Biological Psychiatry

Archival Report

Neonatal Cytokine Profiles Associated with Autism Spectrum Disorder

Paula Krakowiak, Paula E. Goines, Daniel J. Tancredi, Paul Ashwood, Robin L. Hansen, Irva Hertz-Picciotto, and Judy Van de Water

ABSTRACT

BACKGROUND: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that can be reliably diagnosed at age 24 months. Immunological phenomena, including skewed cytokine production, have been observed among children with ASD. Little is known about whether immune dysregulation is present before diagnosis of ASD.

METHODS: We examined neonatal blood spots from 214 children with ASD (141 severe, 73 mild/moderate), 62 children with typical development, and 27 children with developmental delay as control subjects who participated in the Childhood Autism Risks from Genetics and the Environment study, a population-based case-control study. Levels of 17 cytokines and chemokines were compared across groups and in relation to developmental and behavioral domains.

RESULTS: Interleukin (IL)-1β and IL-4 were independently associated with ASD compared with typical development, although these relationships varied by ASD symptom intensity. Elevated IL-4 was associated with increased odds of severe ASD (odds ratio [OR] = 1.40, 95% confidence interval [CI], 1.03, 1.91), whereas IL-1β was associated with increased odds of mild/moderate ASD (OR = 3.02, 95% CI, 1.43, 6.38). Additionally, IL-4 was associated with a higher likelihood of severe ASD versus mild/moderate ASD (OR = 1.35, 95% CI, 1.04, 1.75). In male subjects with ASD, IL-4 was negatively associated with nonverbal cognitive ability ($\beta = -3.63$, SE = 1.33, $p = .04$).

CONCLUSIONS: This study is part of a growing effort to identify early biological markers for ASD. We demonstrate that peripheral cytokine profiles at birth are associated with ASD later in childhood and that cytokine profiles vary depending on ASD severity. Cytokines have complex roles in neurodevelopment, and dysregulated levels may be indicative of genetic differences and environmental exposures or their interactions that relate to ASD.

Keywords: Autism Spectrum Disorder, Blood Spot, Chemokines, Developmental Delays, Neonatal Cytokines, Neurodevelopment

http://dx.doi.org/10.1016/j.biopsych.2015.08.007

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social deficits and restricted or stereotyped behavior patterns that manifest by age 3 years (1–3). The biological basis of ASD is unclear but likely involves a complex interplay between genetic susceptibility and environmental exposures (4). There are no consistent biological markers for ASD, and diagnosis is based on behavioral traits and developmental history (5–7). The most consistently beneficial treatment for ASD is behavioral therapy, which is most effective when administered early in life (8). Therefore, two major goals in autism research are characterization of biological signatures for ASD and early identification of children at risk.

Immunological factors may be involved in ASD, including alterations in the prenatal immune environment that may contribute to the risk of developing ASD (9–11). Children with a diagnosis of ASD have evidence of immune dysfunction that is detected at young ages and near the time of diagnosis (12–17). However, it is unclear whether these immunological differences arise in children before or after diagnosis. Resolving this issue is critical to understanding the temporal relationship between immune anomalies and the development of ASD.

Two studies found differences in cytokine and chemokine levels measured in newborn blood spots of children later given a diagnosis of ASD compared with control subjects (18–20). Abdallah *et al.* (18,19) found decreased levels of many cytokines (i.e., interferon [IFN]-γ, interleukin [IL]-2, IL-4, IL-6, IL-10) in neonatal samples from children with ASD and no differences in chemokine levels. In contrast, Zerbo *et al.* (20) reported increased monocyte chemotactic protein (MCP)-1 and decreased regulated on activation, normal T-cell expressed and secreted (RANTES) in subjects with ASD versus control subjects. Although these studies presented novel data, they were limited by lack of clinical confirmation of ASD and older diagnostic tools owing to the timing of specimen collection. The present study used archived neonatal dried blood spot samples to explore whether early immunological

© 2015 Society of Biological Psychiatry

ISSN: 0006-3223
Biological Psychiatry ■■, 2015; ■:■■■–■■■ www.sobp.org/journal

signs, specifically cytokine profiles, are predictive of neurodevelopmental outcomes in a well-defined study population of children with a confirmed diagnosis of ASD, developmental delay (DD) without ASD, or typical development (TD).

METHODS AND MATERIALS

Participants

We used neonatal blood spots archived by the State of California and obtained for a subset of 303 children (214 with ASD, 27 with DD, and 62 with TD) enrolled in the Childhood Autism Risks from Genetics and the Environment (CHARGE) study (21) between January 2003 and October 2005. The CHARGE study is an ongoing population-based case-control study investigating risk factors for neurodevelopmental disorders, with participants selected from three groups—children with ASD, children with DD, and children with TD—from the general population. Eligible children were 2–5 years old, born in California, living with a biological parent who speaks English or Spanish, and residing in selected regional center catchment areas. Children were identified as described previously (21). The CHARGE protocol was approved by the institutional review boards at the University of California in Davis and Los Angeles and the State of California Committee for the Protection of Human Subjects. Written informed consent was obtained from parents before participation.

