

## Neurological inflammatory disease

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Clinical Reader in Neuroimmunology

## Outline of lecture

- Introduction
- Classification of neuroinflammatory disorders
- CNS inflammation: Multiple sclerosis
- Brief summary of related CNS inflammatory conditions
  - CNS-specific inflammatory syndromes
    - Acute disseminated encephalomyelitis
    - Neuromyelitis optica

## The spectrum of neurological immune-mediated disorders

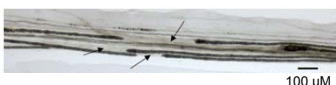
- Target anatomical sites:
  - Muscle and neuromuscular junction
  - Peripheral nervous system
  - Central nervous system
- *Cellular/Molecular targets:*
  - PNS myelin, Schwann cells, axons
  - CNS myelin, oligodendrocytes, axons
  - Ligand- or Voltage-gated ion channels, water channels

## Disorders of muscle and neuromuscular junction

- Inflammatory myopathies
  - Idiopathic Polymyositis
  - Dermatomyositis
  - Inclusion body myositis
- Neuromuscular junction disorders
  - Myasthenia Gravis
  - Lambert-Eaton Myasthenic Syndrome

## Peripheral nerve disorders

- Acquired demyelinating inflammatory neuropathies
  - Acute: Guillain-Barre' Syndrome
  - Chronic Inflammatory Demyelinating Polyneuropathy



## Definition of CNS inflammatory disorders

- Virtually any type of acute injury to the CNS results in some degree of inflammation (e.g. infections, tumours, stroke)
- We consider **neuro-inflammatory** those conditions characterised **primarily** by inflammation
- Aetiologies are unknown or thought to be autoimmune

### Main CNS inflammatory disorders

- MS, neuromyelitis optica, ADEM, Sjogren's, neuro-SLE, Behcet's, sarcoidosis, anti phospholipid antibodies syndrome
- CNS vasculitides

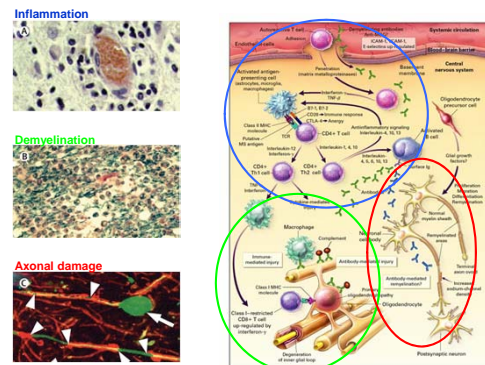
### Multiple sclerosis: definition

- A chronic inflammatory multifocal demyelinating disease of the central nervous system of unknown cause resulting in loss of myelin and oligodendroglial and axonal pathology
- Typically affecting young adults with exacerbating-remitting pattern or chronic progressive evolution

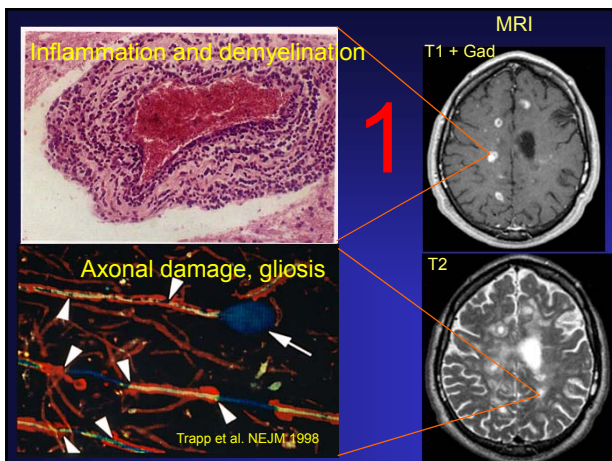
### MS pathogenesis – the working hypothesis

- MS is primarily considered an **inflammatory**, probably **autoimmune demyelinating** disease of the CNS
- A **neurodegenerative component** to the disease is increasingly recognised
- Exact cause of the pathological process remains unknown

