Immune system and Autism Spectrum Disorders

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IACC WORKSHOP 2014:
Immune Disorders

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Disclosures:
Conflict of Interest: None

Research Support:
- The Bart McLean Fund for Neuroimmunology Research
- Peter Emch Fund for Autism Research
- Cure Autism Now/Autism Speaks
- National Institutes of Health, National Institutes of Mental Health (HHSN271200700179)
Gene-Environment Interaction: Etiologic Model of Autism Spectrum Disorders
THE FIRST QUESTIONS:

- What is the level of evidence for immune system involvement in ASD?
- Is there evidence for a common biologic cause of the co-morbidity?
- What are the autism-specific features that affect proper diagnosis and treatment of the co-morbidity?
Homeostasis

INNATE IMMUNITY

Monocyte/macrophage

Activated macrophage

Genetic factors
Trauma
Infections
Malignancy
Autoimmunity
Metabolic others

Highly-specific Response

Adaptive Immunity

APC

T-cell

CD4+ Lymphocyte

B Lymphocyte

T Lymphocyte

Macrophage activation

Antibody production

Cytotoxicity

Non-specific Response
The Neuroimmune System

CENTRAL NERVOUS SYSTEM

Innate immunity
Microglia & astroglia

Adaptive immunity
Specific T cell and antibody responses

Modulation of immune responses
Lymphocyte/monocyte trafficking

Blood Brain Barrier

Astroglia

Neuron

Microglia

Perivascular macrophage

Cytokines
Chemokines
Astroglia

Microglia

Neuron

Perivascular macrophage

B Cell or Plasma cell

Neuroimmune Cross-Talk

IL-1, IL-5, IL-10, and IL-15

CRH Receptor

ACTH Receptor

Cytokines IL-1, IL-6, TNF-α

ICAM-1

BBB

Cytokine Receptors

CRH

GABA(A)-R

Glutamate Receptor

5-HT Receptor

GABA

Glutamate

Serotonin

ACh

Opioid Receptor

Cytokines IL-1, IL-6, TNF-α

ACh Receptor

GABA(A)-R

IFN-α

IFN-α

Glutamate

T-Cell

Monocyte/Macrophage

Dendritic Cell
Key Questions:
Are immune or neuroimmune mechanisms involved in pathogenesis of ASD? What is the evidence?

Adaptive Immunity
- Lymphocyte and Antibody production

Innate Immunity
- Microglia & astroglia activation

NEURODEVELOPMENT
LEARNING AND ADAPTIVE BRAIN FUNCTION
Evidence for immune dysfunction in autism from many avenues

- Immune-genetics
- Brain and CNS Immunity
- Systemic Immunity- Cells and antibodies
- Animal Models
Question:
Are maternal immune mechanisms involved in pathogenesis of ASD?

Adaptive Immunity
- Lymphocyte and Antibody production

Innate Immunity
- Microglia & astroglia activation

Maternal Infection

Auto-immunity
**Neurobiological Trajectories**
- Neuronal migration, cortical & neuronal network organization

**Developmental immune factors**
- Radial Glia & Microglia Modeling Function
- Developmental Cytokine/Chemokine Pathways
- Developmental TLRs and MMPs function
- Developmental complement function

**Developmental Synaptic Plasticity**

**Neuroimmune Factors**
- Neuronal-glial interactions
- Cytokine networks
- Cytokine/neuroglial & neurotransmitter interaction

**Adaptive Synaptic Plasticity**

**NEUROBEHAVIORAL TRAJECTORIES**
- Language & Communication
- Sociability Behavior

**Intra-uterine Brain Development**
- 1st trimester
- 2nd trimester
- 3rd trimester
- First year

**Postnatal brain development**
- Childhood
- Adulthood

**Brain maturation**

**Brain adaptation**

**Critical Pathogenic Period**

**Adaptation Period**

**Genetic Influences**

**Immune & Environmental Factors in Pathogenesis of ASD**
- Neurotoxins
- Maternal Immunity
- Maternal Infection
- Infections
- Neurotoxins
- Host immunity
- Stress

**NEUROBIOLOGICAL TRAJECTORIES**

**NEUROBEHAVIORAL TRAJECTORIES**
What are the known immune mediated factors in ASD pathogenesis?

“Maternal autoimmunity in autism”
Judy Van de Water et. Al.
University of California at Davis,
Davis, California
Maternal Autoantibody Related (MAR) Autism

Autoantibodies present in the circulation of mothers during pregnancy that recognize proteins in the developing fetal brain.
Maternal Anti-Brain Antibodies and ASD: The studies behind the novel immune biomarker for autism risk

