Imperial College London

Neurological inflammatory disease

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Clinical Reader in Neuroimmunology

Outline of lecture

- Introduction
- Classification of neuro-inflammatory disorders
- Inflammatory disorders of muscle and neuromuscular junction (name only)
- PNS inflammation: Demyelinating polyneuropathies
- Systemic inflammatory disorders affecting the CNS (name only)
- CNS-specific inflammatory syndromes
    - Multiple sclerosis
    - Acute disseminated encephalomyelitis
    - Neuromyelitis optica

The spectrum of neurological inflammatory 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: Inflammatory Demyelinating Polyneuropathy - Guillain-Barre' Syndrome and its variants:
    - Miller-Fisher syndrome
    - CIDP
    - Axonal GBS

Guillain-Barre Syndrome (GBS)

- Most frequently acquired demyelinating peripheral polyneuropathy - incidence 1-2 /100,000
- Often preceded by a respiratory or gastrointestinal infection by 1-3 weeks
- Association with Campylobacter jejuni infection (most common), hepatitis, infectious mononucleosis, Mycoplasma pneumoniae,,Cytomegalovirus, vaccination, surgery, lymphoma, pregnancy, HIV, or SLE

Key features

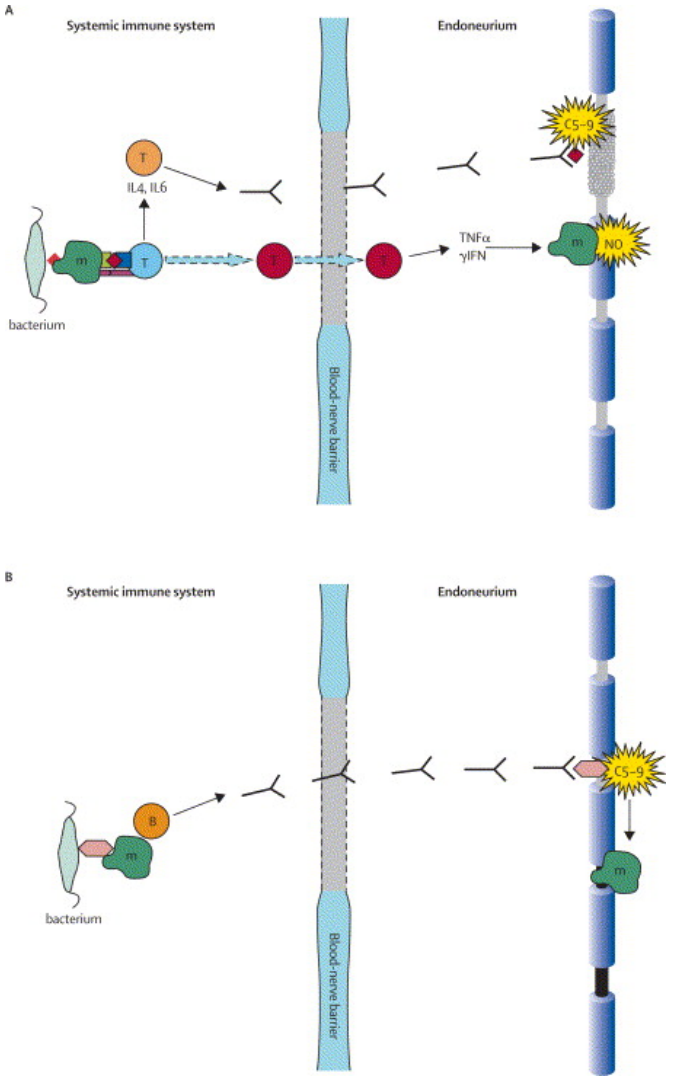
- Onset is progressive
- Ascending weakness +/- paraesthesia over days
- Typically symmetric weakness
- Clinical nadir within 4 weeks
- Occasional severe back pain
- Cranial nerve involvement
- Loss of tendon reflexes
- Respiratory muscle weakness – may req ventilation (20%)
- Autonomic involvement

Investigations

- CSF analysis: cytoalbumin dissociation
- EMG/NCS: abnormalities showing prolonged distal latencies, conduction slowing, block and temporal dispersion of compound action potential

Pathogenesis of GBS

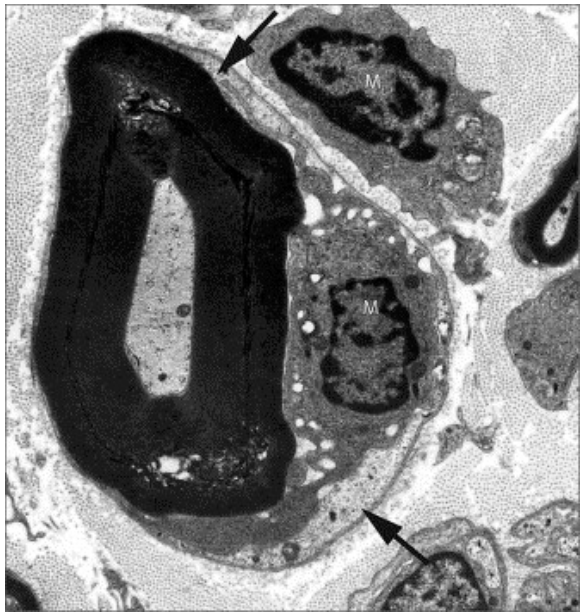
- Working hypothesis: immune response to infectious organisms cross-reacts with neural tissues leading to **attack and injury to PNS myelin**
- Lymphocytic infiltration of spinal roots and peripheral nerves
- Target: PNS myelin (P2, P0?)
- Anti-ganglioside (GM1 or Gd1b) Abs in some patients, >variant forms

