Autism, Gut–Blood-Brain Barrier, and Mast Cells

Theoharis C. Theoharides, BA, MS, MPhil, PhD, MD,†‡§ and Robert Doyle, BS, MD‖*

Autism and autism spectrum disorders (ASDs) are pervasive neurodevelopmental disorders diagnosed in early childhood. They are characterized by varying degrees of dysfunctional communication and social skills, as well as repetitive and stereotypical behaviors.[1,2] The diagnosis has increased considerably over the last few years,[3] but it is not clear whether higher prevalence of the disorder, improved awareness by clinicians, or a combination of these is responsible. Autism spectrum disorder onsets in early childhood. In many cases (25%–50%), a period of seemingly normal development drastically shifts directions as acquired skills are lost or the acquisition of new skills becomes delayed. Recent studies make it clear that vaccines do not seem to be the cause of autism,[4–8] but some other immunologic process may be involved.

Possible involvement of gastrointestinal (GI) pathophysiology in autism and ASD has been suggested[9] because of stomach and bowel symptoms apparently present among many autistic children.[3,10] Unfortunately, studies of autistic children on gluten or casein-free diet[11] were hard to evaluate because of the absence of a control group.[12] Moreover, a nested case-control study showed no association between autism and any defined GI conditions.[13] However, "leaky gut"[14] or food intolerance[15] could possibly permit systemic absorption of substances that could adversely affect brain function. Food intolerance may affect as many as 16% of children[16] and up to 34% of 3-year-old children with significant behavioral responses, especially anxiety and stress.[17,18] Unfortunately, the terms *food allergy*, *food hypersensitivity*, and *food intolerance* are often used interchangeably, although they may refer to different pathologies and not necessarily measuring the same end points, leading to a lot of confusion, both in the literature and among patients.[19] Acceptable forms of testing include serum radioallergosorbent test or skin prick to food antigens, or intestinal biopsy showing inflammation and flattened villi, as in gluten enteropathy. However, many patients may have abdominal cramping and diarrhea without any such positive tests. In this case, serum immunoglobulin G_4 levels may be useful.[20] In addition to exclusion of lactose intolerance or uncooked tuna histamine poisoning,[21,22] rare diseases like carcinoid syndrome or a GI tumor secreting vasoactive intestinal peptide may also need to be excluded.

Abdominal pain may be a "headache equivalent" or "abdominal migraine"[23] that is often precipitated by stress. In 1 study of children migraineurs, the frequency and severity of stress-induced migraines was reduced, along with the unique mast cell marker tryptase, when the children were taught relaxation techniques.[24] Other studies in adults with autism indicated high levels of stress. A comparison of 34 adults with autism and 20 controls matched for age, sex, and intellectual ability showed that adults with autism were 3 times as anxious as controls; there was also a significant difference between the 2 groups with respect to stressors, so that the adults with autism could not cope as well with change and unpleasant events.[25]

*Laboratory of Molecular Immunopharmacology and Drug Discovery, Department of Pharmacology and Experimental Therapeutics, and Departments of †Internal Medicine, ‡Biochemistry, and §Psychiatry, Tufts University School of Medicine, Tufts Medical Center; and ‖Department of Pediatric Psychopharmacology, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Aspects of this work were funded in part by National Institutes of Health Grants NS38326 and AR47652 and Theta Biomedical Consulting and Development Co, Inc (Brookline, MA) to Dr Theoharides.

Address correspondence and reprint request to Theoharis C. Theoharides, BA, MS, MPhil, PhD, MD, Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, 136 Harrison Ave, Boston, MA 02111. E-mail: theoharis.theoharides@tufts.edu.

