Levels of Soluble Adhesion Molecules PECAM-1 and P-Selectin are Decreased in Children with Autism Spectrum Disorder

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Abstract

Background—Although the etiopathology of Autism Spectrum Disorder (ASD) is not clear there is increasing evidence that dysfunction in the immune system affects many children with ASD. Findings of immune dysfunction in ASD include increases in inflammatory cytokines, chemokines and microglial activity in brain tissue and CSF, as well as abnormal peripheral immune cell function.

Methods—Adhesion molecules, such as platelet endothelial adhesion molecule-1 (PECAM-1), intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), P-Selectin, and L-Selectin, function to facilitate leukocyte transendothelial migration. We assessed concentrations of soluble adhesion molecules, sPECAM-1, sICAM-1, sVCAM-1, sP-Selectin, and sL-Selectin in the plasma of 49 participants with ASD, and 31 typically developing controls of the same age, all of whom were enrolled as part of the Autism Phenome Project (APP). Behavioral assessment, the levels of soluble adhesion molecules, head circumference and MRI measurements of brain volume were compared in the same subjects.

Results—Levels of sPECAM-1 and sP-Selectin were significantly reduced in the ASD group compared to typically developing controls (p < 0.02). Soluble PECAM-1 levels were negatively associated with repetitive behavior and abnormal brain growth in children with ASD (p=0.03).

Conclusions—As adhesion molecules modulate the permeability and signaling at the blood brain barrier as well as leukocyte infiltration into the CNS, current data suggests a role for these molecules in the complex pathophysiology of ASD.

Keywords

Autism; PECAM-1; P-Selectin; CD31; CD62-P; Adhesion

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Background

Autism spectrum disorder (ASD) is a collection of behaviorally defined neurodevelopmental conditions including autism, Asperger's syndrome, and pervasive development disorder-not otherwise specified (PDD-NOS) (1). Currently, this disorder is estimated to affect 1 in 110 children in the U.S. (2). Symptoms of ASD generally appear in the first three years of life, and are characterized by repetitive behavior or interests, deficits in both verbal and non-verbal language, and impaired social interaction (1). To date, the etiology of ASD remains largely unknown, and is likely a complex combination of genetic and environmental factors. Recent literature has highlighted the impact of immune function on CNS development and neural function, and there is a growing body of evidence to suggests that atypical immune activity may play a role in the pathophysiology ASD (3–5).

Candidate immune genes and aberrant immune responses in individuals with ASD are consistently reported, reviewed in (6). Several lines of evidence point to ongoing neuroinflammation with marked activation of astroglia and microglia observed in individuals with ASD, as well as increased levels of several inflammatory cytokines and chemokines in the cerebral spinal fluid (CSF)(7). Further studies have demonstrated peripheral immune abnormalities in children with ASD including abnormal circulating levels of cytokines (8–10) and atypical levels of plasma immunoglobulin (11–13). Cellular immune dysfunction is also frequently reported in ASD, including abnormal T cell function (14–19), aberrant NK cell activation (20–22), and abnormal monocyte responses (23–25). Together, these data point to a role for cellular dysfunction and neuroinflammation in ASD and suggests that aberrant neuroimmune interactions may occur in the CNS (7, 8, 12, 13, 17, 21–23, 25–39).

To gain access to the CNS, immune cells must migrate across the blood-brain barrier (BBB), which separates circulating blood from the brain parenchyma. Adhesion molecules facilitate the process of leukocyte migration, and therefore, modulate the permeability of the BBB to immune cells (40). Platelet endothelial adhesion molecule-1 (PECAM-1), intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), P-Selectin, and L-Selectin facilitate leukocyte adhesion and transendothelial migration (41). These molecules are present in membrane bound form, and can also be found in circulating plasma under normal non-inflammatory conditions. However, atypical concentrations of these adhesion molecules in the blood and CSF have been repeatedly reported in neuroinflammatory diseases (42, 43).

Several preceding studies have examined a variety of immune cell effectors in ASD, but few have examined molecules involved in facilitating the migration of immune cells into the CNS in young children with ASD and typically developing controls of the same age. The essential role of adhesion molecules in controlling the process of leukocyte migration across the BBB makes these molecules particularly interesting targets for study. Decreased levels of sPECAM-1, sVCAM-1, and sP-Selectin have been previously reported in high functioning adults with autism (44, 45), but the levels of these molecules have not been examined in children with ASD who are closer to the age of the onset of the disorder. To confirm the finding of lower levels of select adhesion molecules in ASD, and to determine if adhesion molecules were also reduced in children with ASD, sPECAM-1, sICAM-1, sVCAM-1, sP-Selectin, and sL-Selectin were quantified in peripheral blood plasma of children with ASD and typically developing controls as part of the Autism Phenome Project (APP).

