The Pathogenesis of *Chlamydia pneumoniae* in Multiple Sclerosis: Current Thoughts and Future Directions

Seminars in Pathology
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Features of *C. pneumoniae* Infection

- Obligate intracellular pathogen
- Requires host mitochondria as energy source via ATP translocase
- At least nine different species known with different bio-virulence and tropism
- Tissue persistence is a common feature of all chlamydial infections
Life Cycle of *C. pneumoniae*

- Entry of elementary body into macrophages
- Transition from elementary body to replicate body
- Replication of RB’S
- Condensation of RB’s & maturation to EB’s
- Persistent phase
- Release of EB’s
Evidence for *C. pneumoniae* Infection in MS

- Presence of genomic DNA and mRNA in CSF of MS patients but not controls
- Elevated antibody titers to *C. pneumoniae* in MS patients over controls
- Reactivity of OC bands to chlamydial antigens
- Presence of *C. pneumoniae* in brain & CSF
C. pneumoniae and MS
Initial Case Report

- 24-year-old man with MS for 6 months
- Expanded Disability Status Scale = 8.0
- C. pneumoniae isolated from CSF
- CSF PCR for C. pneumoniae positive
- CSF antibodies to C. pneumonia elevated
- Response to anti-chlamydial therapy
- EDSS after 6 months Rx = 3.0
- (Sriram et al, Neurol 1998;50:571-2)
**C. pneumoniae** and MS

**Study Plan**

- **MS Patients:** Patients who satisfied the Poser criteria for the diagnosis of clinically definite MS were recruited.

- **Other Neurologic Disease Controls:** Age and gender matched controls were recruited for OND patients in whom CSF was being obtained for diagnostic studies.

- (Sriram et al, Ann Neurol 1999;46:6-14)
**C. pneumoniae and MS CSF Culture Results**

- Relapsing remitting MS: 8/17 positive (47%)
- Progressive MS: 16/20 positive (80%)
- Overall MS: 24/37 positive (65%)
- OND controls: 3/27 positive (11%)
C. pneumoniae and MS

PCR/S Results

- Relapsing remitting MS: 17/17 (100%)
- Progressive MS: 19/20 (95%)
- Overall MS: 36/37 (97%)
- OND controls: 5/27 (18%)
C. pneumoniae and MS
CSF Oligoclonal Bands

- In 14 of 17 patients with MS, oligoclonal bands were adsorbed either partially or completely from the CSF by elementary body antigens of C. pneumoniae
- (Yao et al, Neurol 2001;56:1168-76)
Adsorption of oligoclonal bands (OCBs) from the CSF of patients with Secondary Progressive Multiple Sclerosis (SPMS, lanes 1 to 6) and Relapsing Remitting MS (RRMS, lanes 7 to 11) patients compared to the CSF of Other Neurological Disease Controls (ONDs, lanes 12 to 20). Equal amounts of Ig were subjected to isoelectric focusing in order to separate Igs by their isoelectric points. The separated Igs were then blotted onto nitrocellulose membranes which had been coated with antigens from EBs of *Chlamydia pneumoniae* with the remaining sites blocked with BSA.
C. pneumoniae and MS CSF Oligoclonal Bands

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Presence of *C. pneumoniae* in CSF of MS Patients

- Electron dense structures resembling *C. pneumoniae* elementary bodies were detected in CSF of MS patients
- These electron dense structures were noted to be positive for *C. pneumoniae* by immunogold staining
- CSF from these MS patients also was PCR positive for *C. pneumoniae* DNA
- (Sriram et al, J Infect Dis 2005;192:1219-28)
Activation Of Brain-Reactive T-Cells
Non-Specific Vs. Molecular Mimicry

(A) Non-specific Activation of Brain-reactive Memory T-Cell

- Antigen-Presenting Cell
- IL-1
- MHC
- Viral peptide
- T-Cell receptor
- IFN-γ
- IL-2

(B) Molecular Mimicry

- Viral peptide-reactive T-Cell
- Viral Peptide-Reactive T-Cell Cross-Reacts With Brain Antigen

- Dividing Brain-Reactive T-Cell
- To CNS
- To CNS
• Immune system T cells normally in the bloodstream become activated against components of the brain myelin