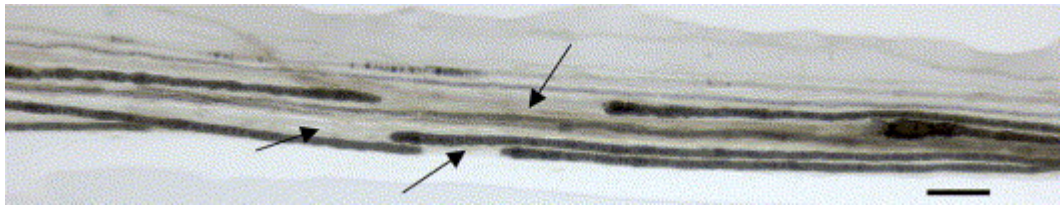


From Hughes and Cornblath, Lancet 2005

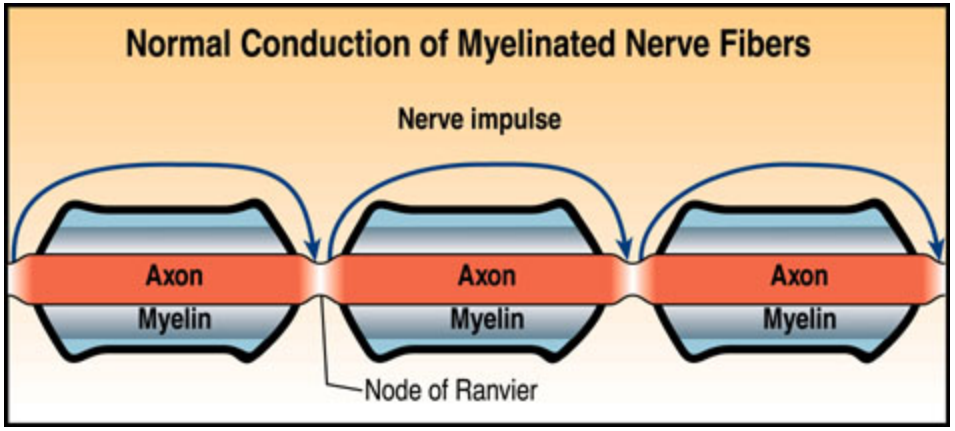
Pathogenesis of GBS

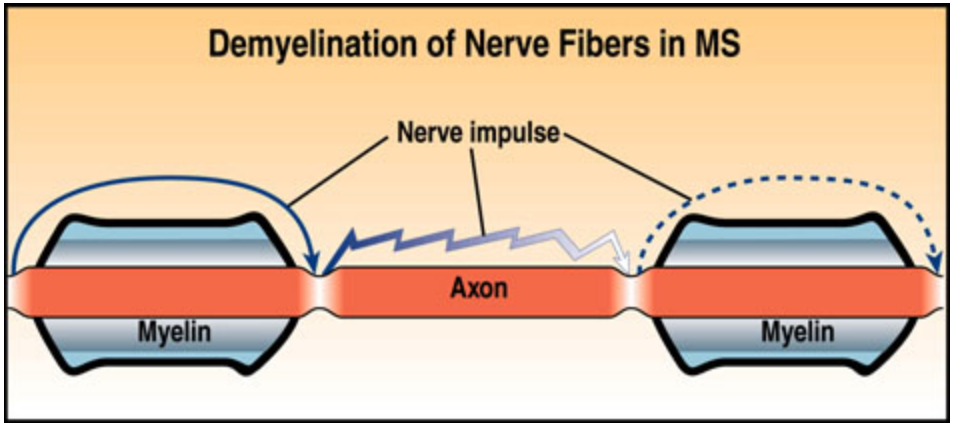
- Macrophage-mediated demyelination starting from nodes of Ranvier and secondary axonal degeneration
- Experimental autoimmune neuritis (EAN) can be induced in animals by inoculation with peripheral myelin (P2, P0, or PMP22) or ganglioside (GM) antigens eliciting both a humoral and a cellular immune response



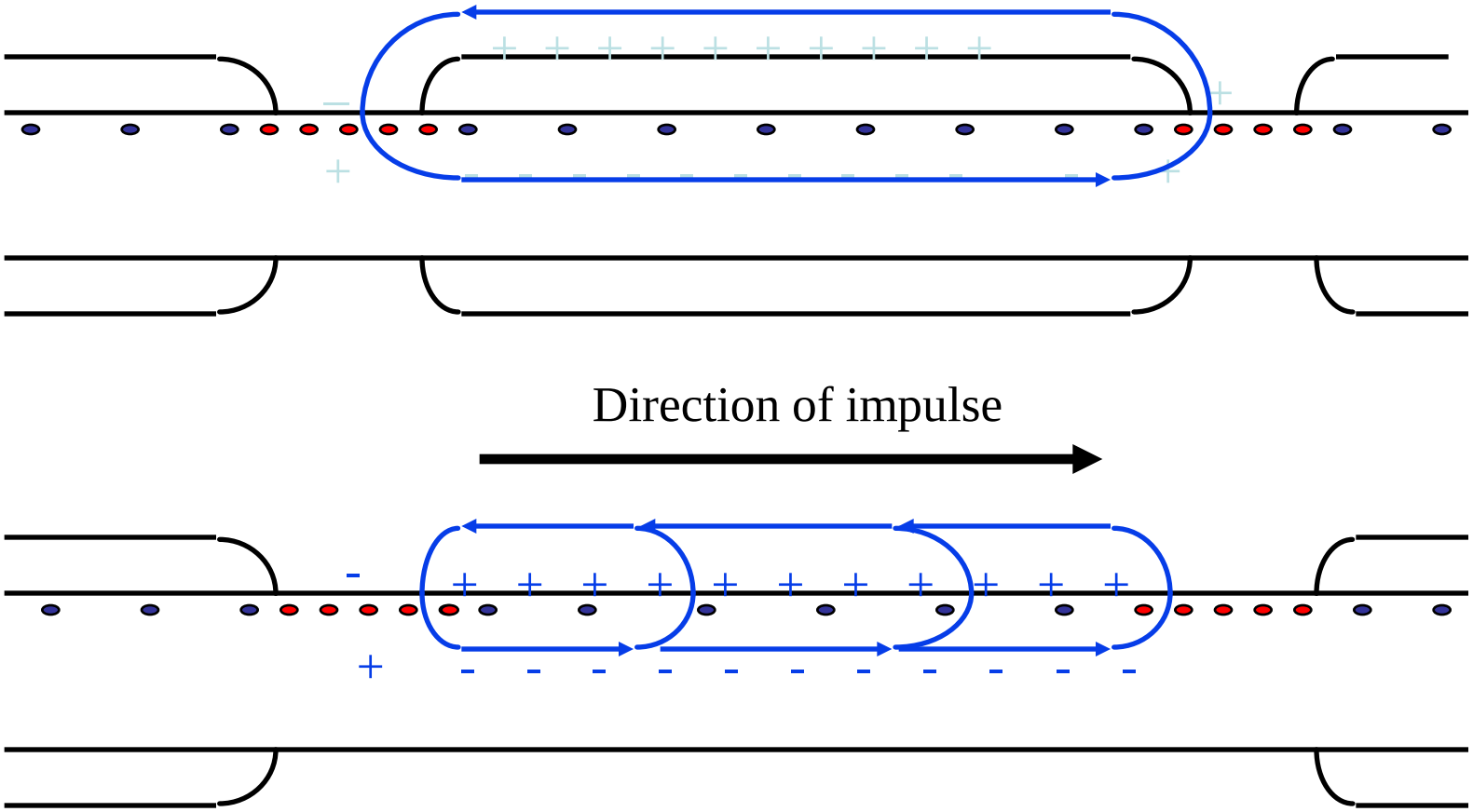


Conduction in myelinated and demyelinated nerve fibres





Courtesy of Dr J Rose


Direction of impulse
Na+ channels
K+ channels

Treatment

- IV Immunoglobulin. Prepared from pooled healthy donor sera. 0.4g/kg/day or 2g/kg total over 5/7. Mode of action unknown, putatively competition with pathogenic auto Ab for binding sites

