Immunologic Factors in the Etiology of Autism

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Neuro-immune interactions

• For the past 60 years, the central nervous system has been considered to be immune privileged.

• The classic paradigm was there is interaction between these two seemingly distinct systems only during disease.
Neuro-immune interactions

- Results from diverse fields now show clear and convincing evidence of bidirectional communication between the nervous and immune systems.
Astroglia

Microglia

Neuron

Perivascular macrophage

B Cell or Plasma cell

Neuroimmune Cross-Talk

Neuron

ACTH Receptor
CRH Receptor
CRH

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

ACTH

Glutamate Receptor

CRHR

GABA

Serotonin

ACh

Cytokine Receptors

GABA(A)-R

Glutamate Receptor

ACh Receptor

CRHR

T-Cell

Monocyte/Macrophage

Dendritic Cell

ICAM-1

BBB

ICAM-1

IL-1, IL-6, and TNF-α

IFN-α

Glutamate

Cytokines IL-1, IL-6, TNF-α

IL-1, IL-6, TNF-α

5-HT Receptor

CRH

ACTH Receptor

ACTH

CRH

GABA(A)-R

Serotonin

ACh

Glutamate

Cytokines IL-1, IL-6, TNF-α

IL-1, IL-6, and TNF-α

IFN-α

Glutamate

Cytokines IL-1, IL-6, TNF-α

IL-1, IL-6, and TNF-α

IFN-α

Glutamate

Cytokines IL-1, IL-6, TNF-α

IL-1, IL-6, and TNF-α

IFN-α

Glutamate
The health and development of the nervous system is largely dependent upon the health and function of the immune system.

What are the factors that influence this cross-talk?
Cross-talk between the Immune and Central Nervous Systems

• Communication between the cells of the immune and nervous systems is possible on several levels.
• Neurons, microglia, and astrocytes can produce cytokines and express cytokine receptors.
• This enables them to direct immune cells as well as respond to various immunological stimuli.
• Cells of the immune system are able to secrete various neurotransmitters and express receptors for many of these molecules.
• This allows immune cells to impact neural processes and respond to neural stimuli.
The neuroimmune system is comprised of different "immune" cells and governed by different mechanisms.
What happens in the developing brain?

We are a long way from knowing everything about the neuro-immune interface during development.
Immune and Nervous System Interactions

• An altered immune response may impact other biological systems including the neuroendocrine and nervous systems, and vice versa.
The neuroimmune interface is critical during gestation and the early post-natal period

• Data suggests that altered cytokine profiles affect neurodevelopmental outcome
  – Both schizophrenia and autism are thought to result in part from changes in the maternal immune profile during gestation.
• These changes include increased inflammatory cytokines and autoantibodies to proteins in the developing fetal brain.
Are neuroimmune mechanisms involved in pathogenesis of ASD?

Adaptive Immunity

Lymphocyte and Antibody production

Innate Immunity

Microglia & astroglia activation

NEURODEVELOPMENT

LEARNING AND ADAPTIVE BRAIN FUNCTION
What is the status of the cellular immune response?

• To analyze this, we look at cytokine levels in the blood, and in culture supernatants after stimulation.
What are cytokines? Molecules that tell other cells what to do.

Activated macrophages secrete a range of cytokines

- **IL-1β**
  - Activates vascular endothelium
  - Activates lymphocytes
  - Local tissue destruction
  - Increases access of effector cells

- **TNF-α**
  - Activates vascular endothelium and increases vascular permeability, which leads to increased entry of IgG, complement, and cells to tissues and increased fluid drainage to lymph nodes

- **IL-6**
  - Lymphocyte activation
  - Increased antibody production

- **CXCL8**
  - Chemotactic factor
  - recruits neutrophils, basophils, and T cells to site of infection

- **IL-12**
  - Activates NK cells
  - Induces the differentiation of CD4 T cells into T_H1 cells

**Local effects**

**Systemic effects**

- **Fever**
  - Production of IL-6
- **Fever**
  - Mobilization of metabolites
  - Shock
- **Fever**
  - Induces acute-phase protein production

*Figure 2-39 Immunobiology, 6/e. (© Garland Science 2005)*
Pro-inflammatory pathways

CNS Environment

Neurotoxicity
Sleep, fever

Almost all aspects of neural development

Oligodendrocyte function
Interneuron migration
Neuromodulation

CCL1 GROα
CXCL12 SDF1
CCL2 MCP1

Immune Environment

IL-1β TNF-α IL-6 TGF-β

Pro-inflammatory pathways
Anti-inflammatory pathways
Leukocyte trafficking
In 2002, we began to collect samples from families as part of the Childhood Autism Risk from Genetics and the Environment (CHARGE) study.

