

## Review Article

# Is a Subtype of Autism an Allergy of the Brain?

Theoharis C. Theoharides, MS, MPhil, PhD, MD

*Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts; Departments of Biochemistry, Tufts University School of Medicine, Boston, Massachusetts; Department of Internal Medicine, Tufts University School of Medicine and Tufts Medical Center, Boston, Massachusetts; and Department of Psychiatry, Tufts University School of Medicine and Tufts Medical Center, Boston, Massachusetts*

### ABSTRACT

**Background:** Autism spectrum disorders (ASDs) are characterized by deficits in social communication and language and the presence of repetitive behaviors that affect as many as 1 in 50 US children. Perinatal stress and environmental factors appear to play a significant role in increasing the risk for ASDs. There is no definitive pathogenesis, which therefore significantly hinders the development of a cure.

**Objective:** We aimed to identify publications using basic or clinical data that suggest a possible association between atopic symptoms and ASDs, as well as evidence of how such an association could lead to brain disease, that may explain the pathogenesis of ASD.

**Methods:** PubMed was searched for articles published since 1995 that reported any association between autism and/or ASDs and any one of the following terms: *allergy, atopy, brain, corticotropin-releasing hormone, cytokines, eczema, food allergy, food intolerance, gene mutation, inflammation, mast cells, mitochondria, neurotensin, phenotype, stress, subtype, or treatment.*

**Results:** Children with ASD respond disproportionately to stress and also present with food and skin allergies that involve mast cells. Brain mast cells are found primarily in the hypothalamus, which participates in the regulation of behavior and language. Corticotropin-releasing hormone is secreted from the hypothalamus under stress and, together with neurotensin, stimulates brain mast cells that could result in focal brain allergy and neurotoxicity. Neurotensin is significantly increased in serum of children with ASD and stimulates mast cell secretion of mitochondrial adenosine triphosphate and DNA, which is increased in these children; these mitochondrial components are misconstrued as innate

pathogens, triggering an autoallergic response in the brain. Gene mutations associated with higher risk of ASD have been linked to reduction of the phosphatase and tensin homolog, which inhibits the mammalian target of rapamycin (mTOR). These same mutations also lead to mast cell activation and proliferation. Corticotropin-releasing hormone, neurotensin, and environmental toxins could further trigger the already activated mTOR, leading to superstimulation of brain mast cells in those areas responsible for ASD symptoms. Preliminary evidence indicates that the flavonoid luteolin is a stronger inhibitor of mTOR than rapamycin and is a potent mast cell blocker.

**Conclusion:** Activation of brain mast cells by allergic, environmental, immune, neurohormonal, stress, and toxic triggers, especially in those areas associated with behavior and language, lead to focal brain allergies and subsequent focal encephalitis. This possibility is more likely in the subgroup of patients with ASD susceptibility genes that also involve mast cell activation. (*Clin Ther.* 2013;35:584–591) © 2013 Elsevier HS Journals, Inc. All rights reserved.

**Key words:** allergy, autism, brain, inflammation, mast cells, mitochondria.

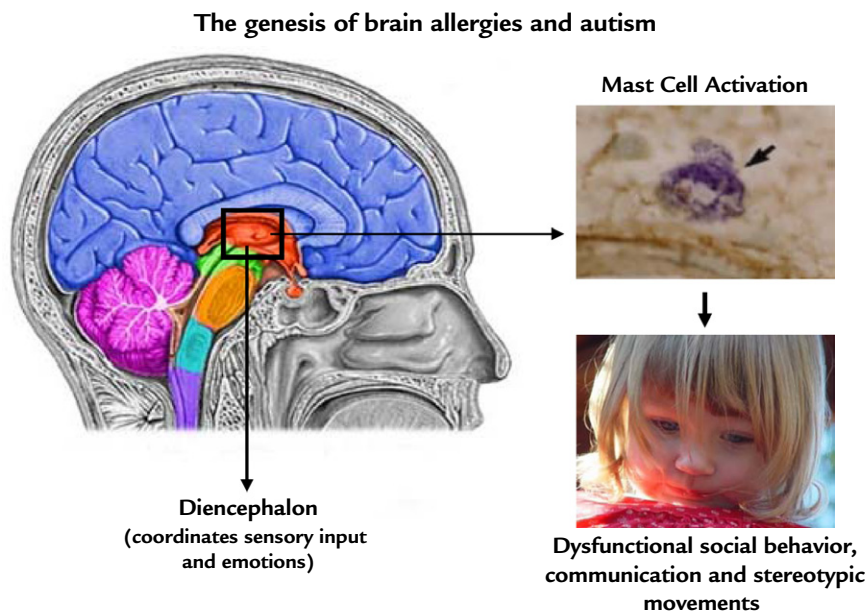
### INTRODUCTION

Autism spectrum disorders (ASDs) are neurodevelopmental disorders that affect almost 1 in 50 children<sup>1–3</sup> and, according to the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) due to