**Epidemiology**
Large population studies to identify potential risk factors for ASD

**Initial finding: Immunology**
Some mothers who have children with autism produce anti-brain antibodies

**Genetics**
MET genetic variant associated with production of the anti-brain antibodies

**MRI**
Enlarged brain volume in male children prenatally exposed to the antibodies

**Basic Science**
The antigenic targets of the antibodies have been identified

**Animal Models**
Animal models (2 monkey, 2 mouse) show behavioral changes after prenatal exposure to the antibodies; monkey model has also reported increased brain volume

**Brain Tissue Studies**
Animal models provide tissue to explore brain pathology (ongoing)

**Translational Potential:**
- Identify kids with this subphenotype and develop tailored behavioral treatment
- Screen women at risk and develop preventative strategies
- Define pathophysiology associated with these antibodies and develop therapeutic interventions
We Have Identified 7 Maternal Antibodies That Bind to Protein Targets Critical to Normal Brain Development*


- **YBX-1**: Neural tube formation, cell division
- **CRMP1**: Cell migration
- **STIP1**: Neuritogenesis
- **Cypin (GDA)**: Dendritic branching
- **CRMP2**: Axon outgrowth, growth cone collapse, basal dendrite patterning
- **LDH**: Metabolism
- **YBX-1**: Transcription regulation

*Neurodevelopment*
### Specific MAR Antigens

<table>
<thead>
<tr>
<th># of Antigens</th>
<th>Antigen</th>
<th>% ASD (N = 241)</th>
<th>N</th>
<th>% TD (N = 147)</th>
<th>N</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>LDH + CRMP2</td>
<td>7%</td>
<td>17</td>
<td>0%</td>
<td>0</td>
<td>0.0004</td>
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<tr>
<td>2</td>
<td>STIP1 + CRMP2</td>
<td>10%</td>
<td>24</td>
<td>1%</td>
<td>1</td>
<td>0.0001</td>
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<tr>
<td>2</td>
<td>CRMP1 + CRMP2</td>
<td>7%</td>
<td>16</td>
<td>0%</td>
<td>0</td>
<td>0.0008</td>
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<tr>
<td>3</td>
<td>LDH + STIP1 + CRMP1</td>
<td>5%</td>
<td>12</td>
<td>0%</td>
<td>0</td>
<td>0.0045</td>
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<td>Cypin + YBX1 + CRMP1</td>
<td>2%</td>
<td>5</td>
<td>0%</td>
<td>0</td>
<td>0.1615</td>
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<tr>
<td>3</td>
<td>Cypin + STIP1 + CRMP1</td>
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<td>18</td>
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<tr>
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<td>Cypin + STIP1 + CRMP2</td>
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<td>0</td>
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<tr>
<td>3</td>
<td>YBX1 + STIP1 + CRMP2</td>
<td>3%</td>
<td>7</td>
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<td>0</td>
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<tr>
<td>4</td>
<td>LDH + Cypin + YBX1 + STIP1</td>
<td>2%</td>
<td>5</td>
<td>0%</td>
<td>0</td>
<td>0.1615</td>
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<tr>
<td>4</td>
<td>LDH + Cypin + STIP1 + CRMP1</td>
<td>2%</td>
<td>5</td>
<td>0%</td>
<td>0</td>
<td>0.1615</td>
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</tbody>
</table>

Several Ab combinations are only found in mothers of ASD children.

All specific combinations combined identify an association of MAR antibodies in ~23% of mothers with children on the ASD spectrum.
What are the effects of these specific maternal autoantibodies?

- Do they have pathologic significance?
- Do they affect how the brain develops?
Studied 181 2-4 YO male children (131 ASD, 50 typically developing (TD) controls) and evaluated total brain volume using structural magnetic resonance imaging (MRI).

The ASD MAR group exhibited a more extreme 12.1% abnormal brain enlargement relative to TD controls.

The remaining ASD children had a smaller 4.4% abnormal brain enlargement relative to TD controls.

Lobar and tissue type analyses revealed that the frontal lobe is selectively enlarged.

MAR autoantibodies may impact brain development leading to abnormal enlargement.
What is the pathology associated with these fetal brain specific auto-antibodies?

– Several passive transfer studies in mice and monkeys show evidence of MAR effects on behavior.

– Our first monkey model pilot study demonstrated behavioral changes in offspring following passive transfer of maternal IgG. Martin, L. A., et al 2008

– Our second monkey study by Bauman et al, Translational Psychiatry 2013, also demonstrated clear differences in social interaction.


