The Immune System, Cytokines, and Biomarkers in Autism Spectrum Disorder

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Abstract  Autism Spectrum Disorder (ASD) is a pervasive neurodevelopmental condition characterized by variable impairments in communication and social interaction as well as restricted interests and repetitive behaviors. Heterogeneity of presentation is a hallmark. Investigations of immune system problems in ASD, including aberrations in cytokine profiles and signaling, have been increasing in recent times and are the subject of ongoing interest. With the aim of establishing whether cytokines have utility as potential biomarkers that may define a subgroup of ASD, or function as an objective measure of response to treatment, this review summarizes the role of the immune system, discusses the relationship between the immune system, the brain, and behavior, and presents previously-identified immune system abnormalities in ASD, specifically addressing the role of cytokines in these aberrations. The roles and identification of biomarkers are also addressed, particularly with respect to cytokine profiles in ASD.

Keywords  Autism Spectrum Disorder · Cytokine · Immune system

Introduction

Autism Spectrum Disorders (ASDs) are complex, pervasive neurodevelopmental conditions with a largely unknown etiology and a significant male bias. ASDs are behaviorally defined and characterized by deficits in social communication and interaction, and the presence of restricted, repetitive patterns of behavior, interests, or activities [1]. Developmental trajectories and patterns of severity vary substantially, with all facets of daily functioning potentially impacted. The vast clinical heterogeneity is a hallmark. Comorbid psychiatric and medical conditions are frequently reported, including social anxiety disorder, attention deficit disorder, immune system abnormalities, gastrointestinal disorders, mitochondrial dysfunction, sleep disorders, and epilepsy [2–6]. The multifaceted nature of the condition has resulted in investigations aiming to characterize biological subtypes of ASD. However, an understanding of the biological mechanisms driving pathophysiology is evolving. Immune system aberrations, including altered cytokine profiles, are believed to have a role in ASD [7, 8]. Accumulating evidence of alterations in central and peripheral immune system functioning supports the proposal that there is a subgroup of individuals with ASD who have some form of immune system dysregulation [9]. Altered cytokine levels may facilitate the identification of ASD subtypes as well as provide biological markers of the response to effective treatments.

The Immune System

The immune system is a complicated group of defense mechanisms that are triggered in order to protect an organism from disease- or illness-causing pathogens,
which include bacteria, viruses, fungi, and parasites. An antigen, considered foreign by the host’s body, is a molecule that stimulates the immune system to produce antibodies which function to identify and neutralize or remove the antigen. Many interconnected organs, molecules, cells, and pathways play roles in a fully functional immune system, which is comprised of two interconnected systems of immunity: innate and adaptive [10]. These two systems work together to protect the body from pathogens.

Innate immunity refers to first-line defense mechanisms that respond to an infection immediately or within hours of the host being attacked by a pathogen. The innate immune response relies on physical barriers, such as the epithelial layers of the skin and mucosal and glandular tissue surfaces connected to the body’s openings, as well as chemical barriers, which include soluble antimicrobial proteins and peptides, and an acidic pH. When pathogens breach these barriers, cellular innate immune responses are triggered through a pathogen-recognition process involving an array of cells with cell surface and intracellular receptors. Groups of pathogens present with characteristic pathogen-associated molecular patterns, which are recognized by pattern recognition receptors expressed by many different immune cells [11]. Some cells are activated to phagocytose and degrade the pathogen, a process through which macrophages and neutrophils engulf and destroy extracellular microbes. Cell receptors can also be activated, causing the cells to produce antimicrobial substances that eliminate the pathogens. Other cellular activation processes lead to the production of cytokines and chemokines, which are proteins that recruit cells, molecules, and fluid to sites of infection. This procedure results in the physiological changes known as inflammation [10]. Other effectors of innate immunity are natural killer (NK) cells that, through cytokine production or cytotoxicity, contain infections until an adaptive immune response is initiated.

When innate immunity is insufficient, for example when features of certain pathogens allow them to evade the defense mechanisms of the innate immune system, the adaptive immune response is activated. The adaptive response is initiated a few days after initial exposure to the pathogen and breach of the physical or chemical barriers. This response is more comprehensive and antigen-specific than the innate response. The adaptive immune system is able to recognize, eliminate, and remember pathogens. The innate immune system is not considered to have this memory component. However, demonstrations of NK cell memory in viral infections indicate that these cells have attributes of both innate and adaptive immunity [12]. There are two types of adaptive immune response, both carried out by lymphocytes. The first type of response is an antibody response carried out by B lymphocytes, or B cells, which, when activated by an antigen, secrete antibodies, also known as immunoglobulins. B cells are formed in the bone marrow. The second type of adaptive immune response is a cell-mediated immune response where activated T cells specifically recognize and neutralize or eliminate antigens. T cells mature in the thymus, which is a lymphoid organ of the immune system. Residual B and T cells remaining after antigen exposure function as memory cells that are activated by subsequent pathogen challenges.

The innate and adaptive immune responses discussed above are characteristic of a regulated immune system contributing to the maintenance of homeostasis and preventing disruptions of normal functions of the body. A regulated immune system requires an optimal balance of pro- and anti-inflammatory signaling. Inflammation may not be a problem in isolation, but if an unregulated or dysregulated immune system responds to physiological changes initiated by pathogens, then the inflammation may be problematic. An aberrant immune system may manifest as upregulation of the inflammatory/immune response or as immune deficiency compromising host defense. Allergies, asthma, and autoimmune disorders are all conditions associated with immune dysfunction.