- Plasma exchange. Mode of action “washing out” auto Abs

GBS variants

- Miller-Fisher Syndrome (Acute disseminated encephalomyeloradiculopathy)
  - Predominant cranial nerves involvement
- Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP)
  - Chronic-relapsing form
  - “The MS of the PNS”
- GBS Axonal Form
  - Directly attacking axonal components?
  - More severe prognosis

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

Some of the many other CNS inflammatory disorders

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

CNS inflammatory disorders

- Systemic autoimmune disorders which can involve the CNS: Sjogren's syndrome, Systemic Lupus Erythematosus, Behcet's, sarcoidosis, anti phospholipid antibodies syndrome; and CNS vasculitides
- **CNS-specific autoimmune disorders:**
  - **MS,**
  - **ADEM**
  - **neuromyelitis optica**

Multiple sclerosis: definition

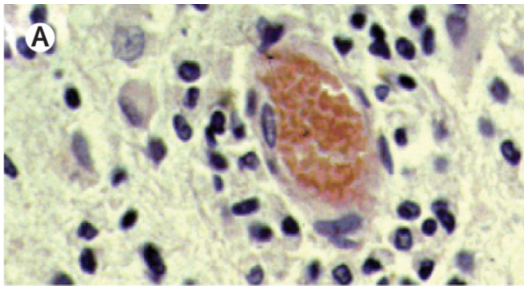
- A chronic inflammatory multifocal demyelinating disease of the central nervous system of unknown cause resulting in loss of myelin and oligodendroglial and axonal pathology
- Typically affecting young adults with exacerbating-remitting pattern or chronic progressive evolution

MS pathogenesis – the working hypothesis

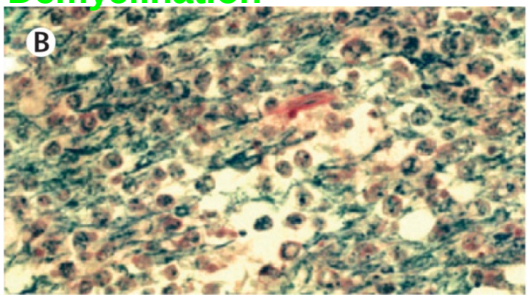
- MS is primarily considered an **inflammatory**, probably **autoimmune demyelinating** disease of the CNS
- A **neurodegenerative component** to the disease is increasingly recognised
- Exact cause of the pathological process remains unknown