### Histopathology of MS – 3 main components



Adapted from Compston and Coles, Lancet 2008 and Noseworthy et al. NEJM 2000



### Basis of the autoimmune hypothesis in MS

1. Immunopathology of lesions
2. Susceptibility associated with immune response genes
3. CSF immunological abnormalities
4. Subtle alteration of blood T cell functions
5. Animal models of autoimmune disease
6. Comorbidity and similarities with other autoimmune diseases
7. Response to immuno-suppressive and -modulatory therapies

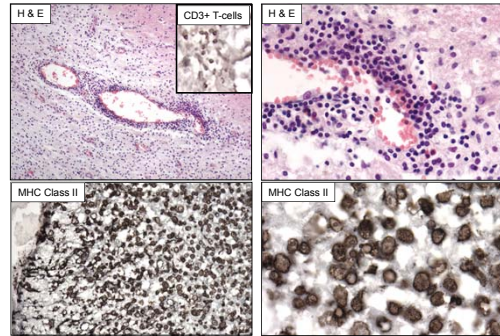
### 1. Immunopathology of CNS

Perivascular cuffs and intra-parenchymal infiltrates of inflammatory cells, mostly **CD4+** and **CD8+ T cells**

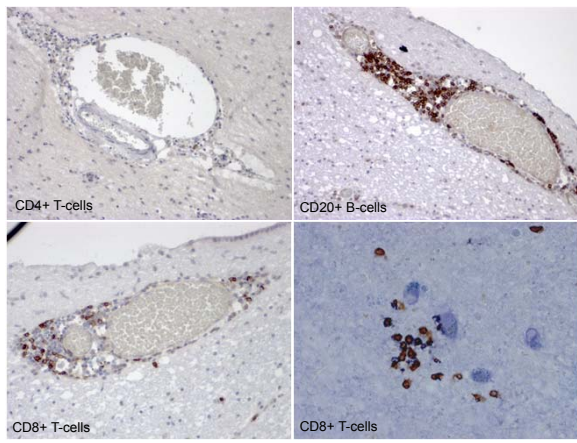
**B-cells** are found in perivascular and meningeal locations where they can aggregate or form part of ectopic lymphoid follicles

Myelin-laden **macrophages** in and around lesions

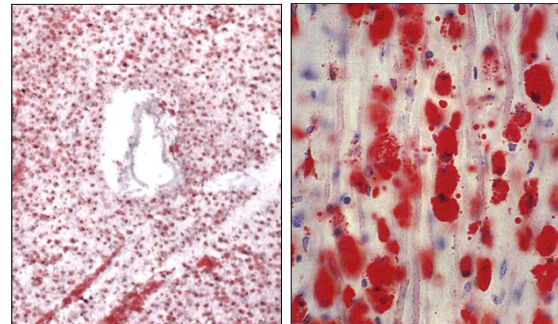
### Inflammation and MS - the earliest events



T-lymphocyte infiltration is only seen during the very early stages of lesion formation and even during active demyelination (above) only few T-cells are found in the brain parenchyma. B-lymphocytes may also be found in small numbers. The majority of inflammatory cells in the MS lesion are monocytes/macrophages.



### Macrophages and MS

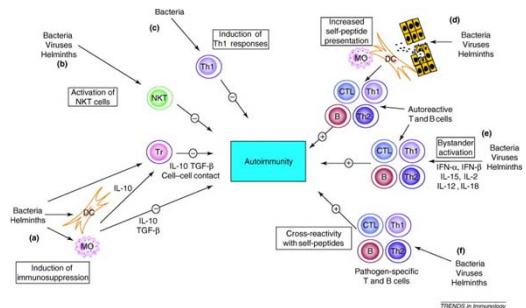


**Macrophages in an active MS plaque.** The foamy macrophages contain numerous lipid droplets, stained here with oil red-O, which represent myelin breakdown products

### How do lymphocytes become activated?

- To migrate into tissues, including CNS, lymphocytes require activation
- The events leading to pathological immune activation in MS are unclear**
- Possible mechanisms induce infection or cross-reactivity with microbial, especially viral antigen(s)
  - Molecular mimicry**
  - Bystander activation**