– Increase in spontaneous grooming behaviors in response to a novel environment in mice exposed intraventricularly to MAR IgG. Comacho et al, Brain behav res. 2014.
**Synaptic Plasticity**
- Synaptic pruning
- Neurotransmitter homeostasis
- Synaptic stripping

**CRITICAL PATHOGENIC PERIOD**

<table>
<thead>
<tr>
<th>Intra-uterine Brain Development</th>
<th>Postnatal brain development</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st trimester</td>
<td>First year</td>
</tr>
<tr>
<td>2nd trimester</td>
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<tr>
<td>3rd trimester</td>
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</table>

**ADAPTATION PERIOD**

<table>
<thead>
<tr>
<th>Brain maturation</th>
<th>Brain adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childhood</td>
<td>Adulthood</td>
</tr>
</tbody>
</table>

**Microglia Astrocytes Neuronal-neuroglia interaction Cytokine/chemokine pathways**
HOW TO STUDY NEURAL AND IMMUNE INTERACTIONS IN ASD?

BRAIN

CSF

Cellular Immune Responses

Cytokines/chemokines networks

Immune & Neurotrophic growth factors

IMMUNITY

BLOOD

Brain Imaging

Neuropathology
Neuroglial Activation and Neuroinflammation in the Brain of Patients with Autism

Diana L. Vargas, MD,1,2 Caterina Nasicmbene, MD,1–3 Chitra Krishnan, MHS,1 Andrew W. Zimmerman, MD,1,4 and Carlos A. Pardo, MD1,2,5

Ann Neurol 2005;57:67–81

Microglial Activation and Increased Microglial Density Observed in the Dorsolateral Prefrontal Cortex in Autism

John T. Morgan, Gursharan Chana, Carlos A. Pardo, Cristian Achim, Katerina Semendeferi, Jody Buckwalter, Eric Courchesne, and Ian P. Everall

BIOL PSYCHIATRY 2010;68:368–376

Transcriptomic analysis of autistic brain reveals convergent molecular pathology

Irina Voineagu1, Xinchen Wang2, Patrick Johnston3, Jennifer K. Lowe1, Yuan Tian1, Steve Horvath4, Jonathan Mill5, Rita M. Cantor4, Benjamin J. Blencowe5 & Daniel H. Geschwind1,4

380 | Nature | Vol 474 | 16 June 2011
Assessment of Neuroglial reactions: Quantification of microglia and astroglia activation

11 cases of autism (age 5-44)
12 controls
Brain regions: Frontal (MFG), cingulate (ACG), and cerebellum

Vargas DL et al. Ann Neurol 2005
Microglia cell density increases in the cerebral cortex in ASD

Morgan JT et al. Biological Psychiatry 2010
Autism: Profiles of Cytokine/Chemokine in the Brain

Vargas DL et al. Ann Neurol 2005
Brain Cytokine-Chemokine-Growth Factors in ASD

Based on Vargas DL et al. Ann Neurol 2005

- CCL-2 (MCP-1)
- IL-6
- TGF-β1
- IGFBP-1
Transcriptomic analysis of autistic brain reveals convergent molecular pathology

Irina Voineagu¹, Xinchen Wang², Patrick Johnston³, Jennifer K. Lowe¹, Yuan Tian¹, Steve Horvath⁴, Jonathan Mill³, Rita M. Cantor⁴, Benjamin J. Blencowe² & Daniel H. Geschwind¹,⁴

380 | NATURE | VOL 474 | 16 JUNE 2011

(a) Autistic M12 Controls
(b) Gene ontology category
1 10⁻³ 10⁻⁴ 10⁻⁵
Gene ontology category
Synapsin
Neurotransmitter transport
Clathrin-coated vesicle
Neuron projection
Alternative splicing
Synaptic vesicle
Axon

(c) Enrichment P value
(d) Autistic M16 Controls
(f) Gene ontology category
Immune response
Response to wounding
Integrin complex
Regulation of cell proliferation
Antigen processing and presentation
Inflammatory response

Differential expression P value

SLC17A6
KCNS1
KCNA5
CPLX2
NEFL
LUZP1
ZADH2
MET
NAAG
ZNF659
EPHN2
SLC6A7
ANK1
PRICLE1
IGSF4B
RYR2
ZBTB16
H2AFY2
MATK
SEMA3C
Microglial Activation in Young Adults With Autism Spectrum Disorder

Katsuaki Suzuki, MD, PhD; Genichi Sugihara, MD, PhD; Yasuomi Ouchi, MD, PhD; Kazuhiko Nakamura, MD, PhD; Masami Futatsubashi, BS; Kiyokazu Takebayashi, MD, PhD; Yuijiro Yoshihara, MD, PhD; Kei Omata, PhD; Kaori Matsumoto, MA; Kenji J. Tsuchiya, MD, PhD; Yasuhide Iwata, MD, PhD; Masatsugu Tsurii, MA; Toshirou Sugiyama, MD, PhD; Norio Mori, MD, PhD
Neuroinflammatory responses in the brain in ASD: what means as co-morbidity?

