How animal models inform potential therapies for immune dysfunction in PANDAS/PANS

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Infection, autoimmunity and behavior: The quest for a link
Autoantibodies disrupt communication between neurons

Access to the brain?

glutamate

NMDAR  AMPAR  NMDAR

Access to the brain?

dopamine

D1R  D2R  D1R
The blood-brain barrier: an important gatekeeper between the blood and the central nervous system

- Maintains brain homeostasis
- Limits CNS entry of
  - pathogens
  - immune cells
  - drugs

Evans blue dye
Abnormal blood-brain barrier function is a prominent feature of many CNS diseases

- Acute traumatic injury
- Stroke
- Exposure to toxic or hypertonic conditions
- Brain infections

- Autoimmune diseases
  - Multiple sclerosis
  - Autoimmune Encephalitis?

- Neurodegenerative diseases
  - Alzheimer’s
  - Huntington’s
  - Parkinson’s

Yu et al. (2015)

Bruce Trapp
Hypothesis 1: Destruction of tight junctions between endothelial cells

Engelhardt & Ransohoff, 2012 *Trends Immunol*
Huppert, J. *et al.*, 2010 *FASEB*
Okada & Khoury, 2012 *JCI*
Risau W. *et al.*, 1990 *JCB*
Hypothesis 2: Selective transport of antibodies from endothelial cells into the brain

Zhang, D. et al., 2012 Brain Behav Immun
Abuqayyas & Balthasar, 2012 Mol Pharmaceutics
Okun, E. et al., 2010 Neuromol Med
Diamond, B. et al., 2013 Ann Rev Immunol
Group A β-hemolytic *Streptococcus pyogenes* causes a plethora of autoimmune diseases.

- Rheumatic fever
- Glomerulonephritis
- Scarlet fever
- Sydenham’s chorea
- PANDAS

Implicated in autoimmunity:
- Heart
- Kidney
- Skin (viral toxin)
- Brain
Neurological symptoms in SC and PANDAS

**SC**
- Chorea
- Hypotonia
- Cardiac involvement
- Hyperactivity
- Obsessions

**PANDAS**
- Emotional lability
- OCD-like symptoms
- Contamination fears
- Choreiform movements
- Tics
- Severe separation anxiety
- Urinary frequency
- Food refusal / anorexia

![Symptom severity over time](chart.png)
*S. pyogenes* activates both arms of the immune system: humoral (antibodies) and cellular (Th17 cells).

*Generation of Th17 cells after multiple intranasal infections*

Patients’ serum autoantibodies bind neurons and activate neuronal signaling.

Kirvan, C. et al. (2006), *J Neuroimmunol*
Kirvan, C. et al. (2003), *Nat Med*
Autoantibodies can bind post-synaptic D1R and D2R receptors

Access to the brain?

dopamine

SC: D2R, D1R or ratio of D2R/D1R titers correlate with symptoms

PANDAS: D2R; Mixed reports

Ben-Pazi, H. et al., 2012 J Mol Neurosci
Ben-Pazi, H. et al., 2013 PLoS One
Brilot, F. et al., 2011 Neurology
Dale, R. C. et al., 2012 Brain
Kirvan, C. et al., 2006 J Neuroimm
Kirvan, C. et al., 2003 Nat Med
Outline

1. What is the role of S. pyogenes-specific Th17 lymphocytes in post-infectious basal ganglia encephalitis?

2. Are Th17 lymphocytes necessary for the development of disease in the brain?
A novel rodent model to understand the impact of the immune system on the brain after S. pyogenes infections

Dileepan T., Smith E. et al., 2016 J Clin Invest
Hypothesis: Dysregulated Th17 immune response to *S. pyogenes* infections is key to understanding “autoimmune” complications associated with this pathogen.
*S. pyogenes*-specific CD4+ T lymphocytes are present in the brain after multiple intranasal infections.

Dileepan T., Smith E. *et al.*, 2016 *J Clin Invest*
Does the route of GAS infections determine entry of Th17 cells into the brain?