### Potential mechanisms for how infections with pathogens might influence the development of autoimmunity

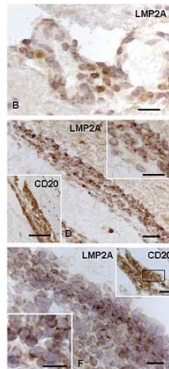


Kamradt et al. 2005

### A possible role of EBV in MS pathogenesis: 1 – histological studies

- EBV almost exclusively infects B cells and uses the B-cell differentiation program to establish a persistent, usually asymptomatic, latent infection in humans.
- Using in situ hybridisation and immunohistochemistry, B cells infected with EBV were detected in post-mortem brain tissue (Serafini et al JEM 2007; JNEN 2010)

controversial



Serafini et al (2010) J Neuropathol Exp Neuro 69(7):677-93

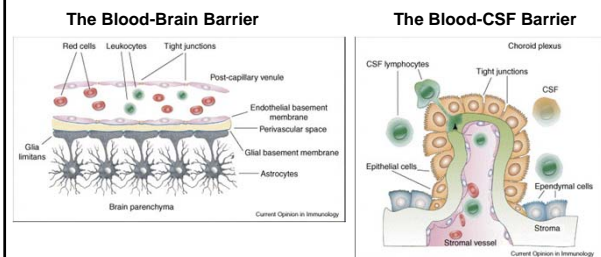
### A possible role of EBV in MS pathogenesis: 2 – serological studies

- Large serological studies show ~100% serological positivity for EBV infection in MS vs. 90% in healthy (Ascherio and Munch, Epidemiology. 11(2):220-224, March 2000)
- Odds ratio of MS comparing EBV seropositive individuals with EBV seronegative individuals was 13.5 (95% CI = 6.3–31.4)
- In longitudinally followed US military personnel, MS risk was 36-fold higher among individuals with anti-EBNA complex IgG titers  $\geq 320$  than among those with titers  $< 20$  (Munger et al. Multiple Sclerosis 2011)

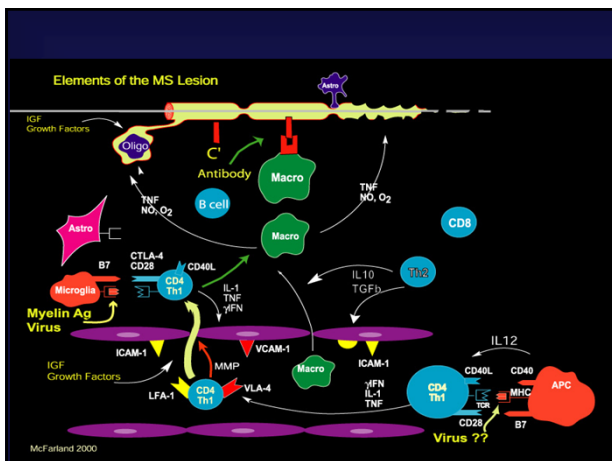
### How do immune cells get to the CNS?

- The CNS is an immune privileged compartment, which is carefully shielded from potentially harmful immune reactions
- There is not a classic lymphatic drainage of the parenchyma
- To get to the CNS leukocytes must pass either of the two physiological barriers:
  1. The Blood-Brain Barrier
  2. The Blood-CSF barrier

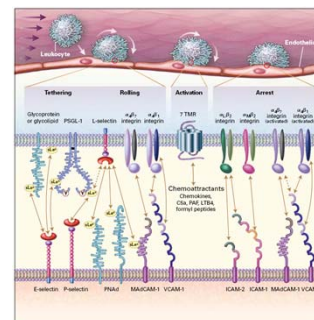
### Physiological barriers shielding the CNS