**Cytokines and the Immune System**

Cytokines are cell-signaling molecules that facilitate communication among cells of both the innate and adaptive immune systems. They are primary regulators of inflammation, coordinating the response to infection and associated immune challenges and are involved in a multitude of biological processes. As part of an integrated network, cytokines stimulate and modulate immune system activity and induce their own synthesis and the synthesis of other cytokines. They are typically soluble molecules although some remain cell-bound. Cytokines can be broadly classified into three groups based on the type of immune response: adaptive immunity, pro-inflammatory signaling, and anti-inflammatory signaling [13]. Chemokines are a subpopulation of cytokines that initiate the recruitment of well-defined leukocyte subsets through chemical stimuli [14]. Cells attracted to the chemokine follow a signal of increasing concentration towards the source of the chemokine, usually an infected or damaged cell. As cell-signal molecules, cytokines bind to receptors on the plasma membrane and elicit effects through the activation of an intracellular signaling cascade. Cytokines can be further classified based on the distance between the cell secreting the signaling ligand and the cell receiving the chemical signal [10]. Endocrine action is when cytokines pass through the bloodstream before reaching the target. Cytokines that act near the secreting cell are paracrine. Autocrine action is when cells can secrete a signal that is
received through its own receptors. Cytokines have been characterized as belonging to one of six groups based on cytokine and cytokine receptor structure, but members may exhibit diverse functionality. The cytokine families and some of the members of each group are detailed in Table 1 [10].

T and B lymphocytes mediate adaptive immunity. However, it is the T helper cells that are required for almost all adaptive immune responses [15]. They help activate B cells to secrete antibodies and macrophages to eliminate pathogens. Naïve T helper cells are differentiated into functional types defined by their pattern of cytokine production and function: Type 1 T helper (Th1), Type 2 T helper (Th2), T-regulator, and Th17 cells [16]. Proliferation and differentiation are functions of the particular cytokine milieu and signaling requirements during T cell receptor activation. Differentiation into either Th1 or Th2 effector cells then determines the nature of the subsequent adaptive immune responses activated by effector cells [17]. Th1 cells produce interferon (IFN)-γ, their signature cytokine and interleukin (IL)-2, while many also produce tumor necrosis factor (TNF)-α. Th2 signature cytokines are IL-4, IL-5, and IL-13, but these cells also make TNF-α, and some produce IL-9 and modest amounts of IL-2 [16]. Immune regulation is thought to require homeostasis between Th1 and Th2 activity [15]. If either Th1 or Th2 dominates, then the other response may be suppressed. Lower proportions of Th1 cells and higher proportions of Th2 cells have been seen in children with ASD compared to healthy controls, providing evidence of an imbalance of Th1- and Th2-like cytokines in ASD [18]. In addition, analysis of peripheral blood mononuclear cells from children with ASD showed increased activation of both the Th1 and Th2 arms of the adaptive immune response, with a Th2 dominance and no compensatory increase in expression of the regulatory cytokine IL-10 [19].

Each cytokine can be produced by a single cell type or multiple cell types. For example, Th1 cells produce IFN-γ, IL-2, and TNF-β, while Th2 cells produce IL-4, IL-5, IL-6, IL-9, and IL-10 [20]. However, granulocyte macrophage colony-stimulating factor (GM-CSF) can be produced by multiple cell types, including macrophages, endothelial cells, and fibroblasts [21]. Similarly, IFN-β can be produced by multiple cell types including fibroblasts and epithelial cells. Cytokines can act on a single or multiple cell types. For example, IL-12 acts on Th1 cells, while IL-1 acts on T cells, B cells, macrophages, endothelial cells, fibroblasts, and epithelial cells, and all interferons act on multiple cell types. Cytokines also exhibit redundancy, meaning multiple cytokines exert the same biological

| Table 1  | Details of six cytokine families and examples of family members. |
|----------|-----------------------------------------------------------------|
| Cytokine Family | Examples | Characteristics |
| Interleukin 1 family | IL-1α, IL-β, IL-RA | Induce responses against infection that are primarily pro-inflammatory IL-RA antagonizes effects of IL-1α and IL-β |
| Hematopoietin (Class I cytokine) family | IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-12p40, IL-12p70, IL-13, IL-15, IL-23, GM-CSF, G-CSF | Largest family of cytokines, all members sharing a 4-helix bundle structure Sequence and functional diversity Diverse effects include proliferation, differentiation, and antibody secretion |
| Interferon (Class II cytokine) family | IFN-γ, IL-10 | Mediate early antiviral responses Activate macrophages, interact with cells of the adaptive immune system, and support the generation of Th1 cells |
| Tumor necrosis factor family | TNF-α, TNF-β1 | Expressed in either soluble or membrane-bound form Cause apoptosis |
| Interleukin 17 family | IL-17 | Primarily pro-inflammatory |
| Chemokine family | IL-8, Eotaxin, IP-10 (CXCL10), MCP-1(CCL2), MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5) | Secondary pro-inflammatory mediators Stimulate recruitment of well-defined leukocyte subsets Promote chemotraction, movement of immune system cells into, within, and out of lymphoid organs |

CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C) ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-γ, interferon-γ; IL, interleukin; IL-1RA, IL-1 receptor antagonist; IP-10, IFNγ-inducible protein 10; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated on activation, normal T cell expressed and secreted; Th, T helper; TNF-α, tumor necrosis factor-α.
action. Furthermore, an individual cytokine can have multiple effects on a target cell. IFN-γ induces antiviral proteins, upregulates major histocompatibility complex Class I antigens, stimulates NK and IL-12 production, and induces antiproliferative effects [22]. Increased levels of IFN-γ and IL-12 can induce inflammation whereas increased levels of transforming growth factor (TGF)-β, IL-4, and IL-10 can downregulate inflammation [23]. The classification of the biological action of cytokines as either pro-inflammatory or anti-inflammatory may be dependent on the amount of cytokine, the nature of the target cell, the nature of the activating signal, the nature of produced cytokines, and the timing and sequence of cytokine action [24].

