution Review Board of the University of California, Irvine.

FITC-labeled anti-IFN-\(\gamma\), PE-labeled anti-IL-2, anti-IL-4, anti-IL-6, and anti-IL-10 monoclonal antibodies, and Per-CP-labeled anti-CD4 and -CD8 and their isotype controls were purchased from Becton Dickinson, Sunnyvale, CA.
Five hundred microliters of whole peripheral blood diluted with 500 μl of phosphate-buffered saline (PBS) was stimulated for 4 h at 37°C with 25 ng/ml phorbol myristate acetate and 1 μg/ml ionomycin in the presence of 10 μg/ml Brefedin A (to inhibit golgi-mediated export of cytokines). Per-CP labeled anti-CD4, anti-CD8 or their isotype controls were added and incubated for 10 min at room temperature in the dark. Cells were incubated with FACS lysis solution (Becton Dickinson) for 10 min. Cells were permeabilized with FACS permeabilizing solution (Becton Dickinson) for 10 min at room temperature. Cells were washed with wash buffer (phosphate-buffered

Fig. 1. (A) Intracellular IL-2 and IFN-γ in CD4⁺ and CD8⁺ T cell subsets in autism (n = 20) and controls (n = 20); P < 0.05. (B) A representative of dual color FACScan analysis of IFN-γ⁺ CD4⁺ and IFN-γ⁺CD8⁺ T cells in an autistic patient and a control.
Fig. 2. Intracellular IL-4 in CD4+ and CD8+ T cell subsets in autism (n = 20) and controls (n = 20); P < 0.05.

saline containing 0.5% bovine serum albumin and 0.1% azide, and incubated with antibodies against each cytokine and their isotype control for 30 min at room temperature in the dark. Cells were washed two times with wash buffer and resuspended in 0.5 ml of 1% paraformaldehyde. Ten thousand cells were acquired and dual/triple color analysis was performed, using FACSscan Research Software. Lymphocyte populations were gated using forward scatter channel (FSC) and side scatter channel (SSC). Quadrant markers were set by staining the cells with FITC-, PE-, or Per-CP-labeled isotype matched control antibodies. Percent CD4+ or CD8+ T cells expressing IL-2, IL-4, IL-6, or IL-10 were determined from quadrant no. 2 dual positive cells using FL-3 and FL-2 channels respectively and percent CD4+ or CD8+ T cells expressing IFN-γ were determined in quadrant no. 2 using FL-3 and FL-1 channels respectively. Data are expressed as % mean ± s.e.m.

Statistical analysis was performed by paired Student t-test.

Fig. 3. Intracellular IL-6 and IL-10 in CD4+ and CD8+ T cell subsets in autism (n = 18) and controls (n = 18).

3. Results

Fig. 1A shows significantly decreased (< 0.05) proportions of IFN-γ CD4+ , IL-2 CD4+ , IFN-γ CD8+ , and IL-2 CD8+ T cell subsets in autism as compared to control group, suggesting a deficiency of Th1 and TC1 subsets. Fourteen of 20 patients and one of 20 controls were outside two standard deviations (SD) of the mean for controls. Fig. 1B shows a representative dual color FACS analysis for IFN-γ CD4+ and CD8+ T cells in a patient and a control. In contrast, IL-4 containing CD4+ and CD8+ T cells were significantly (P < 0.05) increased in autistic group as compared to the control group (Fig. 2), suggesting an increase in Th2 and TC2 subpopulation. Thirteen of 20 patients and one of 20 controls fell outside 2 SD of the mean for controls. However, the proportions of both CD4+ and CD8+ T cells with intracellular IL-6 and IL-10 were comparable in autistic and control groups (Fig. 3).

4. Discussion

In the present study, we have observed decreased proportions of IFN-γ and IL-2 synthesizing CD4+ (Th1) and CD8+ (TC1) T cells in autistic children as compared to controls. In contrast, IL-4 synthesizing CD4+ (Th-2) and CD8 (TC2) T cells were significantly increased in autism. Although proportions of Th1 and TC1 cells synthesizing IL-6 and IL-10 in autism were comparable to controls, a decrease in Th-1 cytokines with increased IL-4 synthesizing cells would suggest a shift from Th1 to Th2. The decrease in IL-2 and IFN-γ would be consistent with depressed cell-mediated immunity and decreased natural killer-cell activity in autistic children (Stubbs et al., 1977; Warren et al., 1986). Furthermore, increased IL-4 containing T-cell subsets are in agreement with elevated levels of serum IgE observed in children with autism (Gupta et al., 1996a). No correlation was observed between the levels of Th1/TC1 or Th2/TC2 and the severity of behavioral disturbances in autistic children. Therefore, it is unlikely that the changes observed in the present study are a consequence of behavior disturbance in the patient group.

This is the first study to examine intracellular cytokines and cellular sources of cytokines in autism. All prior studies have reported cytokines in serum/plasma of autistic patients (Singh et al., 1991, 1993). Normal levels of plasma IL-6 and increased plasma levels of IFN-γ and soluble IL-2 in autism have been reported (Singh et al., 1991; Singh, 1996). There are a number of drawbacks and fallacies in the measurements of serum/plasma cytokine levels, including failure to define cellular source, complexing of cytokines with plasma proteins, and concomitant production of inhibitors of cytokines and soluble cytokine receptors (Whiteside, 1994). The discrepancy between our
data and those reported by Singh (1996) and Singh et al. (1991) could be due to any other factors mentioned above. In addition, children studied by Singh (1996) were much older than those studied in the present study.

In autism, there is evidence to suggest an autoimmune pathogenesis. Singh et al. (1993) and Todd et al. (1988) have reported antibodies to various neural tissue antigens. Todd and Ciaranello (1985) have reported the presence of antibodies to serotonin receptors in autism. Th2 cells have been shown to play a role in the pathogenesis of a number of autoimmune disorders, including Graves disease (Song et al., 1996). Therefore, we suggest a role of Th2-like cytokines in the autoimmune pathogenesis of autism. Although the precise mechanism(s) for a switch from Th1/Th1c to Th2/Th2c remains unclear, increased IL-4, especially in the presence of low levels of IFN-γ induces differentiation to Th2/Th2c cells (Sad et al., 1995). IL-4 is produced by a number of cell types, including NK cells and γδ T cells, in response to allergen or stimulation with parasitic antigens (Romagnani, 1995). NK cell numbers and NK cell activity is reduced in autism (Warren et al., 1987; Gupta et al., 1996a) and therefore unlikely the source of increased IL-4 production. It remains to be determined whether there is an expansion of γδ T cells in autism.

References