Rebenko-Moll et al. 2006

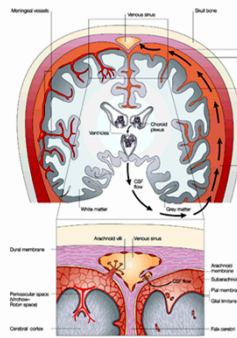


### Essential Molecular Players in the Multistep Adhesion Cascade.



von Andrian and MacKay, NEJM 2000

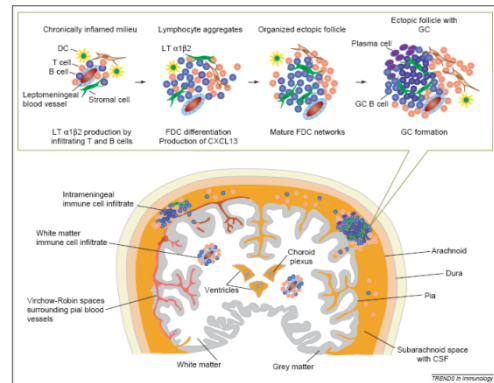
## The blood-CSF route to the CNS



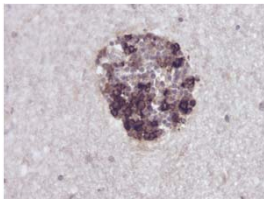
- Choroid plexus
- Subarachnoid space
- Pia
- Brain cortex

(Ransohoff 2003 Nat Rev Immunol)

## A role for B-cells in cortical pathology

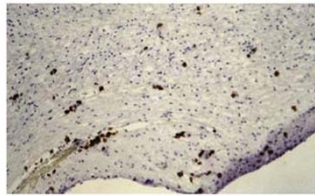
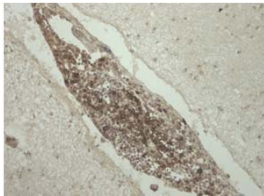


## B-lymphocytes and plasma cells



B-cell follicles are found in perivascular and meningeal locations

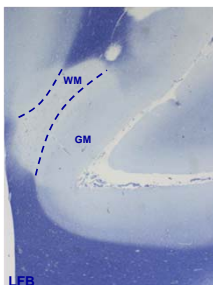
Isolated B-cells and plasma cells are found in the parenchyma in active lesions and in a perivascular location in chronic lesions



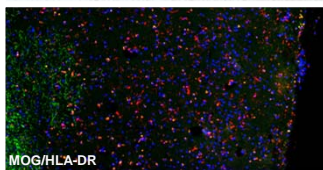
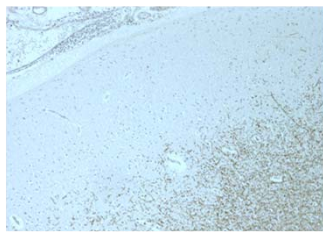
## B cell involvement in MS

- In MS lesions (and CSF) there are clonally expanded B cells
- **Ectopic B cell follicles develop in meninges of patients with SP-MS**
- BAFF (B cell activating factor, also named BLYS), a member of the TNF family, is overexpressed in MS tissue (astrocytes)
- Subpial B cell follicles can restimulate inflammatory T and B cells
- Secretion of inflammatory mediators diffusing to the brain cortex

## Pathology of grey matter lesions



Grey matter lesions are best identified using myelin protein immunostaining



## 2. Susceptibility associated with immune response genes

- Whole genome scans in large populations have confirmed associations with immune genes, including:

HLA-class II (DRB1\*1501, DRB5\*0101; DQw6) **strongest**

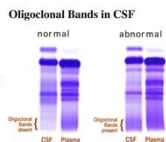
IL-7R

IL-2 R alpha

CD58

### 3. CSF immunological abnormalities

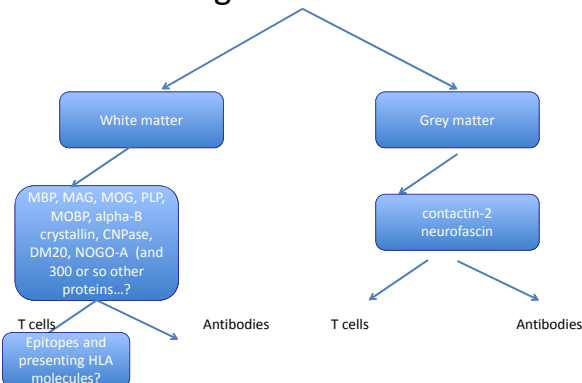
- Leukocyte counts can be normal but are often mildly increased above the normal of  $\approx 3000$  leukocytes/mL
- In MS 80% of total CSF cells are T cells (45% in blood), mainly memory T cells (up to 30% of CSF cells during inflammation), 5% are monocytes,  $\leq 1\%$  B cells, plasma cells
- There can be a minor protein increase
- Increased production of IgG in the CNS
- CSF oligoclonal bands are very frequent detected in MS and stable over years



