The Immunopathogenesis of Secondary Progressive MS

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Disclosures

• Benjamin Segal, MD
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  Honoraria from Industry: Biogen, Innate Therapeutics

• CME Staff Disclosures
  Professional Education Services Group staff have no
  financial interest or relationships to disclose.
Learning Objectives

At the conclusion of this activity, the participant will be able to:

• A. Discuss the immune abnormalities that characterize different stages of multiple sclerosis
• B. Discuss the role of lymphoid neogenesis in progressive MS
• C. Discuss novel MRI outcome measures in MS
Obtaining CME Credit

• If you would like to receive CME credit for this activity, please visit:

http://www.pesgce.com/PVAsummit2011/

• This information can also be found in the Summit 2011 Program on page 8.
Is progressive MS a neuroimmunological or a neurodegenerative disorder?

Is the accumulation of disability during progressive MS the result of the delayed degeneration of axons / death of neurons that were originally damaged by immune mediators during the relapsing remitting phase, or is ongoing neuroinflammation responsible?
Evidence that SPMS is a non-inflammatory disorder

- Loss of responsiveness to immunomodulators (IFNβ, glatiramer acetate)
- Decreased frequency of enhancing lesions on MRI
Evidence that SPMS is an inflammatory disorder

- Efficacy of chemotherapeutic agents in some patients (Mitoxantrone)
- Persistence of oligoclonal bands in the CSF
- Evidence of persistent immune dysregulation in the periphery
- Presence of diffuse T cell infiltration and microglial activation in CNS specimens from individuals with SPMS
Neuropathological Patterns in MS Subsets

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Acute (&lt; 1 yr)</th>
<th>RRMS (&lt;1yr)</th>
<th>SPMS (&gt;1 yr)</th>
<th>PPMS (&gt; 1yr)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>39</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>11</td>
<td>8</td>
<td>8</td>
<td>73</td>
</tr>
</tbody>
</table>

Specimens from 88% of SPMS patients had prominent inflammatory features

Myelin specific Th1 and Th17 effector T cells have both been implicated in MS pathogenesis:

- Th1 cells are induced by IL-12 and produce IFN$_{\gamma}$
- Th17 cells are induced by IL-23 and produce IL-17
IL-12 and IL-23 modulated cells induce qualitatively different types of spinal cord inflammation

### Genes upregulated in PBMCs during SPMS

<table>
<thead>
<tr>
<th>Genes</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-4-integrin</td>
<td>0.1030</td>
</tr>
<tr>
<td>Beta-7</td>
<td>0.1700</td>
</tr>
<tr>
<td>FOXp3</td>
<td>0.2769</td>
</tr>
<tr>
<td>EBI3</td>
<td>0.0698</td>
</tr>
<tr>
<td>GMCSF</td>
<td>0.1419</td>
</tr>
<tr>
<td>IFN-gamma</td>
<td>0.5830</td>
</tr>
<tr>
<td>IL12p35</td>
<td>0.1541</td>
</tr>
<tr>
<td>IL12p40</td>
<td>0.0075**</td>
</tr>
<tr>
<td>IL12Rb1</td>
<td>0.4245</td>
</tr>
<tr>
<td>IL12Rβ2</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>IL17</td>
<td>0.4240</td>
</tr>
<tr>
<td>IL23p19</td>
<td>0.0094**</td>
</tr>
<tr>
<td>IL23R</td>
<td>0.0004**</td>
</tr>
<tr>
<td>RORγT</td>
<td>0.0471*</td>
</tr>
<tr>
<td>Tbet</td>
<td>0.1855</td>
</tr>
</tbody>
</table>

Repeated measurement analysis
IL-12p40 family genes elevated in SPMS
IL-12p40 related proteins are produced in excess in SPMS
The Th1/Th17 balance changes during the transition to SPMS
Longitudinal patterns of MBP-specific cytokine production in SPMS

Th1 dominant (62%)

Mixed (22%)

Th17 dominant (22%)

Healthy control
MBP-specific cytokine patterns in RR and SPMS

<table>
<thead>
<tr>
<th></th>
<th>RRMS</th>
<th>SPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Th1 dominant</strong></td>
<td>44% (4/9)</td>
<td>62% (10/16)</td>
</tr>
<tr>
<td><strong>Th17 dominant</strong></td>
<td>22% (2/9)</td>
<td>19% (3/16)</td>
</tr>
<tr>
<td><strong>Mixed</strong></td>
<td>33% (3/9)</td>
<td>19% (3/16)</td>
</tr>
</tbody>
</table>
Correlation between IFN$_\gamma$/IL-17A ratio and disease duration

Correlation coefficient $r^2 = 0.63$
The Tbet:RORγt ratio is elevated in transitional MS.
A higher % of patients with transitional than RR MS mount Th1 recall responses

<table>
<thead>
<tr>
<th>Cytokine profiles</th>
<th>Patients with relapsing–progressive course (n=10)</th>
<th>Patients with relapsing–remitting course (n=21)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number (%)</td>
<td>number (%)</td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>6 (60)</td>
<td>5 (24)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Type 2</td>
<td>1 (10)</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Type 0</td>
<td>1 (10)</td>
<td>9 (43)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>No cytokine production</td>
<td>2 (20)</td>
<td>7 (33)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Barth H, et al. 2002 *J. Neuroimmunol.* **133**:175
Increased TcR-mediated IFN-γ production in MS is linked to defective regulation by endogenous IL-12.