ISSN: 0271-0749/08/2805-0479
DOI: 10.1097/JCP.0b013e3181845f48

A possible key player in autism and ASD could be the mast cell. Recently, mast cells were shown to be involved in the regulation of the GI pathophysiology,[26,27] migraines,[28] and the blood-brain barrier (BBB).[29] Of note, serum from children with autism was shown to contain a number of autoantibodies against encephalogenic peptides that cross-react with milk butyrophilin; this cross-reactivity may suggest BBB disruption in individuals with autism.[30] Aberrant or immature development of the blood-brain-gut barrier may expose mast cells to triggers resulting in release of vasoactive, inflammatory, and neurosensitizing molecules that could affect brain and intestinal function. Acute stress has been reported to increase salivary cortisol in children with autism, thereby suggesting a heightened hypothalamic-pituitary-adrenal response.[31] Such a response is also supported by a higher plasma adrenocortocotropin hormone level found in adults with Asperger syndrome.[32] Acute stress could stimulate intestinal mast cells, and increase gut permeability.[33] Additionally, acute stress could disrupt the BBB,[34] particularly in brain areas containing mast cells.[35]

The involvement of mast cells in BBB regulation was first hypothesized by Theoharides.[36] Silver et al[37] later showed in animal models that increased BBB permeability occurred in response to the mast cell secretagogue compound 48/80. Subsequent research by Theoharides et al[29] showed that corticotropin-releasing hormone (CRH), typically released under stress, was implicated along with mast cells in BBB dynamics in rodents.[38] Acute stress activated rat dura mast cells,[39] and this effect was abolished by pretreatment with polyclonal antiserum to CRH.[39] Preliminary unpublished experiments (T.C.T.) also used intraluminal administration of ^{99}Technetium gluceptate in mouse colon and assessed its systemic and brain uptake after a 30-minute period of restraint stress. An approximate 5-fold higher label was detected in the blood and 3-fold higher in the brain after stress, suggesting that stress disrupted both the blood-gut-barrier and BBB; this effect was not present in W/W^v mast cell–deficient mice.

Corticotropin-releasing hormone administration in humans causes peripheral vasodilation and flushing, which are mast cell–dependent effects.[40] CRH leads to activation of skin mast cells and vascular permeability in rodents[41] and humans,[42] through activation of receptor 1 (CRH-R1). Similar effects have been shown in the bladder[43] and the intestines.[44] It was also recently shown that CRH could increase permeability in normal human colonic biopsies through activation of subepithelial mast cells.[45]

Mast cells are critical for allergic reactions[46] but are also important in immunity[47] and in inflammation.[48] Mast cell–neuron interactions[49] could involve the "enteric nervous system"[50] and may mediate neurotoxicity[51] and neuroinflammation.[48] In addition to allergic stimulation,[52] many other substances can trigger mast cell secretion[53]: immunoglobulin free light chains,[54] anaphylatoxins, and neuropeptides, such as substance P and neurotensin.[55] Mast cells secrete numerous vasodilatory molecules that include histamine, serotonin, kinins, vascular endothelial growth factor (VEGF), and vasoactive intestinal peptide, as well as proinflammatory, proteases, leukotrienes, prostaglandins, and cytokines, including interleukin (IL-6) and tumor necrosis factor α, which increase BBB permeability.[56] Interestingly, only ASD children with GI symptoms had higher tumor necrosis factor α produced by peripheral blood mononuclear cells in response to gluten gliadin, cow milk protein and soy.[57] Tumor necrosis factor levels in the central nervous system were significantly higher than corresponding serum levels in 10 children with autism, suggesting increased central nervous system production[58] that could affect cognitive functions.[59,60] Another study showed that patients with autism in general had higher urine levels of prostaglandins,[61] which are mainly produced by mast cells.

Mast cells could be involved in ASD through their unique ability to release some mediators "differentially" or "selectively" without anaphylactic degranulation,[62] as was originally shown by Theoharides and Askenase[63] for serotonin release from rodent mast cells.[63] Theoharides et al[64] recently demonstrated that IL-1 can stimulate selective release of IL-6,[64] whereas CRH could stimulate selective release[65] of VEGF,[66] an isoform of which is particularly vasodilatory,[67] from human mast cells. Selective release of IL-6 could have profound effects on brain function[68] and could activate the hypothalamic-pituitary-adrenal axis,[69] whereas selective release of VEGF could lead to increased vascular permeability that could participate in gut-blood-brain barrier disruption.[29]

