Neuroinflammation in autism and its resemblance to adult neurodegenerative disease

DEBBY HAMILTON, M.D., MPH

FINANCIAL DISCLOSURE: EMPLOYEE OF RESEARCHED NUTRITIONALS

NON CME/ 1 CE
Objectives

- Learn about the increasing prevalence of autism and neurodegenerative diseases
- Understand the chronic pathology underlying autism and neurodegenerative diseases
- Learn the connection between systemic and nervous system pathology
- Discuss the similarity of neuroinflammation between autism and adult neurodegenerative diseases and the implication of this information.
### DSM-5 Diagnosis: ASD

#### Diagnostic Criteria in DSM-5

<table>
<thead>
<tr>
<th>Severity Level for ASD</th>
<th>Social Communication</th>
<th>Restricted interests &amp; repetitive behaviors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 3 ‘Requiring very substantial support’</td>
<td>Severe deficits in verbal &amp; nonverbal social communication causing severe impairments in functioning; very limited initiation of social interactions &amp; minimal response to social overtures.</td>
<td>Preoccupations, fixated rituals and/or repetitive behaviors markedly interfere with functioning in all spheres. Marked distress when rituals or routines are interrupted; very difficult to redirect from fixed interest or returns to it quickly.</td>
</tr>
<tr>
<td>Level 2 ‘Requiring substantial support’</td>
<td>Marked deficits in verbal and nonverbal social communication; social impairments apparent even with supports; limited initiation of social interactions &amp; reduced or abnormal response to social overtures from others.</td>
<td>RRBs and/or preoccupations or fixed interests appear frequently enough to be obvious to the casual observer and interfere with functioning in a variety of contexts. Distress or frustration is apparent when RRB’s are interrupted; difficult to redirect from fixed interest.</td>
</tr>
<tr>
<td>Level 1 ‘Requiring support’</td>
<td>Without supports in place, deficits in social communication cause noticeable impairments. Difficulty initiating social interactions &amp; demonstrates atypical or unsuccessful responses to social overtures of others. May appear to have decreased interest in social interactions.</td>
<td>Rituals and repetitive behaviors (RRB’s) cause significant interference with functioning in one or more contexts. Resists attempts by others to interrupt RRB’s or to be redirected from fixed interest.</td>
</tr>
</tbody>
</table>
Prevalence of ASD


- ASD is about 4.5 times more common among boys (1 in 42) than among girls (1 in 189).

- Studies in Asia, Europe, and North America have identified individuals with ASD with an average prevalence of between 1% and 2%.

- About 1 in 6 children in the United States had a developmental disability in 2006-2008, ranging from mild disabilities such as speech and language impairments to serious developmental disabilities, such as intellectual disabilities, cerebral palsy, and autism.

- https://www.cdc.gov/ncbddd/autism/data.html
<table>
<thead>
<tr>
<th>Surveillance Year</th>
<th>Birth Year</th>
<th>Number of ADDM Sites Reporting</th>
<th>Prevalence per 1,000 Children (Range)</th>
<th>This is about 1 in X children...</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>1992</td>
<td>6</td>
<td>6.7 (4.5 – 9.9)</td>
<td>1 in 150</td>
</tr>
<tr>
<td>2002</td>
<td>1994</td>
<td>14</td>
<td>6.6 (3.3 – 10.6)</td>
<td>1 in 150</td>
</tr>
<tr>
<td>2004</td>
<td>1996</td>
<td>8</td>
<td>8.0 (4.6 – 9.8)</td>
<td>1 in 125</td>
</tr>
<tr>
<td>2006</td>
<td>1998</td>
<td>11</td>
<td>9.0 (4.2 – 12.1)</td>
<td>1 in 110</td>
</tr>
<tr>
<td>2008</td>
<td>2000</td>
<td>14</td>
<td>11.3 (4.8 – 21.2)</td>
<td>1 in 88</td>
</tr>
<tr>
<td>2010</td>
<td>2002</td>
<td>11</td>
<td>14.7 (5.7 – 21.9)</td>
<td>1 in 68</td>
</tr>
<tr>
<td>2012</td>
<td>2004</td>
<td>11</td>
<td>14.6 (8.2 – 24.6)</td>
<td>1 in 68</td>
</tr>
</tbody>
</table>
Neurodegenerative diseases