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**Figure.** Brain allergies. Brain mast cells are located close to blood vessels and nerve endings, especially in the hypothalamus of the diencephalon, which participates in the regulation of emotions. On stimulation by allergic, environmental (mercury), infectious (viruses), mitochondrial (adenosine triphosphate and/or DNA), stress (corticotropin-releasing hormone or neurotensin), and toxic (propionic acid) triggers, mast cells release inflammatory and neurotoxic molecules (eg, interleukin 6, tumor necrosis factor, and tryptase), triggering focal brain allergies, while they also increase blood brain barrier permeability, permitting entry of circulating lymphocytes in the brain, and activate microglia cells, leading to focal encephalitis. The microphotograph of the mast cell shown was taken from the rat hypothalamus after staining with toluidine blue (original magnification  $\times 1000$ ; arrow indicates area of degranulation). The photograph of the young, autistic female patient is shown with the parents' permission before improvement with the use of the flavonoid-containing dietary formulation mentioned in the discussion.

be published in May 2013, are characterized by 2 components: deficits in social communication and social interaction and restricted, repetitive behaviors and interests. Allergies and asthma have also reached epidemic proportions in the last 10 years.<sup>4</sup> There is new evidence that the environment contributes significantly to ASD pathogenesis.<sup>5-7</sup> Many patients with ASD also have food allergies<sup>8</sup> and allergic-like symptoms<sup>9</sup> but often without positive objective test results, suggesting mast cell activation by nonallergic triggers.<sup>10,11</sup> A recent epidemiologic study of 92,642 children reported a strong correlation between atopic dermatitis (eczema), attention-deficit/hyperactivity disorder, and autism.<sup>12</sup> Moreover, children with mastocytosis, a spectrum of diseases that present with skin allergies, hyperactivity, and difficulty focusing (brain fog),<sup>13,14</sup> appear to have a 5-fold higher prevalence of

ASD (1 in 10 children) than that reported for the general population.<sup>15</sup>

We propose that brain mast cells in critical brain areas, such as those regulating the autonomic nervous system and emotions (eg, diencephalon) and language (Broca area) are activated by environmental, infectious, or stress triggers, resulting in local brain allergies and focal encephalitis (Figure).<sup>16</sup>

## METHODS

PubMed was searched for articles published since 1995 that reported any association between autism and/or ASD and anyone of the following terms: *allergy, atopy, brain, corticotropin-releasing hormone, cytokines, eczema, food allergy, food intolerance, gene mutation, inflammation, mast cells, mitochondria, neurotensin,*

*phenotype, stress, subtype, treatment, or therapy.* Articles were chosen for relevance, human data, and use of the English language. Any contradictory results are discussed. Articles were excluded if they were published as hypotheses or if they were repetitive.

## RESULTS

### ASD and Brain Inflammation

Substances that originate in the gut or the brain can trigger mast cells to release mediators that could disrupt the blood brain barrier (BBB) and cause allergies in specific brain areas, thus contributing to the pathogenesis of autism.<sup>17</sup> Such mediators include interleukin (IL) 6,<sup>18</sup> which can be released from mast cells selectively.<sup>19</sup> IL-6 expression was elevated in the brains of patients with ASD,<sup>20</sup> and increased serum IL-6 level was linked to the expression of an autistic phenotype in mice.<sup>21,22</sup> Interestingly, patients with mastocytosis, characterized by increased number of activated mast cells, also have high serum IL-6 levels.<sup>23,24</sup> Offspring of maternal immune activation in mice developed increased IL-6 and IL-17, which contributed to ASD-related behaviors.<sup>25</sup> Tumor necrosis factor (TNF) levels were also increased in the cerebrospinal fluid (CSF) of patients with ASD.<sup>20</sup> Brain mast cells were reported to produce TNF.<sup>26</sup> Interestingly, mast cells are the only cell type that stores preformed TNF in secretory granules.<sup>27</sup> A recent diagnosis of mast cell activation syndrome in fact includes the statement “accompanied by neurologic complaints,”<sup>28</sup> implying that brain mast cells could be involved.

