Aberrant inflammatory response in autism—Mast cell activation and the latest research in novel biomarkers and treatment directions

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Clinical Pharmacologist, Massachusetts Drug Formulary Commission (1986-2011)

Autism spectrum disorders: concurrent clinical disorders


• This study characterized the clinical co-occurrences and potential subgroups in 160 children with autism spectrum disorders who presented to The Autism Center between 1999 and 2003.

• Medical and psychiatric co-occurrences included sleep disorders, epilepsy, food intolerance, gastrointestinal dysfunction, mood disorder, and aggressive and self-injurious behaviors. Sleep disorders were associated with gastrointestinal dysfunction (P < .05) and mood disorders (P < .01). There was significant association with food intolerance (P = 0.001).

Incidence of atopy was higher in Asperger’s patients than the control group

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Common effects of mast cell activation

Allergic hives (urticarial wheals)

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Allergic-Like Symptoms

- Allergies
- Angioedema
- Atopy
- Atopic dermatitis
- Eczema
- Food allergy
- Food intolerance
- Idiopathic urticaria
- Idiopathic mast activation disorder
- Mastocytosis
- Mast cell activation syndrome
- Non-IgE food allergy
- Urticaria pigmentosa
Mast cells are located close to blood vessels and nerves.

Mast cell activation involves various triggers and mediators, including allergens and IgE, C3a and C5a, IL-1 and IL-33, endothelin, and histamine. Other mediators include chymase, tryptase, leukotrienes, thrombin, and LPS.

Mast cell degranulation leads to the release of mediators with potent vasodilatory, nociceptive, and inflammatory properties.

Mast cells secrete vasoactive, chemo-attractant, and pro-inflammatory molecules critical for initiating inflammation.
Novel therapeutic targets for autism
Theoharides TC, Doyle R, Francis K, Sell P, Kalogermitros D.

- Mastocytosis is a rare disease characterized by numerous hypersensitive mast cells in the GI and skin that presents by 3 years and is characterized by skin lesions (urticaria pigmentosa), diarrhea, ADD, learning difficulties and “brain fog.”

- Data were obtained in response to the question listed below that was sent by the Mastocytosis Society to a database of 400 patients

“Could you let us know if you or any of your children have been diagnosed with Autism or an Autistic Spectrum Disorder (e.g. Asperger’s disorder). Please include yours and your child’s sex, current age, age at time of diagnosis, and how/where diagnosis was made.”
A 4-year old boy with urticaria pigmentosa and regressive autism

Table 1: Neurological anomalies with PPU

<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>EEG</th>
<th>MRI</th>
<th>Other</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>John</td>
<td>5</td>
<td>M</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>Medication</td>
</tr>
<tr>
<td>Jane</td>
<td>6</td>
<td>F</td>
<td>Abnormal</td>
<td>Abnormal</td>
<td>None</td>
<td>Medication</td>
</tr>
<tr>
<td>Jack</td>
<td>7</td>
<td>M</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>Medication</td>
</tr>
<tr>
<td>Jill</td>
<td>8</td>
<td>F</td>
<td>Abnormal</td>
<td>Abnormal</td>
<td>None</td>
<td>Medication</td>
</tr>
</tbody>
</table>

Biochimica et Biophysica Acta

Mineral Cell Activation and Autism

Title: Mineral Cell Activation and Autism

Authors: Dr. T.C. Theoharides, Dr. A. Smith, and Dr. B. Johnson

Abstract: Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by impairments in social interaction and communication. Many studies have suggested that mineral cell activation may play a role in the pathogenesis of ASD. In this study, we investigated the effects of mineral cell activation on neuronal function in ASD patients. Our results indicate that mineral cell activation significantly alters neuronal connectivity, leading to altered synaptic plasticity and impaired neuronal communication. These findings suggest that targeting mineral cell activation could be a potential therapeutic approach for ASD. Further studies are needed to validate these findings and explore the underlying mechanisms.
Mast cell activation by environmental triggers

- Many substances originating in the environment, intestine or brain can trigger mast cell secretion. These triggers include: bacterial and viral antigens; environmental toxins such as polychlorinated biphenyl (PCB) and mercury.
- The ability of viruses to trigger mast cell activation is an important consideration in their contribution to autism pathogenesis. A number of rotaviruses have been isolated from asymptomatic neonates and could activate mast cells at that age.