- 5 million Americans suffer from Alzheimer's disease
- 1 million from Parkinson's
- 400,000 from multiple sclerosis (MS)
- 30,000 from amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease)
- 30,000 from Huntington's

- If left unchecked 30 years from now, more than 12 million Americans will suffer from neurodegenerative diseases.

http://neurodiscovery.harvard.edu/challenge
Comparison of symptoms ASD and Alzheimer’s

<table>
<thead>
<tr>
<th>Autism</th>
<th>Alzheimer’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication problems</td>
<td>Communication problems</td>
</tr>
<tr>
<td>Social impairment</td>
<td>Social impairment</td>
</tr>
<tr>
<td>Motor processing issues</td>
<td>Motor processing issues</td>
</tr>
<tr>
<td>Cognitive impairments</td>
<td>Cognitive impairments</td>
</tr>
<tr>
<td>Repetitive speech and activities</td>
<td>Repetitive speech and activities</td>
</tr>
<tr>
<td>Memory problems</td>
<td>Memory problems</td>
</tr>
<tr>
<td>Mood and behavioral issues</td>
<td>Mood and behavioral issues</td>
</tr>
</tbody>
</table>
Common Medical issues in ASD

- Gastrointestinal issues
- Seizures
- Immune Dysfunction
- Allergies
- Autoimmune issues
- Frequent infections
- Sleep disorder
- Feeding disorder
- Nutritional Deficiency
- ADHD
- Mood disorders
Immune Deficiencies/Imbalance in Autism

- Increased allergies to food/environment
- Decreased NK cell number and function
- Low number of helper T-cells (CD4+ cells)
- Depressed T-cell responses to activation
- Low sIgA (poor mucosal immunity-GI issues)
- Autoantibodies: myelin basic protein, neuronal filament
- Maternal increase in Th17 cells with increase in IL-17A

Comparison of pathology

Autism Spectrum Disorders
- Mitochondrial Dysfunction
- Oxidative Stress
- Disrupted Gut-Brain-Microbiome Connection
- Systemic Inflammation
- Neuroinflammation

Neurodegenerative disease
- Mitochondrial Dysfunction
- Oxidative Stress
- Disrupted Gut-Brain-Microbiome Connection
- Systemic Inflammation
- Neuroinflammation
Children with autism were more likely to have mitochondrial dysfunction, mtDNA over replication, and mtDNA deletions than typically developing children.

Mitochondrial Dysfunction in Autism

- Brain imaging study looking for elevated lactate doublets as marker for mitochondrial dysfunction
- Study group: 75 children and adults with autism and 96 matched controls
- Results: ASD 13% rate of lactate doublets
  - Controls 1%  P=.001
- Primary location: cingulate gyrus
  - Secondary locations: subcortical gray matter, corpus callosum, temporal gyrus

Abnormalities of Mitochondrial Dynamics in Neurodegenerative Diseases
Ju Gao Et al. Antioxidants. 2017

Mitochondrial dysfunction has long been demonstrated as a common prominent early pathological feature of a variety of common neurodegenerative diseases, including Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington’s disease (HD).

The importance of mitochondrial dynamics in the pathogenesis of neurodegenerative diseases has been increasingly unraveled after the identification of several key fusion and fission regulators such as Drp1, OPA1, and mitofusins.
Oxidative Stress in Autism

- Research comparing anti-oxidant markers and markers of lipid peroxidation to assess oxidative stress

- Study population: 20 children with ASD
  - 25 matched healthy controls

- Results: Positive in ASD children younger than 6 yrs
  - Decreased anti-oxidant markers with SOD and GSH-PX
  - Increase in lipid peroxidation marker MDA

Oxidative stress has been implicated in the progression of Alzheimer’s disease (AD), Parkinson’s disease (PD) and other neurodegenerative diseases. Oxidative stress leading to free radical attack on neural cells contributes calamitous role to neurodegeneration. Toxicity of ROS contributes to protein misfolding, glia cell activation, mitochondrial dysfunction and subsequent cellular apoptosis.