Mast cell activation could also contribute to other immune[70] and neuroinflammatory[71,72] abnormalities that have been reported in patients with ASD. For instance, 1 study showed that duodenal and colonic $CD3^+$ lymphocytes were increased in children with ASD, whereas IL-10–positive cells were decreased,[73] possibly reducing levels of IL-10, which is known to have immunomodulatory effects.[74] Although another study showed that cultured endoscopic biopsies from children with ASD did not produce any more IL-1, IL-6, or IL-8 than those from normal controls,[75] it is conceivable that blind biopsies may have not sampled affected tissue or that such biopsies may not release these cytokines except in severe cases of ASD. Another study of 17 patients with autism showed that there was significant inhibition of macrophage migration to human myelin basic protein, as compared with 13 patients with other mental conditions.[76] Mast cell–derived monocyte chemoattractant protein[77] is a known chemotactic factor for macrophages and other immune cells that could be recruited to brain parenchyma, thus contributing to ASD brain pathology.

Unfortunately, there are no approved treatments for the core symptoms of autism.[78,79] The combined histamine-1 and serotonin receptor (5-HT2) antagonist cyproheptadine may have a role because a double-blind trial of 40 children with autism, randomized to either the antipsychotic haloperidol and cyproheptadine versus haloperidol and placebo, showed significant improvement in the cyproheptadine group.[80] The higher platelet serotonin levels found in more than 40% of patients with autism[81] may explain the apparent effectiveness of cyproheptadine. Although platelet levels of serotonin may or may not correlate with serotonin availability on the brain side of the BBB, such high systemic levels could certainly affect GI function. Cyproheptadine has also been shown to

inhibit mast cell activation[82] and has been used in children with migraines.[83] Additional controlled studies are needed to determine if cyproheptadine might have a role in the treatment of autism.

Oxidative stress is a response to psychological, immune, or infectious origin and could be uncontrolled if the innate ability to neutralize free radicals is impaired. One study of 305 children with autism and 205 controls showed that the ratio of S-adenosylhomocysteine, used as an indicator of methylation ability, was significantly reduced in children with autism.[84] Another study also found reduced plasma levels of S-adenosylmethionine, the main endogenous antioxidant.[85] Both of these studies imply that patients with autism may have excessive free radical production.

Novel medications are needed for effective treatment of the spectrum of autistic disorders. Mast cells could be a unique therapeutic target because they seem to regulate gut-blood-barrier permeability, and they are affected by CRH, which is released under conditions of stress.[48,86] Potentially useful agents could include CRH receptor antagonists[87] and mast cell inhibitors, such as some naturally occurring flavonoids[88,89]; these are found in certain dietary supplements[90] and exhibit antioxidant, anti-inflammatory, and mast cell inhibitory activity,[89] while they can also reduce platelet serotonin secretion.[91]

ADDENDUM

It was recently reported that blood levels of macrophage migration inhibitory factor (MIF), released from mast cells, were higher in family probands with ASD than their unaffected siblings, and that plasma MIF correlated with ASD symptoms.[92] Moreover, the CRH related peptide urocortin (Ucn), released under stress from the brain but also from mast cells, can stimulate endothelial cells of the blood-brain barrier (BBB).[93] Finally, decreased methylation ability due to environmental factors in ASD[94] could compromise the brain's ability to withstand inflammation. These results support the premise discussed above and the proposed novel therapeutic targets for ASD.[95]

ACKNOWLEDGMENT

The authors thank Ms Jessica Christian for word processing skills.

AUTHOR DISCLOSURE INFORMATION

US patents No 6,624,148; 6,689,748; 6,984,667; 7,115,278; and 10/811,825 and EPO 1365777 (awarded to Dr Theoharides) cover the use of methods and compositions of mast cell blockers, such as flavonoids, in neuroinflammatory conditions. These patents and the relevant dietary supplement NeuroProtek have been licensed to Algonot, LLC (algonot.com), Sarasota, FL.