The Immune System, the Nervous System, Behavior, and the Role of Cytokines

The immune system and the nervous system are intricately interconnected. The functional status of the immune system affects a multitude of biological processes, including brain function and development, which can be affected when the innate and adaptive immune responses are dysregulated [25]. Sickness behavior, a term used to describe changes in the subjective experience and behavior occurring in a physically ill person [26], provides an example of how, through multiple mechanisms, the immune system can influence brain function and subsequent behavior [25]. Nonspecific symptoms of sickness behavior include fever, nausea, reduced appetite, fatigue, irritability, and withdrawal from physical and social environments [27]. Sickness behavior is considered an organized and evolved strategy to facilitate the role of fever in fighting infection. It is initiated by pro-inflammatory cytokines that are produced at the site of infection by activated accessory immune cells and is characterized by endocrine, autonomic, and behavioral changes [27]. The brain recognizes cytokines such as the pro-inflammatory cytokines IL-1α, IL-1β, TNF-α, and IL-6 as molecular signals of sickness [28]. Furthermore, TNF-α, IL-6, and IL-1β can cross the blood-brain barrier and act on the hypothalamus where they promote fever and sickness behavior [29]. The similarities of symptom expression in sickness behavior and depression have led to the hypothesis that cytokines and inflammatory factors are involved in the pathophysiology of neuropsychiatric disorders, and this has been a catalyst for extensive research into the pathways and mechanisms through which the immune system influences the brain and behavior [30]. Interestingly, another example of a relationship between symptom and cytokine expression involves immunotherapy in cancer patients, in whom prolonged exposure to the proinflammatory cytokine IL-2 results in dose- and time-related cognitive dysfunction and altered behavior [31].

The literature identifying abnormal cytokine profiles in depression, bipolar disorder, and schizophrenia [32–34] collectively suggests that cytokines induce both sickness behavior and neuropsychiatric symptoms, and that inflammation is a key pivotal factor in psychopathology [35]. In addition, a systematic review evaluating pro-inflammatory markers in almost 4,000 children and adolescents with neuropsychiatric and neurodevelopmental disorders, including ASD, identified preliminary evidence of the role of inflammation in these conditions and an association with a pro-inflammatory state [36]. Furthermore, growth in research into the role of inflammation has led to the redefinition of many diseases, such as heart disease, Alzheimer’s disease, type 1 diabetes, type 2 diabetes, and obesity, as inflammatory disorders [13]. A possible mechanism for the role of inflammation in these disorders is the alteration of the structural and functional integrity of the central nervous system (CNS) by cytokines, thereby contributing to the pathology of neuro-inflammation and neuropsychological disorders.

Peripheral cytokine signals are thought to access the brain through three pathways: humoral (with antibody involvement), neural, and cellular [30]. These communication pathways involve at least five mechanisms: (1) passage of cytokines through leaky regions of the blood-brain barrier; (2) active transport via saturable cytokine-specific transport molecules on brain endothelium; (3) activation of endothelial cells, which release second messengers within the brain parenchyma; (4) transmission of cytokine signals via afferent nerve fibers, including the vagus; and (5) entry into the brain parenchyma of peripherally-activated monocytes which release cytokines.

Cytokines may influence behavior through effects on neurotransmitter function, neuroendocrine activity, neurogenesis, and alterations to brain circuitry [30]. For example, cytokines have been shown to increase the release and decrease reuptake of the excitatory neurotransmitter glutamate, which can result in the pathological process of excitotoxicity [37]. This type of mechanism could support a model for some types of ASD; the model postulates an increased excitation/inhibition ratio in key neural systems, such as sensory, mnemonic, social, and emotional systems [38].

An alternate communication pathway has recently been proposed based on the groundbreaking work by Louveau and colleagues who identified functional lymphatic vessels in the CNS that carry fluid and immune cells from the cerebrospinal fluid, and in doing so discovered a pathway for immune cells to exit the CNS [39]. While the anatomy and functional importance of these pathways and systems have yet to be characterized in humans, this work provides
a new perspective on the possible etiology of neuroinflammatory and neurodegenerative conditions. These findings also provide an impetus for further consideration of the relationship between immune responses and behaviors in other conditions that are characterized by immune system dysfunction, such as ASD.

An understanding of the pathways and mechanisms through which the immune system affects behavior is primarily based on findings in animal models. For example, mice deficient in T cells have cognitive deficits [25]. Interestingly, an altered activation profile for T cells has been identified in ASD, with these perturbations of T cell function possibly modulating behavior and core features of ASD [40]. Recently, social behavior as operationalized within an animal model, has been shown to be influenced by meningeal immunity [41]. Mice deficient in adaptive immunity, specifically an absence of interferon IFN-γ, display both hyper-connectivity in the prefrontal cortex (PFC) and significant social deficits. This is particularly interesting, given that hyperactivity in the PFC in the context of social stimuli is known to be a feature of social impairment in ASD [42]. Filiano and colleagues demonstrated that CNS neurons respond to IFN-γ derived from meningeal T cells, elevating tonic GABAergic inhibition [41]. This process prevents aberrant hyper-excitability in the PFC and restores social behaviors through IFN-γ. Previously, IFN-γ released from T cells was thought to predominantly stimulate and modulate immune responses to infection. Interestingly, NK cells, recognized as major producers of cytokines including IFN-γ [12] in physiological and pathological conditions, are dysfunctional in ASD [43]. While a novel finding, the regulation of neural activity and social behavior through IFN-γ provides further evidence for the interconnectedness of the immune system, the nervous system, and behavior.