Balashov K et al. PNAS 1997;94:599-603
Normalized IL-12p35 levels in PBMCs of SPMS patients versus healthy controls
Enhanced secretion of cytokines by MO from SPMS patients

DC from SP MS donors express elevated levels of IL-12

DC from SPMS donors stimulate T cells to produce IFN\(\gamma\)

Th17 cells convert to Tbet⁺ IFNγ producers during EAE

Gated on eYFP+ cells

Hirota et al. *Nature Immunology* 2011 12:255
IL-17 production by PBMC is highest at the onset of MS

Summary: Evolution of the autoreactive Th repertoire during the transition from RRMS to SPMS

- PBMCs from MS patients over-express IL-12 and contain elevated frequencies of MBP-specific cytokine producing cells
- The majority of SPMS patients in our study exhibited Th1 dominant MBP-specific responses that were stable over time
- This Th1 “skewing” was more prominent in SPMS than in RRMS

Hypothesis: The autoreactive T cell response evolves over the course of MS to favor TH1 immunity, in part, as a consequence of Th17 plasticity
Therapeutic Implications

Targeting Th1 cells and their factors in individuals with transitional and progressive forms of MS
CXCR3 is preferentially expressed on Th1 cells from subjects with transitional MS
CXCR3 expression correlates with the Th1:Th17 ratio
SPMS is associated with a shift of the autoimmune response from a peripheral to a CNS driven process.
Enhancing lesions in SPMS
Enhancing lesions in a subset of SPMS
IFN-γ production in SPMS patients with or without enhancing lesions
ROI Selection in Corpus Callosum

Genu  Body  Splenium
MS enhancing group P04

1st scan

6th(last) scan
Longitudinal patterns: IL-12p40 monokine subunits and downstream cytokines
Longitudinal patterns: IL-12p40 monokine subunits and downstream cytokines
The CNS as a Lymphoid Organ

Prineas described “clusters of plasma cells together with...reticular cells surrounding collagen-free channels containing lymphocytes and macrophages” and “lymphatic-like capillaries” in plaques in brain specimens from patients with MS.

Science 203:1123-1125
Lymphoid Follicles in Progressive MS

Magliozzi, et al. Brain 130: 1089-1104
Characteristics of SPMS patients with meningeal follicles

- Younger age of onset
- Frequent relapses in first three years from diagnosis
- Relatively high level of disability based on age
- Shorter lifespan
Normalized Lymphotoxin-β level

enhancing lesions: - +
CXCL13+ Lymphoid like follicles in meninges of mice with EAE

CNS

LN

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Spleen</th>
<th>Spinal Cords</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chronic

LFB-PAS

RCA

GFAP

Trichrome

CXCL13 -/-  WT
Summary II: The role of CXCL13 driven lymphoid neogenesis in the CNS during SPMS

• A subset of SPMS patients (approximately 40%) experience progressive axonal loss/ demyelination in the corpus callosum association with episodic cytokine dysregulation and CNS inflammatory activity.

• CNS inflammation tends to precede peripheral spikes in cytokine expression, suggesting that the autoimmune attack is mounted within the CNS

• Meningeal B cell follicles expressing the chemokine CXCL13 are present in SPMS patients with a more aggressive clinical course
Summary II (cont’d)

• Meningeal B cell follicles are most frequently located in deep sulci, in direct apposition to cortical plaques

• CXCL13 deficiency or LTβ receptor antagonism abrogates relapses and chronic progression of EAE

• Drugs designed to dissociate meningeal lymphoid follicles (such as anti-CXCL13, CXCR5-Fc fusion proteins, pr Lymphotoxinβ receptor fusion proteins) might be therapeutic in some patients with SPMS (intrathecal administration?)
HUMAN B CELL-ATTRACTING CHEMOKINE 1 (BCA-1; CXCL13) IS AN AGONIST FOR THE HUMAN CXCR3 RECEPTOR

Chung-Her Jenh f1, Mary Ann Cox, William Hipkin, Tianhong Lu, Catherine Pugliese-Sivo, Waldemar Gonsiorek, Chuan-Chu Chou, Satwant K. Narula and Paul J. Zavodny

Cytokine
Volume 15, Issue 3, August 2001, Pages 113-121
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Ashok Srinivasan, M.D.
Lymphoid Chemokines and Chronic Inflammation

- Ectopic expression of CXCL13 in non-lymphoid organs leads to chronic inflammation and the formation of lymph node-cell like structures ("lymphoid neogenesis")

- TNF family molecules (TNF\(\alpha\), LT-\(\alpha\) and LT-\(\beta\), that have been implicated in the pathogenesis of EAE and MS, stimulate production of CXCL13
CXCL13 in MS lesions

- p < 0.001
- Control CNS vs. active, inactive MS lesions

Activated T cells but not APCs from progressive MS patients are responsible for increased IL-12 production.

Balashov K et al. PNAS 1997;94:599-603
Neurite density is diminished in SPMS cords with follicles
Longitudinal time course for Lymphotoxin B expression for Patient 9

Data is normalized to 4 Housekeeping genes
Longitudinal time course for Lymphotoxin B expression for Patient 12

Data is normalized to 4 Housekeeping genes
LTβR-Ig Tx inhibits clinical EAE

RunX3 normalized to GAPDH

Th1                      Th2                     Th17