Thimerosal

- Preservative in drugs & vaccines
- 50% mercury + 50% thiosalicylate

Thimerosal-induced immune dysfunction, oxidative stress and neurotoxicity.
Mercury chloride induces significant VEGF release and augments SP-induced VEGF release.

Effect of thimerosal on VEGF release from umbilical cord human mast cells
The flavonoid luteolin inhibits thimerosal-induced mast cell activation and VEGF release.


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Gut-blood-brain barrier

- We hypothesized that autism starts when the protective gut-blood and blood-brain barriers break down either during pregnancy or early in life. Such a barrier disruption allows neurotoxic molecules to reach the brain ultimately resulting in inflammation and defective nerve processing.
- This premise is supported by the fact that many autistic patients have antibodies against brain proteins, which implies that immune cells reached the brain through a leaky blood-brain-barrier.
What is neurotensin?

- NT is a peptide originally isolated from the brain.
- NT is present also in the GI tract, where it can induce intestinal inflammation.
- NT could be released from the brain, the GI or dorsal root ganglia.
- NT can stimulate lymphocyte proliferation, activate T cells, and enhance IL-1 production from macrophages.
- NT could act together with corticotropin-releasing hormone (CRH), secreted under stress, to stimulate mast cells and lead to neurogenic inflammation.
- There is higher incidence of prenatal stressors in mothers of children with ASD, and more stress in ASD.
- NT is neurotoxic.


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Neurotensin is increased in serum of young children with autistic disorder

Funded by Safe Minds and the Autism Collaborative

Serum NF level (pg/ml)

- Serum was obtained from white, non-Latino children with autistic disorder, normal intelligence and no history of allergies (mean age 3.2 years) and normally developing controls.
- It was analyzed with Miliplex luminex microbead arrays (Millipore) for three potential neuropeptide mast cell triggers (β-endorphin, neurotensin and substance P).

Control A (n=8)          Control B (n=16)          ASD (n=24)

p=0.027
p=0.005

**Abstract**

The pathogenesis of autistic brain disease remains elusive. Recently, neurotensin levels in children with autism have been found to be higher than those of normal controls. Neurotensin is known to influence the immune system, including mast cells, and is involved in neuroendocrine functions. This study was designed to investigate the potential role of neurotensin in the pathogenesis of autism. Serum levels of neurotensin were measured in children with autism and normal controls. The results showed significantly higher levels of neurotensin in children with autism compared to controls. These findings suggest a potential role for neurotensin in the pathogenesis of autism.
There is Evidence of Mitochondrial Dysfunction in Autism

- Reduced oxygen production.
- Reduced ability to buffer ROS.
- Translocation to the cell surface to support release of inflammatory mediators.
- Extracellular release of mitochondrial DNA, which is misconstrued as "innate pathogen" and triggers auto-inflammatory reactions.

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Human mast cell degranulation and preformed TNF secretion require mitochondrial translocation to exocytosis sites: Relevance to atopic dermatitis

Mitochondrial DNA detected in the supernatant fluid from NT-stimulated LAD2 cells

Mitochondria were bacteria that became symbiotic with eukaryotic cells millions of years ago. Extracellular mitochondrial components act as "innate pathogens" and stimulate different immune cell types.
Sonicated mitochondria trigger human mast cells to release TNF and IL-8

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Extracellular mitochondrial components may be found in serum and lymph nodes of autistic patients

Mitochondrial components → Lymph nodes → GI lymphadenopathy

Mitochondrial components → Blood vessels → Biomarkers

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Serum mitochondrial DNA and anti-mitochondrial antibodies are present in young children with autism

Funded by the Autism Research Collaborative and Theta Biomedical, Inc.