Diagnostic Confirmation

Children were assessed at the University of California, Davis, MIND (Medical Investigation of Neurodevelopmental Disorders) Institute at study enrollment (2–5 years old). Cognitive function and adaptive function were evaluated in all children with Mullen Scales of Early Learning (MSEL) (22) and Vineland Adaptive Behavior Scales (VABS) (23), respectively. Diagnosis of ASD was confirmed with the Autism Diagnostic Interview–Revised (24) and the Autism Diagnostic Observation Schedule (6,25) using criteria described by Risi *et al.* (26) and in accordance with DSM-5 (3). Children with ASD (n = 214) were subdivided into children exhibiting severe ASD symptoms (ASD_{sev} [n = 141]) and children exhibiting mild/moderate symptoms (ASD_{mild} [n = 73]) using Autism Diagnostic Observation Schedule comparison scores ≥7 and <7, respectively (25).

Control subjects were screened for ASD using the Social Communication Questionnaire (27); children with a Social Communication Questionnaire score ≥15 were evaluated with the Autism Diagnostic Interview–Revised and Autism Diagnostic Observation Schedule and reclassified if they met criteria for ASD. Control subjects with TD had no prior diagnosis of ASD or DD and composite scores ≥70 on the MSEL and VABS. Control subjects with DD had composite scores <70 on the MSEL and VABS.

Behavioral and Developmental Assessments

Aberrant Behavior Checklist. The Aberrant Behavior Checklist (28) measures maladaptive behavior using the following subscales: Irritability (15 items), Lethargy/Social Withdrawal (16 items), Stereotypy (7 items), and Hyperactivity (16 items). Each item is rated on a 4-point Likert scale ranging from 0 (not at all a problem) to 3 (problem severe in degree).

Mullen Scales of Early Learning. The MSEL is a standardized assessment of cognitive development in young children and includes the following scales: Visual Reception (nonverbal cognitive ability), Fine Motor, Receptive Language (language comprehension), and Expressive Language (language production). Developmental quotients ([age equivalent/chronological age] × 100) were calculated for each scale and composite to overcome the floor effect.

Vineland Adaptive Behavior Scales. The VABS is a standardized assessment that measures personal and social skills needed for everyday living on the following domains: Communication, Daily Living Skills, Socialization, and Motor Skills. Developmental quotients were calculated as described in the preceding paragraph.

Blood Spot Specimens

In California, capillary blood is collected at birth by heel prick, spotted onto standardized filter paper, and tested for various disorders as part of the Genetic Disease Screening Program. The remaining specimen is stored through the California Department of Public Health (29).

Blood Spot Elution

Three 3-mm punches of each dried blood spot specimen were placed together into a single well in a 96-well plate and stored at −80°C until elution. The blood spot elution protocol was as follows: 200 μL of elution buffer (phosphate-buffered saline, .5% bovine serum albumin, and protease inhibitors [Complete Protease Inhibitor Cocktail, Roche Diagnostics Corporation, Indianapolis, Indiana]) was added to each well, and plates were placed on a plate shaker overnight at 4°C. The eluates were analyzed immediately after elution.

Cytokine and Chemokine Measurement

Blood spot cytokine and chemokine levels were measured using Luminex Multiplex (EMD Millipore, Billerica, Massachusetts) technology. Cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, and tumor necrosis factor (TNF)-α were measured using high-sensitivity Bio-Plex Precision Pro Human Cytokine Assays (Bio-Rad, Hercules, California). Chemokines IL-8, MCP-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, eotaxin, interferon-inducible protein (IP)-10, and RANTES were measured using MILLIPLEX human chemokine kits (EMD Millipore) according to the manufacturer's directions. Briefly, 25 μL of eluate was incubated with the kit beads with shaking overnight at 4°C. The beads were then washed using a vacuum manifold, and biotin-conjugated detection antibodies were added for a 1-hour incubation. After washing, the beads were incubated with streptavidin-phycoerythrin for 30 min, the plates were read on a Bio-Plex 100 system (Bio-Rad), and the signal was analyzed with Bio-Plex Manager software (Bio-Rad) using a 5-point standard curve. Because eluted blood spots contain rehydrated components of whole blood, including plasma and lysed cells, the cytokine and chemokine profiles are derived from plasma and cellular sources. Total protein was measured in each eluted blood spot sample using BCA Protein Assay (Pierce, Rockford, Illinois). Cytokine and chemokine concentrations were normalized for total protein content and presented in pg/mg total protein.

متن کامل مقاله ← دریافت فوری

ISIArticles
مرجع مقالات تخصصی ایران

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان دانلود رایگان ۲ صفحه اول هر مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات