Expert Opinion

Autism: an emerging 'neuroimmune disorder' in search of therapy

Theoharis C Theoharides[†], Duraisamy Kempuraj & Lyn Redwood
[†] *Tufts University School of Medicine, Department of Pharmacology and Experimental Therapeutics, Boston, Massachusetts, USA*

Background: Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by difficulties in communication and by repetitive and stereotypic behaviors, as well as by social impairment, attention, cognitive, and learning defects. ASDs present in early childhood and their prevalence has increased significantly to 1/150 children. Despite a number of theories, the actual reasons for this increase are still not clear. There is no reliable screening test, and no definite pathogenesis or curative therapy. Consequently, there is a major gap hampering development of effective treatments. *Objective*: To review recent publications on ASDs pathogenesis and treatment with emphasis on neuroimmune processes and new therapeutic approaches. *Methods*: Mostly original papers (450) on epidemiology, possible pathogenesis or treatment of ASDs in Medline from 1990 to May 2009 were reviewed. All authors contributed to this review. *Results/conclusion*: Increased oxidative stress and immune dysregulation are present in ASDs. Mast-cell activation may contribute to gut–blood–brain barrier disruption and brain inflammation. No effective treatments have emerged. Well-designed clinical trials with nonpsychotropic drugs were few and ASD characteristics varied considerably, making conclusions difficult. Psychotropic drugs are often used for stereotypic and aggressive behaviors. Unique combinations with antioxidant and anti-inflammatory flavonoids hold promise. New potential translational research areas and possible treatments are suggested.

Keywords: autism, blood–brain barrier, cytokines, flavonoids, inflammation, intestine, mast cells, mastocytosis, oxidative stress, psychotropic agents, treatment

Expert Opin. Pharmacother. (2009) ***10****(13):2127-2143*

1. Introduction

Autistic disorder, Asperger's disorder and atypical autism, also known as pervasive developmental disorder not otherwise specified (PDD-NOS), constitute the autism spectrum disorders (ASDs). These are neurodevelopmental disorders diagnosed in early childhood, with a male to female ratio of 4 : 1 [1,2]. ASDs are characterized by varying degrees of dysfunctional communication and social abilities, repetitive and stereotypic behaviors, and by defects in attention, cognition, learning and sensory functioning [1,3]. Cognitive impairment can vary in ASDs, with Asperger's disorder being high functioning whereas mental retardation may be present in as much as 75% of the time in autistic disorder. A period of normal development may be suddenly followed by loss of acquired skills and delay in acquisition of new ones [4].

Prevalence estimates indicate that ASDs have increased considerably from 4/10,000 before 1980 to as many as 90/10,000 [2-8]. A recent review of 43 studies published since 1966 concluded that a best estimate is 60 – 70/100,000 or

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1/150 [9]; however, the same study could not point to a true increase in incidence. A recent paper evaluated ASD in California and concluded that there had been a 7- to 8-fold increase in cases since 1990. Changes in diagnostic criteria, inclusion of milder cases and earlier age at diagnosis could not account for more than a 2.2-fold increase at best, leaving the majority of ASD cases unexplained [10]. Environmental factors might also be involved, and the prevalence of ASDs in New Jersey has been found to be double that in West Virginia [2]. The role of mercury in vaccines has been debated extensively; whereas many epidemiological studies do not support a causal association between vaccines and autism [11-15], others provide evidence to the contrary [16,17]. For instance, 87% of children included in the US Vaccine Adverse Event Reporting System (VAERS) had ASDs [18]. Moreover, a paper based on computerized medical records in the Vaccine Safety Datalink concluded that there was 'significantly increased rate ratios for ASDs with mercury exposure from thimerosal-containing vaccines' [19]. Population-based studies cannot determine a 'causal relationship' if they involve subpopulations of patients or vulnerable individuals.

Recent reviews that have summarized the current findings in autism research focus mostly on neural processes [20,21] or genetics [22-26]. Rett and fragile X syndromes, as well as tuberous sclerosis, are associated with ASDs, but explain only a small percentage of cases. There is no biochemical or genetic screening test. One recent study suggested the use of novel probes that identified a partial duplication in the *ASMT* gene, observed in 6% of children with ASDs as compared to 2% of controls, to screen children suspected of ASDs [27]. Recent studies indicate genome-wide abnormalities. Association studies have identified single nucleotide polymorphisms in genes encoding neuronal cell adhesion molecules and calcium channels. However, no gene abnormality alone can explain the apparent increase in prevalence over the last 20 years [28-30].

1.1 Oxidative stress

Considerable evidence indicates that oxidative stress might be unregulated in patients with ASDs, possibly due to decreased ability to neutralize free radicals (**Table 1**). One study of autistic children (n = 305) and controls (n = 205) reported that plasma *S*-adenosylhomocysteine, which was used as an indicator of methylation ability, was significantly lower in autistic children [31]. Another study found reduced plasma levels of the key endogenous antioxidant *S*-adenosylmethionine (SAMe) [32].