• They cause local inflammation in scattered regions of the brain and spinal cord once they cross the barrier between the bloodstream and CNS
Immune Mechanism In Demyelination

Activated macrophage/microglia

Class II MHC

Antigen

T-Cell receptor

Activated CD4+ T-Cell

IFNγ

IL-1

TNF/IL

Oligodendrocyte

Axon

Myelin

Axon

Myelin
Mechanisms of Demyelination

Phagocytosis Vs. Apoptosis

Activated Phagocytic Macrophage

Apoptotic Oligodendrocyte

Fragmentation

Axon
Evidence for Primary Oligodendrocytic Death as the Initial Event in Multiple Sclerosis

• In early (17-hour) lesions of multiple sclerosis, there is relatively little loss of myelin throughout the lesion
• In early lesions, myelin sheaths show only a slight reduction in staining intensity
• In early lesions, all normal appearing oligodendrocytes are replaced by apoptotic oligodendrocytes that have shrunken nuclei with annular/compact condensation of nuclear chromatin
• Macrophages, T cells, and other mononuclear cells as well as enlarged astrocytes are absent in these early lesions

Apoptosis of Oligodendrocytes in an MS Patient Dying of Acute Worsening of MS (Barnett and Prineas)
Figure 1. Alternative views of the mechanisms of lesion formation in MS. (a) Activated T cells migrate into the CNS (1) and initiate inflammatory events, including recruitment of blood macrophages, activation of local microglia and release of toxins. This leads to myelin destruction, oligodendrocyte death and clearance of damaged tissue by phagocytes (2). (b) Viruses, glutamate and other agents can cause extensive oligodendrocyte apoptosis in tissue foci (1). As a consequence, large amounts of myelin debris are generated (2), overwhelming the physiological mechanisms of elimination of apoptotic leftovers and, thus, triggering inflammation. Subsequently, T cells and macrophages invade the CNS (3) and initiate a stereotyped autoimmune attack of myelin (4), as described in (a).
Breeches of the CNS barriers: Role of Circumventricular Organs

- In certain regions of the brain the blood brain barrier is deficient
- Dyes injected intravenously can be seen defusing into tissues around these specialized regions
- These regions are located in and around the third ventricle and the floor of the fourth ventricle
- These regions consist of loose fenestrated vascular tissue with endothelial cells known as ependymal cells or tanycytes that appear different from those in the rest of the CNS
- These overlying ependymal cells are non-ciliated
- The function of these specialized areas is not fully understood

Breeches of the CNS barriers: Role of Circumventricular Organs

- Recently, the choroid plexus has been considered as an alternative entry site for circulating lymphocytes into the CSF
- The choroid plexus belongs to the circumventricular organs (CVOs) localized in the walls of the ventricles
- Other CVOs, which are similar to the choroid plexus lack an endothelial BBB, are also considered as possible entry sites for immune cells into the CNS parenchyma or the CSF

Summary of the Distribution of Pathological CNS Lesions in MS

- Plaques follow the course of the third and fourth ventricles and are seen in 100% of patients.
- Most periventricular plaques (seen adjacent to the 3rd and 4th ventricles) are old suggesting they may be the initial lesions.
- Cortical plaques are as common as periventricular plaques but are less extensive and only appear with late disease progression.
- Plaques in spinal cord are more common in the cervical cord than in the thoracic and lumbar cords.
Figure 1  A, immunohistochemical (IHC) staining of the brain periventricular region from 2 patients with multiple sclerosis [left 20×] and middle [100×] and from a control individual with other neurological disease [right [20×]], by use of monoclonal antibody (MAb) 807. Note the granular staining of cytoplasm of ependyma. B, IHC staining of a mouse lung infected with Chlamydia pneumoniae, by use of MAb 807 [left] and isotype control antibody [right]. Note the granular staining of C. pneumoniae–infected cells.
Figure 2. Six panels showing immunohistochemical (IHC) staining of ependyma from patient 1 with multiple sclerosis (table 2), stained with CF-2 antibody (upper left), anti-hsp60 antibody (upper middle), and monoclonal antibody (MAb) 807 (upper right). Their respective isotype-matched antibody controls are shown in the lower panels. The staining in these sections shows the signal as arising in either the nuclear or perinuclear region of the cell. In the panel showing staining with MAb 807, weak, diffuse staining is seen in some of the subependymal cells (20×).
The Pathogenesis of *Chlamydia pneumoniae*