Methods

Subjects and behavioral assessments

Eighty study participants aged between 2–4 years of age were recruited as part of the Autism Phenome Project (APP). The study protocol was approved by the Institutional Review Board for the UC Davis School of Medicine, and parents of each subject provided written informed consent for their child to participate. Participants consisted of 49 children with ASD, (median age 2.91 years; interquartile range 2.66–3.41 years; 42 males) and 31 typically developing (TD) controls children (median age 3.13 years; interquartile range 2.85–3.27; 20 males). Of the 49 children with ASD, 43 were diagnosed with autism and 6 with PDD-NOS. Diagnostic instruments included the Autism Diagnostic Observation Schedule – Generic (ADOS-G) (46) and the Autism Diagnostic Interview – Revised (ADI-R) (47). All diagnostic assessments were conducted or directly observed by trained, licensed clinical psychologists who specialize in autism and had been trained according to research standards for these tools.

Inclusion criteria for ASD were taken from the diagnostic definition of ASD in young children formulated and agreed upon by the Collaborative Programs of Excellence in Autism (1). Inclusion criteria for TD controls included developmental scores within two standard deviations of the mean on all subscales of the MSEL. Exclusion criteria for TD controls included a diagnosis of Mental Retardation, Pervasive Developmental Disorder or Specific Language Impairment, or any known developmental, neurological, or behavioral problems. TD children were screened and excluded for autism with the Social Communication Questionnaire (48) (scores > 11) (SCQ - Lifetime Edition). All participants were native English speakers, ambulatory, and had no suspected vision or hearing problems. The exclusion criteria for all subjects consisted of the presence of Fragile X or other serious neurological (e.g., seizures), psychiatric (e.g., bipolar disorder) or known medical conditions including autoimmune disease and inflammatory bowel diseases/celiac disease. All subjects were screened via parental interview for current and past physical illness. Children with known endocrine, cardiovascular, pulmonary, liver, or kidney disease were excluded from enrollment in the study. The ASD and TD children had similar vaccination histories with all except 2 children with ASD up-to date for the number and type of vaccinations. No differences were noted for time from last vaccine in the two groups.

Measurement of sPECAM-1, sICAM-1, sVCAM-1, P-Selectin, and L-Selectin

Peripheral blood plasma was collected in acid-citrate-dextrose Vacutainers (BD Biosciences, San Jose, CA) on the last day after behavioral assessments were performed. No participant presented with a cold, fever or other common illness, if such a condition occurred the blood draw were delayed until the child's health status was stable for 48 hours. The plasma was collected immediately by centrifugation and stored in aliquots at 80 °C until the date of assay. Levels of sPECAM-1 were determined by Human sPECAM-1 enzyme-linked immunosorbant assay kit (ELISA) (Bender MedSystems, Vienna, Austria), with a sensitivity of 0.06 ng/ml, a mean intra-assay coefficient of variation of 1.7%, and a mean inter-assay coefficient of variation of 7.4%. Levels of sP-Selectin were determined sP-Selectin Quantikine® ELISA kits (R&D Systems, Minneapolis, MN) with sensitivities of 0.1ng/ml, a mean intra-assay coefficient of variation of 5.2%, and a mean inter-assay coefficient of variation of 8.9%. Levels of sICAM-1 were determined sICAM-1 Quantikine® ELISA kits (R&D Systems) with sensitivities of 0.6 ng/ml, a mean intra-assay coefficient of variation of 4.6%, and a mean inter-assay coefficient of variation of 5.5%. Levels of VCAM-1 were determined sICAM-1 Quantikine® ELISA kits (R&D Systems) with sensitivities of 0.5 ng/ml, a mean intra-assay coefficient of variation of 3.1%, and a mean inter-assay coefficient of variation of 7.0%. Levels of L-Selectin were determined sICAM-1 Quantikine® ELISA kits

(R&D Systems) with sensitivities of 0.3 ng/ml a mean intra-assay coefficient of variation of 3.9%, and a mean inter-assay coefficient of variation of 9.2%. All standards and samples were assayed in duplicate. Two kits were used for each molecule measured, with ASD and typically developing control samples evenly distributed between the two kits. All assays were performed according to the protocols recommended by the manufacturers. Optical densities were determined on a Wallac Victor3 multilabel-plate reader (PerkinElmer, Boston, MA).