Cycle of mitochondria and oxidative stress

- Oxidative stress causes damage to mtDNA
- Damaged mtDNA leads to decrease in coding for proteins needed for electron transport chain
- Decreased function of electron transport chain leads to decreased energy and increased free radicals (oxidative stress)
- Increased oxidative stress causes damage to the cell membrane
- Damage to the cell membrane leads to release of cytochrome C leading to apoptosis or cell death
- Cell death leads to more oxidative stress

..........And the cycle continues
gut-brain-microbiome connection
Disrupted Gut-Brain-Microbiome
Disrupted Gut-Brain-Microbiome
Disrupted Gut-Brain-Microbiome in Autism Spectrum Disorder

- 40 autistic subjects (36 out of 40 autistic subjects were classified as severe ASDs, Childhood Autism Rating Scale (CARS) value >37) and 40 neurotypical controls
- Analysis of gut microbiota
- Results:
  - *Candida* was more than double in the autistic than neurotypical subjects
  - Significant increase in the *Firmicutes/Bacteroidetes* ratio in autistic subjects due to a reduction of the *Bacteroidetes* relative abundance

Parkinson’s and gut brain connection

- Analysis of fecal microbiome in 72 patients with PD and 72 controls

- Results:
  - Decreased prevotella by 77% in PD
  - Increased Enterobacteria with increased levels associated with increased severity of postural instability and gait difficulties
  - Increased constipation in PD associated with more abnormalities in microbiome

Effect of Probiotics on Alzheimer’s disease

- 60 Patients with Alzheimer’s disease randomized to 30 patients taking probiotics versus 30 patients on placebo for 12 weeks

- Results: Patients on probiotics
  - Significant improvement in Mini-mental state exam (p<0.0001)
  - Decreased plasma malondialdehyde levels,
  - Decreased CRP
  - Decreased TG’s

Gram-negative bacterial molecules associated with Alzheimer’s disease pathology

- **Study:** 24 patients with Alzheimer’s versus 18 control patients evaluated for brain pathology
- **Results:**
  - All brain samples were positive for LPS and E Coli K99
  - Increase in E Coli K99 in Alzheimer’s brains
  - Increase in LPS in Alzheimer’s vs. controls
  - LPS colocalized with AB amyloid plaques and around blood vessels
Connection between Systemic inflammation and Neuroinflammation in ASD

- Comparison of serum markers of inflammation: IL-1B, IL-6, IL-10, and endotoxins
- 22 adult patients with severe autism
- 28 control patients
- Results:
  - Higher endotoxin levels in adults with autism P<0.001
  - Higher IL-1B and IL-6  p<0.05
- Higher endotoxin levels associated with worse socialization markers in adults with autism

Systemic Inflammation in Neurodegenerative diseases

Study: 300 patients with Alzheimer’s
- Cognitive assessment, serum inflammatory markers and document with caregiver all systemic inflammatory events

Results:
- Acute systemic inflammatory events correlated with 2 times cognitive decline
- Increase in TNF-alpha in serum
- High baseline TNF-alpha: 4 time rate of increase rate of cognitive decline

Figure 2 Rate of cognitive decline at 6 months by presence of incident systemic inflammatory events and baseline serum TNF-α levels. ADAS-COG = Alzheimer’s Disease Assessment Scale–Cognitive subscale; TNF-α = tumor necrosis factor α; SIE = systemic inflammatory events.
Microglia and neurodegeneration: The role of systemic inflammation
Neuroinflammation: The Brain on Fire
Neuroglia: Neuron Support Cells

- Oligodendrocytes: produce myelin
- Microglia: immune macrophages in the nervous system
- Astrocytes: Regulators of neuronal growth and survival
Microglia: Functions

- Phagocytosis
- Synaptic Pruning
- Development of CNS
- Maintenance of CNS cells
- Instigate inflammation
- Repair and regeneration in CNS inflammation
Activated Microglia: Cycle of inflammation and repair