Such deficiencies might, in certain cases, be associated with mercury toxicity, which was recently shown to be tightly bound to, and inactivate, thioredoxin [33]. In fact, the glutathione redox index was found to be decreased and oxidized glutathione to be increased both in the cytosol and the mitochondria of lymphoblastoid cell lines obtained from patients with autism disorder as compared to controls [34]. Moreover, exposure to thimerosal further exacerbated these imbalances [34]. Two other studies support a possible association between mercury exposure and increased oxidation stress. In one study, patients with ASDs (n = 38) were shown to have decreased plasma levels of reduced glutathione, cysteine, and sulfate, but increased plasma levels of oxidized glutathione as compared to age-matched neurotypical controls ($p < 0.001$) [35]. In another study, patients with severe ASDs (n = 28) had significantly increased urinary porphyrins associated with mercury toxicity (pentacarboxyporphyrin, precoproporphyrin, and coproporphyrin) as compared to patients with mild ASD using the Childhood Autism Rating Scale [36]. Decreased plasma levels of reduced glutathione, cysteine, and sulfate were again noted, suggesting increased oxidation stress and reduced detoxification ability, especially of mercury. Finally, cerebellar homogenate levels of the oxidative stress marker 3-nitrotyrosine were elevated by about 70% in autistic subjects (n = 9, mean age 12 years), as was cerebellar mercury, as compared to control cerebellar tissue (n = 10, mean age 15 years) [37].

Erythrocyte superoxide dismutase (SOD) and the endogenous antioxidants plasma glutathione peroxidase and erythrocyte glutathione peroxidase (GSH-Px) were also significantly reduced in autistic children (n = 45) compared to controls (n = 41) [38]. The parents of autistic children also had decreased serum methylation capacity and reduced glutathione-dependent antioxidant ability [39]. Serum levels of the antioxidant proteins transferrin and ceruloplasmin were also significantly reduced in children with ASDs compared to their nonautistic siblings [40]. Moreover, there was a strong correlation between the reduced levels of these proteins and loss of previously acquired language skills in autistic children [40]. Another study showed increased urine levels of isoprostane F(2α)-VI, 2,3-dinor-thromboxane B2 and 6-keto-prostaglandin F1α, indicating increased levels of lipid peroxidation [41].

Two recent studies have reported mitochondrial dysfunction. One showed evidence of increased mitochondrial metabolism and oxidized mitochondrial proteins in temporocortical gray matter in postmortem samples from six autistic patients as compared to six matched controls [23]. The other reported that 11/21 patients with a primary diagnosis of ASD had definite mitochondrial disease, and the rest probable mitochondrial disease, depending on the criteria [42]. This evidence implies that autistic patients might have excessive reactive oxygen species (ROS) production and reduced methylation capacity [43].

1.2 Immune dysregulation in autistic spectrum disorders

Increasing evidence suggests that there are some generalized immune abnormalities in at least a subgroup of patients with ASDs (**Table 2**) [44-47]. Children with autism (n = 35) were reported to have 11 genes, associated with natural killer (NK) cells, that were upregulated 1.5-fold as compared to children (n = 14) who did not meet the criteria for autism

Table 1. Increased oxidative stress in autistic spectrum disorders.

Increased oxidative stress	Ref.
Decreased plasma and erythrocyte glutathione peroxidase, and superoxide dismutase	[38]
Decreased cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cell lines	[34]
Decreased plasma *S*-adrenosyhomocysteine and *S*-adenosylmethionine	[31,32]
Decreased serum antioxidant transferrin and ceruloplasmin	[40]
Increased urine phospholipid peroxidation products	[41]
Increased temporocortical gray matter mitochondrial metabolism, oxidized mitochondrial proteins and excessive Ca^{2+}	[23]
Evidence of mitochondrial disease	[42]

Table 2. Neuroimmune findings in autistic spectrum disorders.

Neuroimmune findings	Ref.
Reactive astrogliosis and activated microglia	[44,57]
Circulating antibodies to brain proteins	[69,70]
High CSF TNF-α	[50]
Increased brain IL-6, IL-8, and TNF	[52]
High CSF and brain levels of MCP-1 and TNF; MCP-1 is chemotactic for mast cells, which release TNF-α and IL-6	[59]
High TNF production from peripheral leukocytes	[87]
Reduced serum TGF-β1, which is inhibitory for mast cell maturation and activation	
Increased serum macrophage MIF, also released from mast cells	[56]
Increased circulating IL-6 levels that cross the placenta at midgestation and induce the release of fetal stress hormones resulting in fetal brain injury and autistic-like behavior after maternal mouse viral infection	[62]
Increased IL-6 in the placenta and brain leading to autistic-like behavior in mice after maternal viral poly(I : C) injection	[63]

CSF: Cerebrospinal fluid; IL: Interleukin; MCP-1: Macrophage chemoattractant protein-1; MIF: Migration inhibitory factor; TGF-β1: Transforming growth factor-beta 1; TNF: Tumor necrosis factor.

($p < 0.02$) [48]. NK-cell-related molecules such as perforin, granzyme B, and interferon gamma (INF-γ), were significantly upregulated in 52 children with ASDs ($p < 0.01$) as compared to 27 controls [49]. Cerebrospinal fluid (CSF) levels of tumor necrosis factor (TNF) were significantly higher than corresponding serum levels in ten autistic children [50]. Moreover, TNF-receptor II was elevated in 35 children with ASDs as compared to controls ($p < 0.02$) [51]. Expression of TNF, along with interleukin-6 (IL-6) and IL-8, was recently shown to be increased in the brains of ASD patients as compared to matched controls [52]. TNF and other cytokines can affect cognitive functions in humans [53]. Inhibition of macrophage migration in response to human myelin basic protein was higher in 17 autistic patients than in 13 patients with other mental conditions [54]. Preliminary evidence showed that peripheral blood mononuclear cells from ASD children ($n = 19$) secreted more IL-1β and IL-8 than age-matched controls when stimulated with lipopolysaccharide (LPS) following exposure to the common environmental contaminant 2,2′,4,4′-tetrabrominated biphenyl (BDE-47) [55]. Plasma levels of macrophage migration inhibitory factor (MIF) were higher in family probands with ASDs than in their unaffected siblings, and correlated with ASD symptoms [56]. Postmortem brains, microglia, and the CSF of patients with ASDs contained high levels of macrophage chemoattractant protein-1 (MCP-1) [57], which is also a potent chemoattractant for mast cells [58]. Conversely, plasma-transforming growth factor-beta (TGF-β1) was significantly lower in 75 children with ASDs than in 68 controls [59]. TGF-β1 inhibits human intestinal mast-cell functions [60] and downregulates the expression of high-affinity IgE receptor (FcεRI) on mast cells [61].