- Effects of chronic *Chlamydia* infection on host cells
  - Impaired host cell function and signaling
  - Host cell production of nitric oxide
  - Host cell production of growth factors
  - Host cell production of cytokines
  - Host cell production of chemokines
  - Inhibition of host cell apoptosis
Pattern of Progression of CNS Damage in Multiple Sclerosis Following Infection of Ependymal Cells in Circumventricular Organs (CVO)

- CNS damage may be caused by the migration of a pathogen(s) into the CVO

- Infection of ependymal cells and other regions around the CVO may be an early and critical event in MS

- In MS, lesions are maximal in areas around CVO and decrease away from it

- In MS, lesions in the thoracic cord are 50% less than lesions in the cervical cord

- The immunopathology of CNS lesions may result from an inflammatory response caused by a distant pathogen located in the CVO region
Monoclonal Antibody (mAb) Therapy for Multiple Sclerosis

• Approved mAbs
  – Natalizumab targets an integrin present on all leukocytes except neutrophils
  – This blocks binding of these leukocytes to the vascular cell adhesion molecule and thus prevents migration of these leukocytes into the tissue
  – Currently restricted because a number of patients with MS developed progressive multifocal leukoencephalopathy

(Lutterotti and Martin. Lancet Neurol. 7:538-547, 2008)
Monoclonal Antibody (mAb) Therapy for Multiple Sclerosis

• Investigational mAbs
  – Daclizumab targets the interleukin-2-receptor site present on upregulated T cells
  – This blocks the proliferation of autoreactive T cells
  – Clinical results to date have not been impressive

(Lutterotti and Martin. Lancet Neurol. 7:538-547, 2008)
Monoclonal Antibody (mAb) Therapy for Multiple Sclerosis

- Investigational mAbs
  - Rituximab targets CD20 present on B cells and lyses these cells
  - This reduces peripheral B cells by 100% and CSF B cells by 90%
  - Rituximab has been approved in the USA and Europe for the treatment of B-cell non-Hodgkin’s lymphoma as well as for the treatment of rheumatoid arthritis when anti-TNF-alpha therapy has failed
  - Clinical results to date have been good; study results from MS patients with primary-progressive disease should be published soon

(Lutterotti and Martin. Lancet Neurol. 7:538-547, 2008)
Monoclonal Antibody (mAb) Therapy for Multiple Sclerosis

• Investigational mAbs
  – Alemtuzumab targets the CD52 cell-surface glycoprotein present on T cells, B cells, monocytes and eosinophils
  – This mediates the cytotoxic effects of its target cells and rapidly produces a profound leucopenia
  – Alemtuzumab is licensed in the USA and Europe for the treatment of fludarabine-resistant chronic lymphocytic leukemia
  – The therapeutic benefit in MS has been impressive
  – Currently there are concerns about long-term safety

(Lutterotti and Martin. Lancet Neurol. 7:538-547, 2008)
Heat Shock Protein mRNA Studies in Multiple Sclerosis

- Two investigators have found mRNA from *Chlamydia pneumoniae* in some patients (50% and 64%) with active relapsing-remitting multiple sclerosis.
- The PCR tests used in these studies were not very sensitive.
- A more sensitive PCR for HSP mRNA might be useful clinically to identify infected MS patients.

Future Direction

• PCR-EIA test for *Chlamydia pneumoniae* MOMP
• PCR-EIA test for *C. pneumoniae* HSP mRNA
• Evaluation of these more sensitive PCR methods in MS patients using a multi-collaborative approach
• Investigation of the *in-vivo* effect of *C. pneumoniae* LPS and HSP-60 on oligodendrocytes in the mouse brain
• Susceptibility testing method for *C. pneumoniae* using HSP-60 mRNA and MOMP
$y = -0.3462x + 17.3189$

$r = 0.9649$, $p < 0.000001$
Collaborators

VUMC/Neurology    VUMC/Pathology

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