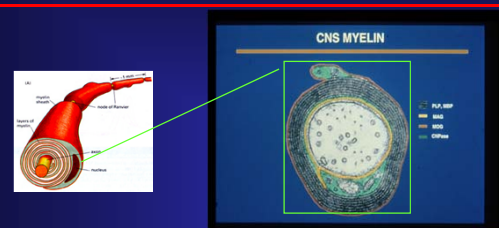
### 4. Subtle abnormalities of blood T cell functions

- Slightly increased frequency and reduced requirements for activation of T cells responding to myelin antigens
- Prevalence of T helper 1 cytokine secretion by *myelin* antigen-specific T cells
- Likely role of Th17 cells in driving inflammation
- Reduced activity of (CD4+/CD25+) regulatory T cells

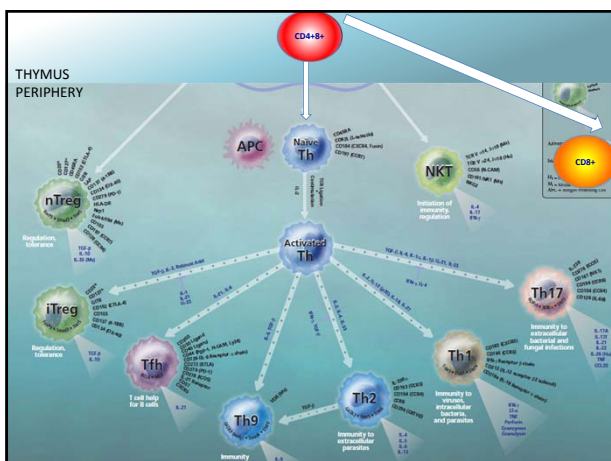
### CNS antigens – but which?



### Candidate target myelin antigens in MS



- Myelin basic protein (MBP)**; 170 aa, several isoforms
- Proteolipid protein (PLP)**; 272 aa, two isoforms
- Myelin oligodendroglia glycoprotein (MOG)**; target of T- and B cell response
- A few others



### Is CNS immunopathology initiated by Th17 cells?

- IL-17A expressed at relatively high levels in circulating leukocytes and cerebrospinal fluid mononuclear cells of patients with MS, particularly during relapses
- IL-17A transcripts were elevated in MS plaques compared to brain tissues from control subjects by microarray
- presence of IL-17A+ cells in active areas of MS lesions by immunocytochemistry
- CD8+, as well as CD4+, T cells equally immunostained for IL-17 in MS tissues
- In a patient with aggressive relapsing remitting MS, transcripts encoding retinoic acid-related orphan nuclear hormone receptor C (RORC; the hallmark transcription factor of Th17 differentiation) were upregulated in an acute lesion compared to normal appearing white matter

## Pathogenic CD8 T cells?

- CD8 are the most frequent T-cell subset seen in acute and chronic lesions, outnumbering CD4<sup>+</sup> T cells 3- to 10-fold in chronically inflamed MS plaques
- there is specific enrichment of highly differentiated CD8<sup>+</sup> rather than CD4<sup>+</sup> T cells in the CSF in relapsing-remitting and possible MS patients
- CD8 T cells show oligoclonal expansions in MS brains, blood, and CSF that have not been reported for CD4<sup>+</sup> T cells

## MS as a disease of regulatory T cell dysfunction?

### Loss of Functional Suppression by CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cells in Patients with Multiple Sclerosis

Vissia Viglietta, Clare Baecher-Allan, Howard L. Weiner, and David A. Hafler

Laboratory of Molecular Immunology, Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

- In patients with multiple sclerosis, the function of peripheral CD4<sup>+</sup> T<sub>Reg</sub> cells seems to be impaired... J Exp Med 199, 971

Research article

Patients with relapsing-remitting multiple sclerosis have normal Treg function when cells expressing IL-7 receptor  $\alpha$ -chain are excluded from the analysis