Maternal viral infection in mice has been found to result in substantial increases in circulating IL-6 levels, which cross the placenta at midgestation and induce the release of fetal stress hormones resulting in fetal brain injury [62]. Viral poly (I : C) injection in mice to mimic viral infection also raised IL-6 in the placenta and brain, leading to autistic-like behavior that could also be induced by a single IL-6 injection [63]. It is intriguing that acute stress can increase serum IL-6 levels, but not in mast-cell-deficient mice [64]. IL-6 can be involved in disruption of the blood–brain barrier (BBB) [65,66].

1.3 Disruption of the blood–brain barrier in autistic spectrum disorders

A number of studies suggest that the BBB is breached in ASDs. Serum from autistic children contained a number of autoantibodies against encephalogenic peptides that cross-react with milk butyrophilin [67]. Another study showed that plasma from 21% of patients with ASDs had antibodies against one cerebellar protein ($p = 0.001$) [68]. Moreover, more autistic children than their unaffected control siblings ($p < 0.01$) had serum autoantibodies to human brain, especially the cerebellum and cingulate gyrus [69]. In another study, plasma from 37% of children with autism ($n = 62$) contained antibodies with reactivity against three hypothalamic proteins, as compared to 13% in normally developing controls ($n = 63$, $p = 0.004$) [70]. Autoantibodies against brain proteins also appear to be present in mothers of ASD children. Serum from 100 mothers with ASD children contained significantly more antibodies that recognized embryonic brain tissue [71]. Specific patterns of antibody reactivity to

adult rat brain proteins were present in mothers of ASD children for 2 – 18 years after the birth of the affected children [72]. In one study of 61 mothers with ASD children, 11.5% had antibodies against fetal, but not adult, brain, as compared to 62 mothers of normal children ($p = 0.0061$) and to 40 mothers with children identified as having non-ASD developmental delays ($p = 0.04$) [73]. It is not known when or how the BBB becomes disrupted in patients with ASDs.

1.4 Allergic symptomatology in autistic spectrum disorders

A number of papers indicate that many children with ASDs have allergic symptoms, often in the absence of positive skin testing (**Table 3**), and may comprise a subgroup of patients with ASD. In a National Survey of Children's Health, parents of autistic children reported symptoms of allergies more often than those of other children, with food allergies being the most prevalent complaint [5]. A recent study of 362 ASD patients in Italy reported that the strongest association was with history of allergies [74]. A case series study also reported higher rate of food intolerance in ASD children [75]. In another study, there were significantly higher levels of IgA antibodies to casein, lactalbumin, and beta lactoglobulin in autistic children (n = 36) than controls (n = 20), with marked improvement in behavioral symptoms after an 8-week elimination of the antigen involved [76]. Food intolerance may affect as many as 16% of children in general [77] and up to 34% of 3-year-old children, leading to significant behavioral responses [78]. Unfortunately, the terms 'food allergy' [79] and 'food intolerance' [80] are often used interchangeably, even though the latter is not IgE mediated. Mast cells are involved in food-related and other gastrointestinal (GI) pathology [81], such as in inflammatory bowel disease [82], and irritable bowel syndrome [82,83], in which they can increase intestinal permeability, which might also explain other GI complaints in ASD patients [6,84]. A paper reporting on mitochondrial dysfunction in ASDs patients also concluded that GI complaints was the most common non-neurogenic dysfunction in 64% of the ASD subjects investigated [42]. Another recent paper also reported an apparent association between ASD children with language regression and GI symptoms [85], whereas another reported that from 172 patients with pervasive developmental disorder (PPD), those 22.7% with GI problems also had greater symptom severity, especially irritability and anxiety [86]. Peripheral blood mononuclear cells from ASD children with GI symptoms produced higher TNF-α in response to gluten gliadin, cow-milk protein, and soy [87]. However, 'autistic enterocolitis' has been disputed, and a nested case-control study found no association between autism and any GI disease [88].

A paper reporting terminal ileal lymph nodular hyperplasia (LNH) in 129/144 (90%) of ASD children as compared to 8/27 (30%) in controls ($p = 0.0003$) [89] has been disputed. Yet a separate study independently reported LNH to be present in 60 – 70% of autistic patients, with ileocolitis present in 76% [90]. Nevertheless, it is difficult to document a 'leaky gut' [91] that could permit the systemic absorption of substances adversely affecting brain function. It would be interesting to investigate levels of heparan sulfate, a reduction of which was associated with increased intestinal permeability [92,93], especially as ASDs patients appear to have low sulfation capacity [94] and might not produce sufficient quantities of sulfated proteoglycans.

