ASD: NEUROINFLAMMATION -- OR IMMUNE DYSREGULATION?

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OUTLINE

• Review immune findings in Autism Spectrum Disorder
  • Immunogenetics: gene expression profiles, HLA
  • Maternal and child autoantibodies: causes or effects?
  • Microglial activation: neurotoxic or neuroprotective?
  • Neuroglial functions: a broader perspective
• Prenatal insults: reprogramming -- arrested development?
• Immunomodulatory therapies -- Rx at which level?
• Cellular metabolic and synaptic dysfunction: the “fever effect”

HLA-DR4 in Families With Autism
Li-Ching Lee, PhD, Andrea A. Zachary, PhD, Mary S. Lefler, PhD,
Craig J. Neuschaffer, PhD, Karla J. Matteo, PhD, John D. Tyler, PhD, and
Andrew W. Zimmerman, MD

Pediatric Neurology 2006;35:303-307

Odds Ratio (95% C.I.)

<table>
<thead>
<tr>
<th>DR4 - Tennessee Mothers</th>
<th>3.54 (1.74, 6.47)</th>
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<tbody>
<tr>
<td>Fathers</td>
<td>1.57 (0.44, 5.42)</td>
</tr>
<tr>
<td>Children</td>
<td>4.20 (1.37, 13.27)</td>
</tr>
</tbody>
</table>

AGRE (USA) Mothers: 1.10 (0.47, 2.49)
Fathers: 0.94 (0.40, 2.20)
Children: 0.94 (0.40, 2.20)

Cytokines-plasma of children

<table>
<thead>
<tr>
<th>Study Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated levels of IL-1b, IL-6, IL-8 and IL-12p40. Associated with regression.</td>
<td>Ashwood et al., 2011b</td>
</tr>
<tr>
<td>Increase in chemokine MCP-1, Rantes and Eotaxin levels in ASD subjects compared to age-matched typically developing controls. An association between increases chemokines levels with aberrant behaviors.</td>
<td>Ashwood et al., 2011c</td>
</tr>
<tr>
<td>In male ASD subjects, an increase in cytokines IL-1beta, IL-1RA, IL-5, IL-8, IL-12(p70), IL-13, IL-17 and GRO-alpha.</td>
<td>Suzuki et al., 2011</td>
</tr>
<tr>
<td>Increase in leptin levels in ASD subjects compared to age-matched controls.</td>
<td>Ashwood et al., 2008b</td>
</tr>
<tr>
<td>Increase in macrophage migration inhibitory factor (MIF) in ASD subjects compared to age-matched controls.</td>
<td>Grigorova et al., 2008</td>
</tr>
<tr>
<td>Decrease in TGF-beta in subjects with ASD compared to controls.</td>
<td>Ashwood et al., 2008a; Okada et al., 2007</td>
</tr>
<tr>
<td>Increase in IL-12 and IFN-gamma in ASD subjects compared to age-matched controls.</td>
<td>Singh, 1996</td>
</tr>
</tbody>
</table>
**Cytokine/Chemokines- activated cells**

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<tbody>
<tr>
<td>In isolated PBMCs stimulated with PHA, increase in GM-CSF, TNF-alpha and IL-13. A decrease in IL-12p40 in ASD subjects vs. controls.</td>
<td>Ashwood et al., 2011d</td>
</tr>
<tr>
<td>Stimulation of TLR on monocytes - ASD vs. age-matched controls. Increase in IL-1beta, IL-6, TNF-alpha, with stimulation of TLR4. Increase in IL-1beta, IL-6, GMCSF, TNF-alpha with TLR9.</td>
<td>(Enstrom et al., 2010)</td>
</tr>
<tr>
<td>Increase in IFN gamma in NK cells from subjects with ASD.</td>
<td>(Enstrom et al., 2009b)</td>
</tr>
<tr>
<td>Increase production of cytokines from Th1 and Th2 cytokines in ASD subjects vs age-matched controls.</td>
<td>(Molloy et al., 2006)</td>
</tr>
<tr>
<td>Increase in IL-12 and TNF-alpha in ASD subject with GI symptoms.</td>
<td>(Crosby et al., 2005)</td>
</tr>
<tr>
<td>Increase in IFN gamma and TNF alpha in isolated PBMCs from ASD subjects compared to age-matched controls stimulated with LPS.</td>
<td>(Crosby et al., 2002)</td>
</tr>
<tr>
<td>Unstimulated whole blood from ASD vs. age-matched controls – Increase in IFN-gamma and IL-1RA with TLR9.</td>
<td>(Crosby et al., 2002)</td>
</tr>
<tr>
<td>Unstimulated PBMC: ASD subjects: higher levels of TNF-alpha, IL-1beta, and IL-6 vs. controls. PBMCs stimulated with LPS, PHA and IFN-gamma produced increases in IL-12 and IL-1beta.</td>
<td>(Crosby et al., 2002)</td>
</tr>
</tbody>
</table>

**T cells in Autism**

- When peripheral blood T cells were stimulated, GM-CSF, TNFα, and IL-13 were significantly increased whereas IL-12p40 was decreased in ASD relative to TD controls.
- Increased pro-inflammatory or TH1 cytokines were associated with greater impairments in core features of ASD as well as aberrant behaviors.
- In contrast, production of GM-CSF and TH2 cytokines were associated with better cognitive and adaptive function.