The current evidence:
- Increased innate immunity responses in the brain
  - Microglial & astroglial responses
  - Cytokine & chemokine increases or disregulation
- Differential transcriptome expression for synaptic and immune related genes
- In-vivo evidence of microglial “activation” (PET scanning)

Brain pathology
- Abnormal brain development?
  Response to intrauterine injury? (e.g. infection, maternal antibodies)
  Abnormal cortical function? (e.g. epilepsy)

Peripheral Immune responses?
- Host autoimmunity?
- Systemic inflammation?
- Microbial translocation?
What are the triggering factors in neuroinflammation in the brain of ASD patients?

Observations in the neuropathology of ASD:
Excess neurons, white matter and enlarged frontal lobe
What is abnormal in the white matter?

Filipek et al. 1991
Piven et al. 1995, 1996
Courchesne E et al. 2001-2004
Sparks et al. 2002
Aylward et al 2002
Herbert et al 2003-2005
Hardan A, 2006
Others…

Schumann CM,
J Neurosci 2010
Loss of mTOR-Dependent Macroautophagy Causes Autistic-like Synaptic Pruning Deficits

Guomei Tang,1 Kathryn Gudsnuk,2 Sheng-Han Kuo,1 Marisa L. Cotrina,3,7 Gorazd Rosoklija,4,8 Alexander Sosunov,3
Mark S. Sonders,1 Ellen Kanter,1 Candace Castagna,1 Ai Yamamoto,1 Zhenyu Yue,6 Ottavio Arancio,3
Bradley S. Peterson,4,8 Frances Champagne,2 Andrew J. Dwork,3,4,8 James Goldman,3 and David Sulzer1,4,5,8,*

Neuron 83, 1131–1143, September 3, 2014

A
Pial surface
Layer I
Layer II, III
Layer IV
Layer V
Layer VI
20X
5μm
C (8yrs)
C (10yrs)

B

C

D

E

Spine density (110 μm)

Age:

<table>
<thead>
<tr>
<th>Age</th>
<th>C (2-8y)</th>
<th>C (13-18y)</th>
<th>A (3-9y)</th>
<th>A (13-19y)</th>
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</table>

Spines / 10μm

Age (y)

Spines / 10μm

C (2-8) C (13-18) A (3-9) A (13-19)

2-9 y 13-19 y
Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner

Dorothy P. Schafer,1 Emily K. Lehman,1,5 Amanda G. Kautzman,1,5 Ryuta Koyama,1 Alan R. Mardini,3 Ryo Yamasaki,4 Richard M. Ransohoff,4 Michael E. Greenberg,3 Ben A. Barres,2 and Beth Stevens1,5

1Department of Neurology, F.M. Kirby Neurobiology Center, Children’s Hospital, Harvard Medical School, Boston, MA 02115, USA
2Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA
3Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA
4Neuroinflammation Research Center, Department of Neurosciences, Lerner Research Institute, and Mellen Center for MS Treatment and Research, Neurological Institute, Cleveland Clinic, Cleveland, OH 44195, USA

Neuron 74: 691-705, 2012
Microglia is a critical cell from the innate immune system for modeling neuronal networks and circuits during development and adaptive brain plasticity.

How Many Cell Types Does It Take to Wire a Brain?
Richard M. Ransohoff and Beth Stevens
Science 333, 1391 (2011);

Neuronal-Microglia-Astrocyte Interactions. Kettenmann H, Neuron 2103
HOW TO STUDY NEURAL AND IMMUNE INTERACTIONS IN ASD?

BRAIN

- Neuropathology
- Brain Imaging

IMMUNITY

- Cellular Immune Responses
- Cytokines/chemokines networks
- Immune & Neurotrophic growth factors

CSF

BLOOD
• Prospective study of patients with ASD
  – Non-regressive vs. “regressive”
  – Clinical, behavioral and cognitive assessment
  – Neuroimaging
  – Blood/CSF studies for evaluation of immune activation
CNS Environment

- Neurotoxicity
- Neurotransmission
- Oligodendrocyte function
- Interneuron migration
- Tissue repair
- Neuromodulation

Immune Environment

- Pro-inflammatory pathways
- Tissue repair
- Anti-inflammatory pathways
- CCL1 GRO-alpha
- CXCL12 SDF-1
- CCL2 MCP-1
- Leukocyte trafficking
NIMH Study of Immunological Factors in Autism:
Profile of cytokines and chemokines in serum & CSF
Prevalence of Immune Related Proteins in the CSF of patients with ASD
Autism and the Immune Response

What we know:

• Various immune system abnormalities have been reported in children with autistic disorders by a number of different laboratories.