REFERENCES

1. Frith U. Autism. *Sci Am*. 1993;268:108–114.
2. Fombonne E, Zakarian R, Bennett A, et al. Pervasive developmental disorders in Montreal, Quebec, Canada: prevalence and links with immunizations. *Pediatrics*. 2006;118:e139–e150.
3. Johnson CP, Myers SM. Identification and evaluation of children with autism spectrum disorders. *Pediatrics*. 2007;120:1183–1215.
4. Stehr-Green P, Tull P, Stellfeld M, et al. Autism and thimerosal-containing vaccines: lack of consistent evidence for an association. *Am J Prev Med*. 2003;25:101–106.
5. D'Souza Y, Fombonne E, Ward BJ. No evidence of persisting measles virus in peripheral blood mononuclear cells from children with autism spectrum disorder. *Pediatrics*. 2006;118:1664–1675.
6. DeStefano F. Vaccines and autism: evidence does not support a causal association. *Clin Pharmacol Ther*. 2007;82:756–759.
7. Baker JP. Mercury, vaccines, and autism: one controversy, three histories. *Am J Public Health*. 2008;98:244–253.
8. Fombonne E. Thimerosal disappears but autism remains. *Arch Gen Psychiatry*. 2008;65:15–16.
9. White JF. Intestinal pathophysiology in autism. *Exp Biol Med (Maywood)*. 2003;228:639–649.
10. Levy SE, Souders MC, Ittenbach RF, et al. Relationship of dietary intake to gastrointestinal symptoms in children with autistic spectrum disorders. *Biol Psychiatry*. 2007;61:492–497.
11. Elder JH, Shankar M, Shuster J, et al. The gluten-free, casein-free diet in autism: results of a preliminary double blind clinical trial. *J Autism Dev Disord*. 2006;36:413–420.
12. Alpert M. The autism diet. *Sci Am*. 2007;296:19–20.
13. Black C, Kaye JA, Jick H. Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database. *BMJ*. 2002;325:419–421.
14. Liu Z, Li N, Neu J. Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr*. 2005;94:386–393.
15. Gurney JG, McPheeters ML, Davis MM. Parental report of health conditions and health care use among children with and without autism: National Survey of Children's Health. *Arch Pediatr Adolesc Med*. 2006;160:825–830.
16. Venter C, Pereira B, Voigt K, et al. Prevalence and cumulative incidence of food hypersensitivity in the first 3 years of life. *Allergy*. 2008;63:354–359.
17. Teufel M, Biedermann T, Rapps N, et al. Psychological burden of food allergy. *World J Gastroenterol*. 2007;13:3456–3465.
18. Vatn MH. Food intolerance and psychosomatic experience. *Scand J Work Environ Health*. 1997;23(suppl 3):75–78.
19. Ortolani C, Pastorello EA. Food allergies and food intolerances. *Best Pract Res Clin Gastroenterol*. 2006;20:467–483.
20. Kruszewski J, Raczka A, Klos M, et al. High serum levels of allergen specific IgG-4 (asIgG-4) for common food allergens in healthy blood donors. *Arch Immunol Ther Exp (Warsz)*. 1994;42:259–261.
21. Morrow JD, Margolies GR, Rowland J, et al. Evidence that histamine is the causative toxin of scombroid-fish poisoning. *N Engl J Med*. 1991;324:716–720.
22. McLauchlin J, Little CL, Grant KA, et al. Scombrotoxic fish poisoning. *J Public Health (Oxf)*. 2006;28:61–62.
23. Lundberg PO. Abdominal migraine—diagnosis and therapy. *Headache*. 1975;15:122–125.
24. Olness K, Hall H, Roznieckl JJ, et al. Mast cell activation in children with migraine before and after training in self-regulation. *Headache*. 1999;39:101–107.
25. Gillott A, Standen PJ. Levels of anxiety and sources of stress in adults with autism. *J Intellect Disabil*. 2007;11:359–370.
26. Rijnierse A, Nijkamp FP, Kraneveld AD. Mast cells and nerves tickle in the tummy. Implications for inflammatory bowel disease and irritable bowel syndrome. *Pharmacol Ther*. 2007;116:207–235.
27. Farhadi A, Fields JZ, Keshavarzian A. Mucosal mast cells are pivotal elements in inflammatory bowel disease that connect the dots: stress, intestinal hyperpermeability and inflammation. *World J Gastroenterol*. 2007;13:3027–3030.
28. Theoharides TC, Donelan J, Kandere-Grzybowska K, et al. The role of mast cells in migraine pathophysiology. *Brain Res Rev*. 2005;49:65–76.
29. Theoharides TC, Konstantinidou A. Corticotropin-releasing hormone and the blood-brain-barrier. *Front Biosci*. 2007;12:1615–1628.
30. Vojdani A, Campbell AW, Anyanwu E, et al. Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, *Chlamydia pneumoniae* and *Streptococcus* group A. *J Neuroimmunol*. 2002;129:168–177.