The CHARGE study population was sampled from three strata of children, ages 2-5 yrs:

1) Children with autism (currently includes over 800 families)
2) Typically developing children selected from the general population without autism or other developmental disabilities (currently over 500 families enrolled)
3) Children with developmental disabilities without autism (currently 350 families enrolled)
Do we see changes in cytokines in the plasma of children with ASD?

• Several studies have described alterations in cytokine profiles associated with autism.

• The regulatory cytokine TGFβ has been linked to ASD in multiple studies.
  – Other studies describe decreased levels of TGFβ in blood samples from individuals with ASD.
  – Ashwood et al found that lower TGFβ correlated with more severe behavioral scores in ASD children.
  – Lower TGFβ in peripheral blood, TGFβ levels in post-mortem brain and cerebrospinal fluid samples were higher in ASD subjects than controls.
Other cytokine/chemokines altered in ASD

• MIF (macrophage inhibitory factor) a pro-inflammatory immune regulator that is constitutively expressed in brain tissues, and has important impacts on neural and endocrine systems.
  – Autism subjects with the highest levels of plasma MIF were found to have the most severe behavior.
Plasma levels of IL-6, IL-8, IL-1β and IL-12p40 are significantly higher in the ASD (n=97) group when compared to TD (n=87) and DD (n=39) controls.
Altered T cell responses in children with autism: association with behavior

- 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.


*Brain Behavior and Immunity 2010; 24(1):64-71*

- Concentration (log-scale) of IL-1β (A), IL-6 (B), and TNFα (C) in monocyte cell culture supernatants following stimulation with TLR 2 ligands.

- Autism (white bars); typically developing controls (grey bars). * P < 0.05.
Assessment of Adaptive or specific immune reaction: Quantification of lymphocyte infiltration, immunoglobulin deposition or complement activation

There was no evidence of any infiltration by T- or B-lymphocytes or immunoglobulin deposition in any of the brain regions studied.

11 cases of autism
12 controls

What about in the brain itself?
Autism: Cytokine Profile in Brain

Vargas DL et al. Ann Neurol 2005
Cytokine profile in autism

- Subsets of pro-inflammatory and anti-inflammatory cytokines are increased in the brain of autistic patients.
- Cytokines are produced in the brain by neurons and neuroglial cells.

Vargas DL et al. Ann Neurol 2005
Autism: Chemokine Profile in Brain

Vargas DL et al. Ann Neurol 2005
Assessment of cytokines and chemokines in CSF: Expression profile and quantification

7 autism cases
7 control cases

Cerebrospinal fluid
- CSF-
  • Cells
  • Proteins
  • Immunoglobulins
  • Oligoclonal bands

Cytokines
Chemokines
Autism: In-vivo markers of inflammation in the cerebrospinal fluid were found.

Vargas DL et al. Ann Neurol 2005
Leptin, a newly discovered cytokine

- Adipokines such as leptin are examples of molecules that interface between immune and metabolic regulation.
- Leptin signals the brain that sufficient food is stored as fat.
- In the following study, we compared plasma leptin levels in a population of well-defined children with autism and age-matched controls, with and without developmental disabilities.
The cytokine leptin was analyzed in children with early onset vs regressive autism.

Can begin to separate phenotypes based on biologic outcomes.

Plasma Leptin Levels are Elevated in Autism: Association with an Early Onset Phenotype? Ashwood et al, JADD, 2008
What does this mean?

• Leptin controls intracellular metabolism.
• Decreased leptin leads to increased infections.
• Increased leptin, see increased frequency of autoimmunity.
What might be the outcome of immune dysregulation such as elevated inflammatory cytokines?

• One potential outcome is the increased susceptibility towards the generation of autoantibodies.

• We have examined plasma from children with an ASD for autoantibodies to brain proteins using:
  – Immunohistochemistry
  – Western blot analysis
Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders

Sharifia Wills a,e, Maricel Cabanlit a,e, Jeff Bennett b,d, Paul Ashwood c,d,e, David G. Amaral b,d, Judy Van de Water a,d,e,*

Autoantibodies from children with ASD-

Immunohistochemical and Western blot analysis of autoantibody localization in cerebellum of Rhesus monkeys
• The Golgi cell of the cerebellum was strongly reactive when probed with antibodies from children with autism.