Histopathology of MS – 3 main components

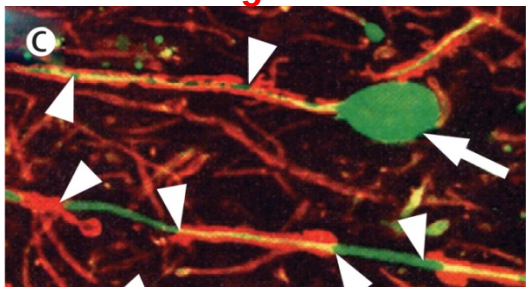
Inflammation

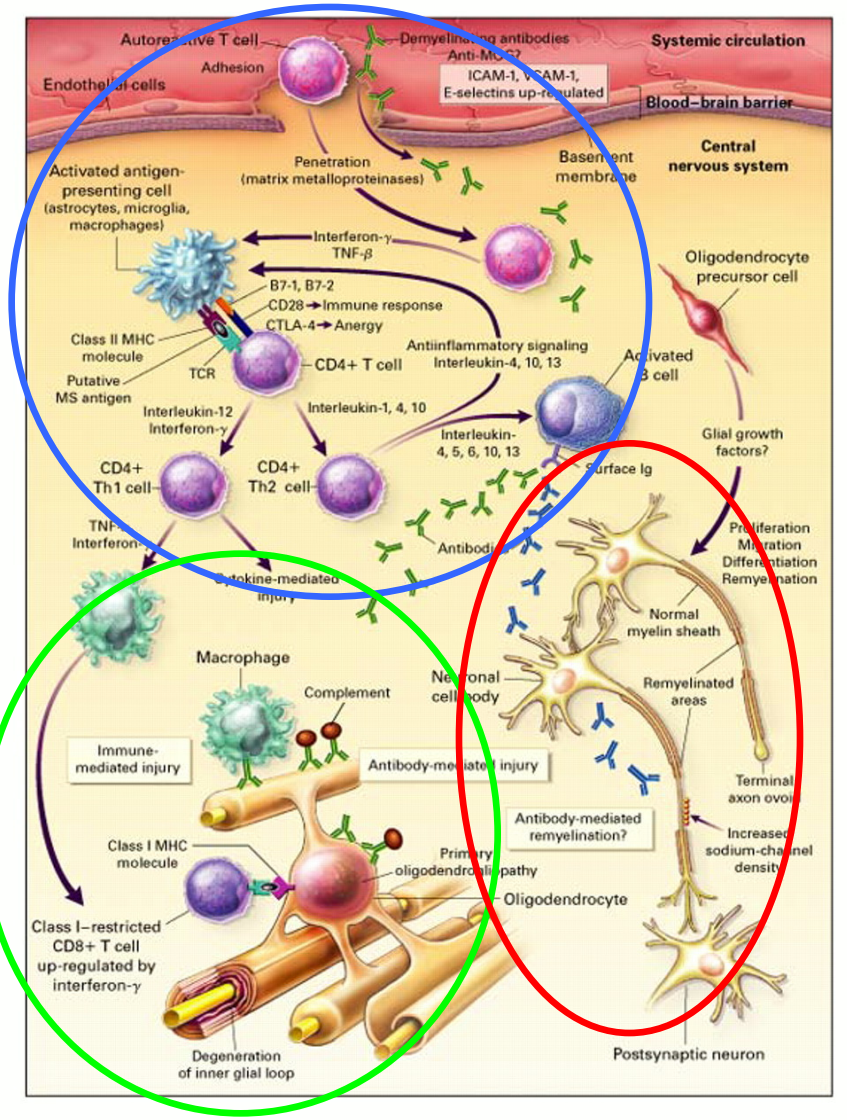


Demyelination



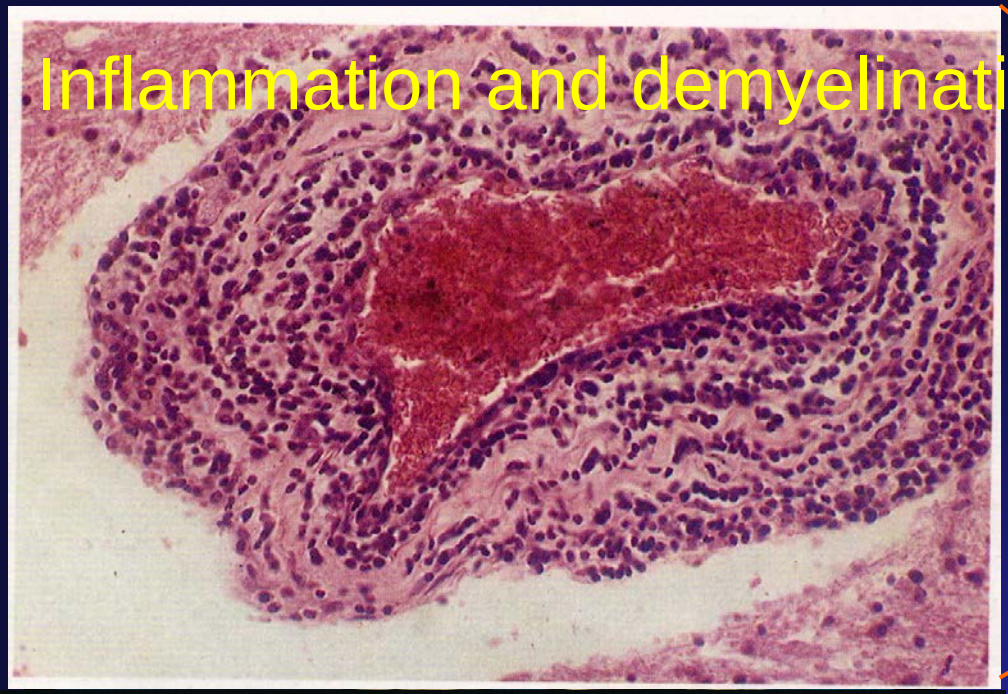
Axonal damage



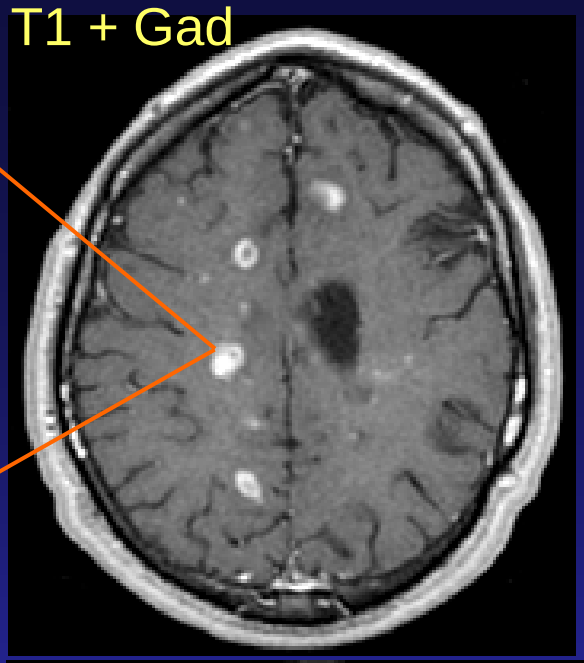


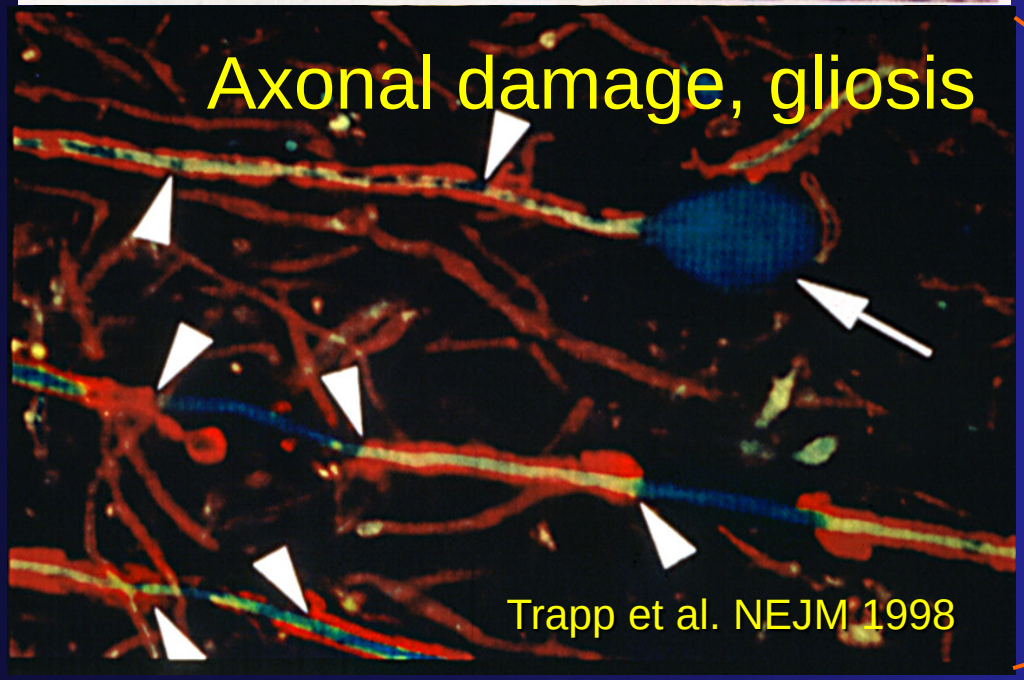
Adapted from Compston and Coles, Lancet 2008 and Noseworthy et al. NEJM 2000

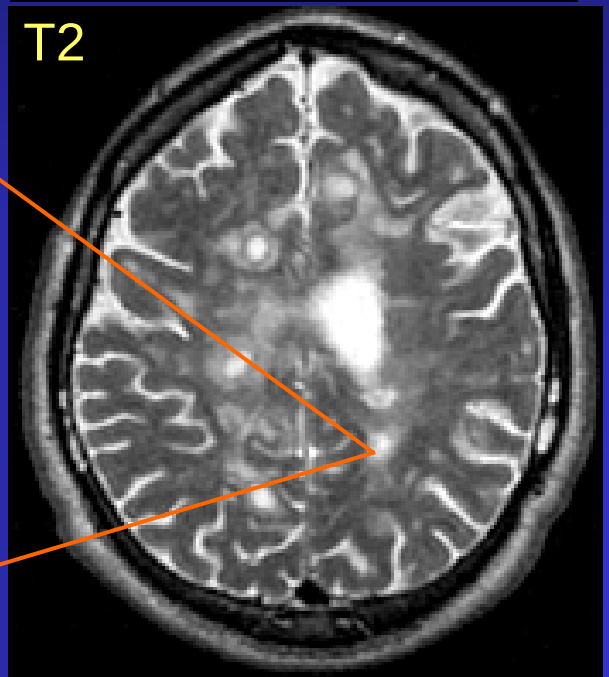
MRI


Inflammation and demyelination

1


T1 + Gad


Axonal damage, gliosis
Trapp et al. NEJM 1998


T2

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. Response to immuno-suppressive and -modulatory therapies

