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Review

Mast cell activation and autism ☆

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ABSTRACT

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by varying degrees of dysfunctional communication and social interactions, repetitive and stereotypic behaviors, as well as learning and sensory deficits. Despite the impressive rise in the prevalence of autism during the last two decades, there are few if any clues for its pathogenesis, early detection or treatment. Increasing evidence indicates high brain expression of pro-inflammatory cytokines and the presence of circulating antibodies against brain proteins. A number of papers, mostly based on parental reporting on their children's health problems, suggest that ASD children may present with "allergic-like" problems in the absence of elevated serum IgE and chronic urticaria. These findings suggest non-allergic mast cell activation, probably in response to environmental and stress triggers that could contribute to inflammation. *In utero* inflammation can lead to preterm labor and has itself been strongly associated with adverse neurodevelopmental outcomes. Premature babies have about four times higher risk of developing ASD and are also more vulnerable to infections, while delayed development of their gut–blood–brain barriers makes exposure to potential neurotoxins likely. Perinatal mast cell activation by infectious, stress-related, environmental or allergic triggers can lead to release of pro-inflammatory and neurotoxic molecules, thus contributing to brain inflammation and ASD pathogenesis, at least in a subgroup of ASD patients. This article is part of a Special Issue entitled: Mast cells in inflammation.

1. Prevalence and characteristics of autism spectrum disorders

Autism spectrum disorders (ASD) are pervasive developmental disorders that include autistic disorder, Asperger's disorder and pervasive developmental disorder-not otherwise specified (PDD-NOS) [1]. They are characterized by stereotypic behaviors, variable deficits in language and social skills and a wide range of other behavioral problems. ASD manifest during childhood and at least 30% present with sudden clinical regression of development around 3 years of age [2,3]. Over the last 20 years, there has been an impressive rise in ASD with current prevalence estimates being about 1/100 children [4,5].

In the majority of cases, the cause of ASD is unknown [6], although some possible autism susceptibility genes have been identified [7] and

Abbreviations: ASD, autism spectrum disorders; BDNF, brain-derived neurotrophic factor; BBB, blood–brain barrier; CGRP, calcitonin-gene related peptide; CRH, corticotropin-releasing hormone; CSF, cerebrospinal fluid; FcεRI, high affinity IgE receptor; GI, gastrointestinal; IFN, interferon; LPS, lipopolysaccharide; M-CHAT, Modified Checklist for Autism in Toddlers; MCP-1, chemoattractant protein-1; MIF, macrophage inhibitory factor; NGF, nerve growth factor; NK cells, natural killer cells; NT, neurotensin; PCB, polychlorinated biphenyl; PDD-NOS, pervasive developmental disorder-not otherwise specified; SP, substance P; TGF-β1, transforming growth factor-beta1; TLR, toll-like receptor; TNF, tumor necrosis factor; UP, urticaria pigmentosa; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide

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gene interactions with environmental factors have been suspected [8]. Recent reviews have focused mostly on genomic screens that suggest there are multiple gene interactions in autism; however, no gene abnormality alone can explain the apparent increase in ASD prevalence. Increasing evidence suggests that there are different ASD endophenotypes, even within the ASD spectrum [9].

2. Immune dysregulation

The concept of some immune abnormality in ASD has been debated since the 1990s, when a study reported reduced numbers of CD4+ CD45RA+ lymphocytes (subpopulation responsible for induction of suppressor T cells or regulatory T cells) in autistic subjects ($n=36$) compared to healthy age-matched controls ($n=35$), indicating a functional deficit in the innate immune response [10]. Measurement of natural killer (NK) cell activity in blood samples of autistic children ($n=1027$) revealed that 45% of the subjects exhibited low NK cell activity compared to the controls ($n=113$). The correlation of this finding with low intracellular glutathione, IL-2 and IL-15 levels may indicate the underlying cause for NK cell dysfunction in a subset of autistic children [11]. Gene expression of perforin, granzyme B and interferon-γ (IFNγ) in peripheral blood NK cells of ASD patients ($n=52$) was decreased compared to the control group ($n=27$) under similar stimulation conditions, indicating depressed cytotoxicity [12].

In contrast to possibly depressed cell-mediated immunity, the role of pro-inflammatory molecules appears to be increased in autism. Peripheral blood mononuclear cells from ASD patients ($n=71$) secreted more tumor necrosis factor (TNF) in response to lipopolysaccharide (LPS) as compared to controls ($n=40$) [13]. Plasma levels of IL-12 and IFNγ were increased in autistic individuals [14] and IFNγ plasma levels were later found to be positively correlated with the generation of nitric oxide in autism [15]. IL-6 expression was elevated in the brains of deceased ASD patients [16]; it was detected at low levels in the cerebrospinal fluid (CSF) in subjects with autism ($n=35$) as compared to control subjects with other neurologic disorders, but only TNF receptor II was significantly elevated in the serum [17]. TNF levels were elevated in CSF of patients with ASD but were not elevated in the serum [18]. Elsewhere, there was significant increase in the serum concentration of IFNγ, and a trend towards increased production of IL-6 and TNF in *whole blood* of autistic children [19]. Macrophage inhibitory factor (MIF), a molecule shown to enhance immunity through different mechanisms, was higher in the plasma of probands with ASD than their unaffected siblings and correlated with severity of ASD symptoms [20].

