Early Career Investigator Commentary

Biological Psychiatry: Celebrating 50 Years

Cytokines and Chemokines in Novel Roles: Exploring Their Potential as Predictors of Autism Spectrum Disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by the primary symptoms of impaired social interaction, communication deficits, and repetitive, stereotyped behaviors. These symptoms are caused by a mixture of both genetic (>50%) and environmental factors and start to manifest from 2 years of age. Currently, the most effective approach to treating ASD is social stimulation, and its success depends on initiating treatment as early as possible. Therefore, the prediction of an ASD risk factor at the earliest possible age would allow for more timely behavioral intervention and may considerably improve the chances of counteracting the symptoms of ASD.

No molecular signature with predictive potential is known for ASD. However, immune dysregulation is a common feature of this condition, and alterations in circulating cytokine/chemokine levels have been well described (1). Therefore, they might be promising candidates for predicting ASD risk. In this issue of *Biological Psychiatry*, Heuer *et al.* (2) studied an assortment of circulating cytokines and chemokines from neonatal blood as predictive biomarkers for ASD risk. Specifically, Heuer *et al.* (2) obtained newborn bloodspots from children with ASD, children with developmental delay (DD), and general population (GP) control subjects from a large population study that was conceived to investigate archived biological samples for markers of susceptibility to ASD. Groups were matched by sex, birth season, birth year, and maternal age, among other factors. Those subjects classified as having ASD were further categorized into either early onset (EO) or regressive (Reg) or according to the presence of intellectual disability (YesID) or the absence of intellectual disability (NoID). At the time when all children were between 4.5 and 9 years of age, blood samples were analyzed for 42 immune markers using a Luminex multiplex platform. Comparisons between groups were examined using logistic regression and partial least squares discriminant analyses.

Heuer *et al.* (2) found that increased levels of interleukin (IL)-6 and IL-8 were associated with a global ASD phenotype when compared with the GP. Particularly, only increased IL-8 was associated with both YesID and NoID subgroups within ASD, whereas cytokine IL-12p70 and chemokines eotaxin-1 and granulocyte chemotactic protein 2 were associated specifically with the ASD-NoID subgroup, and IL-4 was associated with the ASD-YesID subgroup. When analyzing EO and Reg subgroups within ASD, Heuer *et al.* (2) found that ASD-EO was associated with increased IL-8 while ASD-Reg was linked to an increase in B cell-attracting chemokine 1, eotaxin-3, and IL-10.

When comparing ASD with DD control subjects, increased chances of developing overall ASD were linked to higher levels of IL-12p70, interferon (IFN)-γ, and eotaxin-1 as well as lower levels of tumor necrosis factor alpha. Regarding NoID and YesID subgroups, both subgroups were associated with increased levels of IL-12p70 and eotaxin-1, while NoID was associated only with increased levels of IL-13, granulocyte chemotactic protein 2, and T cell chemoattractant with angiogenic properties, and YesID was correlated only with higher levels of IFN-γ. When evaluating EO and Reg subgroups, both subgroups were associated with an increase of eoxtaxin-1. The odds of developing ASD-EO were associated only with increased levels of IL-12p70, IFN-γ, and T cell chemoattractant with angiogenic properties, while an ASD-Reg phenotype was associated with increased levels of IFN-γ. Here, a decreased risk of ASD-Reg was associated with higher levels of monocyte chemotactic protein 4. Lastly, within the ASD group, elevated levels of eotaxin-1 and cutaneous T cell–attracting chemokine were associated with greater odds of developing ASD-NoID compared with ASD-YesID. Importantly, comparisons between DD and GP control groups revealed no differences in any of the analyzed immune markers.

Data were also subjected to a partial least squares discriminant multivariate analysis that replicated the main individual findings obtained with the logistic regression approach but that also revealed that no combinations of immune markers were able to predict a particular case status.