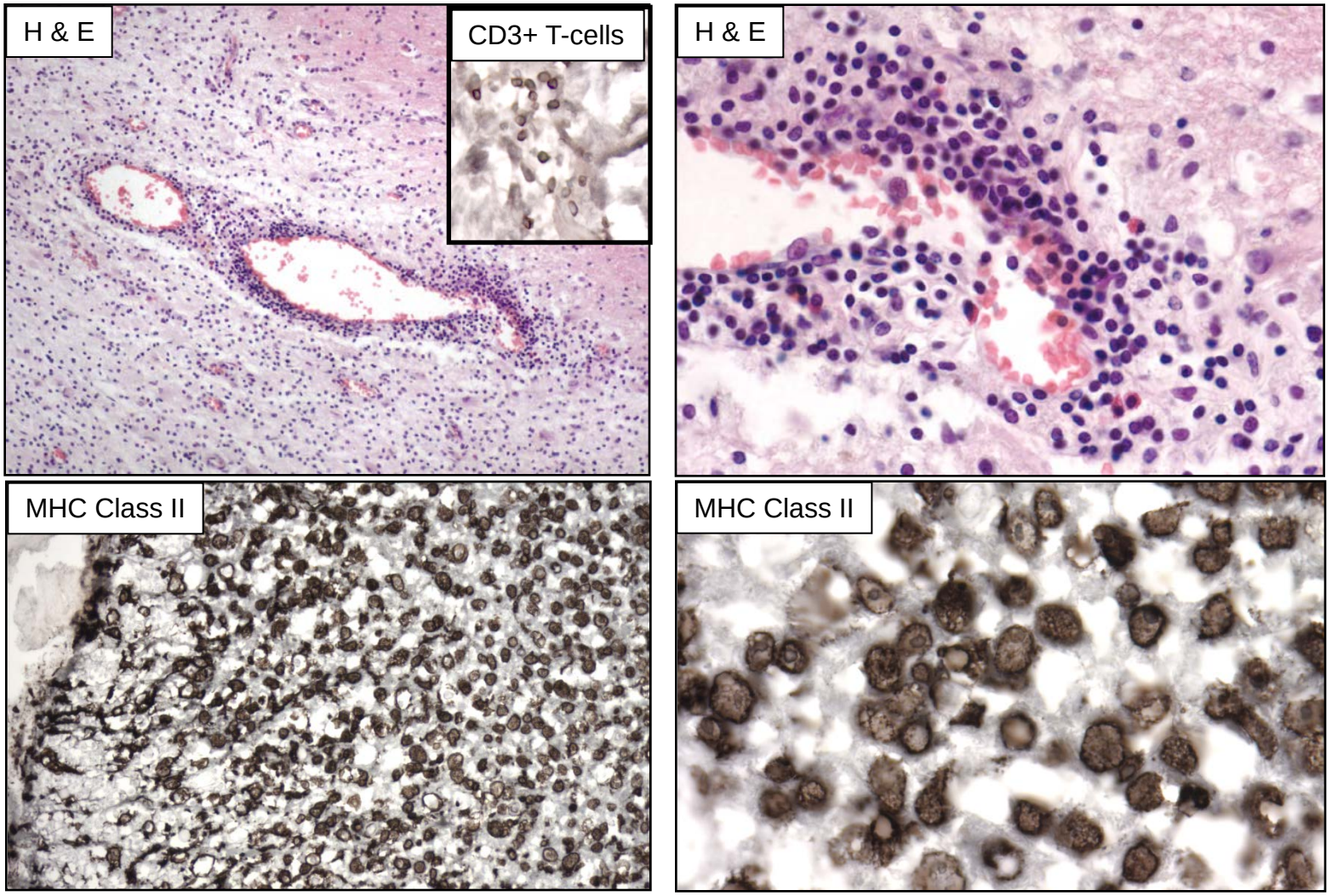
1. Immunopathology of CNS

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

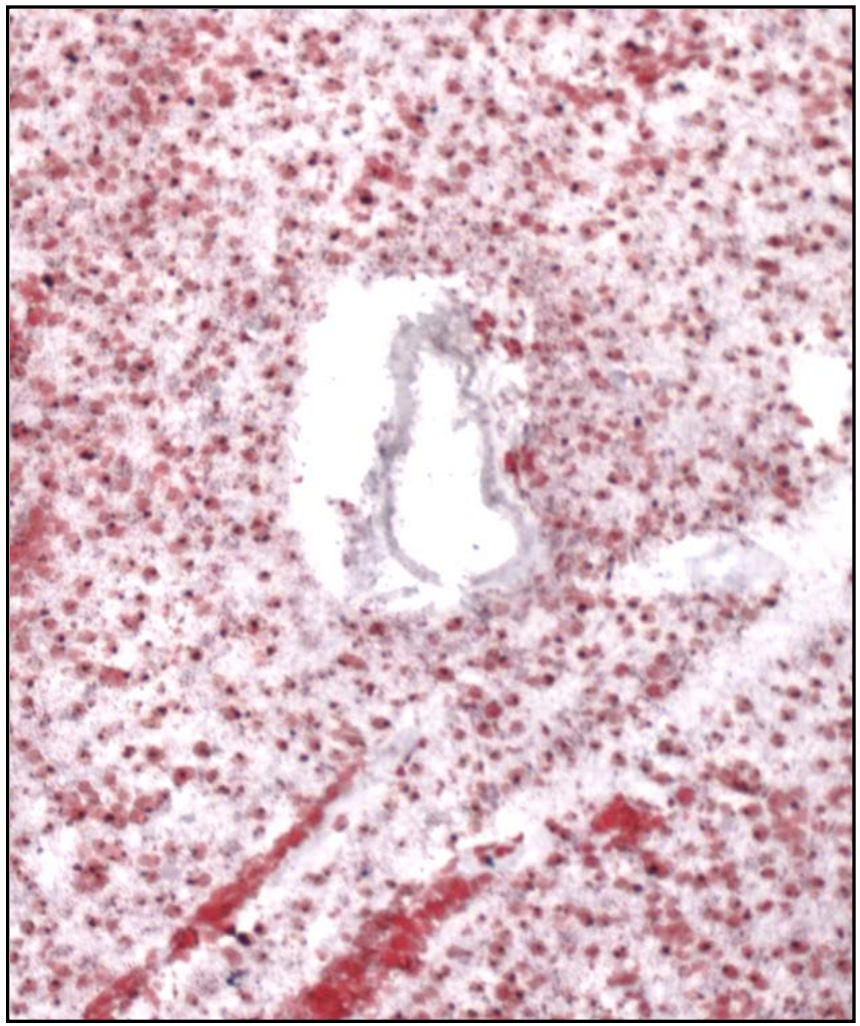
Myelin-laden **macrophages** in and around lesions