We recently showed that levels of the peptide neurotensin (NT), which is present in both the brain and gut, were elevated in the serum of young autistic patients [21]. NT can stimulate lymphocyte proliferation [22], activate T cells [23], enhance IL-1 production from macrophages [24], and trigger mast cell activation [25]. Unlike NT, substance P (SP) was not elevated as also previously reported [26,27]; β-endorphin was also not elevated, even though it had been reported to be increased in the CSF of a small group of children ($n=9$) with infantile autism [28]. We also recently showed that NT can stimulate mast cells to release mitochondrial DNA extracellularly and that such DNA was significantly elevated in the serum of autistic children [29].

With respect to other neuropeptides, archived neonatal blood was analyzed with immunoaffinity chromatography, and serum levels of vasoactive intestinal peptide (VIP) and calcitonin-gene related peptide (CGRP) were reported to be higher in children with ASD ($n=69$) and those with mental retardation without ASD ($n=60$); in contrast, levels of substance P (SP) and nerve growth factor (NGF) were similar to those of controls [26]. Nevertheless, the same authors using Luminex immunoaffinity arrays later showed no difference in any of these peptides between autistic subjects and controls [27].

There may be a persistently inappropriate immune response of autistic subjects to antigenic stimuli, also observed in their unaffected siblings, suggesting a particular genetic background influenced by environmental triggers [30]. A number of papers have reviewed family or personal history of immune disorders in many children with ASD [31,32], prompting the suggestion that ASD may have a "neuroimmune" component [31–33].

3. "Allergic symptoms" in children with ASD

Many ASD children suffer from "allergic-like" symptoms [34], although their exact prevalence remains unknown compared to the general population. Many of the "allergic-like" symptoms reported by ASD children could be consistent with chronic idiopathic or chronic autoimmune urticaria [35]. A case-control study, nested within a cohort of infants born in California between 1995 and 1999, examined the association of "immune-related conditions" with ASD using health records and reported that prevalence of maternal psoriasis, asthma, hay fever and atopic dermatitis during the second trimester of pregnancy correlated with >2-fold elevated risk of ASD in their children [36]. Increased allergic problems (i.e., atopic dermatitis, asthma and rhinitis, as well as high serum IgE, number of eosinophils and positive skin tests) were present in 70% of Asperger patients ($n=15$) compared to 7% of age-matched healthy controls ($n=15$) [37]. In a National Survey of Children's Health, parents of autistic children ($n=483$) reported more symptoms of allergies (also anxiety/depression), with food allergies being the most prevalent complaint, than those of healthy control children ($n=84{,}789$) [38]. Nevertheless, there are limitations relevant to the subjective nature of parents' perception about allergies, since these were not confirmed by a clinician. A link between allergies and autism is also suggested by a recent preliminary study of children with ASD ($n=245$), which indicated that the strongest association of autism was with a history of allergies [39].

There is also evidence of non-IgE-mediated "allergic symptoms." In a hospital-based case-control study, based on questionnaires completed by the parents and scored blindly by an allergist, 30% of autistic children ($n=30$) had a family history of allergic features compared to 2.5% of age-matched "neurologic controls" ($n=39$) ($p<0.005$); however, there was no difference in serum IgE or skin prick tests to 12 common antigens between autistic subjects and controls [40], suggesting non-allergic mast cell activation. There was also no difference in IgG, IgA or IgM levels [40]. One study reported elevated IgG4 levels in children with autistic disorder ($n=114$) compared to normally developing children ($n=96$) [41]. However, the significance of this finding is not apparent because high levels of IgG4 antibodies to foods during infancy are associated with tolerance later in life [42], while many ASD children are in fact intolerant to foods. Moreover, testing for IgG4 against foods is not recommended for diagnosis of food hypersensitivity. Another study investigated the prevalence of atopic and non-IgE-mediated disorders in ASD children (a) with frequent infections and behavioral problems ($n=26$) and (b) without frequent infections ($n=107$), compared to non-ASD controls ($n=43$). Even though the prevalence of atopic disorders in ASD subjects was similar to that of the controls, non-IgE-mediated food allergy was observed at a significantly higher rate in both ASD subgroups compared to controls [43].

One representative case is that of a 12-year-old Caucasian male with a history of gastrointestinal (GI) complaints, diarrhea and frequent rashes at various parts of the body since birth (Fig. 1), often precipitated by certain foods. Exhaustive clinical testing including immune function, autoimmune indices, serum IgE, tryptase, number of eosinophils, tissue transglutaminase and gliadin antibodies, viral antibody titers were negative. This child was developing normally until 2.5 years of age, at which point he exhibited developmental delay and was diagnosed with regressive autism. At about 8 years old,