• Both enhanced autoimmunity and reduced immune function have been shown.

• Development of ‘autism’ animal models with immune basis
## Studies on Plasma cytokines

<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 <strong>MCP-1</strong>, <strong>Rantes</strong> and <strong>Eotaxin</strong> 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 <strong>IL-1beta</strong>, <strong>IL-1RA</strong>, <strong>IL-5</strong>, <strong>IL-8</strong>, <strong>IL-12(p70)</strong>, <strong>IL-13</strong>, <strong>IL-17</strong> and <strong>GRO-alpha</strong>.</td>
<td>(Suzuki et al., 2011)</td>
</tr>
<tr>
<td>Increase in <strong>leptin</strong> levels in ASD subjects compared to age-matched controls.</td>
<td>(Ashwood et al., 2008b)</td>
</tr>
<tr>
<td>Increase in <strong>macrophage migration inhibitory factor (MIF)</strong> in ASD subjects compared to age-matched controls.</td>
<td>(Grigorenko et al., 2008)</td>
</tr>
<tr>
<td>Decrease in <strong>TGF-beta</strong> in subjects with ASD compared to controls.</td>
<td>(Ashwood et al., 2008a; Okada et al., 2007)</td>
</tr>
<tr>
<td>Increase in <strong>IL-12</strong> and <strong>IFN-gamma</strong> in ASD subjects compared to age-matched controls.</td>
<td>(Singh, 1996)</td>
</tr>
</tbody>
</table>
# Serum Cytokines/Chemokines

<table>
<thead>
<tr>
<th>Studies in chronological order</th>
<th>Immune Measure</th>
<th>Behavior Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashwood et al. (2008)</td>
<td>Low serum levels of TGFβ1</td>
<td>Stereotypy, Irritability, Lethargy, and Hyperactivity</td>
</tr>
<tr>
<td>Grigorenko et al. (2008)</td>
<td>High serum levels of MIF</td>
<td>Impaired Sociability</td>
</tr>
<tr>
<td>Kajizuka et al. (2010)</td>
<td>High serum levels of PDGF</td>
<td>Stereotypy</td>
</tr>
<tr>
<td>Ashwood et al. (2011a)</td>
<td>High serum chemokines CCL2/MCP-1, CCL5/RANTES and eotaxin</td>
<td>Lethargy, Stereotypy, Hyperactivity, Impaired Communication and Socialization</td>
</tr>
<tr>
<td>Ashwood et al. (2011b)</td>
<td>High serum levels of cytokines IL-1β, IL-6, IL-8 and IL-12p40</td>
<td>Lethargy, Stereotypy, and Hyperactivity, and Impaired Communication</td>
</tr>
<tr>
<td>Study Description</td>
<td>Reference</td>
<td></td>
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<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------</td>
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<tr>
<td>In isolated PBMCs stimulated with PHA, increase in <strong>GM-CSF, TNF-alpha</strong> and <strong>IL-13</strong>. A decrease in <strong>IL-12(p40)</strong> in ASD subjects vs. controls.</td>
<td>(Ashwood et al., 2011d)</td>
<td></td>
</tr>
<tr>
<td>Stimulation of TLR on monocytes - ASD vs. to age-matched controls. Increase in <strong>IL-1beta, IL-6, TNF-alpha</strong>, with stimulation of TLR2. Increase in <strong>IL-1beta</strong>, with stimulation of TLR4. Decrease in <strong>IL-1beta, IL-6, GMCSF, TNF-alpha</strong> with TLR9.</td>
<td>(Enstrom et al., 2010)</td>
<td></td>
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<tr>
<td>Increase in <strong>IFN-gamma</strong> in NK cells from subjects with ASD.</td>
<td>(Enstrom et al., 2009b)</td>
<td></td>
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<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>
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<tr>
<td>Increase in <strong>IL-12</strong> and <strong>TNF-alpha</strong> in ASD subject with GI symptoms.</td>
<td>(Jyonouchi et al., 2005)</td>
<td></td>
</tr>
<tr>
<td>Increase in <strong>IFN-gamma</strong> and <strong>TNF-alpha</strong> in isolated PBMCs from ASD subjects compared to age-matched controls stimulated with LPS.</td>
<td>(Jyonouchi et al., 2002)</td>
<td></td>
</tr>
<tr>
<td>Unstimulated whole blood from ASD vs. age-matched controls – increase in <strong>IFN-gamma</strong> and <strong>IL-1RA</strong> with higher <strong>IL-6</strong> and <strong>TNF-alpha</strong>.</td>
<td>(Croonenberghs et al., 2002)</td>
<td></td>
</tr>
<tr>
<td>Unstimulated PBMC- ASD subjects: higher levels of <strong>TNF-alpha, IL-1beta, and IL-6</strong> vs. controls. PBMCs stimulated with LPS, PHA and tetanus produced increase levels of <strong>IL-12</strong> and <strong>IL-1beta</strong>.</td>
<td>(Jyonouchi et al., 2002)</td>
<td></td>
</tr>
</tbody>
</table>
## Dynamic Cellular Responses