Patients with ASD exhibit neuroimmune dysfunction.<sup>29</sup> We reported that neurotensin (NT) was increased in young children with autism<sup>30</sup> and was proposed as a possible therapeutic target for autism also due to its ability to induce neurotoxicity.<sup>31</sup> We also reported that NT and corticotropin-releasing hormone (CRH), secreted under stress, synergistically stimulate mast cells, leading to increase vascular permeability<sup>32</sup> and contributing to BBB disruption.<sup>33</sup> We further found that NT stimulates mast cell secretion of vascular endothelial growth factor.<sup>34</sup> In addition, NT increases expression of CRH receptor 1 (CRHR-1),<sup>35</sup> activation of which by CRH increases allergic stimulation of human mast cells.<sup>36</sup> Furthermore, NT is increased in the skin after acute stress, stimulates skin mast cells, and increases vascular permeability in rodents.<sup>37</sup> Finally, NT stimulates rodent peritoneal mast cells to secrete histamine and elevates histamine plasma levels through activation of specific NTR.<sup>38–40</sup>

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### Brain Mast Cells

Mast cells are considered the “immune gate to the brain.”<sup>41</sup> The richest source of mast cells in the brain is the hypothalamus,<sup>42</sup> part of the diencephalon that relays sensory information among brain regions, connects structures of the endocrine and nervous systems, and communicates with the limbic system to regulate emotions. Moreover, the highest concentration of NT receptors is in the hypothalamus,<sup>43</sup> and Broca area,<sup>44</sup> which regulates language, known to be lost in many children with ASD. Stress activates brain mast cells, leading to BBB disruption,<sup>45</sup> which is important in brain inflammation.<sup>46</sup> Patients with ASD are susceptible to stress,<sup>47</sup> and prenatal stress has been linked to an increased risk of autism.<sup>48–50</sup> Moreover, CRH, secreted under stress, can activate mast cells<sup>33</sup> and is responsible for mast cell-dependent BBB<sup>42</sup> disruption.<sup>33,45</sup> This process could worsen by activation of Fcγ receptors (FcγRI) on neurons that could contribute to brain cell death after injection of the kainic acid.<sup>51</sup> Moreover, Fcε receptors (FcεRI), typically thought to be expressed only by mast cells and basophils, were recently identified on neurons,<sup>52</sup> implying that allergic triggers may even affect the neurons directly once the BBB has been disrupted to permit entry of immunoglobulins. We had found that rat brain mast cells did not synthesize FcεRI until induction of experimental allergic encephalomyelitis.<sup>53</sup>

Mast cell–microglial interactions are important in neuroinflammatory diseases.<sup>54,55</sup> Microglia are the innate brain immune cells that are increasingly implicated in a number of neuropsychiatric diseases.<sup>56</sup> In fact, abnormal microglial growth and activation were recently reported in the brain of patients with ASD.<sup>57,58</sup> It is therefore of interest that mast cell tryptase induced microglia activation.<sup>59</sup> Mast cells are responsible for eliciting neutrophil infiltration that promotes inflammation.<sup>60</sup> Increasing evidence indicates that mast cells participate in innate and acquired immunity<sup>61</sup> and inflammation.<sup>62</sup>

### Extracellular Mitochondrial Components

Brain gene clusters associated with increased inflammation and mitochondrial dysfunction appear to be the main findings in neuropsychiatric disorders.<sup>63</sup> We recently found that mast cell activation

leads to mitochondrial translocation to the cell surface.<sup>64</sup> and secretion of extracellular mitochondrial adenosine triphosphate (ATP) and DNA.<sup>35</sup> We also found that serum of children with autism had increased levels of extracellular mitochondrial DNA,<sup>65</sup> and such extracellular mitochondrial components could augment allergies and eczema.<sup>36</sup> Extracellular mitochondrial ATP and DNA are misconstrued by the body as innate pathogens and induce a strong autoinflammatory response<sup>35</sup> because mitochondria were bacteria that became symbiotic with eukaryotic cells.<sup>66</sup> Neurotensin triggers mast cells to secrete mitochondrial DNA that acts as innate pathogen to stimulate mast cells and other immune cells, leading to autoinflammation and contributing to autism pathogenesis.<sup>67</sup>

Mast cells also express Toll-like receptors (TLRs), including TLR-9, which can be activated by bacterial DNA sequences, leading to release of different cytokines,<sup>68</sup> and allow mast cells to participate in immunity against bacteria.<sup>69</sup> Extracellular ATP has been found to trigger and maintain inflammation in asthmatic airways.<sup>70</sup> Moreover, extracellular ATP was recently considered a universal alarm signal released from cells under stress and capable of affecting neighboring cells.<sup>71</sup> Mitochondrial DNA was also reported to be directly neurotoxic and alter behavior in mice.<sup>72</sup>