**Immune System Deregulation in ASD**

Evidence suggesting a pathophysiological relationship between the immune system and ASD was first presented over 40 years ago [44]. Subsequent research investigating the complex relationship between the immune system and ASD symptomatology has identified numerous potential interactions and proposed associated mechanisms at both the systemic and cellular levels [7, 45]. One of these areas of research focuses on the prenatal period. Maternal immune activation refers to the defensive response of the mother’s immune system to an invading pathogen. A large population-based study found that acute immune activation caused by maternal viral infection during the first trimester increases the risk of ASD in children [46]. Furthermore, a recent meta-analysis of >40,000 ASD cases showed that maternal infection during pregnancy is associated with an increased risk of ASD in the offspring, with hospitalization during infection heightening the risk [47]. Moderators of this risk include the type of infectious agent, the timing of infectious exposure, and the site of infection. A recent review has proposed that maternal infection leads to the release of pro-inflammatory cytokines and activation of Th17 cells in the mother’s bloodstream and that the immune status and genetic predisposition of the fetus determine its vulnerability to maternal immune activation, a process considered a disease primer [48]. Peripheral cytokine profiles at birth, including elevated IL-1β and IL-4, are associated with an ASD diagnosis later in childhood and vary with ASD symptom severity [49]. Elevation of IL-1β and IL-4 may reflect a prenatal immune challenge, and an association with both ASD risk and cognitive developmental outcomes suggests the possibility of a global impact of early cytokine dysregulation [49]. Familial autoimmunity has also been implicated in the pathogenesis of ASD, with an increased risk of ASD in children with a maternal history of rheumatoid arthritis and celiac disease, and an increased risk of infantile autism has been identified in children with a family history of type 1 diabetes [50].

Another area of focus on immune involvement in the pathogenesis and maintenance of ASD is the postnatal period. Altered cytokine profiles have been consistently linked to ASD in children during this period [7]. In high-functioning male children with ASD, the plasma levels of IL-1β, IL-1 receptor antagonist (IL-1RA), IL-5, IL-8, IL-12(p70), IL-13, and IL-17 are elevated relative to matched controls [51]. IL-1β, a pro-inflammatory cytokine, activates neutrophils and macrophages to phagocytose invading pathogens [52]. IL-1RA inhibits the activities of IL-1β, suggesting that the levels of IL-1RA might be a function of a negative feedback regulator role in response to the elevation of IL-1β [51]. IL-1β is involved in the production of IL-17 [53] and IL-17 is a potent mediator of the production of IL-8, a chemokine with important roles in the innate immune response. IL-5 and IL-13 stimulate B cells to secrete immunoglobulins including IgE, which is a mediator of allergic inflammation. IL-12(p70) is a pro-inflammatory cytokine that enhances Th1 and NK cell responses [54]. In addition to elevated expression of IL-1β, as identified by Suzuki and colleagues, IL-6, IL-12, TNF-α, and IL-23 are also elevated in ASD compared to healthy controls, suggesting a dysregulated immune response [55]. TNF-α is a central regulator of inflammation and is elevated in the cerebrospinal fluid of children with ASD [56]. IL-6 is typically regarded as a pro-inflammatory cytokine and has been identified as a cytokine the brain recognizes as a molecular signal of sickness [28]. However, it also has regenerative or anti-inflammatory activity, and is involved in the regulation of metabolic and neural processes [57].
Finally, a further example of immune abnormalities in the postnatal period involves activation of the monocytic and Th1 arm of the immune response, via increased IL-1RA and increased IFN-γ, respectively, and this has been found in children with ASD [58].

Immune-mediated mechanisms have also been hypothesized as reflecting a chronic state of specific cytokine activation [59]. Immunocytochemical studies have identified marked activation of microglia and astroglia associated with the increased production of two cytokines by neuroglia, macrophage chemoattractant protein (MCP)-1, and TGF-β1 [59]. In addition, a unique profile of pro-inflammatory cytokines has been identified in cerebrospinal fluid [59]. Another post-mortem study also demonstrated significant increases in pro-inflammatory and Th1 cytokines relative to matched controls [60]. Elevation of IL-6 in ASD, both centrally and peripherally, has been frequently reported [59–62]. In a mouse model with elevated IL-6 in the brain, Wei and colleagues have shown that IL-6 can modulate autism-like behaviors through impairments of synapse formation, dendritic spine development, and neuronal circuit balance [63]. Acute and chronic psychological stress and alterations in sleep duration and quality, a commonly reported comorbidity in ASD [64], increase the concentrations of IL-6 [65]. This evidence for abnormal cytokine profiles in ASD suggests that immune system disturbances may be active and continuous contributors to the presentation of ASD. It is this accumulation of evidence that has acted as the catalyst for efforts to characterize possible subgroups of ASD patients who present with immune system abnormalities or dysfunction and altered patterns of symptom presentation [9, 66].

Associations between changes in peripheral cytokine expression and the severity of behavioral impairments and associated symptoms have been identified in children with ASD (Table 2). Reduced levels of the regulatory cytokine TGF-β1 are associated with reduced adaptive behavior and worsening behavioral symptoms [67, 68]. While TGF-β1 is involved in cell growth and differentiation, organ development, migration, and apoptosis, its major role is to control inflammation. This negative correlation with behavioral impairment suggests that there is an ongoing inflammatory process in children with ASD who present with worsening behavioral profiles [67]. Increased levels of the chemokines MCP-1, RANTES (regulated on activation, normal T cell expressed and secreted), and eotaxin are associated with more impaired behaviors and adaptive functioning [69]. Chemokines are expressed in the developing brain and regulate neuronal cell migration, proliferation, and differentiation. They are also involved in communication between neurons and microglia [70]. Elevated IL-1β and IL-6 have been associated with increased stereotypical behaviors [62]. Dysregulation of IL-1β, a pro-inflammatory cytokine expressed early in an immune response, is implicated in impairments in memory and learning [71]. IL-1β induces and inhibits neural progenitor cell proliferation in the CNS, which can contribute to region-specific deviant brain growth in ASD [72]. As previously highlighted, IL-6 is elevated in most inflammatory states and has been implicated in a wide range of conditions. Elevation of IL-8 and IL-12p40 is also associated with greater impairment of aberrant behaviors including lethargy and stereotypy as measured by the Aberrant Behavior Checklist (ABC) [62]. In addition, as the expression of IL-8 decreases, cognitive and adaptive ability improves [62]. IL-8 is a chemoattractant cytokine, attracting and activating neutrophils in regions of inflammation [73] and hence may contribute to the pathogenesis of inflammatory diseases [74].