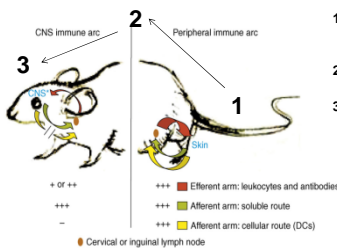
Laure Michel,<sup>1,2</sup> Laureline Berthelot,<sup>1</sup> Ségolène Pettré,<sup>1</sup> Sandrine Wierzbicki,<sup>1,2</sup> Fabienne Lefèvre,<sup>1</sup> Cécile Braudeau,<sup>1</sup> Sophie Brouard,<sup>1</sup> Jean-Paul Souillou,<sup>1</sup> and David-Alex Laplaud<sup>1,2</sup>

"...we conclude that CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup> Tregs from MS patients and healthy individuals exhibit similar suppressive functions. J Clin Invest 118, 3411

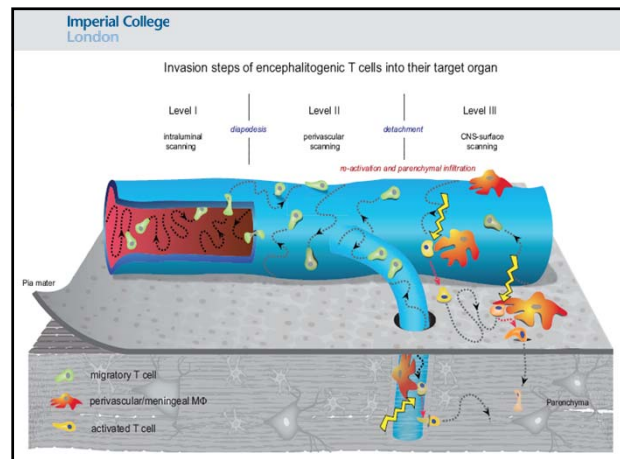
## 5. Animal models of autoimmune disease

### Experimental allergic encephalomyelitis (EAE):

- Induced by peripheral immunisation with myelin protein antigens
- Mediated by CD4<sup>+</sup> myelin-specific T cells



- 1) Autoreactive myelin-specific effector T cells are primed in peripheral lymph nodes and
- 2) Migrate into uninfamed CNS to initiate tissue inflammation.
- 3) Antigen re-encounter in CNS propagates inflammation and damage



## 6. Comorbidity and similarities with other autoimmune diseases

Patients with MS have increased incidence of some autoimmune conditions (best evidence is for thyroiditis) and asymptomatic positivity for autoantibodies

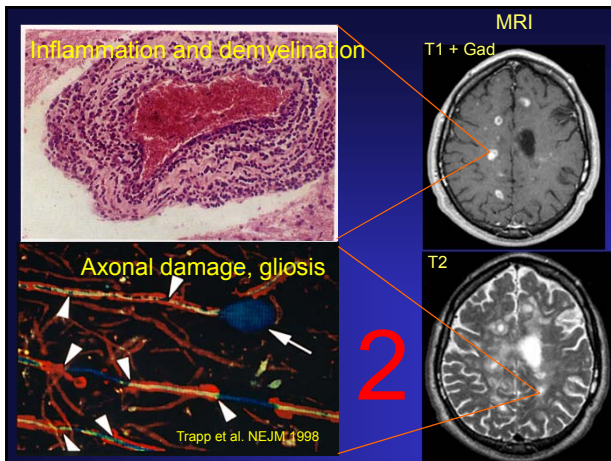
MS share with Rheumatoid Arthritis, Systemic lupus Erythematosus, autoimmune thyroiditis and other autoimmune disorders features such as:

- higher incidence in females,
- young adult onset,
- initially relapsing course

## 7. Response to immuno-suppressive and -modulatory therapies

MS acute relapses are improved by high-dose corticosteroid administration

The clinical course of MS is attenuated by immuno-modulatory treatment (e.g. interferon beta), by treatments blocking immune cell entry to the CNS (anti-alpha-4 integrin blockade: natalizumab) and by immuno-suppressive and cytotoxic agents (e.g. Mitoxantrone)



### Mechanisms of neuronal loss in MS

#### INDIRECT

- neuronal changes due to inflammation/demyelination induced axonal mitochondrial insufficiency

#### DIRECT (mostly immune-mediated)

- direct effect of inflammatory mediators produced by immune cells on neuronal perikarya in grey matter lesions
- direct effect of low level release of inflammatory mediators by **activated microglia** in normal appearing tissues