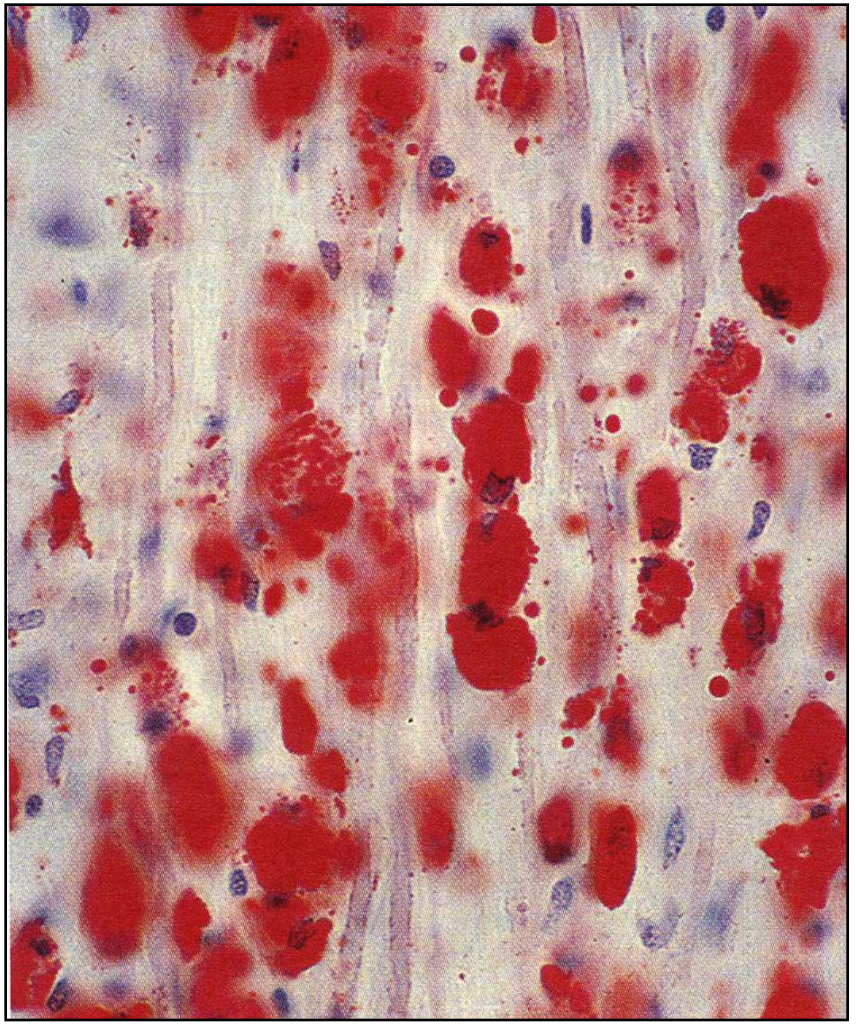
Inflammation and MS - the earliest events



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



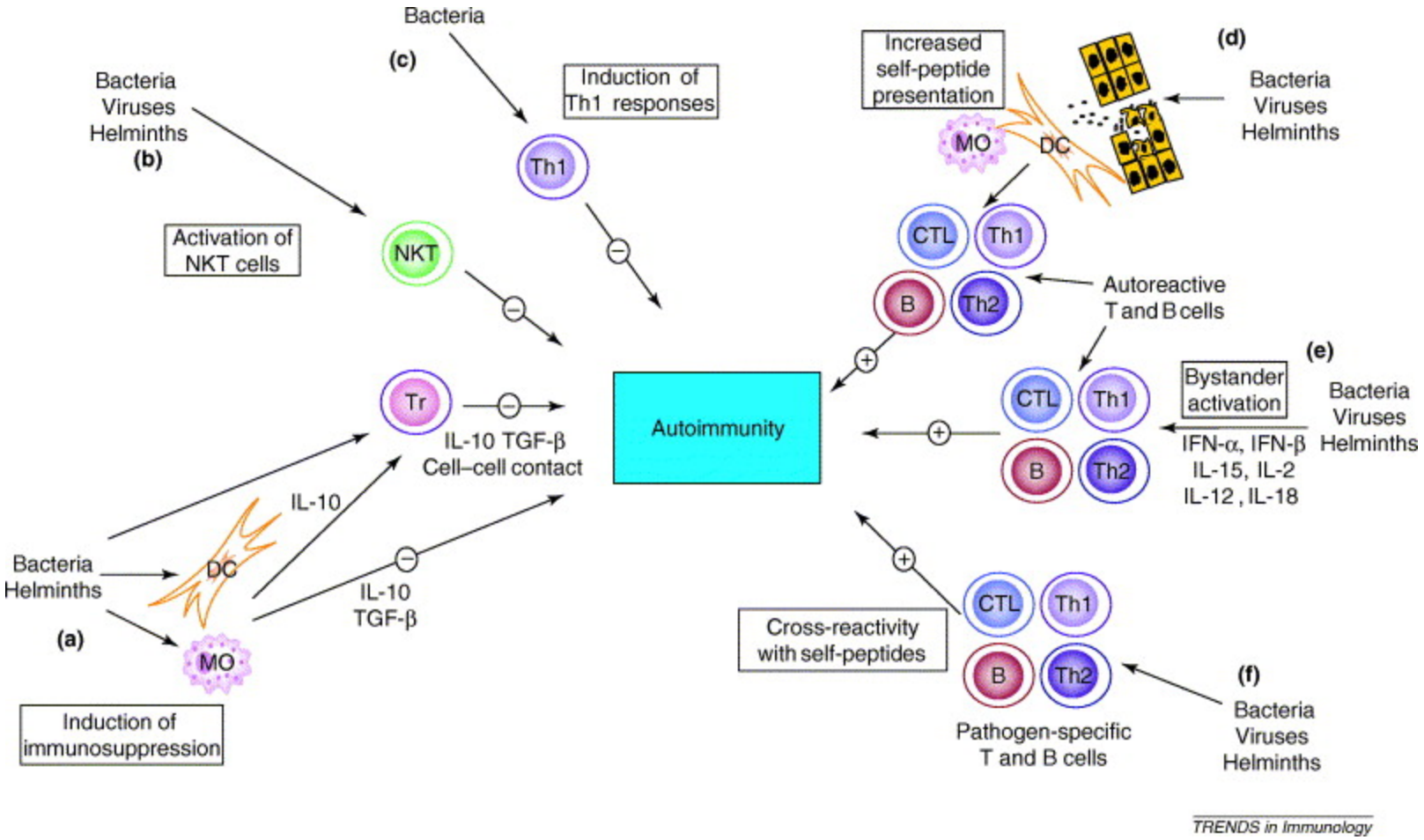


Macrophages in an active MS plaque. The foamy macrophages contain numerous lipid droplets, stained here with oil red-O, which represent myelin breakdown products