Statistical Analysis

As the data was non-parametrically distributed statistical analysis to compare levels of soluble adhesion between ASD and TD groups was conducted with Mann-Whitney U-test. Correlation analysis was performed with Spearman analysis. All analyses were two-tailed, and values of $p < 0.05$ were considered statistically significant. Unadjusted P values are presented (49). All analyses were conducted with GraphPad Prism statistical software (GraphPad Software Inc., San Diego, CA).

Results

Median plasma levels of sPECAM-1 were reduced approximately 25% lower in the ASD group (median 62.4 ng/ml; interquartile range 48.0–85.1 ng/ml) compared with the TD control group (81.5 ng/ml; 72.4–99.2 ng/ml; $p = 0.01$) (Figure 1-A). Plasma levels of sP-Selectin were also significantly reduced, by approximately a 25%, in the ASD group (40.0 ng/ml; 31.4–53.5 ng/ml) in comparison with the TD control group (52.1 ng/ml; 39.3–70.2 ng/ml; $p = 0.01$) (Figure 1-D). There were no significant differences in plasma levels of sICAM-1, sVCAM-1, or sL-Selectin between the ASD participants and TD controls (Table 1). We did not observe any statistical significant differences in adhesion molecule levels based on sex or age in either group (data not shown).

We then examined whether there were associations between the levels of sPECAM-1 and sP-Selectin and clinical behavioral and medical variables among children with ASD. The Repetitive Behavior Scale-Revised (RBSR) is a standard clinical tool for measuring repetitive behavior in children. A negative association was observed between sPECAM-1 levels and scores on the RBSR (r = -0.35; $p = 0.03$) (Figure 1-F) such that, as sPECAM-1 levels decreased repetitive behaviors became more pronounced in the ASD group. There was no association in the TD group alone ($p = 0.83$). The potential relationships between PECAM-1 and P-selectin levels were also examined in ADOS and ADI-R scores, however no significant correlations were observed. Abnormal brain size and birth head circumference has previously been linked to sPECAM-1 levels in adults with high functioning autism disorder. In this study we found that birth head circumference was significantly associated with sPECAM-1 levels in TD children (r = 0.44; $p = 0.04$) but not in children with ASD (r = 0.17; $p = 0.58$).

Conclusions

For the first time, we show that levels of soluble sPECAM-1 and sP-Selectin, two molecules which mediate leukocyte migration, are significantly decreased in young children with ASD compared to typically developing controls of the same age. This finding is consistent with previous reports of decreased levels of both sPECAM-1 and sP-Selectin in adults with high functioning autism (44, 45). In addition, we observed significant associations between PECAM-1 levels and higher repetitive behavior scores in children with ASD. Repetitive, restricted, and stereotyped behavior is one of the core features of ASD, and this data suggests a potential relationship between PECAM-1 levels, and the severity of repetitive behaviors. Moreover, head circumference was associated with increased sPECAM-1 levels

in typically developing children but not children with ASD, suggesting that sPECAM-1 may play a role in normal brain growth, and that this relationship is dysregulated in ASD.

Recent literature demonstrates that immune cells migrate into the CNS under normal non-inflammatory conditions, and that this process is required for normal neurodevelopment and cognitive function (50). Immune deficient mice display cognitive impairments and reconstitution of the mice with T cells by adoptive transfer improves behavior (51–53). In particular IL-4 producing T_H2 cells are important for cognitive function (54). Moreover, immune cell activity during an inflammatory episode in the CNS can play a protective effect against further damage and be beneficial, and depletion of immune cells results in exacerbation of neuroinflammation (55–58). In ASD, behavioral improvements have been observed in individuals with fever (59, 60), likely due in part to increased immune cell activity and interactions of immune cells with the blood brain barrier (BBB) and CNS. To further extend these observations, it has recently been shown that increased activation of T cells with a T_H2 profile is associated with improved expressive language, fine motor skills and visual reception as determined by Mullens assessments (9); while abnormal circulating lymphocyte numbers and phenotypes are directly associated with better executive function in ASD (61). These findings suggest an intimate relationship between immune cells, and CNS function with a close controlled trafficking of lymphocytes key to the development of typical behaviors.