In a small study of ASD children (n = 6), there was at least one manifestation of allergy in all patients and more than two symptoms in 50%; however, the serum or IgE was not elevated, suggesting non-IgE-dependent mechanisms [95]. In another study, 30% of autistic children (n = 30) had a family history of atopy as compared to 2.5% age-matched 'neurologic controls' (n = 30), but there was no difference in serum IgE or in skin-prick tests to 12 common antigens [96], again implicating triggers other than IgE. Autistic patients had higher urine levels of prostaglandins [41], which are mainly produced by mast cells. Collectively, these results suggest that nonallergic mast-cell activation might be involved in at least a subgroup of patients with ASDs. It was recently shown that serum levels of IgG4, associated with atopic phenotype, were increased in children with ASDs [49].

Many of the symptoms that characterize ASD patients are also present in patients with mastocytosis, a spectrum of rare disorders involving proliferation and activation of mast cells in the skin and other organs [97-99]. Such symptoms include: allergies, behavioral problems, 'brain fog', diarrhea, food intolerance, and skin reactions, often in the absence of positive skin testing (**Box 1**).

The Mastocytosis Society [100] has produced a video together with the American Academy of Allergy, Asthma and Immunology, entitled 'Mast-cell activation symptomatology'. This is being distributed free to physicians to sensitize them to the fact that allergies may be only one aspect of mast-cell activation. A preliminary report indicated that the prevalence of ASDs might be 10-fold higher in mastocytosis patients than in the general population (in which the prevalence is 1/150) [101]. Moreover, serum IL-6 is elevated in most mastocytosis patients [102] and might be relevant to findings discussed earlier linking elevated IL-6 to autistic phenotype in mice.

These results do not indicate a cause-and-effect relationship, but do suggest an increased risk associated with mast cell activation. Such a risk could extent to the period of gestation. A nested case control study of ASD children (n = 407) and controls (n = 2095) showed that there was a > 2-fold risk of ASDs in mothers who had a diagnosis of allergies or asthma during the second trimester of pregnancy [103]. In one study of families of autistic patients (n = 61) and healthy controls (n = 61), almost 50% of autistic patients had two or three family members with autoimmune diseases as compared to 26% of controls; however, there was no increased incidence of allergies [104].

Mast cells are crucial for allergic reactions [105] but are also important in both innate and acquired immunity [106,107], as

Table 3. Allergic symptoms and autistic spectrum disorders.

Evidence	Ref.
In a nested control study, there was over 2-fold higher risk of children with ASDs (n = 407) born to mothers with diagnosis of allergies or asthma during the second trimester of pregnancy as compared to controls (n = 2095)	[103]
ASD children had a significant family history of atopy (30%) as compared to controls (2.5%)	[104]
ASD children had at least one manifestation of allergy and 50% of ASD children may have two or more symptoms	[95]
In a National Survey of Children's Health, parents of autistic children reported symptoms of allergies more often than other children, with food allergies showing the greatest difference	[5]
Non-IgE-mediated food intolerance was significantly more common in ASD children (n = 133) than controls (n = 81)	[75]
A study of 362 ASD patients in Italy showed that the strongest association was with history of allergies	[74]
Serum levels of IgG4, associated with atopic phenotype, were increased in children with autism disorder	[49]
ASD and mastocytosis children share common symptoms such as atopic dermatitis, food intolerance, anxiety, 'brain fog', and neurobehavioral problems	[97]
The prevalence of ASDs in children with mastocytosis appears to be 10-fold higher than the general population	[101]

ASD: Autistic spectrum disorder; Ig: Immunoglobulin.

Box 1. Mast-cell triggers relevant to autistic spectrum disorders.

Allergens
Antibody-free light chains
Bacteria (TLR 2 and 4)
CRH
Mercury
NT
Opioids
IL-1
IL-33
ROS
SP
Toxins
VIP
Viruses (TLR 3, 5, 7, and 9)

CRH: Corticotropin-releasing hormone; IL: Interleukin; NT: Neurotensin; ROS: Reactive oxygen species; SP: Substance P; TLR: Toll-like receptors; VIP: Vasoactive intestinal peptide.

well as in inflammation [108]. Functional mast-cell–neuron interactions occur in the brain [109,110] and the GI tract [111]. In addition to allergic stimulation [112], many other substances originating in the gut or the brain can trigger mast-cell secretion (**Figure 1**) [105]. These include (**Box 1**) immunoglobulin-free light chains [113]; bacterial and viral products; and neuropeptides such as corticotropin-releasing hormone (CRH), myelin basic protein (MBP) [114], neurotensin (NT) [115], substance P (SP) [116], and vasoactive intestinal peptide (VIP) [117]. Oxidative stress can also trigger or augment mast-cell activation [118]. Our recent evidence also indicates that mercuric chloride and thimerosal (100 nM) can induce the release of vascular endothelial growth factor (VEGF) from human mast cells [119]. Thimerosal had previously been shown to induce platelet aggregation with subsequent serotonin and prostaglandin release [120], and to enhance the allergic stimulation of human basophils [121]. Such findings might be relevant to the recent report that patients with severe ASDs had evidence of significantly increased urinary porphyrins consistent with mercury intoxication [36]. Mast cells can also interact with T cells [107,122,123] and superactivate these through TNF [114]. Once activated, mast cells secrete numerous vasoactive, neurosensitizing and proinflammatory molecules that are relevant to ASDs. These include histamine, serotonin, kinins, proteases, VEGF, MCP-1, MIF, and prostaglandin D_2 (PGD_2), as well as cytokines, such as IL-6, IL-8, IL-9, IL-13, IL-17, and TNF, which are known to increase permeability of the BBB [65,66].