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**

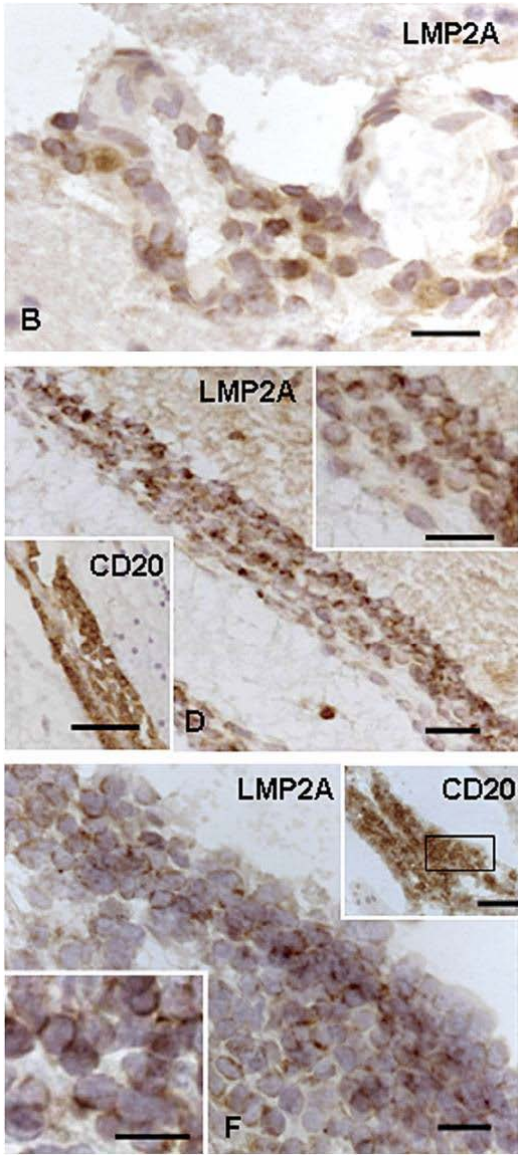
Potential mechanisms for how infections with pathogens might influence the development of autoimmunity



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 Neurol 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 ≥320 than among those with titers <20 (Munger et al. Multiple Sclerosis 2011)

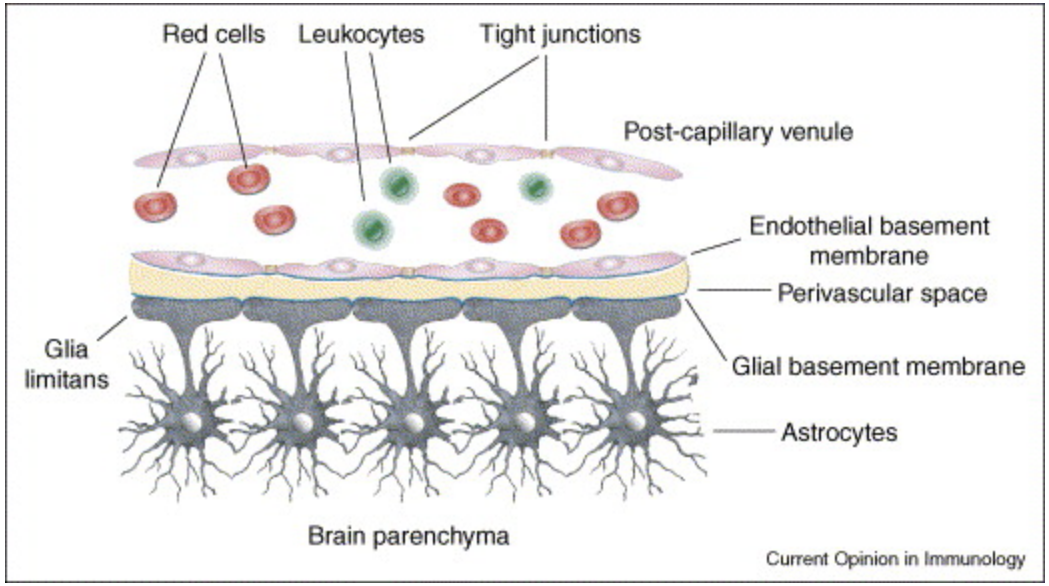
How do immune cells get to the CNS?

- The CNS is an immune privileged compartment, which is carefully shielded from potentially harmful immune reactions

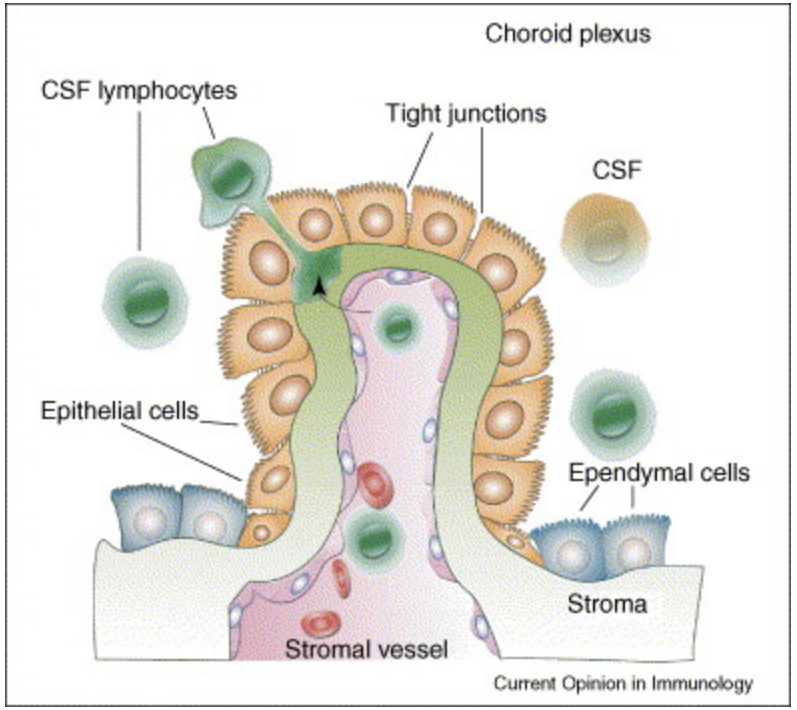
- To get to the CNS leukocytes must pass either of the two physiological barriers:
  1. **The Blood-Brain Barrier**
  2. **The Blood-CSF barrier**