• Intense Golgi cell staining was observed in ~21% of patients with ASD compared with 0% of normal controls.
Western blot of monkey cerebellum

- Blot was run with plasma from children with autism and the presence of bands determined

*Goines, et al, Submitted BBI, 2010*
Western blot analysis of monkey cerebellum

<table>
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<th>Child IgG Targets in Cerebellum</th>
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<td></td>
<td>TD</td>
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<th>AU vs. TD</th>
<th>AU vs. ASD</th>
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<td>0.043</td>
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Significant behavioral associations for children with and without IgG reactivity towards the 45 kDa protein.

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<tr>
<th>Test</th>
<th>ASD Mean Score (SD)</th>
<th>45+</th>
<th>45-</th>
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<th>AU Mean Score (SD)</th>
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<th>TD Mean Score (SD)</th>
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<th>45-</th>
<th></th>
<th>All Children Mean Score (SD)</th>
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<td>ABC 2: Lethargy</td>
<td>8.2 (7)</td>
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<td>12.9 (9.6)</td>
<td>12.3 (7.1)</td>
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<td>3.3 (3.5)</td>
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<td>100.8 (17.7)</td>
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Significant behavioral associations for children with and without IgG reactivity towards the 62 kDa protein.

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<td>Mean Score (SD)</td>
<td>Mean Score (SD)</td>
<td>Mean Score (SD)</td>
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<td>Total ABC</td>
<td>68.1 (40.9)</td>
<td>38.7 (25.)</td>
<td>48.3 (25.6)</td>
<td>53.1 (26.1)</td>
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<td>ABC 2: Lethargy</td>
<td>16.6 (11.9)</td>
<td>7.5 (5.9)</td>
<td>10.3 (7.5)</td>
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<td>ABC 3: Stereotypy</td>
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<td>VABS</td>
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<td>70.5 (13.5)</td>
<td>65.1 (15.8)</td>
<td>62.1 (11.1)</td>
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Neuroimmune Cross-talk in the developing brain

Astroglia

Microglia

Neuron

Perivascular macrophage

Permeable BBB

Cytokines
- IL-1, IL-6, TNF-α

ICAM-1

Maternal anti-fetal brain antibodies

B Cell or Plasma cell

Circulation

Monocyte/Macrophage

Dendritic Cell

T-Cell

Cytokine Receptors

B Cell or Plasma cell

CRH

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ACTH

GABA(A)-R

Glutamate Receptor

5-HT Receptor

CrH Receptor

ACh

GABA(A)-R

IL-1, IL-6, TNF-α

IFN-α

Glutamate

Neuroimmune Cross-talk in the developing brain
## Results of First Study

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<th>Study population</th>
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<tr>
<td>TD (n=62)</td>
<td>0 (0%)</td>
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<tr>
<td>DD (n=40)</td>
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Abbreviations:  
AU = Autism;  
TD = Typically Developing;  
DD = Developmental delays
## Expanded Study: Specificity Remains

<table>
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<tr>
<th>Study Population</th>
<th>37&amp;73kD*</th>
<th>39&amp;73kD</th>
<th>37&amp;73kD or 39&amp;73kD</th>
<th>37 &amp; 39 &amp; 73kD</th>
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<td>AU (n=204)</td>
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<td>ASD (n=71)</td>
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<td>9 (13%)</td>
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<td>19 (7%)</td>
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<td>TD (n=183)</td>
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<tr>
<td>AU vs ASD</td>
<td>0.766</td>
<td>0.0525</td>
<td>0.209</td>
<td>0.344</td>
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</table>

Abbreviations: AU = Autism; TD = Typically Developing; DD = Developmental delays. Submitted: J. Autoimmunity
Autoantibodies can cross the placenta and bind to proteins present in the developing fetal brain.

Fetal brain protein antigens

Maternal IgG Autoantibody

Altered Neurodevelopment
Putting it all together...

- Environmental perturbation
- Genetic susceptibility

Immune function

Autoantibody production

Oxidative stress

Immune dysregulation

Leptin

IL-6

TNF-α
The UC Davis Team

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Luke Heuer
Robert Boyce
Paula Goines
Marjannie Eloi
Lori Haapanen

UCD Children’s Health Center and C.H.A.R.G.E
Dr. Isaac Pessah, Director
Dr. Irva Hertz-Picciotto
Dr. Robin Hansen

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