31. Jansen LM, Gispen-de Wied CC, van der Gaag RJ, et al. Differentiation between autism and multiple complex developmental disorder in response to psychosocial stress. *Neuropsychopharmacology.* 2003;28: 582–590.
32. Tani P, Lindberg N, Matto V, et al. Higher plasma ACTH levels in adults with Asperger syndrome. *J Psychosom Res*. 2005;58:533–536.
33. Theoharides TC, Letourneau R, Patra P, et al. Stress-induced rat intestinal mast cell intragranular activation and inhibitory effect of sulfated proteoglycans. *Dig Dis Sci*. 1999;44:87S–93S.
34. Belova I, Jonsson G. Blood-brain barrier permeability and immobilization stress. *Acta Physiol Scand*. 1982;116:21–29.
35. Esposito P, Gheorghe D, Kandere K, et al. Acute stress increases permeability of the blood-brain-barrier through activation of brain mast cells. *Brain Res*. 2001;888:117–127.
36. Theoharides TC. Mast cells: the immune gate to the brain. *Life Sci.* 1990;46:607–617.
37. Silver R, Silverman A-J, Vitkovic L, et al. Mast cells in the brain: evidence and functional significance. *Trends Neurosci.* 1996;19:25–31.
38. Esposito P, Chandler N, Kandere-Grzybowska K, et al. Corticotropin-releasing hormone (CRH) and brain mast cells regulate blood-brain-barrier permeability induced by acute stress. *J Pharmacol Exp Ther.* 2002;303:1061–1066.
39. Theoharides TC, Spanos CP, Pang X, et al. Stress-induced intracranial mast cell degranulation. A corticotropin releasing hormone-mediated effect. *Endocrinology.* 1995;136:5745–5750.
40. Kubler A, Rothacher G, Knappertz VA, et al. Intra- and extracerebral blood flow changes and flushing after intravenous injection of human corticotrophin-releasing hormone. *Clin Invest.* 1994;72:331–336.
41. Theoharides TC, Singh LK, Boucher W, et al. Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its pro-inflammatory effects. *Endocrinology.* 1998;139:403–413.
42. Crompton R, Clifton VL, Bisits AT, et al. Corticotropin-releasing hormone causes vasodilation in human skin via mast cell-dependent pathways. *J Clin Endocrinol Metab.* 2003;88:5427–5432.
43. Spanos C, Pang X, Ligris K, et al. Stress-induced bladder mast cell activation: implications for interstitial cystitis. *J Urol*. 1997;157:669–672.
44. Castagliuolo I, LaMont JT, Qiu B, et al. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am J Physiol*. 1996;271:884–892.
45. Wallon C, Yang P, Keita AV, et al. Corticotropin releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies in vitro. *Gut*. 2008;57:50–58.
46. Theoharides TC, Kalogeromitros D. The critical role of mast cell in allergy and inflammation. *Ann NY Acad Sci*. 2006;1088:78–99.
47. Galli SJ, Kalesnikoff J, Grimbaldeston MA, et al. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol*. 2005;23:749–786.
48. Theoharides TC, Cochrane DE. Critical role of mast cells in inflammatory diseases and the effect of acute stress. *J Neuroimmunol*. 2004; 146:1–12.
49. Roznieckі JJ, Dimitriadou V, Lambracht-Hall M, et al. Morphological and functional demonstration of rat dura mast cell–neuron interactions in vitro and in vivo. *Brain Res*. 1999;849:1–15.
50. Stead RH, Dixon MF, Bramwell NH, et al. Mast cells are closely apposed to nerves in the human gastrointestinal mucosa. *Gastroenterology.* 1989;97:575–585.
51. Purcell WM, Atterwill CK. Mast cells in neuroimmune function: neurotoxicological and neuropharmacological perspectives. *Neurochem Res*. 1995;20:521–532.
52. Blank U, Rivera J. The ins and outs of IgE-dependent mast-cell exocytosis. *Trends Immunol*. 2004;25:266–273.
53. Janiszewski J, Bienenstock J, Blennerhassett MG. Picomolar doses of substance P trigger electrical responses in mast cells without degranulation. *Am J Physiol*. 1994;267:C138–C145.
54. Redegeld FA, Nijkamp FP. Immunoglobulin free light chains and mast cells: pivotal role in T-cell–mediated immune reactions? *Trends Immunol*. 2005;24:181–185.