Extracellular nucleic acids are now considered as sensors of cell damage and are involved in autoimmunity.<sup>73</sup> However, our findings are different from those of damaged associated molecular patterns, which include mitochondrial DNA and are released after major trauma in humans<sup>74</sup> or shock-injured rat tissues<sup>75</sup> and can activate TLR-9 receptors on human peripheral polymorphonuclear cells, leading to inflammation.<sup>74</sup> In both these cases, the damaged associated molecular patterns came from dying cells. A recent study reported that treatment with the antitrypanosomiasis, nonspecific, antipurinergic drug suramin reversed ASD-like behavior in offspring of mice treated with the polyinosinic-polycytidylic acid during pregnancy.<sup>76</sup> They concluded that this drug blocked ATP released due to the viral infection. This was not a novel theory<sup>35</sup> as it was claimed, and the natural flavonoid luteolin had previously been reported to inhibit ASD-like behavior and brain oxidative changes in the same mouse model.<sup>77</sup>

### Possible Treatment Targets

Activation of susceptibility genes is being increasingly invoked to explain ASD.<sup>78,79</sup> High risk of developing ASD has been associated with gene mutations, leading to decreased phosphatase and tensin homolog.<sup>80,81</sup> This protein is an upstream inhibitor of the mammalian target of rapamycin, which leads to microglial and mast cell proliferation<sup>82,83</sup>, as well as mast cell chemotaxis<sup>84</sup> and activation.<sup>85</sup>

Rapamycin and its analogues are mammalian target of rapamycin inhibitors<sup>86</sup> and are being tried for treatment of ASD.<sup>87-89</sup> Our preliminary results indicate that the natural flavonoid luteolin,<sup>90</sup> with potent antioxidant, free radical scavenger and antiinflammatory effects, is more potent than rapamycin that inhibits human mast cell TNF release.<sup>16</sup> Obviously, luteolin is not specific for mast cell inhibitory activity.<sup>91</sup> However, luteolin inhibits microglia IL-6 release,<sup>92,93</sup> microglial-induced hippocampal neuron apoptosis,<sup>94</sup> and also decreased cognitive decline in rats.<sup>95</sup> Luteolin inhibited mast cell-dependent stimulation of activated T cells<sup>96</sup> and activated peripheral blood mononuclear cells from patients with multiple sclerosis.<sup>97</sup> Recently, a luteolin-containing formulation was found to have considerable benefits in children with ASD.<sup>98,99</sup>

### DISCUSSION

Many recent studies stress the importance of mast cells in both innate and acquired immunity<sup>100</sup> and inflammation.<sup>62</sup> Brain mast cells can be activated by a number of neurohormonal, infectious, and environmental triggers, leading to secretion of numerous molecules that could cause brain allergy in key brain regions. Extracellular mitochondrial components could also act as innate pathogens, turning brain allergy into focal encephalitis. Detecting increased serum or CSF levels of mitochondrial DNA and/or ATP could be used for diagnosis. Preventing brain mast cell activation and secretion of TNF and extracellular mitochondrial components may serve as a novel therapeutic approach. Unfortunately, no drugs are clinically available that can block mast cell secretion. The so-called mast cell stabilizer disodium cromoglycate (cromolyn) is effective in rats<sup>101</sup> but has been recently reported not to inhibit human mast cells.<sup>102-104</sup> Luteolin may be a reasonable alternative.

Even though luteolin is not specific, it certainly inhibits microglia and mast cells, the main immune cells involved in neuroinflammation, and is more tol-

erable that nonsteroidal anti-inflammatory drugs.<sup>105</sup> Our unpublished findings indicate that a structural analogue of luteolin particularly rich in a specific natural source is more potent than luteolin with particular affinity for mast cells.

## CONCLUSIONS

Activation of brain mast cells by allergic, environmental, immune, neurohormonal, stress, and toxic triggers, especially in those areas associated with behavior and language, could lead to focal brain allergies and subsequent focal encephalitis. This possibility is more likely in the subgroup of patients with ASD with susceptibility genes that also involve mast cell activation.

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## CONFLICTS OF INTEREST

Dr. Theoharides is the inventor of US patent No. 8,268,365 for treatment of neuroinflammatory conditions, as well as US patent applications No. 12/534,571 and No. 13/009,282 for the diagnosis and treatment of ASD. Dr Theoharides is also the inventor of the dietary supplement NeuroProtek®, which has the US trademark No. 3,225,924.

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**Address correspondence to:** Theoharis C. Theoharides, MS, PhD, MD, Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111. E-mail: theoharis.theoharides@tufts.edu