<table>
<thead>
<tr>
<th>Studies in chronological order</th>
<th>Immune Measure</th>
<th>Behavior Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onore et al. (2009)</td>
<td>PHA induced IL-23 production</td>
<td>Impaired Sociability</td>
</tr>
<tr>
<td>Enstrom et al. (2010)</td>
<td>High LPS induced IL-1B production in purified monocytes</td>
<td>Impaired Sociability and Communication</td>
</tr>
<tr>
<td>Ashwood et al. (2011)</td>
<td>Higher T lymphocyte production of IFNγ, IL-8, TNFα lower IL-13, IL-10, IL-5</td>
<td>Hyperactivity, Stereotypy, Lethargy, and Impaired Communication and Sociability</td>
</tr>
<tr>
<td>Han et al. (2011)</td>
<td>Higher T lymphocyte, cytotoxic lymphocyte and total lymphocyte numbers</td>
<td>Stereotypy, executive function</td>
</tr>
<tr>
<td>Breece et al. (2012)</td>
<td>Increased frequency of myeloid dendritic cells</td>
<td>Stereotypy, Regression, GI symptoms, amygdala volume</td>
</tr>
</tbody>
</table>
Immune dysregulation in ASD- evidence from response to environmental toxicant

• Differential Immune response in the presence of the toxicant BDE-49
• ASD vs. TD Subjects demonstrate opposite response to co-culture with polybrominated diphenyl ether (BDE)-49
• Is this an example of genetic susceptibility for immune dysregulation in the context of environment?
Non-activated immune cells- Differential response to BDE-49

Mean fold change was derived from log_{10} transformed pg/ml cytokine production (Treatment-Baseline/Baseline). (t-test,*p<=0.05)
Are there systemic factors such as GI pathology involved in ASD immune abnormalities?

The Gut Microbiota Modulate the Immune System

The Gut Microbiota Influence the CNS Function

Integration of the microbiota into the gut-brain axis

- The ability of the brain to influence the intestinal microbiota
- Perturbation of normal habitat via stress-induced changes in gastrointestinal:
  - Physiology
  - Epithelial function
  - Mucin production
  - EE cell function
  - Motility
- Release of neurotransmitters

The ability of the microbiota to influence brain and behavior

- Activation of neural afferent circuits to the brain
- Activation of mucosal immune responses
- Production of metabolites that directly influence the CNS

Collins SM & Bercik P.

M J Barnes, F Powrie
Science 2011;331:289-290
**Microbial Translocation:**
The innate and adaptive immune systems coordinate containment of intestinal microbes

**Lipopolysaccharide (LPS)**, a component of Gram negative bacteria cell walls, **LPS-binding protein (LBP)**, and **anti-endotoxin core immunoglobulin** levels have been shown to be reliable biomarkers of MT along with markers of immune activation such as sCD14 and proinflammatory cytokines.