Other cellular markers of immune dysfunction identified in children with ASD include significantly higher absolute numbers of B cells and NK cells, and increased markers of cellular activation compared to healthy controls [75]. These findings suggest an immune response activation that leads to an increased frequency of NK cells and activated B cells and T cells. Increased levels of the IgG4 subclass have been identified in children with ASD [76]. The IgG4 subclass has features and biological function different from other subclasses of IgG, acting as a blocking antibody that binds strongly to antibody receptors rather than a protective antibody. A correlation between the severity of behavioral measures and reduced levels of immunoglobulin has also been found, suggesting suboptimal humoral function in children with ASD [77]. Furthermore, an elevated prevalence of other immune-related comorbidities, including autoimmune diseases, allergies, and psoriasis, has been found in children with ASD compared to healthy controls [6]. Overall, these relationships between immune dysfunction and behavioral symptoms associated with an ASD presentation suggest an ongoing relationship impacting the severity of the condition in children with an ASD diagnosis.

Altered Cytokine Profiles as Potential Biomarkers in ASD

To facilitate improved communication about measurements of disease and treatment effects, an expert working group convened by the National Institutes of Health Director’s Initiative on Biomarkers and Surrogate Endpoints (USA) proposed the following definition of a biological marker or biomarker: a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [78]. Additional applications of biomarkers have been proposed,
such as use as a diagnostic tool for the identification of those patients with a disease or abnormal condition, and for the prediction and monitoring of the clinical response to an intervention. Specifically, with respect to using biomarkers to guide better treatment of schizophrenia and other psychotic disorders, Banati and Hickie have proposed clinically useful properties of biomarkers, including diagnostically non-specific, quantitative, longitudinal, plausibly linked to underlying pathophysiology, and predictive of risk of impairment [79]. They also highlight the clinical importance of the role of biomarkers in guiding treatment selection, and demonstrating a correlation between active interventions and the short-term clinical response.

Numerous biomarkers have been proposed for ASD, including biochemical, morphological, immunological, hormonal, neurophysiological, neuroanatomical, and neuropsychological markers [80]. A recent clinical trial in ASD involving an immunomodulatory pharmacological intervention demonstrates a correlation between an active intervention and a relevant short-term clinical response. Greater improvement in symptoms of irritability, hyperactivity, stereotypic behavior, social withdrawal, and inappropriate speech was achieved when risperidone was used adjunctively with pentoxifylline, an immune-modulating drug and pro-inflammatory cytokine inhibitor [81]. Furthermore, another adjunct treatment trial in children with ASD using risperidone and pioglitazone showed reductions in the severity of symptoms of irritability, social withdrawal, and hyperactivity in the adjunct treatment group compared to the risperidone-only group, indicating positive effects of pioglitazone [82]. Pioglitazone is a

| Cytokine | n (ASD) | Medium | Measures | Finding | Study |
|----------|---------|--------|----------|---------|-------|
| TGF-β1   | 75      | Plasma | ABC, VABS| Reduced levels associated with reduced adaptive behavior and worsened behavioral symptoms including stereotypy, irritability, and hyperactivity | Ashwood et al. [67] |
| Eotaxin  | 80      | Plasma | ABC, MSEL, VABS| Increased levels associated with increased severity of lethargy, stereotypy, and hyperactivity (ABC) | Ashwood et al. [69] |
|          |         |        |          | Increased levels associated with greater impairments in visual reception, fine motor skills, and receptive and expressive language (MSEL) | |
|          |         |        |          | Increased levels associated with greater impairments in communication, daily living skills, socialization, and motor skills (VABS) | |
| MCP-1    | 80      | Plasma | ABC, MSEL, VABS| Increased levels associated with greater impairments in visual reception, fine motor skills and expressive language (MSEL) | Ashwood et al. [69] |
|          |         |        |          | Increased levels associated with greater impairments in daily living skills (VABS) | |
| RANTES   | 80      | Plasma | ABC, MSEL, VABS| Increased levels associated with increased severity of lethargy, stereotypy, and hyperactivity (ABC) | Ashwood et al. [69] |
|          |         |        |          | Increased levels associated with greater impairments in visual reception, fine motor skills, and expressive language (MSEL) | |
|          |         |        |          | Increased levels associated with greater impairments in communication and socialization (VABS) | |
| IL-1β    | 97      | Plasma | ADI-R, ABC, MSEL, VABS| Increased levels associated with increased stereotypy (ABC) | Ashwood et al. [62] |
| IL-4     | 97      | Plasma | ADI-R, ABC, MSEL, VABS| Increased levels associated with greater impairments in non-verbal communication (ADI-R) | Ashwood et al. [62] |
| IL-6     | 97      | Plasma | ADI-R, ABC, MSEL, VABS| Increased levels associated with increased stereotypy (ABC) | Ashwood et al. [62] |
| IL-8     | 97      | Plasma | ADI-R, ABC, MSEL, VABS| Increased levels associated with increased hyperactivity, stereotypy, and lethargy (ABC) | Ashwood et al. [62] |
|          |         |        |          | Decreased levels associated with improved visual reception, receptive language, and expressive language (MSEL) | |
|          |         |        |          | Decreased levels associated with improved daily living skills (VABS) | |
| IL-12p40 | 97      | Plasma | ADI-R, ABC, MSEL, VABS| Increased levels associated with increased stereotypy and lethargy (ABC) | Ashwood et al. [62] |
| TGF-β1   | 30      | Plasma | CARS    | Reduced levels associated with increasing severity | El Gohary et al. [68] |

ABC, Aberrant Behavior Checklist; ADI-R, Autism Diagnostic Interview—Revised; IL, interleukin; MCP-1, monocyte chemotactic protein-1; MSEL, Mullen Scales of Early Learning; RANTES, regulated on activation, normal T cell expressed and secreted; TGF-β1, transforming growth factor-β1; VABS, Vineland Adaptive Behavior Scales; WAIS-R, Wescheler Adult Intelligence Scale-Revised.
peroxisome proliferator-activated receptor, which inhibits the production of pro-inflammatory cytokines and chemokines by microglia [83, 84]. Furthermore, risperidone was found to be more effective when given adjunctively with celecoxib, a nonsteroidal anti-inflammatory drug, with significant improvements in the irritability, social withdrawal, and stereotypy subscales of the ABC [85]. However, in a recent open-label study of risperidone treatment for children and adolescents with ASD, the plasma levels of eosin and MCP-1 showed statistically significant decreases after treatment, although these changes were not significantly associated with changes in severity measures [86]. Eotaxin and MCP-1 are pro-inflammatory and are elevated in brain specimens from patients with ASD [59]. Overall, the results of these trials suggest that treating immune-related symptoms may contribute to behavioral changes in ASD.