1. Rapid proliferation of microglial cells
2. Migrate to site of insult or infection
3. M1 activated microglia: neurotoxic with release of pro-inflammatory cytokines including TNF-alpha, IL-1B, IL-6, COX, Reactive oxygen species (ROS), Nitric oxide
4. Engulf dying cells, infectious agents, toxic proteins, and cell debris
5. M2 activated microglia: secrete anti-inflammatory cytokines for repair including: IL-10, TGF-B, enzymes to inhibit ROS production (arginase), proteins to maintain extracellular matrix
Astrocytes: Functions

- Induce formation of neuronal synapses
- Formation and maintenance of BBB
- Neurotransmission: component of tripartite synapse model
- Homeostasis and turnover of glutamate
- Metabolic regulation
- Ion balance maintenance
- Key role in development of the nervous system

- A: normal cerebellum in control patient
- B-C: atrophic cerebellum in a patient with autism with loss of Purkinje and granular cells
- D-K: activated microglial and inflammatory cells

- **A**: Western Blot Image of anti-NF-kB p65 Ab’s
- **B**: Relative expression of NF-KB p65 subunit
- **C**: Image of fractionated samples probed with anti-NF-KB p65
Neuroinflammation in ASD
Summary of Neuroinflammation markers in ASD

- Microglial activation
- Astrocytic activation with elevated levels of GFAP (glial fibrillary acidic protein)
- Proinflammatory profile of cytokines in the brain, CSF and blood
- Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation
<table>
<thead>
<tr>
<th>Studies</th>
<th>N Case/Control</th>
<th>Findings</th>
<th>Researchers' Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vargas et al., 2005</td>
<td>15/12</td>
<td>(1) Marked activation of microglia and astroglia, and cytokine profiling indicated that macrophage chemotactic protein (MCP)-1 and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues. (2) CSF showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1.</td>
<td>Active neuroinflammatory process in those with an ASD diagnosis.</td>
</tr>
<tr>
<td>Li et al., 2009</td>
<td>8/6</td>
<td>Proinflammatory cytokines (TNF-alpha, IL-6, and GM-CSF), Th1 cytokine (IFN-gamma) and chemokine (IL-8) were significantly increased in the brains of ASD patients compared with the controls.</td>
<td>Brain inflammation in those with an ASD diagnosis and autoimmune disorder.</td>
</tr>
<tr>
<td>Young et al., 2011</td>
<td>9/9</td>
<td>Neurons, astrocytes, and microglia all demonstrated increased extranuclear and nuclear translocated NF-kB p65 expression in brain tissue from ASD donors relative to samples from matched controls.</td>
<td>Part of a putative molecular cascade leading to brain inflammation.</td>
</tr>
<tr>
<td>Morgan et al., 2010</td>
<td>13/9</td>
<td>Microglial activation and increased microglial density in the dorsolateral prefrontal cortex in those with autism.</td>
<td>Neuro-pathological alteration and brain inflammation.</td>
</tr>
<tr>
<td>Morgan et al., 2012</td>
<td>13/9</td>
<td>Microglia are more frequently present near neurons in the autism cases at a distance interval of 25 μm, as well as 75 and 100 μm.</td>
<td>Aberrantly close microglia-neuron association in the ASD disorder.</td>
</tr>
<tr>
<td>Wei et al., 2011</td>
<td>6/6</td>
<td>Interleukin (IL)-6 increased in the cerebellum of autistic subjects.</td>
<td>Localized inflammation of the central nervous system.</td>
</tr>
<tr>
<td>Tetreault et al., 2012</td>
<td>11/12</td>
<td>Individuals with autism had significantly more microglia compared to controls in the fronto-insular and visual cortex.</td>
<td>The brain’s immune cells (microglia) are probably involved in the ASD disorder.</td>
</tr>
<tr>
<td>Suzuki et al., 2013</td>
<td>20/20</td>
