

## 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.<sup>1,2</sup> The diagnosis has increased considerably over the last few years,<sup>3</sup> 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,<sup>4–8</sup> but some other immunologic process may be involved.

Possible involvement of gastrointestinal (GI) pathophysiology in autism and ASD has been suggested<sup>9</sup> because of stomach and bowel symptoms apparently present among many autistic children.<sup>3,10</sup> Unfortunately, studies of autistic children on gluten or casein-free diet<sup>11</sup> were hard to evaluate because of the absence of a control group.<sup>12</sup> Moreover, a nested case-control study showed no association between autism and any defined GI conditions.<sup>13</sup> However, “leaky gut”<sup>14</sup> or food intolerance<sup>15</sup> could possibly permit systemic absorption of substances that could adversely affect brain function. Food intolerance may affect as many as 16% of children<sup>16</sup> and up to 34% of 3-year-old children with significant behavioral responses, especially anxiety and stress.<sup>17,18</sup> 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.<sup>19</sup> 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<sub>4</sub> levels may be useful.<sup>20</sup> In addition to exclusion of lactose intolerance or uncooked tuna histamine poisoning,<sup>21,22</sup> 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”<sup>23</sup> 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.<sup>24</sup> 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.<sup>25</sup>

\*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.

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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.

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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,<sup>26,27</sup> migraines,<sup>28</sup> and the blood-brain barrier (BBB).<sup>29</sup> 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.<sup>30</sup> 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.<sup>31</sup> Such a response is also supported by a higher plasma adrenocorticotropin hormone level found in adults with Asperger syndrome.<sup>32</sup> Acute stress could stimulate intestinal mast cells, and increase gut permeability.<sup>33</sup> Additionally, acute stress could disrupt the BBB,<sup>34</sup> particularly in brain areas containing mast cells.<sup>35</sup>

The involvement of mast cells in BBB regulation was first hypothesized by Theoharides.<sup>36</sup> Silver et al<sup>37</sup> 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<sup>29</sup> showed that corticotropin-releasing hormone (CRH), typically released under stress, was implicated along with mast cells in BBB dynamics in rodents.<sup>38</sup> Acute stress activated rat dura mast cells,<sup>39</sup> and this effect was abolished by pretreatment with polyclonal antiserum to CRH.<sup>39</sup> Preliminary unpublished experiments (T.C.T.) also used intraluminal administration of <sup>99m</sup>Tc 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<sup>v</sup> mast cell-deficient mice.

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

Mast cells are critical for allergic reactions<sup>46</sup> but are also important in immunity<sup>47</sup> and in inflammation.<sup>48</sup> Mast cell–neuron interactions<sup>49</sup> could involve the “enteric nervous system”<sup>50</sup> and may mediate neurotoxicity<sup>51</sup> and neuroinflammation.<sup>48</sup> In addition to allergic stimulation,<sup>52</sup> many other substances can trigger mast cell secretion<sup>53</sup>: immunoglobulin free light chains,<sup>54</sup> anaphylatoxins, and neuropeptides, such as substance P and neurotensin.<sup>55</sup> 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  $\alpha$ , which increase BBB permeability.<sup>56</sup> Interestingly, only ASD children with GI symptoms had higher tumor necrosis factor  $\alpha$  produced by peripheral blood mononuclear cells in response to gluten gliadin, cow milk protein and soy.<sup>57</sup> 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<sup>58</sup> that could affect cognitive functions.<sup>59,60</sup> Another study showed that patients with autism in general had higher urine levels of prostaglandins,<sup>61</sup> 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,<sup>62</sup> as was originally shown by Theoharides and Askenase<sup>63</sup> for serotonin release from rodent mast cells.<sup>63</sup> Theoharides et al<sup>64</sup> recently demonstrated that IL-1 can stimulate selective release of IL-6,<sup>64</sup> whereas CRH could stimulate selective release<sup>65</sup> of VEGF,<sup>66</sup> an isoform of which is particularly vasodilatory,<sup>67</sup> from human mast cells. Selective release of IL-6 could have profound effects on brain function<sup>68</sup> and could activate the hypothalamic-pituitary-adrenal axis,<sup>69</sup> whereas selective release of VEGF could lead to increased vascular permeability that could participate in gut-blood-brain barrier disruption.<sup>29</sup>

Mast cell activation could also contribute to other immune<sup>70</sup> and neuroinflammatory<sup>71,72</sup> abnormalities that have been reported in patients with ASD. For instance, 1 study showed that duodenal and colonic CD3<sup>+</sup> lymphocytes were increased in children with ASD, whereas IL-10–positive cells were decreased,<sup>73</sup> possibly reducing levels of IL-10, which is known to have immunomodulatory effects.<sup>74</sup> 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,<sup>75</sup> 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.<sup>76</sup> Mast cell–derived monocyte chemoattractant protein<sup>77</sup> 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.<sup>78,79</sup> The combined histamine-1 and serotonin receptor (5-HT<sub>2</sub>) 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.<sup>80</sup> The higher platelet serotonin levels found in more than 40% of patients with autism<sup>81</sup> 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<sup>82</sup> and has been used in children with migraines.<sup>83</sup> 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.<sup>84</sup> Another study also found reduced plasma levels of S-adenosylmethionine, the main endogenous antioxidant.<sup>85</sup> 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.<sup>48,86</sup> Potentially useful agents could include CRH receptor antagonists<sup>87</sup> and mast cell inhibitors, such as some naturally occurring flavonoids<sup>88,89</sup>; these are found in certain dietary supplements<sup>90</sup> and exhibit antioxidant, anti-inflammatory, and mast cell inhibitory activity,<sup>89</sup> while they can also reduce platelet serotonin secretion.<sup>91</sup>

### 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.<sup>92</sup> 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).<sup>93</sup> Finally, decreased methylation ability due to environmental factors in ASD<sup>94</sup> could compromise the brain's ability to withstand inflammation. These results support the premise discussed above and the proposed novel therapeutic targets for ASD.<sup>95</sup>

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### 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.

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