## MT markers in ASD

<table>
<thead>
<tr>
<th>Subjects</th>
<th>LPS (mean rank)</th>
<th>LBP (mean rank)</th>
<th>IgM (mean rank)</th>
<th>IgG (mean rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism (n=57)</td>
<td>42.56</td>
<td>40.57</td>
<td>45.27</td>
<td>43.40</td>
</tr>
<tr>
<td>Typical (n=33)</td>
<td>50.58</td>
<td>51.38</td>
<td>45.89</td>
<td>49.12</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.161</td>
<td>0.056</td>
<td>0.913</td>
<td>0.418</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>LPS (mean rank)</th>
<th>LBP (mean rank)</th>
<th>IgM (mean rank)</th>
<th>IgG (mean rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism – Regressors (n=23)</td>
<td>29.46</td>
<td>26.36</td>
<td>29.79</td>
<td>32.84</td>
</tr>
<tr>
<td>Autism-Non Regressors (n=34)</td>
<td>28.91</td>
<td>31.57</td>
<td>27.83</td>
<td>23.33</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.974</td>
<td>0.240</td>
<td>0.661</td>
<td><strong>0.034</strong></td>
</tr>
</tbody>
</table>
Analysis of the 16S rRNA in plasma of ASD vs. control patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>St.Dev.</th>
<th>N</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct1</td>
<td>Autism</td>
<td>27.87</td>
<td>1.92</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Typical</td>
<td>27.47</td>
<td>1.80</td>
<td>34</td>
</tr>
<tr>
<td>Ct2</td>
<td>Autism</td>
<td>27.29</td>
<td>2.01</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Typical</td>
<td>27.00</td>
<td>1.81</td>
<td>34</td>
</tr>
<tr>
<td>Ct3</td>
<td>Autism</td>
<td>27.53</td>
<td>1.98</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Typical</td>
<td>27.2</td>
<td>1.61</td>
<td>34</td>
</tr>
</tbody>
</table>
Circulating levels of MT markers, LPS, LPB, or anti-endotoxins, did not differ significantly between children with autism and age-matched typical controls, nor did a history of regression correspond to evidence of circulating MT markers.

There is not a valid rationale for the empiric use of antibiotics in the treatment of hypothetical “leaky gut” or “occult” infections in children with autism.
How to modify microglial activation?

- **Minocycline hydrochloride**, is a broad spectrum tetracycline antibiotic.
- It is the most lipid-soluble of the tetracycline-class antibiotics with greatest penetration into brain.
- Minocycline has been used in many neurological disorders:
  
  
  
  
  
A pilot open-label trial of minocycline in patients with autism and regressive features

Carlos A Pardo1, Ashura Buckley2, Audrey Thom3, Li-Ching Lee3, Arun Ashagiri1, David M Neville3 and Susan E Swedo2

Table 1 Patient sample description

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pretreatment age (months)</th>
<th>Sex</th>
<th>Age at onset of regression (months)</th>
<th>Non-verbal IQ/DQ</th>
<th>CGI-baseline</th>
<th>CGI-post-treatment</th>
<th>Vineland-ABC baseline</th>
<th>Vineland-ABC post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99</td>
<td>F</td>
<td>27</td>
<td>30</td>
<td>6</td>
<td>5</td>
<td>55</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>M</td>
<td>13</td>
<td>60</td>
<td>5</td>
<td>4*</td>
<td>68</td>
<td>73*</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>M</td>
<td>12</td>
<td>33</td>
<td>5</td>
<td>5</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>153</td>
<td>M</td>
<td>19</td>
<td>77</td>
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<td>5</td>
<td>56</td>
<td>55</td>
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<tr>
<td>5</td>
<td>91</td>
<td>M</td>
<td>8</td>
<td>58</td>
<td>3</td>
<td>3</td>
<td>60</td>
<td>56</td>
</tr>
<tr>
<td>6</td>
<td>66</td>
<td>M</td>
<td>18</td>
<td>103</td>
<td>4</td>
<td>4</td>
<td>70</td>
<td>71</td>
</tr>
<tr>
<td>7</td>
<td>128</td>
<td>F</td>
<td>15</td>
<td>25</td>
<td>5</td>
<td>5</td>
<td>55</td>
<td>46</td>
</tr>
<tr>
<td>8</td>
<td>112</td>
<td>M</td>
<td>14</td>
<td>33</td>
<td>5</td>
<td>5</td>
<td>53</td>
<td>56</td>
</tr>
<tr>
<td>9</td>
<td>49</td>
<td>M</td>
<td>18</td>
<td>59</td>
<td>4</td>
<td>4</td>
<td>66</td>
<td>63</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>M</td>
<td>22</td>
<td>90</td>
<td>4</td>
<td>3</td>
<td>70</td>
<td>66</td>
</tr>
<tr>
<td>11</td>
<td>83</td>
<td>M</td>
<td>18</td>
<td>32</td>
<td>5</td>
<td>5</td>
<td>48</td>
<td>57</td>
</tr>
</tbody>
</table>

*Denotes child that discontinued the study at 3 months.