55. Carraway R, Cochrane DE, Lansman JB, et al. Neurotensin stimulates exocytotic histamine secretion from rat mast cells and elevates plasma histamine levels. *J Physiol*. 1982;323:403–414.
56. Kim KS, Wass CA, Cross AS, et al. Modulation of blood-brain barrier permeability by tumor necrosis factor and antibody to tumor necrosis factor in the rat. *Lymphokine Cytokine Res*. 1992;11:293–298.
57. Jyonouchi H, Geng L, Ruby A, et al. Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention. *Neuropsychobiology.* 2005;51:77–85.
58. Chez MG, Dowling T, Patel PB, et al. Elevation of tumor necrosis factor–alpha in cerebrospinal fluid of autistic children. *Pediatr Neurol*. 2007;36:361–365.
59. Reichenberg A, Yirmiya R, Schuld A, et al. Cytokine-associated emotional and cognitive disturbances in humans. *Arch Gen Psychiatry.* 2001;58:445–452.
60. Tobinick EL, Gross H. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. *J Neuroinflammation*. 2008;5:2.
61. Yao Y, Walsh WJ, McGinnis WR, et al. Altered vascular phenotype in autism: correlation with oxidative stress. *Arch Neurol*. 2006;63: 1161–1164.
62. Theoharides TC, Kempuraj D, Tagen M, et al. Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunol Rev.* 2007;217:65–78.
63. Theoharides TC, Bondy PK, Tsakalos ND, et al. Differential release of serotonin and histamine from mast cells. *Nature*. 1982;297: 229–231.
64. Kandere-Grzybowska K, Letourneau R, Kempuraj D, et al. IL-1 induces vesicular secretion of IL-6 without degranulation from human mast cells. *J Immunol*. 2003;171:4830–4836.
65. Cao J, Papadopoulou N, Kempuraj D, et al. Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH leads to selective secretion of vascular endothelial growth factor. *J Immunol*. 2005;174:7665–7675.
66. Boesiger J, Tsai M, Maurer M, et al. Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E–dependent upregulation of Fcε receptor I expression. *J Exp Med*. 1998;188:1135–1145.
67. Grutzkau A, Kruger-Krasagakes S, Baumeister H, et al. Synthesis, storage and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: Implications for the biological significance of $VEGF_{206}$. *Mol Biol Cell*. 1998;9: 875–884.
68. Theoharides TC, Weinkauf C, Conti P. Brain cytokines and neuropsychiatric disorders. *J Clin Psychopharmacol*. 2004;24:577–581.
69. Mastorakos G, Chrousos GP, Weber JS. Recombinant interleukin-6 activates the hypothalamic-pituitary-adrenal axis in humans. *J Clin Endocrinol Metab*. 1993;77:1690–1694.
70. Ashwood P, Wills S, Van de WJ. The immune response in autism: a new frontier for autism research. *J Leukoc Biol*. 2006;80:1–15.
71. Pardo CA, Vargas DL, Zimmerman AW. Immunity, neuroglia and neuroinflammation in autism. *Int Rev Psychiatry.* 2005;17:485–495.
72. Cohly HH, Panja A. Immunological findings in autism. *Int Rev Neurobiol*. 2005;71:317–341.
73. Ashwood P, Anthony A, Torrente F, et al. Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10. *J Clin Immunol*. 2004;24:664–673.
74. Conti P, Kempuraj D, Kandere K, et al. IL-10, an inflammatory/inhibitory cytokine, but not always. *Immunol Lett*. 2003;86:123–129.
75. DeFelice ML, Ruchelli ED, Markowitz JE, et al. Intestinal cytokines in children with pervasive developmental disorders. *Am J Gastroenterol*. 2003;98:1777–1782.
76. Weizman A, Weizman R, Szekely GA, et al. Abnormal immune response to brain tissue antigen in the syndrome of autism. *Am J Psychiatry.* 1982;139:1462–1465.
77. Conti P, Pang X, Boucher W, et al. Impact of Rantes and MCP-1 chemokines on in vivo basophilic mast cell recruitment in rat skin injection model and their role in modifying the protein and mRNA levels for histidine decarboxylase. *Blood*. 1997;89:4120–4127.
78. Myers SM. The status of pharmacotherapy for autism spectrum disorders. *Expert Opin Pharmacother.* 2007;8:1579–1603.