PECAM-1 and P-Selectin both facilitate leukocyte migration across the endothelial barrier, including the BBB. P-Selectin is constitutively expressed in platelets and endothelial cells, but only transported to the cell surface in response to inflammatory signals, such as the presence of histamine or tumor necrosis factor-alpha in response to injury or infection (62, 63). P-Selectin binds its receptor, P-Selectin Glycoprotein Ligand-1 (PSGL-1) with high affinity, which is expressed on virtually all leukocytes (64). Large amounts of soluble P-Selectin are reportedly produced by shedding from the surface of endothelial cells and platelets (65, 66). PECAM-1 is also expressed on endothelial cells, and is heavily enriched within the intercellular junctions of vasculature. PECAM-1 is also expressed in the majority of leukocytes and platelets, including granulocytes, myeloid lineage cells, and lymphocytes (67). PECAM-1, binds itself in a homophilic manner facilitating adhesion transendothelial migration of leukocytes (68). Soluble PECAM-1 exists in a 90kd cleaved form, and a 120kd form produced by mRNA alternative splicing, both of which are present at approximately equal levels in plasma (69). Although the primary source of soluble PECAM-1 under homeostatic conditions in humans has not been conclusively determined the literature suggests a sizeable source of soluble PECAM-1 is from endothelial cells (69, 70).

PECAM-1 and P-Selectin are important for normal transendothelial migration, and pre-exposure of leukocytes to anti-PECAM-1 blocking antibodies prevents transendothelial migration of leukocytes *in-vitro* (68, 71). Similarly, in an animal model of neuroinflammation, intravenous injection of anti-PECAM-1 antibodies markedly reduces T cell infiltration into the CNS (72). Like PECAM-1, P-Selectin is essential for facilitating immune cell migration into the CNS. *In-vivo* administration of blocking antibodies specific for P-Selectin, or its ligand, PSGL-1, result in greatly reduced T cell infiltration in animal models (73, 74). The data from our current study suggest a link between sPECAM-1 levels and normal brain growth and cognition in TD children but that is aberrant in children with ASD. Decreased sPECAM-1 was also associated with increased repetitive behaviors. This data suggests that reduced levels of either PECAM-1 or P-Selectin may result in reduced immune cell-endothelial cell interactions and possible reduced access to the CNS. Fewer immune-endothelial cell interactions could effect a number of physiological processes that impact neurodevelopment and or behavior (7, 26–28, 38, 39, 51, 52, 68, 71, 72, 75–81).

In conclusion, adhesion molecules, sPECAM-1 and sP-Selectin, play a crucial role in regulating immune cell access to the CNS. Data for the current studies shows that there are decreased sPECAM-1 and sP-Selectin levels in young children with ASD. These reduced levels were associated with increased repetitive behaviors, abnormal brain growth and impaired cognition. These findings are novel and further investigations aimed at determining the interactions between immune cells and behavior in children with ASD are needed. Although we have demonstrated that the lower PECAM-1 levels previously reported in adults is also present in children between the ages of 2–5 years, little is known about possible fluctuations of these levels during development. The results from this study warrant follow-up in a replication study to measure soluble PECAM-1 levels longitudinally. In addition to further human studies, animal models of deficient PECAM-1 production can also be utilized. The B6.129P2-Pecam1^{tm1Mak} mouse is virtually devoid of PECAM-1, and has been utilized in a number of studies to demonstrate the immunological and vascular impact of PECAM-1 deficiency (82, 83). However, to our knowledge, neurodevelopment and behavior have not been thoroughly examined in the B6.129P2-Pecam1^{tm1Mak} mouse. Examination of this mouse model, and heterozygotes of this mouse line, may be a promising tool to examine the relationship between low PECAM-1 levels, immune cell neuroendothelial interactions and behavior. Such future studies may elucidate the physiological relevance of low sPECAM-1 levels in ASD described herein.