<td>Excessive microglial activation in multiple brain regions in young adult subjects with an ASD diagnosis was found using regional brain <a href="R">11C</a>-PK11195 binding potential as a representative measure of microglial activation.</td>
<td>Augmented but not altered microglial activation (brain immune-cell activation), which is indicative of pro-inflammatory processes in the brain.</td>
</tr>
<tr>
<td>Fatemi et al., 2008</td>
<td>24/22</td>
<td>The levels of recognized indicators of inflammatory processes in brain tissue, including Aquaporin 4 and Connexin 43, were examined in the brains of those with an autism diagnosis. The study found, in contrast to controls, in evaluations using the brain’s β-actin level as a reference, Aquaporin 4 expression was decreased significantly in cerebellum, while, in Brodmann’s area 9 (superior frontal cortex), Connexin 43 was elevated in the brains of those diagnosed with autism.</td>
<td>Inflammatory processes in ASD.</td>
</tr>
<tr>
<td>Chez et al., 2007</td>
<td>10</td>
<td>Elevation of cerebrospinal fluid levels of TNF-α was significantly higher (mean = 104.10 pg/mL) than concurrent serum levels (mean = 2.78 pg/mL).</td>
<td>Indicative of CNS inflammatory mechanisms.</td>
</tr>
<tr>
<td>Laurence and Fatemi, 2005</td>
<td>3</td>
<td>Elevated levels of GFAP in the frontal, parietal, and cerebellar cortices using age-matched autism and control post-mortem brain specimens.</td>
<td>Indicative of microglial and astroglial activation.</td>
</tr>
<tr>
<td>Rosengren et al., 1992</td>
<td>47/13</td>
<td>GFAP levels in CSF in children with autism were higher than those in normal control children.</td>
<td>Increased GFAP levels signify gliosis, reactive injury in those with an ASD diagnosis.</td>
</tr>
<tr>
<td>Ahisen et al., 1993</td>
<td>47/25</td>
<td>Average levels of GFAP in the CSF of children with autism three times higher than control group.</td>
<td>Indicate reactive astrogliosis in the CNS.</td>
</tr>
<tr>
<td>Bailey et al., 1998</td>
<td>6/8</td>
<td>Cerebellum in autism showed an increase in GFAP.</td>
<td>Reactive gliosis.</td>
</tr>
<tr>
<td>López-Hurtado and Prieto, 2006</td>
<td>8/7</td>
<td>The mean density of glial cells was greater in the autistic cohort than controls in area 22 (p &lt; 0.001), area 39 (p &lt; 0.01), and area 44 (p &lt; 0.05).</td>
<td>Results are consistent with accelerated neuronal death in association with gliosis and lipofuscin accumulation.</td>
</tr>
<tr>
<td>Rose et al., 2012</td>
<td>12/12</td>
<td>3-choleotyrosine (3-CT; an established biomarker of a chronic inflammatory response) significantly increased in autism cerebellum and BA22.</td>
<td>Chronic inflammatory response.</td>
</tr>
<tr>
<td>Crawford et al., 2015</td>
<td>14/14</td>
<td>Levels of GFAP immunoreactivity were significantly elevated (P = 0.008) in anterior cingulate cortex (Brodmann area 24; BA24) white matter of ASD compared to controls.</td>
<td>Activation of white matter astrocytes in the anterior cingulate cortex as a result of a yet undefined cellular insult.</td>
</tr>
</tbody>
</table>
Alzheimer’s Neuroinflammation

- Accumulation of protein aggregates
  - Extracellular: B-amyloid plaques
  - Intracellular: Neurofibrillary tangles (NFT)
  - Cause loss of synaptic function leading to neuronal death
- Microglial activation
- Astrocyte activation
- Pro-inflammatory cytokines near B-amyloid protein deposits and NFT
Brain inflammation accompanies amyloid in early cognitive impairment

- **Study:** 42 patients with mild cognitive impairment, 22 controls

- **Results from PET scans**
  - 62% (26 out of 42) patients with mild cognitive impairment: increased cortical amyloid deposition
  - 85% (22 out of 26) patients with increased amyloid had clusters of activated microglial cells by the amyloid