79. Broadstock M, Doughty C, Eggleston M. Systematic review of the effectiveness of pharmacological treatments for adolescents and adults with autism spectrum disorder. *Autism*. 2007;11:335–348.
80. Akhondzadeh S, Erfani S, Mohammadi MR, et al. Cyproheptadine in the treatment of autistic disorder: a double-blind placebo-controlled trial. *J Clin Pharm Ther*. 2004;29:145–150.
81. Hranilovic D, Bujas-Petkovic Z, Vragovic R, et al. Hyperserotonemia in adults with autistic disorder. *J Autism Dev Disord*. 2007;37: 1934–1940.
82. Theoharides TC, Kops SK, Bondy PK, et al. Differential release of serotonin without comparable histamine under diverse conditions in the rat mast cell. *Biochem Pharmacol*. 1985;34:1389–1398.
83. Worawattanakul M, Rhoads JM, Lichtman SN, et al. Abdominal migraine: prophylactic treatment and follow-up. *J Pediatr Gastroenterol Nutr*. 1999;28:37–40.
84. James SJ, Melnyk S, Jernigan S, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141:947–956.
85. James SJ, Cutler P, Melnyk S, et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J Clin Nutr*. 2004;80:1611–1617.
86. Theoharides TC, Donelan JM, Papadopoulou N, et al. Mast cells as targets of corticotropin-releasing factor and related peptides. *Trends Pharmacol Sci*. 2004;25:563–568.
87. Gravanis A, Margioris AN. The corticotropin-releasing factor (CRF) family of neuropeptides in inflammation: potential therapeutic applications. *Curr Med Chem*. 2005;12:1503–1512.
88. Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacol Rev*. 2000;52:673–751.
89. Kempuraj D, Madhappan B, Christodoulou S, et al. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *Br J Pharmacol*. 2005;145:934–944.
90. Theoharides TC, Bielory L. Mast cells and mast cell mediators as targets of dietary supplements. *Ann Allergy Asthma Immunol*. 2004;93:S24–S34.
91. Beretz A, Cazenave JP, Anton R. Inhibition of aggregation and secretion of human platelets by quercetin and other flavonoids: structure-activity relationships. *Agents Actions*. 1982;12:382–387.
92. Grigorenko EL, Han SS, Yrigollen CM, et al. Macrophage migration inhibitory factor and autism spectrum disorders. *Pediatrics*. 2008;22: e438–e445.
93. Pan W, Kastin AJ. *Prog Neurobiol*. 2008;84:148–156.
94. Deth R, Muratore C, Benzecry J, et al. How environmental and genetic factors combine to cause autism: a redox/methylation hypothesis. *Neurotoxicology*. 2008;29:190–201.
95. Theoharides TC, Doyle R, Francis K, et al. Novel therapeutic targets for autism. *Trends Pharmacol Sci*. 2008;29:375–382.