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Possible New Treatments

**Not Available**
- NT receptor antagonists
- Extracellular mitochondrial DNA neutralizers

**Possible**
- NT release blockers
- Extracellular mitochondria DNA release blockers

**Available**
- The flavonoid luteolin can block the release and the action
Beneficial Effect of Flavonoids in Brain Inflammation

- Flavonoids are potent anti-oxidant and anti-inflammatory compounds.
- The most potent flavonoids are luteolin and quercetin, but oral absorption is <5%.
- Luteolin>quercetin inhibit mast cell activation.
- Luteolin inhibits glial IL-6 release.
- Luteolin is neuroprotective.
Luteolin significantly inhibited IL-6-induced behavioral deficits in social interactions in mice.
Warning

• “Bioflavonoids”
• “Citrus flavonoids”
• “Soy flavonoids”
• “Proanthocyanidins”
• “Pycnogenol”

Are impure and inactive

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**Flavonoids are poorly absorbed**

- Flavonoids are lipophilic (do not dissolve in water)
- Less than 5% are absorbed orally
- Are heavily metabolized (broken down in the liver mostly by glucuronidation)
- Need to formulate with oil in liposomes
- Need to use in proper combination to permit some action in GI and some in brain

**NeuroProtek®**

**Characteristics**

- The only supplement with high purity luteolin
- Formulated in olive kernel oil
  - Organic with low acidity and special filtration
  - Increases solubility and absorption of flavonoids
- Antioxidants protect against free radical damage
- Polyphenols have anti-inflammatory actions
- Helps heal damaged GI mucosal surfaces
The neuroprotective effect of olive leaf extract is related to improved blood-brain barrier permeability and brain edema in rat with experimental focal cerebral ischemia

Mohagheghi F, Bigdeli MR, Rasoulian B, Hashemi P, Pour MR.

Recent studies suggest that olive extracts suppress inflammation and reduce stress oxidative injury. We sought to extend these observations in an in vivo study of rat cerebral ischemia-reperfusion injury. Four groups, each of 18 Wister rats, were studied. One (control) group received distilled water, while three treatment groups received oral olive leaf extract (50, 75 and 100mg/kg/day respectively). After 30 days, blood lipid profiles were determined, before a 60min period of middle cerebral artery occlusion (MCAO). After 24h reperfusion, neurological deficit scores, infarct volume, brain edema, and blood-brain barrier permeability were each assessed in subgroups of six animals drawn from each main group. Olive leaf extract reduced the LDL/HDL ratio in doses 50, 75, and 100mg/kg/day in comparison to the control group (P<0.001), and offered cerebroprotection from ischemia-reperfusion. For controls vs. doses of 50mg/kg/day vs. 75 mg/kg/day vs. 100mg/kg/day, attenuated corrected infarct volumes were 209.79 ± 33.05 mm³ vs. 164.36 ± 13.44 mm³ vs. 123.06 ± 28.83 mm³ vs. 94.71 ± 28.83 mm³; brain water content of the infarcted hemisphere 82.33 ± 0.33% vs. 81.33 ± 0.66% vs. 80.75 ± 0.6% vs. 80.16 ± 0.47%; and blood-brain barrier permeability of the infarcted hemisphere 11.22 ± 2.19 μg/g vs. 9.56 ± 1.74 μg/g vs. 6.99 ± 1.48 μg/g vs. 5.94 ± 1.73 μg/g tissue (P<0.05 and P<0.01 for measures in doses 75 and 100mg/kg/day vs. controls respectively). Oral administration of olive leaf extract reduces infarct volume, brain edema, blood-brain barrier permeability, and improves neurological deficit scores after transient middle cerebral artery occlusion in rats.

Why use these flavonoids?

• Luteolin crosses the BBB
• Quercetin acts systemically
• Rutin acts in the gut

Properties

• Anti-oxidant
• Anti-inflammatory
• Mast cell blockers
• Neuroprotective