In view of the fact that allergy symptoms in a subgroup of ASD patients do not appear to be triggered by IgE, it is noteworthy that mast cells can be stimulated by nonallergic triggers to release some mediators 'selectively', without degranulation (**Table 4**) [124]. It was first shown that mast cells could release serotonin without histamine [125]. Activation of Toll-like receptors (TLR) by microorganisms also leads to mast-cell release of different cytokines [126]. For instance, bacterial LPS activates TLR-4 and induces selective release of TNF from rodent mast cells [127,128]. Fetal rat-skin-derived mast cells express viral TLR-3, activation of which by double-stranded RNA induces release of TNF and IL-6, as well as regulating upon activation, normal T-cell expressed and secreted (RANTES) and macrophage inflammatory protein (MIP), but without degranulation [129]. Activation of TLR-9 selectively produced IL-6 [130]. The ability of viruses to trigger mast-cell activation could contribute to the pathogenesis of ASDs, as they affect children at the critical age of 3 – 5 years [131]. It is therefore interesting that a number of rotaviruses were isolated from asymptomatic neonates [132]. Enteroviruses could also induce seizures [133], which are reported more often in children with ASDs [134], and mastocytosis [135].

IL-1 can stimulate human mast cells to selectively release IL-6 [136], whereas CRH could stimulate selective release of VEGF [137]. Release of IL-6 could have profound effects on brain function [138], whereas the release of VEGF could increase BBB permeability [139].

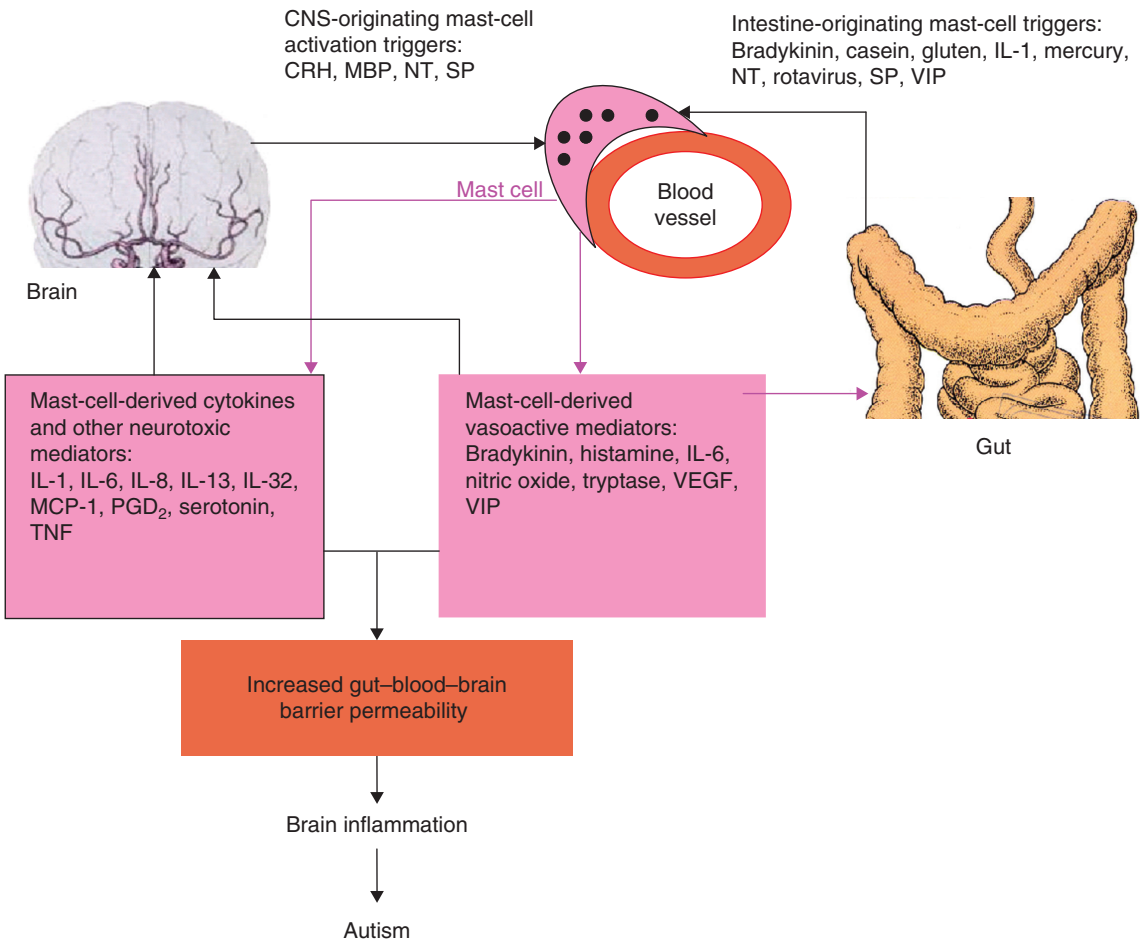

Figure 1. Schematic depiction of the proposed role of mast cells in gut–blood–brain barrier disruption, brain inflammation, and autistic spectrum disorders. Intestinal-derived mast-cell triggers such as neuropeptides, stress hormones, and toxins stimulate mast cells to release inflammatory and vasoactive molecules that disrupt the gut–blood–brain barrier, permitting entry of enterotoxic molecules in the brain. Neurogenic inflammation through the release of mast-cell mediators intensifies and propagates this response.
CNS: Central nervous system; CRH: Corticotropin-releasing hormone; IL: Interleukin; MBP: Myelin basic protein; MCP-1: Macrophage chemoattractant protein-1; NT: Neurotensin; PGD_2: Prostaglandin D2; SP: Substance P; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor; VIP: Vasoactive intestinal peptide.