Unresolved

### Take home points

- MS is an inflammatory, likely autoimmune demyelinating and axonal disorder of the CNS
- Initiated by CD4 and/or CD8 T cells infiltrating the CNS, propagated by T cells, B cells, and plasma cells, with macrophages as common effector arm and a possible role of microglia
- Axonal degeneration is at least initially strictly dependent on inflammation, but may progress independently later on

### Recommended reading

#### Books:

- McAlpine's Multiple Sclerosis, Fourth Edition, Churchill Livingstone, 2005 (relevant sections)

#### Review and original articles:

- Compston & Coles (2008) Multiple Sclerosis. Lancet.
- Prineas et al (2001) Immunopathology of secondary progressive MS. Ann Neurol 50:646-657.
- Peterson et al (2001) Transected neurites, apoptotic neurons, and reduced inflammation in cortical MS lesions. Ann Neurol 50:389-400.
- Kutzelnigg et al (2005) Cortical demyelination and diffuse white matter injury in MS. Brain 128:2705-2712.
- Magliozzi et al (2007) Meningeal B-cell follicles in secondary progressive MS associate with early onset of disease and severe cortical pathology. Brain 130:1089-1104.

### Some of the many other CNS inflammatory disorders

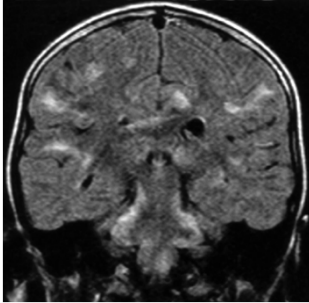
- **CNS-specific inflammatory syndromes**
  - Acute disseminated encephalomyelitis
  - Neuromyelitis optica
- **Systemic immune diseases affecting the CNS**
  - Neurosarcoidosis
  - Systemic lupus erythematosus
  - Anti-phospholipid syndrome
  - Sjogren's syndrome

### Acute disseminated encephalomyelitis (ADEM)

- Childhood age of onset
- Usually antecedent infection or immunization
- Molecular targets unknown
- Monophasic
- Fever, headache, meningism
- Seizures, coma
- Multifocal neurological deficits
- Bilateral optic neuritis
- Usually good recovery with marked resolution of lesions at follow up



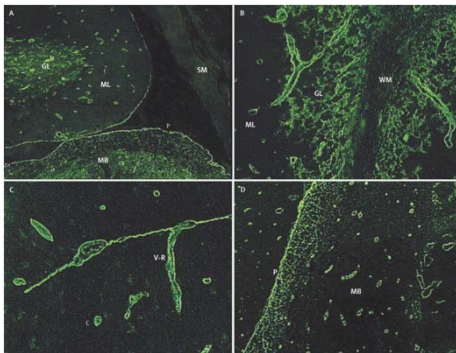
Coronal FLAIR sequence of a boy aged 8 showing multiple areas of high signal within the white matter, typical of ADEM



### Neuromyelitis optica (NMO) – a newly recognized CNS channelopathy

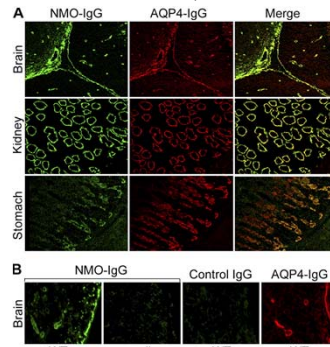
- NMO (Devic's disease) is a clinically defined severe CNS demyelinating syndrome characterized by optic neuritis and acute myelitis
- Characteristic immunopathology: IgG, IgM and complement deposited in a vasculogenic pattern suggest role for autoantibody
- IgG specific for NMO in serum of 73% of patients; binds to the **aquaporin-4** water channel

Immunofluorescence pattern of bound NMO-IgG in mouse CNS



Lennon et al., Lancet 2004

### Immunofluorescence reveals NMO-IgG colocalization with AQP4 in mouse tissues



Lennon et al., JEM 2005

### Take-home points

- MS: inflammatory + demyelinating + degenerative disease of the CNS
- Role of T, B cells and macrophages in pathogenesis
- Molecular targets not clearly identified
- Differential diagnosis includes
  - ADEM
  - NMO
 Which have different evolution and underlying pathologies