**Note:** Effect size was calculated for the within-subjects effect using the following formula: ES = (M_{post} - M_{baseline}) / SD_{baseline}. CGI, Clinical Global Impression.
### Table 3: Treatment effect on cytokines, chemokines, metalloproteinases and growth factors

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cerebrospinal fluid (CSF)</th>
<th>Serum</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z$</td>
<td>$P$</td>
<td>$Z$</td>
</tr>
<tr>
<td>TNFα</td>
<td>-0.45</td>
<td>0.66</td>
<td>-1.78</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.36</td>
<td>0.72</td>
<td>-1.75</td>
</tr>
<tr>
<td>CCL2 (MCP-1)</td>
<td>-1.78</td>
<td>0.075</td>
<td>-1.27</td>
</tr>
<tr>
<td>CCL3 (MIP-1α)</td>
<td>-0.18</td>
<td>0.86</td>
<td>-0.76</td>
</tr>
<tr>
<td>CCL5 (RANTES)</td>
<td>-0.73</td>
<td>0.47</td>
<td>0.66</td>
</tr>
<tr>
<td>CXCL8 (IL-8)</td>
<td>-0.45</td>
<td>0.66</td>
<td>-1.99</td>
</tr>
<tr>
<td>BDNF</td>
<td>-2.03</td>
<td><strong>0.042</strong></td>
<td>-0.76</td>
</tr>
<tr>
<td>Truncated-BDNF/α-2 M°</td>
<td>-2.19</td>
<td><strong>0.028</strong></td>
<td>-1.25</td>
</tr>
<tr>
<td>Mature-BDNF/α-2 M°</td>
<td>-0.87</td>
<td>0.386</td>
<td>-1.33</td>
</tr>
<tr>
<td>CD40L</td>
<td>-0.89</td>
<td>0.37</td>
<td>-1.33</td>
</tr>
<tr>
<td>GDNF</td>
<td>-0.37</td>
<td>0.71</td>
<td>-1.83</td>
</tr>
<tr>
<td>HGF</td>
<td>-2.19</td>
<td><strong>0.028</strong></td>
<td>-1.25</td>
</tr>
<tr>
<td>Leptin</td>
<td>-1.48</td>
<td>0.14</td>
<td>-0.27</td>
</tr>
<tr>
<td>MMP-1</td>
<td>-1.07</td>
<td>0.29</td>
<td>-1.07</td>
</tr>
<tr>
<td>MMP-3</td>
<td>-0.15</td>
<td>0.88</td>
<td>-1.89</td>
</tr>
<tr>
<td>MMP-7</td>
<td>-1.89</td>
<td>0.09</td>
<td>-0.05</td>
</tr>
<tr>
<td>MMP-8</td>
<td>-1.38</td>
<td>0.17</td>
<td>-1.38</td>
</tr>
</tbody>
</table>

*Selected analytes, Mann–Whitney U-test.

Quantified by Luminex technique. In CSF only the truncated-BDNF form appears to be detectable in immunoblotting experiments.

Quantified by immunoblotting and densitometry analysis. Significance was calculated based on ratio BDNF isoform/α-2 M°.
IMMUNOTHERAPIES IN AUTISM: Are these good options??

**CNS:**
- Innate immunity
  - Microglia, astroglia, cytokines, chemokines
- Adaptive immunity
  - Specific T cell and antibody responses

**Peripheral Immune System**

**Brain NeuroImmunity**

**Immunomodulators**
- e.g. Steroids (e.g. Prednisone)

**Immunosuppressants**
- e.g. Cyclophosphamide, Methotrexate, tacrolimus, etc

**CNS:**
- Immunoglobulins (IVIG)
- Minocycline, COX2 inhibitors, NSAIDs

**Peripheronal Immune System**

**Brain NeuroImmunity**
Autism and the Immune Response

Overall Conclusions

- Various neuroimmune system abnormalities have been reported in children with autistic disorders:
  - Neuroglial and innate immune responses may reflect homeostatic mechanisms rather than pathogenic immunological reactions
- Maternal antibodies have been demonstrated in subsets of mother of children with autism, a finding that suggest a potential role of maternal-immune mediated mechanisms in neurodevelopmental disarrangements
Autism and the Immune Response
Overall Conclusions

- Systemic immune abnormalities such as variable expression of cytokines and chemokines, increase of NK cells and decreased immunoglobulin G levels may reflect a dysfunctional immune response rather than an autoimmune or immunologically pathogenic process.

- This dysregulation may inform as to dysfunction in pathways common to both the immune and nervous systems.

- Gastrointestinal symptoms are not associated with microbial translocation or represent inducers of immune activation.

- There is no role at this time for the use of immunological therapies, immunosuppressants or antibiotics to “modify” neurological or behavioral abnormalities in autism.