Table 4. Triggers inducing selective release of ASD-relevant mast-cell mediators.

Trigger	Mediator released
CRH	VEGF
IL-1	IL-6
IL-33	IL-13
LPS	TNF
SCF	IL-6
TLR-9	IL-6

CRH: Corticotropin-releasing hormone; IL: Interleukin; LPS: Lipopolysaccharide; SCF: Stem cell factor; TLR: Toll-like receptors; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor.

1.5 Stress, the blood–brain barrier, mast cells, and brain inflammation

Anxiety is one of the most common presenting problems for at least a subgroup of children with ASDs, who may also be more prone to stress [140]. A comparison of 34 adults with autism and 20 controls – matched for age, gender and intellectual ability – showed that patients were three times as anxious as controls and were significantly less likely to cope with stressful triggers [141]. Autistic patients might also have increased activity of the hypothalamic–pituitary–adrenal (HPA), as indicated by elevated salivary cortisol response in children [142], and higher plasma levels of adrenocorticotropin hormone (ACTH) in adults with Asperger's syndrome [143].

Increased anxiety and aggression might be related to significantly increased plasma levels of testosterone and

dehydroepiandrosterone (DHEA) [144], as well as to serum levels of free testosterone (214%) and DHEA (192%) in patients with ASDs (n = 70) in comparison with controls [145]. Acute stress could activate brain mast cells [146], an effect that was abolished by pretreatment with polyclonal antiserum to CRH [146]. Subsequently, CRH was reported to activate brain mast cells and increase BBB permeability in rodents [147], particularly in brain areas containing mast cells [148]. Mast cells were hypothesized to regulate permeability of BBB almost 20 years ago [149]. It was later shown that increased BBB permeability in animal models occurred in response to the mast-cell secretogogue compound 48/80 [150]. The direct effect of CRH was documented by intradermal administration leading to increased vascular permeability in rodents [151] and humans [152], through activation of CRH receptor-1 (CRH-R1). In fact, normal human cultured mast cells express high-affinity CRH-R1, activation of which leads to selective release of VEGF, which is also vasoactive [137]. The most common manifestation of mastocytosis is urticaria pigmentosa (UP), which is characterized by small brown–red maculopapules on the skin [153]. It is interesting that lesional skin mast cells from a patient whose UP worsened dramatically after stress expressed CRH-R1 and had high serum levels of CRH [154], suggesting that mast cells might respond to local release of CRH typically secreted from the hypothalamus under stress.

Acute restraint stress in rodents also stimulated intestinal mast cells, and flattened villi, consistent with increased gut permeability [155]. Acute stress through CRH and mast-cell activation induced mucin release in the intestines [156], and CRH was involved in intestinal inflammation [157] and motility [158]. In fact, CRH increased permeability in normal human colonic biopsies through activation of subepithelial mast cells [159]. Gonadotropin-releasing hormone (GnRH) agonists may be useful because they block CRH-induced anxiety and stress-induced behaviors in mice through a sex-steroid-independent mechanism [160,161]. Neonatal maternal deprivation (NMD) increased gut paracellular permeability through CRH stimulating intestinal mast cells [162]. NMD stress in rats induced long-term alterations of colonic nerve–mast-cell interactions [163]. Moreover, stress of caregivers during 6- to 18-month-old children was significantly associated with higher serum IgE and TNF, as well as with a predisposition to atopic symptomatology in these children [164]. Aberrant, dysfunctional, or immature development of the gut–blood barrier [165] could expose local mast cells to intestinal triggers leading to release of mast-cell-derived vasoactive, inflammatory, and neurosensitizing molecules that could contribute to brain inflammation and ASD-like behavior (**Figure 1**).

2. Treatment

Unfortunately, there are no approved effective treatments for autism [166,167]. Most behavioral [168] or pharmacological [169,170] treatments do not address the core symptoms of ASDs. One paper reviewed all randomized, controlled, clinical trials of pharmacological interventions and concluded that no treatment met the criteria for even 'probably efficacious' [167]. A recent paper reviewed all studies conducted in children with ASDs and concluded that there was minimal success in treating the core deficits; only risperidone has been approved for use in children [171]. It has been estimated that psychotropic drug treatments for outpatient children and adolescents with ASDs range from 25 to 55% (**Table 5**) [172-174].

A recent paper reported that the use of psychotropic prescriptions for children with ASDs increased from 39% in the period 1996 – 2000 to 79% for the period 2001 – 2005 [174]. The mean number of psychotropic drug classes prescribed was 2.4, raising concerns of long-term safety [174]. The efficacy of antidepressants varies significantly in ASDs [175,176]. Novel targets and medications are needed for effective treatment of the core symptoms of ASDs.