Autoantibodies in Children

- Intense Golgi cell staining in ~21% of patients with ASD compared with 0% of normal controls (BBI, 2008). This staining pattern correlates with a ~52 kDa protein by blot (Wills et al, 2008).

- In addition to antibodies against the cerebellum, there is intense reactivity to proteins in the thalamus and hypothalamus (Cabanlit et al, ANYAS, 2007).
The glial perspective of autism spectrum disorders

Fares Zeniæ-Chulià,1,2,3 Alla B. Salmila,4 Natália A. Melnivskaya,4 Mami Noda,4 Alois Werthenski,2,3,5 José Claudio Fonseca Moreira,6

Genes
Immune System
Cytokines
TNFα
Microglial Activation
GI Tract
CNS

Neuroinflammation

Beneficial
Neither?
Harmful

Normal Development
**MICROGLIAL COLONIZATION OF THE HUMAN CEREBRAL CORTEX**

Rezaie P & Male D: Microscopy Res Tech 1999;45:359

rcv = radiating cortical vessel
rg = radial glial astrocytes
m = neuronal progenitors
mp = cortical plate

**IMMUNE DYSREGULATION IN ASD**

Hsiao, EY: Int Rev Neurobiol 2013;113:269

**IMMUNOMODULATION?**

- IVIG
- Prednisone
- Minocycline
- Pioglitazone
- Infliximab
- Experimental models?
  - VPA
  - fraX mice
  - Bone marrow transplant
  - Stem cells

**Fever in Children with Autism**

30 children with autism, with fever;
30 age-matched with autism, afebrile.

Overall, 83% of children improved on at least one domain of the A.B.C. during fever: irritability, stereotypy, hyperactivity, and inappropriate speech.

Curran LX et al, Pediatrics 2007

**Results: Mean ABC Subscale Scores and RM-ANOVA Findings (n=30 pairs)**
Biochemical and Metabolic Abnormalities in ASD Compared to Neurotypical Children

- Oxidative Stress Increased and Antioxidant Enzymes Lower
- Total Glutathione Levels are Lower
- Ratio of Oxidized to Reduced Glutathione Levels is Higher
- Capacity for Reducing Glutathione is Impaired
- Enhanced Lipid Peroxidation
- Increased Neuroinflammation in Selected Brain Regions
  Elevated NO Synthesis
- Impaired Mitochondrial Function and Energy Generation
- Nrf2 Levels are Depressed and Genes under its Control are Lower

Prenatal Infection!

Mitochondria!

- Antioxidant response
- Immune response

Metabolism of Granulocytes in Children with ASD

<table>
<thead>
<tr>
<th></th>
<th>ASD</th>
<th>Neurotypical</th>
<th>p</th>
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<tbody>
<tr>
<td>Resting O₂ Uptake</td>
<td>0.38±0.09</td>
<td>1.1±0.3</td>
<td>.05</td>
</tr>
<tr>
<td>NADH Oxidase</td>
<td>4.6±3.1</td>
<td>10.9±4.9</td>
<td>.01</td>
</tr>
<tr>
<td>Succinate Oxidase</td>
<td>1.6±1.2</td>
<td>5.2±2.0</td>
<td>.001</td>
</tr>
<tr>
<td>ATPase</td>
<td>46±28</td>
<td>123±54</td>
<td>.005</td>
</tr>
<tr>
<td>Nrf2 Gene Express Level</td>
<td>0.45±0.01</td>
<td>1.00±0.03</td>
<td>.01</td>
</tr>
</tbody>
</table>

[Nmold x (min x mg protein)]

[Napoli et al, Pediatrics 2014]
POSSIBLE CAUSES OF NEUROINFLAMMATION AND IMMUNE DYSREGULATION IN AUTISM

A. EXOGENOUS: e.g., infections, valproate, “environmental factors”

B. AUTOIMMUNE ACTIVATION

C. ALTERED (IMMUNE) GENE EXPRESSION AND BRAIN DEVELOPMENT

D. CELLULAR METABOLIC DYSFUNCTION

E. All of the above.

COLLABORATORS

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