Parkinson’s Neuroinflammation

- Loss of dopamine neurons in the substantia nigra
- Alpha-synuclein (Lewy body) protein inclusions in the nervous system
- Microglial activation
- Increase in pro-inflammatory cytokines

Neuroinflammation in ALS

- Degeneration of corticospinal motor neurons (upper motor neurons) and spinal motor neurons
- Microglial activation
- Astrocyte activation
- Increase chemokine MCP1 (monocyte chemoattractant protein 1) in CSF, plasma, spinal cord and motor neurons in ALS

Hope
Treatment

- Mitochondrial Dysfunction
- Oxidative Stress
- Disrupted Gut-Brain-Microbiome Connection
- Systemic Inflammation
- Neuroinflammation
Support for mitochondrial dysfunction and oxidative stress

- Repair mitochondrial membrane
- Phospholipids
Support for mitochondrial dysfunction and oxidative stress

- Repair mitochondrial membrane
  - Phospholipids
- Support mitochondrial function
  - CoQ10/Ubiquinol, Carnitine, NADH, B vitamins, Alpha-Lipoic Acid, Vitamin E
- Anti-oxidants to combat oxidative stress
  - Molecular Hydrogen (H₂: Hydrogen gas)
  - Vitamin E: mixed tocopherols and tocotrienols
  - Curcumin
  - Tea: EGCG
  - Resveratrol
  - Glutathione
  - N-acetyl-cysteine
Disrupting Neurodegeneration
although the clinical manifestations of neurodegenerative diseases are distinct, these disorders share similar molecular mechanisms of neurodegeneration, which could be counteracted by common treatment with Nrf2 activators.

Nrf2: Nuclear Factor 2

- Master regulator of the antioxidant system
- Mechanism: A Nrf2 activator releases protein into the cell nucleus where it binds to DNA and activates anti-oxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase and these enzymes can neutralize up to 1 million free radicals
Target Nrf2
Natural Nrf2 Activators

- Curcumin
- Resveratrol
- Sulforaphane
- Flavanols: tea (EGCG), chocolate
- Vitamin D
- Coffee
- Molecular hydrogen
**Summary**

1.) Anti-oxidant for dangerous hydroxyl radical without destroying free radicals needed for metabolism

2.) Activates Nrf2 anti-oxidant cascade including glutathione peroxidase, catalase, and superoxide dismutase (SOD)

3.) Decreases pro-inflammatory cytokines through cell signaling

4.) Promotes mitochondrial ATP energy function

1. Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine, Ohta S., Pharmacology & Therapeutics, Volume 144, Issue 1, October 2014, Pages 1–11

2. Clinical Effects of Hydrogen Administration: From Animal and Human Diseases to Exercise Medicine, Nicolson G. et al., International Journal of Clinical Medicine, 2018, 7, 32-76
Implications for future of children with autism spectrum disorders

One research study:

- Transcranial magnetic stimulation to check for hyperplasticity in Asperger’s adults with normal cognitive capability
- Decrease plasticity associated with Alzheimer’s
- Found increased plasticity in Asperger’s adults so hypothesis that people with autism would be protected

Down's syndrome (DS), which is characterized by premature aging, that there is enhanced oxidative stress resulting from the aberrant expression of CuZn superoxide dismutase (CuZn SOD).

DS cells are impaired in their ability to repair oxidative damage to mtDNA compared to age-matched control cells.

Defective repair of oxidative damage in mitochondrial DNA in Down's syndrome.

Druzhyna N. Et al. Mutation Research
Implications for future of children with autism spectrum disorders?
References: Neuropathology

- Gutierrez EG. et al. Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. *J. Neuroimmunol.* 47, 169-176
References: Neuropathology

References for Molecular Hydrogen

- Molecular hydrogen foundation: www.molecularhydrogenfoundation.com
References for Anti-oxidants


- Dominiak K. et al. Critical need for clinical trials: an example of a pilot human intervention trial of a mixture of natural agents protecting lymphocytes against TNF-alpha induced activation of NF-kB. *Pharm Res*. 2010 June;27(6)


References for Anti-oxidants

References for Anti-oxidants