2.1 Antipsychotics

Almost 20% of ASD children are prescribed some antipsychotic drug [171,174,177]. These drugs [178], especially the newer atypical compounds [179,180], are used often in ASDs, mostly to control irritability and stereotypic – sometimes self-injurious – behavior [178-182]. Such drugs include haloperidol, risperidone, and olanzapine (**Table 5**). A recent review discussed 21 randomized, placebo-controlled trials using psychopharmacologic agents; of the various drugs used, only methylphenidate [183] for aggressive behavior, and risperidone for hyperactivity [184,185] produced significant improvement in more than one study [182]. Haloperidol is limited by tardive dyskinesia, and risperidone by considerable weight gain [177]. Long-term safety with all of these drugs remains unknown. Interestingly, some of the older antipsychotics used in ASDs [177,181] also have antiserotonergic and mast-cell-blocking effects [186].

2.2 Serotonin receptor antagonists

The combined histamine-1 and serotonin receptor ($5\text{-}HT_2$) antagonist, cyproheptadine, produced significant improvement over that of the antipsychotic haloperidol in a double-blind trial of 40 children with autism, who were randomized to either haloperidol and cyproheptadine vs haloperidol and placebo [187]. The apparent benefit of cyproheptadine may be related to the higher platelet serotonin levels reported in over 40% of patients with autism [188]. Although high platelet serotonin might not reflect availability in the brain, it could affect the neuroenteric plexus that utilizes serotonin [189].

2.3 Alternative and nutritional interventions

Patients with ASDs are commonly placed in a variety of nutritional regiments [190]. Although many studies have used omega-3 fatty acids, vitamins, zinc, and various herbal extracts, as well as avoidance of certain food substances [190,191], they lacked appropriate controls [191,192]. Moreover, most

Table 5. Psychotropic agents showing most benefit in autistic spectrum disorders.

Drug	Actions	Results	Adverse effects	Ref.
Cyproheptadine+	Serotonin and histamine-1 receptor antagonist	Symptoms ↓	Sedation, weight ↑	[187]
Haloperidol	Dopamine receptor antagonist	Repetitive behavior ↓	Tardive dyskinesia	[177]
Methylphenidate	Central nervous system stimulant	Hyperactivity ↓		[182,183]
Naltrexone	Opioid receptor	Self-injuries ↓	Sedation, transient	[207]
Olanzapine	Dopamine and serotonin receptor antagonist	Symptoms ↓	Weight ↑	[177,180]
Risperidone	Dopamine receptor antagonist	Aggression ↓ Irritability ↓	Weight ↑	[183-185]

+ with or without haloperidol; ↓ decrease; ↑ increase.

studies were open label, not controlled, and did not include sufficient number of patients.

2.4 Antioxidant and anti-inflammatory agents

A recent open-label study reported significant reversal in indexes of oxidative stress as evidenced by increased serum levels of cysteine and glutathione in 40 children with ASDs treated for 3 months with methyl B_{12} and folinic acid [193].

Some naturally occurring flavonoids, such as quercetin and the closely structurally related luteolin, have potent antioxidant and anti-inflammatory activity [194]; they also reduce platelet serotonin secretion [195]. Luteolin also inhibits IL-6 release from microglia cells [196] and the IL-1-mediated release of IL-6, IL-8, and MCP-1 from astrocytes [197]. Quercetin also inhibited and reversed acute stress-induced autistic-like behavior and the associated reduced brain glutathione levels in mice [198].

Quercetin and luteolin can also inhibit the release of histamine, leukotrienes, and PGD_2 [199], as well as IL-6, IL-8, TNF, and tryptase from human cultured mast cells [200]. Moreover, quercetin inhibits mast-cell activation stimulated by IL-1 [201], and mast-cell-dependent stimulation of activated T cells [114].

Quercetin is safe [202] but flavonoids are difficult to absorb in powder form and are extensively metabolized [194], mostly through glucuronidation (~ 50%), sulfation (~ 30%), and some methylation [203,204]. A unique dietary formulation, NeuroProtek® (**Table 6**), largely overcomes the problem of absorption by combining the natural lipophilic flavonoids (luteolin and quercetin) in olive kernel extract to increase absorption and minimize metabolism in order to get to the brain and inhibit inflammation. Chondroitin sulfate is included to correct any intestinal barrier disruption, which was recently shown to be associated with decreased levels of the structurally related heparan sulfate [92,93]. Chondroitin sulfate also inhibits GI mucosal damage due to acute stress [155] and histamine release from mast cells [205].

Disodium cromoglycate (cromolyn) is a potent inhibitor of rodent mast-cell histamine secretion, but weak inhibitor of human mast cells [105]. Nevertheless, it is often used to treat GI symptoms in mastocytosis patients [153]. The histamine-1 receptor antagonist ketotifen has been reported to also partially inhibit mast-cell activation and is often used for treating symptoms associated with mastocytosis [153]. A newer histamine-1 receptor antagonist, rupatadine, available in Europe and Latin America but not yet in the USA, also inhibits mast-cell release of inflammation mediators [206]. These drugs may be more appropriate for the subgroup of ASD patients with allergic symptoms (**Table 6**).

The opioid receptor antagonist naltrexone was used in a number of studies and had some benefit, mostly in reducing self-injurious behavior [207]. Naltrexone's beneficial effect may be partially related to blockade of mast cell activation by internal opioids [208].

3. Expert opinion

Much useful evidence has been produced over the last few years on ASDs [209], but we now need to move from informational to transformational research if we are to develop novel effective treatments. Increased effort should be directed at understanding what might contribute to the pathogenesis of autism, rather than the neurological sequellae. Mast cells and CRH are involved in the regulation of BBB pathophysiology [139] and inflammation [105]. Enteroviruses, neuropeptides, stress hormones, and toxins could contribute through mast-cell activation, especially in subpopulations that are made vulnerable by their age and genetic make-up.