Previous publications have attempted to address the potential of cytokines as predictors of ASD (3–5), and some of the cytokines found to be differential are coincident with the ones found by Heuer *et al.* (2) However, this approach has been performed with a higher-sensitivity assay and with more cases studied. This has permitted the detection and analysis of a greater number of cytokines and chemokines and facilitated a focus on subgroups within ASD, particularly whether they could be discriminated by specific biomarkers. In this sense, Heuer *et al.* (2) go a step further toward predicting the odds of developing ASD: this study allows for the more precise determination of an ASD subgroup, and therefore early interventions can more accurately target the particular ASD behavioral phenotype.

Given the pleiotropic nature of cytokines and chemokines, it will be a key aspect to explore whether multiple cytokine/chemokine profiles do predict an ASD predisposition—and, if possible, which type. It is compelling to think that because several immune features are changed in ASD (1) this will be

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reflected in alterations of the overall pattern of circulating immune molecules rather than individual ones. This pattern may in turn differ according to the ASD subtype and may be possibly masked when examining heterogeneous groups within ASD. Therefore, special attention should be given to clinical ASD subcategorization when analyzing several cytokines and chemokines acting together.

Among the risk factors of developing ASD, those based on prevalence statistics have been characterized—for example, gender, co-occurrence of non-ASD developmental diagnoses, or siblings with ASD, according to the Centers for Disease Control and Prevention. Moreover, some have addressed ASD risk by acknowledging early behavioral features that may have predictive potential. For example, atypical social orienting and reduced sustained attention while watching video stimuli have been coded by a computer vision analysis in toddlers between 16 and 31 months of age (6). This is supported by a large body of evidence showing that most of the abnormalities that are present in ASD are likely to have arisen before or shortly after birth. In this sense, the heterogeneity of ASD uncovers a need for the identification of biological measures as a means of describing subsets within ASD and thereby facilitating the targeting of individualized therapies. Therefore, the differentially regulated cytokines and chemokines identified by Heuer *et al.* (2) could be combined with other predictive components that have already been described (7) to establish an ASD risk factor based on biological parameters as well as behavioral abnormalities.

An interesting concept discussed by Heuer *et al.* (2) is that maternal cytokines can undergo alterations that may predict ASD prenatally. It is well known that during pregnancy there is a differential modulation of the immune system that leads to changes in the response to pathogens. This is reflected in adjusted cytokine signaling pathways that are responsible for promoting a healthy pregnancy (8). At the same time, certain infections during pregnancy can have detrimental effects on the developing fetus. In this sense, maternal immune activation is associated with an increased risk of ASD in offspring, particularly through dysregulation of IL-6 (9) and IL-17a (10). Therefore, further studies regarding the maternal cytokine profile in ASD should not only reveal cytokine candidates to use as predictive biomarkers, but also clarify their role in contributing to inflammation-induced ASD-like phenotypes, and ultimately look for therapeutic targets to reduce this likelihood. Because of its broad potential benefits, continuing this line of research is well worth the challenge.

Cytokines and chemokines have been studied as a predictive tool in a variety of conditions, from immune-related conditions like neonatal sepsis and pneumonia to heart failure, Alzheimer's disease, and some types of cancer. They have even been able to predict preterm delivery. Although cytokines have been shown to play key roles in several psychiatric conditions, including ASD and schizophrenia, whether they can act as predictors is a major question that deserves further attention. Nevertheless, and due in part to their pleiotropicity, additional factors considered when using cytokines as predictive tools would improve the chances of accuracy and robustness. Finally, because cytokines are present in children who have already been diagnosed with ASD, there is value in exploiting their potential as follow-up biomarkers to monitor the progression of ASD after behavioral interventions.

In this context, this approach introduces a different way of conceiving of cytokines and chemokines: given the fact that they are usually associated with poorer prognosis of an ongoing ASD condition, the current challenge is, through the detection of their early abnormal profiles, to develop a certain probability of the patient's developing ASD at a later stage, to guide treatment selection, and to best counteract behavioral symptoms of people with ASD.

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Article Information

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