Acknowledgments

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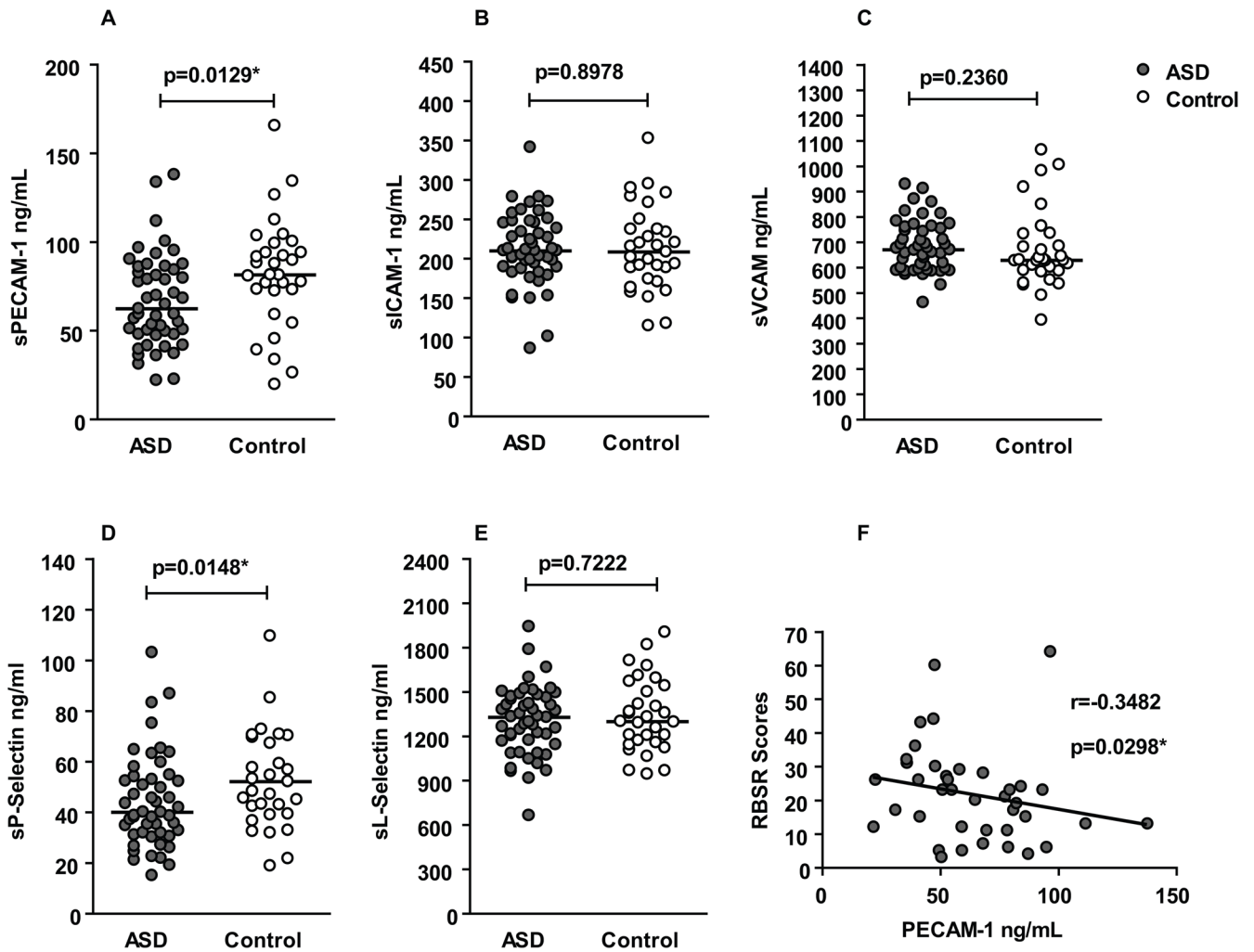
Figure 1. Levels of soluble adhesion molecules in ASD and association with repetitive behavior
(**A**) Median levels (black horizontal bar) of sPECAM-1 are significantly lower in ASD (grey dots) in comparison with typically developing controls (white dots). (**B, C**) There was no difference in the median levels of sICAM-1, sVCAM-1 or sL-Selectin between ASD participants and controls. (**D**) sP-Selectin levels are significantly lower in the ASD group as compared with controls. (**E**) There was no difference in sL-Selectin levels between the two groups. (**F**) Correlation between sPECAM-1 levels and RBSR scores in ASD subjects. RBSR scores correlate negatively with PECAM-1 levels.

Table 1

Descriptive statistics

Descriptive statistics of study participants, with median and interquartile ranges of sPECAM-1, sICAM-1, sVCAM-1, sP-Selectin, and sL-Selectin levels and the statistical significance between groups presented.

	ASD (n=49)	Control (n=31)	P-Value
Male/Female	42 Male/7 Female	20 Male/11 Female	
Age	2.9 years (2.7–3.4 years)	3.1 years (2.8–3.3 years)	
sPECAM-1*	62.4 ng/ml (47.9–85.1 ng/ml)	81.5 ng/ml (72.4–99.2 ng/ml)	0.0129
sICAM-1	210 ng/ml (188.5–245.5 ng/ml)	208.5 ng/ml (174.5–237.4 ng/ml)	0.8978
sVCAM-1	670.2 ng/ml (595.9–756.5 ng/ml)	628.9 ng/ml (586.3–732.1 ng/ml)	0.2360
sP-Selectin*	40 ng/ml (31.4–53.5 ng/ml)	52.1 ng/ml (39.3–70.2 ng/ml)	0.0148
sL-Selectin	1328.00 ng/ml (1156–1465 ng/ml)	1300.00 ng/ml (1157–1543 ng/ml)	0.7222