The clinical trials of immune-modulating agents, taken together with previous examples of immune system perturbations in children with ASD, suggest that cytokines are worthy of consideration as potential biomarkers of a subgroup of individuals with an ASD diagnosis and more severe behavioral outcomes. However, the validity of peripheral sampling of blood cells as a relevant biomarker and as a surrogate for a CNS sample is still debated. Given that peripheral blood cells comprise the major cellular components of the immune system, they could be considered suitable for the assessment of immune-related markers [87]. In addition, accessibility and speed of sampling for regular assessments and analysis of peripheral blood sampling are significant advantages over, for example, cerebrospinal fluid sampling and brain imaging.

A National Research Council (USA) report on precision medicine, which is the tailoring of medical treatment to the individual characteristics of each patient, highlights a critical need for the deconstruction of current diagnostic groups using biomarkers to help identify the subgroups for which treatment is highly effective [88, 89]. In addition to physical signs and symptoms, it is recommended that conditions should also be defined by their underlying molecular causes and other factors, which would represent an emergence of a new taxonomy based on biomedical research and an extensive patient data network. A data network would be needed to integrate current research on the molecular composition of conditions with clinical data on individual patients in an effort to drive precision medicine. Precision medicine also refers to the classification of individuals into subpopulations that differ in their susceptibility to a particular condition in the biology and/or prognosis of those conditions they may develop, or in their response to a specific treatment [89]. Similarly, the long-term aim of the Research Domain Criteria (RDoC) project of the National Institute of Mental Health (USA) is precision medicine for psychiatry through the adoption of a diagnostic system based on a comprehensive understanding of the biological and psychosocial bases of conditions, unhindered by the limitations of diagnostic categories [88].

A robust biological system is one that maintains its state and functions against external and internal perturbations [90]. It is possible that a robust immune system is protective and that while the mechanisms leading to abnormal function have yet to be established, it is tempting to consider that a regulated and functional immune system, both pre- and postnatally, is a prerequisite for a normal functioning brain [91]. Consistent with the RDoC agenda, the non-specific association found between inflammation and neuropsychiatric disorders, including ASD, and the identification of common reliable inflammatory markers across those different disorders warrant further investigations to determine if these processes play a role in the etiology of symptom dimensions or symptom domains that overlap or are shared by different conditions [35]. A diagnosis of ASD continues to be behaviorally defined. However, the body of research and accumulating evidence with respect to immune system perturbations in ASD suggest that a broader approach should be taken in order to understand biological systems as they pertain to ASD and associated behaviors.

Conclusion

The hallmark heterogeneity of ASD is a key reason for the focus of researchers on the identification of potential biological measures as a means of describing subsets within ASD, and thereby facilitating the targeting of more individualized therapies. Previously discussed cytokine aberrations in ASD have highlighted a possible relationship between cytokine aberration and ASD. Altered cytokine levels may facilitate the identification of ASD subtypes that share similar traits and profiles, as well as provide biological markers that facilitate monitoring of the benefits of active treatments over the time-course of clinical trials. Biological markers, as objective measures of the response to treatment in clinical trials, will assist with the identification of efficacious interventions. Furthermore, the identification of objective markers of a pathological state related to a subgroup in ASDs may assist in reducing the heterogeneity of participants in clinical trials, and this may lead to the identification of more targeted treatments for autism-related symptoms.

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References

1. APA. Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition. Washington, DC: American Psychiatric Association, 2013.

2. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. J Am Acad Child Adolesc Psychiatry 2008, 47: 921–929.

3. Mannion A, Leader G. An investigation of comorbid psychological disorders, sleep problems, gastrointestinal symptoms and epilepsy in children and adolescents with autism spectrum disorder: A two year follow-up. Res Autism Spectr Disord 2016, 22: 20–33.

4. Mannion A, Leader G. Comorbidity in autism spectrum disorder: A literature review. Res Autism Spectr Disord 2013b, 7: 1595–1616.

5. Bauman ML. Medical comorbidities in autism: Challenges to diagnosis and treatment. Neurotherapeutics 2010, 7: 320–327.

6. Zerbo O, Leong A, Barcellos L, Bernal B, Fireman B, Croen LA. Immunemediated conditions in autism spectrum disorders. Brain Behav Immun 2015, 46: 232–236.

7. Goines P, Van de Water J. The immune system’s role in the biology of autism. Curr Opin Neurol 2010, 23: 111–117.

8. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. Mol Psychiatry 2015, 20: 440–446.

9. Mead J, Ashwood P. Evidence supporting an altered immune response in ASD. Immunol Lett 2015, 163: 49–55.

10. Owen JA, Pun J, Stranford SA, Jones PP, Kuby J. Kuby Immunology. New York: W.H. Freeman, 2013.

11. D’Elia RV, Harrison K, Oyston PC, Lukaszewski RA, Clark GC. Targeting the “cytokine storm” for therapeutic benefit. Clin Vaccine Immunol 2013, 20: 319–327.

12. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Mantovani A. Innate or adaptive immunity? the example of natural killer cells. Science 2011, 331: 44–49.

13. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 2014, 1843: 2563–2582.