**Subcutaneous**
- PTx i.v.
- GAS or PBS
- CFA emulsion

**Experiment day**
- 0
- 14
- 28
- 30

**Intranasal**
- Weekly i.n. GAS or PBS

**Experiment day**
- 0
- 7
- 14
- 21
- 28
- 30

Platt M. *et al.* (unpublished)
Only intranasal GAS infections promote entry of Th17 cells into the brain

Platt M. et al. (unpublished)
# The route of inoculation determines immune outcome

<table>
<thead>
<tr>
<th></th>
<th>Subcutaneous</th>
<th>Intranasal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T cell phenotype</strong></td>
<td>Th1</td>
<td>Th17, Th1</td>
</tr>
<tr>
<td><strong>T cells in the CNS</strong></td>
<td>Few</td>
<td>Many</td>
</tr>
<tr>
<td><strong>Microglia activation</strong></td>
<td>Some</td>
<td>Robust</td>
</tr>
<tr>
<td><strong>Glomerular synapse loss</strong></td>
<td>N/A</td>
<td>Degraded</td>
</tr>
<tr>
<td><strong>Anti-GAS titer</strong></td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td><strong>BBB permeability</strong></td>
<td>Adjuvants</td>
<td>High</td>
</tr>
<tr>
<td><strong>Behavioral deficits</strong></td>
<td>Perseveration</td>
<td>Mild motor deficits</td>
</tr>
</tbody>
</table>

Platt M. *et al.* (unpublished)
S. pyogenes CD4+ T cells in the brain are associated with neuroinflammation

Dileepan T., Smith E. et al., 2016 J Clin Invest
Do Th17 cells in the brain affect the integrity of blood vessels?

Intranasal

Weekly i.n. GAS or PBS

i.v. tracer, sac

Experiment day

0  7  14  21  28  30

Biocytin-TMR

GAS or PBS

Dileepan T., Smith E. et al., 2016 J Clin Invest
The integrity of the blood-brain barrier is compromised after multiple *S. pyogenes* infections.

Dileepan T., Smith E. *et al.*, 2016 *J Clin Invest*
GAS-specific Th17 lymphocytes may disrupts blood-brain barrier tight junctions

Dileepan T., Smith E. et al., 2016 J Clin Invest
Are Th17 cells present in tonsils from PANDAS children?

- *S. pyogenes* subtypes may be important for disease pathogenesis

- Genetic risk factors for SC and PANDAS

- Repeated infections with *S. pyogenes* or other pathogens that generate Th17 cells.

- Presence of both Th17 cells and autoantibodies is important for disease pathogenesis

Dileepan T., Smith E. *et al.*, 2016 *J Clin Invest*
Novel pathway for T cell entry into the CNS

Dileepan T., Smith E. et al., 2016 J Clin Invest
Outline

1. What is the role of S. pyogenes-specific Th17 lymphocytes in post-infectious basal ganglia encephalitis?

2. Are Th17 lymphocytes necessary for the development of disease in the brain?
Visualizing the presence of Th17 lymphocytes in the brain using $ROR\gamma t^+/GFP$ mice

Martin Lipp & Gerd Müller, Lymphoid organogenesis: getting the green light from RORt

GAS or PBS

**Intranasal**

Platt M. *et al.* (unpublished)
Th17 cells are a subset of total CD4\(^+\) T lymphocytes in the CNS after multiple *S. pyogenes* infections

Platt M. *et al.* (unpublished)
RORγtc-/− mice have fewer CD4+ T cells in the brain after multiple *S. pyogenes* infections

Platt M. *et al.* (unpublished)
Neuroinflammation is present in the brain in the absence of Th17 cells after multiple infections

Platt M. et al. (unpublished)
Blood-brain barrier is still damaged in the absence of Th17 lymphocytes after multiple *S. pyogenes* infections.

Platt M. et al. (unpublished)
Th17 cells are not required to damage synapses after multiple *S. pyogenes* infections

Platt M. *et al.* (unpublished)
*S. pyogenes*-infected mice have blunted olfactory perception regardless of the presence or absence of Th17 cells

Platt M. et al. (unpublished)
Th17 and Th1 cells are present in the CNS after multiple GAS infections.
How can OCD/PANDAS research in animals inform clinical diagnosis and treatment?

- Test whether cytokine profiles characteristic of a Th17/Th1 phenotype are present in CSF from PANDAS patients.

A “signature profile” for autoimmunity to ascertain diagnosis of PANDAS/PANS should be established.

- Develop novel, dynamic contrast-enhanced MRI imaging tools to detect blood-brain barrier dysfunction during periods of disease flares.

- Develop new immunotherapies targeted against Th17/Th1 cells, in conjunction with IVIG or plasmapheresis.

Cutforth T., Agalliu, D et al., 2016 J Future Neurology
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