Immune Dysregulation in Autism: What’s the connection?

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Objectives

- Review current research on the heterogeneity of autism spectrum disorders in terms of immune regulation
- Link immune markers to behaviors
- Discuss potential applications to early identification and treatment

Autism Spectrum Disorders

- A group of disorders exhibiting a complex and wide variability of symptoms that point to multiple etiologies and yet share similar core behavioral symptomatology

Autism Core Characteristics

- Social Impairment
- Communication Impairment
- Repetitive Behaviors
ASD: Heterogeneity

- ASD are more common in males than females (4:1) across ethnic groups.
- ASD are associated with intellectual disability although the rates may be decreasing with earlier identification and treatment.
  - Autistic disorder 30.8% normal intelligence
  - PDD NOS 92% normal
    - (Chakrabarti & Fombonne, 2001)
  - 55.4% IQ above 70 (42-77% range 7 states, F>M)
    - (ADDMNS, 2002)

- 30% of individuals with autism have epilepsy.
- 20% of individuals with autism have big heads and brains …
- but 15% have small brains.
- Some have early symptoms, some lose acquired developmental skills and regress.
- Some have chronic GI symptoms.

Interactive models of ASD

Genes
G₁, G₂, G₃, ………..Gₙ

Environment
Factor₁, Factor₂,………Factorᵢ

Autism Type A
Autism Type B
Autism Type C
The Problem with Heterogeneity

- Research on autism has been too fragmentary to allow determination of the biomedical and behavioral characteristics that define different subtypes (endophenotypes) and mechanisms of autism.

Phenotyping Studies at the MIND

- CHARGE (Childhood Autism Risks from Genetics and the Environment)
- MARBLES (Markers of Autism Risk-Learning Early Signs)
- APP (Autism Phenome Project)

CHARGE Study

- Evaluate interactions between genetic susceptibility factors and environmental exposures
- Identify autism sub-types/comorbidities
- Case-control study of autism, developmental delay/intellectual disability, and general population
- 24-54 months of age
- Born in California
- English and Spanish
Behavioral Phenotyping: Pattern of Onset in CHARGE

Pattern of Onset in CHARGE

- Most children with regression lost social interest and engagement (82%) rather than language (54%)
- Defining regression based on language loss significantly underestimates the frequency of developmental regression and misclassifies children with social regression only into early onset

Pattern of Onset in CHARGE

Clinical features

- Regression v. Early Onset
  - No differences in demographic factors
  - No differences in sleep problems
  - No differences in seizures
  - No differences in G.I. symptoms
  - Differences in communication measures (children with regression scoring lower) although very small effect size and clinical significance remains to be determined due to young age

Dysregulated Gene Expression

11 genes differentially expressed in A-R and A-E compared to control children
- Natural Killer (NK) cells upregulated in ASD
  - Gregg et al, 2008
- NK cells in ASD vs controls at rest had increased production of perforin, granzyme B, and IFN-gamma but reduced production (cytotoxicity) when stimulated
In functional studies of NK cells: A reduction in cytototoxic activity of cells obtained from ASD subjects was observed at cellular ratios above 1:1, which was significant at the 25:1 NK:K562 ratio (p = 0.02).

Leptin

- Regulates food intake but not just important in regulating weight
- Hormone that links nutritional status with neuroendocrine and immune functions.
- Affects thymic homeostasis and the secretion of acute phase reactants IL-1 and TNFα
- Acts as a pro-inflammatory cytokine, promotes TH1 cell differentiation and may play a role in the onset of autoimmunity

Leptin profiles in early onset vs regressive

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Cellular Immune Function in Autism

• Th-17 cells, subset involved in autoimmune and chronic inflammatory diseases
  – Secrete IL-6, IL-17, tumor necrosis factor-alpha
  – IL-23 is a survival factor for Th-17 cells
• Plasma samples 40 ASD (20/20) and 20 TD
  – IL-17 levels similar
  – IL-23 decreased in ASD (5.05 pg/ml) vs TD (12.48), largely driven by early onset sample (4.25 vs 5.78)


Reduced Levels of Immunoglobulin in Children with Autism Correlates with Behavioral Symptoms

• Heuer, Luke*1,7,8, Ashwood, Paul*2,7,8, Schauer, Joseph1,7,8, Goines, Paula1,7,8, Hertz-Picciotto, Irva3,7,8, Hansen, Robin4,7,8, Croen, Lisa A5, Pessah, Isaac N6,7,8, Van de Water, Judy**1,7,8.

Reduced levels of IgG and IgM are indicative of an underlying defect in the adaptive immune system of children with autism.

- **Total IgG**
  - Autism: 2.5±0.5 mg/dL
  - Typical: 7.5±2.5 mg/dL
  - **Total IgM**
  - Autism: 2.0±0.5 mg/dL
  - Typical: 4.0±0.5 mg/dL
  - **Total IgA**
  - Autism: 0.5±0.5 mg/dL
  - Typical: 1.5±0.5 mg/dL
  - **Total IgE**
  - Autism: 0.5±0.5 IU/mL
  - Typical: 2.0±0.5 IU/mL

This study provides a novel association between immune dysfunction and behavioral parameters in autism.
Immunohistochemical and Western blot analysis of autoantibody localization in cerebellum

- 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 - Human cerebellum

The presence of the ~52 kDa band corresponds to Golgi staining by IHC (p=0.04).

Maternal antibodies to fetal brain protein

- In addition to looking at the immune system of children with ASD, we have found a specific subset of antibodies to fetal brain proteins (not adult) in the blood of their mothers.
- These antibodies occur most frequently in mothers who have children with regressive autism in one study and early onset in a different sample.
  - Croen et al, Biol Psychiatry, 2008
Western blot analysis of human fetal brain probed with maternal serum taken mid pregnancy.

- Lane A represents a subject with autism with the early onset phenotype with the 39 kDa: 73kDa band pattern.
- Lane B represents a subject with regressive autism and the 37kDa: 73kDa band pattern.
- This study further defined a phenotypic difference associated with maternal antibody band pattern.

<table>
<thead>
<tr>
<th>Prevalence (%)</th>
<th>AU (n=61)</th>
<th>37kD &amp; 73kD</th>
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<tbody>
<tr>
<td>AU Reg (n=36)</td>
<td>6 (17%)</td>
<td></td>
</tr>
<tr>
<td>AU EO (n=25)</td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>TD (n=62)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>DD (n=40)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Significance (p-value)</td>
<td>AU vs TD 0.0061*</td>
<td>AU vs DD 0.0401*</td>
</tr>
</tbody>
</table>
Conclusions

- Autism Spectrum Disorders are heterogeneous in terms of behavior constellations/pattern of onset and biological correlates
- Immune system dysregulation seems to be important in understanding biologic links to behavior in autism
- Genes related to immune function are differentially regulated in many children with ASD, particularly NK cell genes, which show evidence of reduced cytotoxicity when stimulated
- Other aspects of chemokine and cytokine dysfunction are present in children with ASD, some of which seem related to behavioral phenotypes/pattern of onset (leptin, IL-23, BDNF)
Conclusions

- Reduced levels of IgG and IgM represent another underlying defect in the immune system of children with autism.
- Autoantibodies reactive to fetal brain are present in both children with ASD and their mothers and are related to pattern of onset.
- Exposure of monkeys to IgG from mothers of children with ASD and brain reactive antibodies during pregnancy is associated with increased stereotypies and activity levels.
- Further study is needed to determine the extent to which immune dysfunction is related to the development of different ‘autisms’ and to develop diagnostic biomarkers for early identification and treatment.

Thank you!

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