14. Graves DT, Jiang Y. Chemokines, a family of chemotactic cytokines. Crit Rev Oral Biol Med 1995, 6: 109–118.

15. Depino MA, de los Angeles Robinson-Agramonte M. Understanding on neuroimmunology in autism spectrum disorder. Translational approaches to autism spectrum disorder. Springer International Publishing, 2015: 155–180.

16. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations. Annu Rev Immunol 2010, 28: 445–489.

17. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. 4th edition. New York: Garland Science; 2002. Helper T Cells and Lymphocyte Activation.

18. Gupta S, Aggarwal S, Rashanravan B, Lee T, Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. J Neuroimmunol 1998, 85: 106–109.

19. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dieger K, Manning-Courtenay P, et al. Elevated cytokine levels in children with autism spectrum disorder. J Neuroimmunol 2006, 172: 198–205.

20. Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol 2000, 85: 9–21.

21. Shi Y, Liu CH, Roberts AI, Das J, Xu G, Ren G, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don’t know. Cell Res 2006, 16: 126–133.

22. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol 2004, 75: 163–189.

23. Xu N, Li X, Zhong Y. Inflammatory cytokines: potential biomarkers of immunologic dysfunction in autism spectrum disorders. Mediators Inflamm 2015, 2015: 10.

24. Cavaillon JM. Pro- versus anti-inflammatory cytokines: Myth or reality. Cell Mol Biol (Noisy-le-grand) 2001, 47: 695–702.

25. Filiano AJ, Gadani SP, Kipnis J. Interactions of innate and adaptive immunity in brain development and function. Brain Res 2015, 1617: 18–27.

26. Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 1988, 12: 123–137.

27. Dantzer R, O’Connor JC, Freund FG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008, 9: 46–56.

28. Dantzer R. Cytokine, sickness behavior, and depression. Immunol. Allergy Clin North Am 2009, 29: 247–264.

29. Dantzer R. Cytokine-Induced Sickness Behavior: Where Do We Stand? Brain Behav Immun 2001, 15: 7–24.

30. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. Pharmacology & therapeutics 2011, 130: 226–238.

31. Denicoff KD, Rubinow DR, Papa MZ, Simpson C, Seipp CA, Lotze MT, et al. The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. Ann Intern Med 1987, 107: 293–300.

32. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. Biol Psychiatry 2010, 67: 446–457.

33. Miller BJ, Buckley P, Seabol W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry 2011, 70: 663–671.

34. Modabbernia A, Taslimi S, Brietzke E, Ashrafi M, Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. Biol Psychiatry 2013, 74: 15–25.

35. Capuron L, Castanon N. Role of inflammation in the development of neuropsychiatric symptom domains: evidence and mechanisms. Curr Top Behav Neurosci 2017, 31: 31–44.

36. Mitchell RH, Goldstein BL. Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. J Am Acad Child Adolesc Psychiatry 2014, 53: 274–296.

37. Tillieux S, Hermans E. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. J Neurosci Res 2007, 85: 2059–2070.

38. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003, 2: 255–267.

39. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske EK, et al. Structural and functional features of central nervous system lymphatic vessels. Nature 2015, 523: 337–341.

40. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Altered T cell responses in children with autism. Brain Behav Immun 2011, 25: 840–849.