Some of the most apparent problems in the studies reviewed were: the widely ranging ages, the severity and duration of ASDs, the heterogeneity of types of ASDs, lack of appropriate power, lack of objective end points, and lack of easy-to-use, validated, post-treatment follow-up instruments. Major efforts should be directed at testing novel pathogenetic hypotheses

Table 6. Suggested regimens for autistic spectrum disorder patients with allergic symptomatology*.

Drug/formulation	Actions	Suggested dose	Ref.
Disodium cromoglycate (Gastrocrom®)	'Mast cell stabilizer'	100 – 400 mg/day dissolved in water	[153]
Cyproheptadine (Periactin®)	Serotonin and histamine-1 receptor antagonist	1 – 4 mg/day	[187]
Ketotifen (Zaditen®)	Histamine-1 receptor antagonist, anti-eosinophil	1 – 4 mg/day	[212]
Rupatadine (Rupafin®)	Histamine-1 receptor and platelet activating factor antagonist; mast cell inhibitor, anti-eosinophil	20 mg/day	[206]
NeuroProtek®	Contains flavonoids and a proteoglycan	Two capsules/20 kg body weight/day	[155,196,197,199,200]

*Doses will vary depending on age and weight of patient.

with defined objective end points that may subsequently be used in clinical trials.

3.1 Notes in press

In spite of the fact that antidepressants are increasingly prescribed to children with ASDs as discussed earlier (Section 2), a recent, multicenter, randomized study of 150 children and adolescents using the selective serotonin re-uptake inhibitor (SSRI) citalopram or placebo for 12 weeks, reported no benefit and significant adverse effects [214]. An accompanying editorial justifiably concluded that "perhaps this evidence will change this practice" of widely prescribing SSRIs to children with ASDs [215].

3.2 The future

Areas for future research could include:

- Expression – in peripheral lymphocytes from patients with ASDs – of proteins, such as moesin [210] and the mitochondrial uncoupling protein 2 (UCP2) [211] recently shown to have an inverse relationship to immune activation. Low UCP2 expression may also be related to recent evidence of mitochondrial dysfunctions in patients with ASDs [213].
- Reactivity of circulating basophils from patients with ASDs to environmental pollutants and toxins.
- Investigation of ASD patient heterogeneity based on allergic symptomatology, with the radio-allergo-sorbent test (RAST) and skin tests, as well as serum levels of mast-cell triggers and mediators.
- Blood levels of mast-cell triggers and mediators in groups of patients with ASDs (e.g., different age groups, with and without allergies).
- Blood levels of CRH, endorphins, and key neuropeptides in subgroups of ASD patients.
- Blood levels of CRH and other mast-cell triggers and mediators in premature infants with follow-up of how many will develop ASDs.
- Epidemiologic study of ASDs in patients with mastocytosis.
- Evidence of BBB disruption in ASD patients using gadolinum-enhanced magnetic resonance imaging, as already done successfully in patients with multiple sclerosis.
- Evidence of intestinal barrier disruption in ASD patients by measuring blood levels of heparan sulfate, chondroitin sulfate, and zonulin.
- Human mast-cell and basophil activation by microbial, stress and toxic triggers, with and without allergic stimulation.
- The effect of microbial, stress hormones, and toxic triggers on gut–blood–brain permeability in mice, including appropriate knockout mice.
- Double-blind clinical trial of the effect of the flavonoid-containing formulation NeuroProtek® in patients with ASDs.

Acknowledgments

Thanks to J Christian for her patience and word-processing skills. We also thank J Hogan and B Zhang for their help with some of the references, as well as J Adams for useful comments.

Declaration of interest

T Theoharides' research on ASDs discussed in this review was supported by Theta Biomedical Consulting and Development Co., Inc. (Brookline, MA). His research on relevant mast-cell pathophysiology has been supported by NIH grants NS55681, and AR47652, as well as grants from the Mastocytosis Society, as well as the National Autism Association, Safe Minds and the Defeat Austism NOW Coalition (USA). He is also a member of the Scientific Advisory Board of the Mastocytosis Society (www.tmsforacure.org). T Theoharides also has US patents no. 6,624,148; 6,689,748; 6,984,667; 7,115,278; EPO 136577 and pending applications covering methods and compositions for blocking mast-cell activation. These patents and the dietary supplement NeuroProtek® have been licensed to Algonot, LLC (Algonot.com).

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Affiliation

Theoharis C Theoharides[†1] MS MPhil PhD MD FAAAI, Duraisamy Kempuraj[2] MS MPhil PhD & Lyn Redwood[3] RN MSN
[†]Author for correspondence
[1]Director,
Molecular Immunopharmacology and Drug Discovery Laboratory,
Tufts University School of Medicine,
Tufts Medical Center,
Department of Pharmacology and Experimental Therapeutics, and Departments of Biochemistry, Internal Medicine, and Psychiatry,
Molecular Immunopharmacology and Drug Discovery Laboratory,
136 Harrison Avenue,
Boston, MA 02111, USA
Tel: +1 617 636 6866; Fax: +1 617 636 2456;
E-mail: theoharis.theoharides@tufts.edu
[2]Tufts University School of Medicine,
Department of Pharmacology and Experimental Therapeutics,
136 Harrison Avenue,
Boston, MA 02111, USA
[3]Coalition for Safe Minds and the National Autism Association,
16033 Bolsa Chica St, #104 142,
Huntington Beach, CA 92649, USA