41. Filiano AJ, Xu Y, Tutisston NJ, Marsh RL, Baker W, Smirnov I, et al. Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour. Nature 2016, 535: 425–429.
42. Forbes CE, Graffman J. The role of the human prefrontal cortex in social cognition and moral judgment. Annu Rev Neurosci 2010, 33: 299–324.
43. Enstrom AM, Lit L, Onore CE, Gregg JP, Hansen R, Pessah IN, et al. Altered gene expression and function of peripheral blood natural killer cells in children with autism. Brain Behav Immun 2009, 23: 124–133.
44. Stubbs EG, Crawford ML. Depressed lymphocyte responsiveness in autistic children. J Autism Child Schizophren 1977, 7: 49–55.
45. Meltzer A, Van de Water J. The role of the immune system in autism spectrum disorder. Neuropsychopharmacology 2017, 42: 284–298.
46. Atladottir HO, Thorsen P, Østergaard L, Schendel DE, Lemcke S, Abdallah M, et al. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J Autism Dev Disord 2010, 40: 1423–1430.
47. Jiang HY, Xu LL, Shao L, Xia RM, Yu ZH, Ling ZX, et al. Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. Brain Behav Immun 2016, 58: 165–172.
48. Estes ML, McCallister AK. Maternal immune activation: Implications for neuropsychiatric disorders. Science 2016, 353: 772–777.
49. Krakowiak P, Goines PE, Tancredi DJ, Ashwood P, Hansen RL, Hertz-Picciotto I, et al. Neonatal cytokine profiles associated with autism spectrum disorder. Biol Psychiatry 2017, 81: 442–451.
50. Atladottir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, et al. Association of family history of autoimmune diseases and autism spectrum disorders. Pediatrics 2009, 124: 687–694.
51. Suzuki K, Matsuzaki H, Iwata K, Yamanoe Y, Shimmura C, Kawai S. Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. Biopolymers 2017, 124: 687–694.
52. Verma ND, Hall BM, Plain KM, Robinson CM, Boyd R, Tran GT, et al. Interleukin-12 (IL-12p70) Promotes Induction of Highly Potent Th1-Like CD4(+)CD25(-) T Regulatory Cells That Inhibit Allograft Rejection in Unmodified Recipients. Front Immunol 2014, 4: 190.
53. Ricci S, Businaro R, Ippoliti F, Lo Vasco VR, Massoni F, Onofri E, et al. Altered cytokine and BDNF levels in autism spectrum disorder. Neurotox Res 2013, 24: 491–501.
54. Chez MW, Dowling T, Patel PB, Khanna P, Kominsky M. Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. Pediatr Neurol 2007, 36: 361–365.
55. Scheller J, Chalarias A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011, 1813: 878–888.
56. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 2005, 57: 67–81.
57. Li X, Chauhn A, Shiekh AM, Patil S, Chauhn V, Li X-M, et al. Elevated immune response in the brain of autistic patients. J Neuroimmunol 2009, 207: 111–116.
58. Wei H, Zou H, Sheikh AM, Malik M, Dobkin C, Brown WT, et al. IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. J Neuroinflammation 2011, 8: 52.
59. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 2011a, 25: 40–45.
60. Wei H, Chadman KK, McCloskey DP, Sheikh AM, Malik M, Brown WT, et al. Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim Biophys Acta 2012, 1822: 831–842.
61. Miano S, Giannotti F, Cortesi F. Sleep disorders and autism spectrum disorder. In: Mazzone L, Vitiello B (Eds.). Psychiatric symptoms and comorbidities in autism spectrum disorder. Springer International Publishing, 2016: 111–128.
62. Rohleder N, Aringer M, Boentert M. Role of interleukin-6 in stress, sleep, and fatigue. Ann N Y Acad Sci 2012, 1261: 88–96.
63. McDougle CJ, Landino SM, Vahabzadeh A, O’Rourke J, Zurcher NR, Finger BC, et al. Toward an immune-mediated subtype of autism spectrum disorder. Brain Res 2015, 1617: 72–92.
64. Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, et al. Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. J Neuroimmunol 2008, 204: 149–153.
65. El Gohary TM, El Aziz NA, Darweesh M, Sadad ES. Plasma level of transforming growth factor beta 1 in children with autism spectrum disorder. Egyptian Journal of Ear, Nose, Throat and Allied Sciences 2015, 16: 69–73.
66. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. J Neuroimmunol 2011b, 232: 196–199.
67. Biber K, Vinet J, Boddeke HWGM. Neuron-microglia signaling: Chemokines as versatile messengers. J Neuroimmunol 2008, 198: 69–74.
68. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. Neurotoxicol Teratol 2013, 36: 67–81.
69. Courchesne E. Brain development in autism: Early overgrowth followed by premature arrest of growth. Ment Retard Dev Disabil Res Rev 2004, 10: 106–111.
70. Bickel M. The role of interleukin-8 in inflammation and mechanisms of regulation. J Periodontol 1993, 64: 456–460.
71. Qazi BS, Tang K, Qazi A. Recent advances in underlying pathologies provide insight into interleukin-8 expression-mediated inflammation and angiogenesis. Int J Inflam 2011, 2011: 908468.
72. Ashwood P, Corbett BA, Kantor A, Schulman H, Van de Water J, Amaral DG. In search of cellular immunophenotypes in the blood of children with autism. PLoS One 2011c, 6: e19299.
73. Enstrom A, Krakowiak P, Onore C, Pessah IN, Hertz-Picciotto I, Hansen RL, et al. Increased IgG4 levels in children with autism disorder. Brain Behav Immun 2009, 23: 389–395.
74. Heuer L, Ashwood P, Schauer J, Goines P, Krakowiak P, Hertz-Picciotto I, et al. Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. Autism Res 2008, 1: 275–283.
75. Biomarkers Definitions Working Group. Biomarkers and surrogates: Preferred definitions and conceptual framework. Clin Pharmacol Ther 2001, 69: 89–95.
76. Banati R, Hickie IB. Therapeutic signposts: using biomarkers to guide better treatment of schizophrenia and other psychotic disorders. Med J Aust 2009, 190: S26.
80. Ruggeri B, Sarkans U, Schumann G, Persico A. Biomarkers in autism spectrum disorder: the old and the new. Psychopharmacology (Berl.) 2014, 231: 1201–1216.

81. Akhondzadeh S, Fallah J, Mohammadi MR, Imani R, Mohammadi M, Salehi B, et al. Double-blind placebo-controlled trial of pentoxifylline added to risperidone: effects on aberrant behavior in children with autism. Prog. Neuropsychopharmacol. Biol Psychiatry 2010, 34: 32–36.

82. Ghaleiha A, Rasa SM, Nikoo M, Farokhnia M, Mohammadi M-R, Akhondzadeh S. A pilot double-blind placebo-controlled trial of pioglitazone as adjunctive treatment to risperidone: Effects on aberrant behavior in children with autism. Psychiatry Res 2015, 229: 181–187.

83. Kobayashi T, Notoya K, Naito T, Unno S, Nakamura A, Martel-Pelletier J, et al. Pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, reduces the progression of experimental osteoarthritis in guinea pigs. Arthritis Rheum 2005, 52: 479–487.

84. Mandrekar-Colucci S, Karlo JC, Landreth GE. Mechanisms underlying the rapid peroxisome proliferator-activated receptor-$
\gamma$-mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease. J Neurosci 2012, 32: 10117–10128.

85. Asadabadi M, Mohammadi MR, Ghanizadeh A, Modabbernia A, Ashrafi M, Hassanzadeh E, et al. Celecoxib as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. Psychopharmacology (Berl.) 2013, 225: 51–59.

86. Choi JE, Widjaja F, Careaga M, Bent S, Ashwood P, Hendren RL. Change in plasma cytokine levels during risperidone treatment in children with autism. J Child Adolesc Psychopharmacol 2014, 24: 586–589.

87. Hayashi-Takagi A, Vawter MP, Iwamoto K. Peripheral biomarkers revisited: integrative profiling of peripheral samples for psychiatric research. Biol Psychiatry 2014, 75: 920–928.

88. Insel TR. The NIMH research domain criteria (RDoC) project: precision medicine for psychiatry. Am J Psychiatry 2014, 171: 395–397.

89. National Academy of Sciences. The national academies collection: reports funded by national institutes of health. Washington (DC): National Academies Press (US), 2011.

90. Kitano H. Biological robustness. Nat Rev Genet 2004, 5: 826–837.

91. Derecki N, Privman E, Kipnis J. Rett syndrome and other autism spectrum disorders—brain diseases of immune malfunction? Mol Psychiatry 2010, 15: 355–363.