Physiological barriers shielding the CNS

The Blood-Brain Barrier

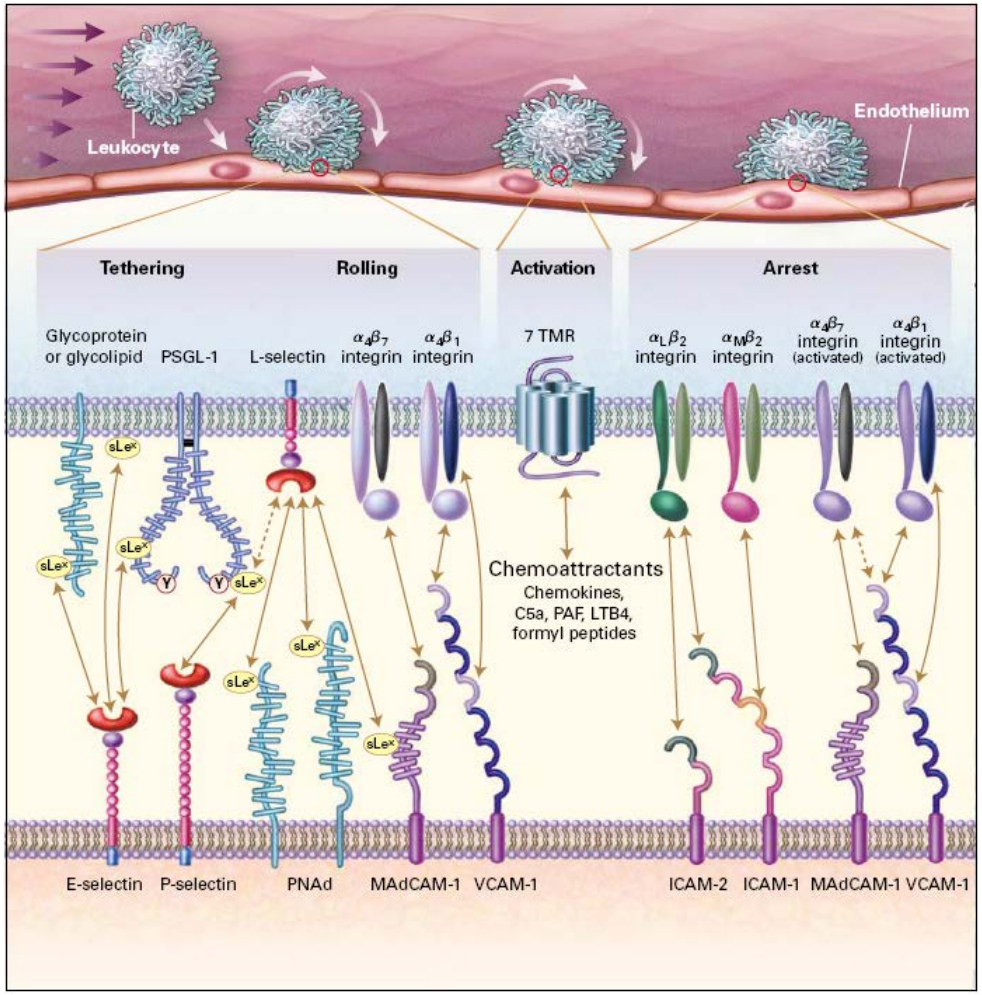


The Blood-CSF Barrier



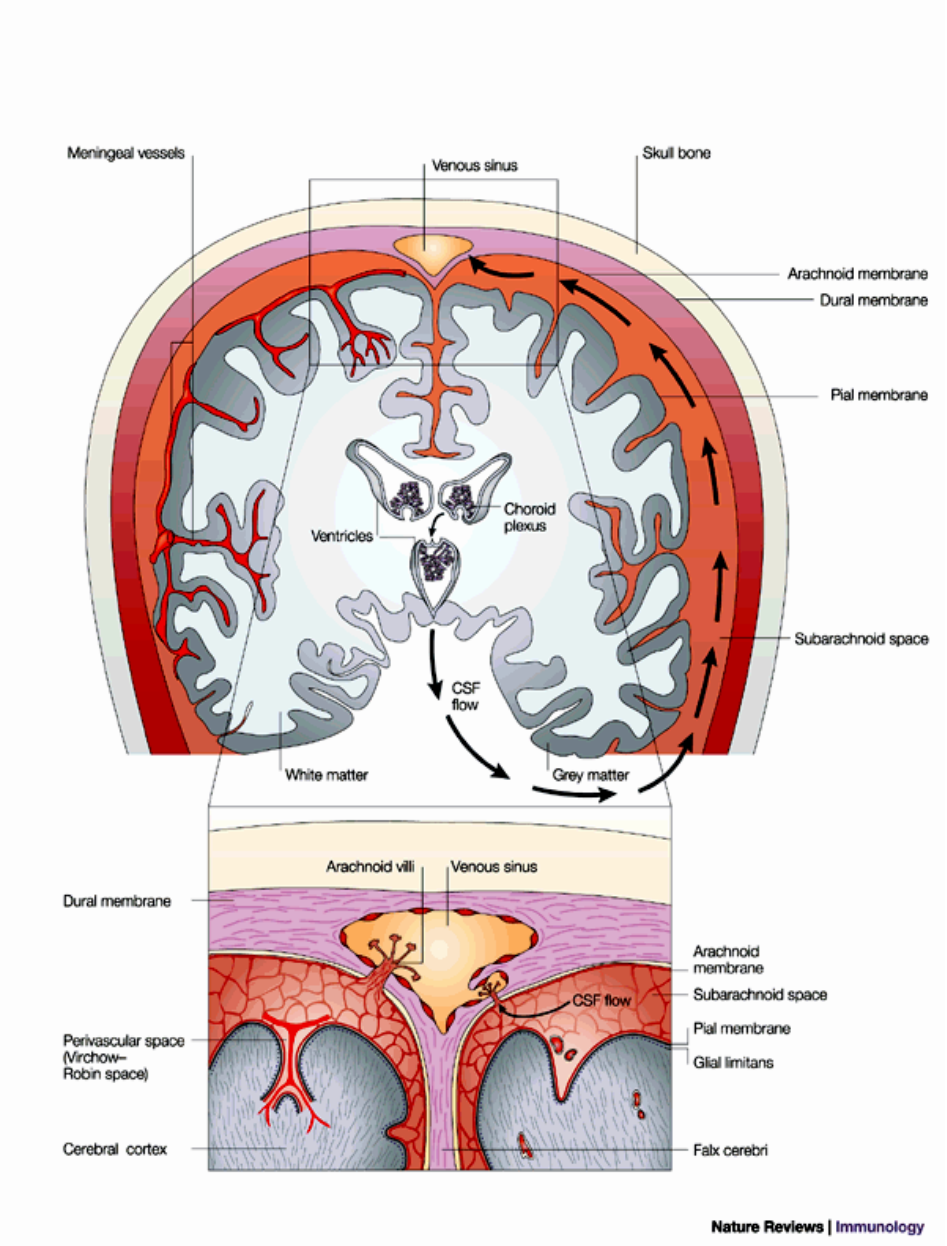
Rebenko-Moll et al. 2006

Essential Molecular Players in the Multistep Adhesion Cascade.



von Andrian and MacKay, NEJM 2000

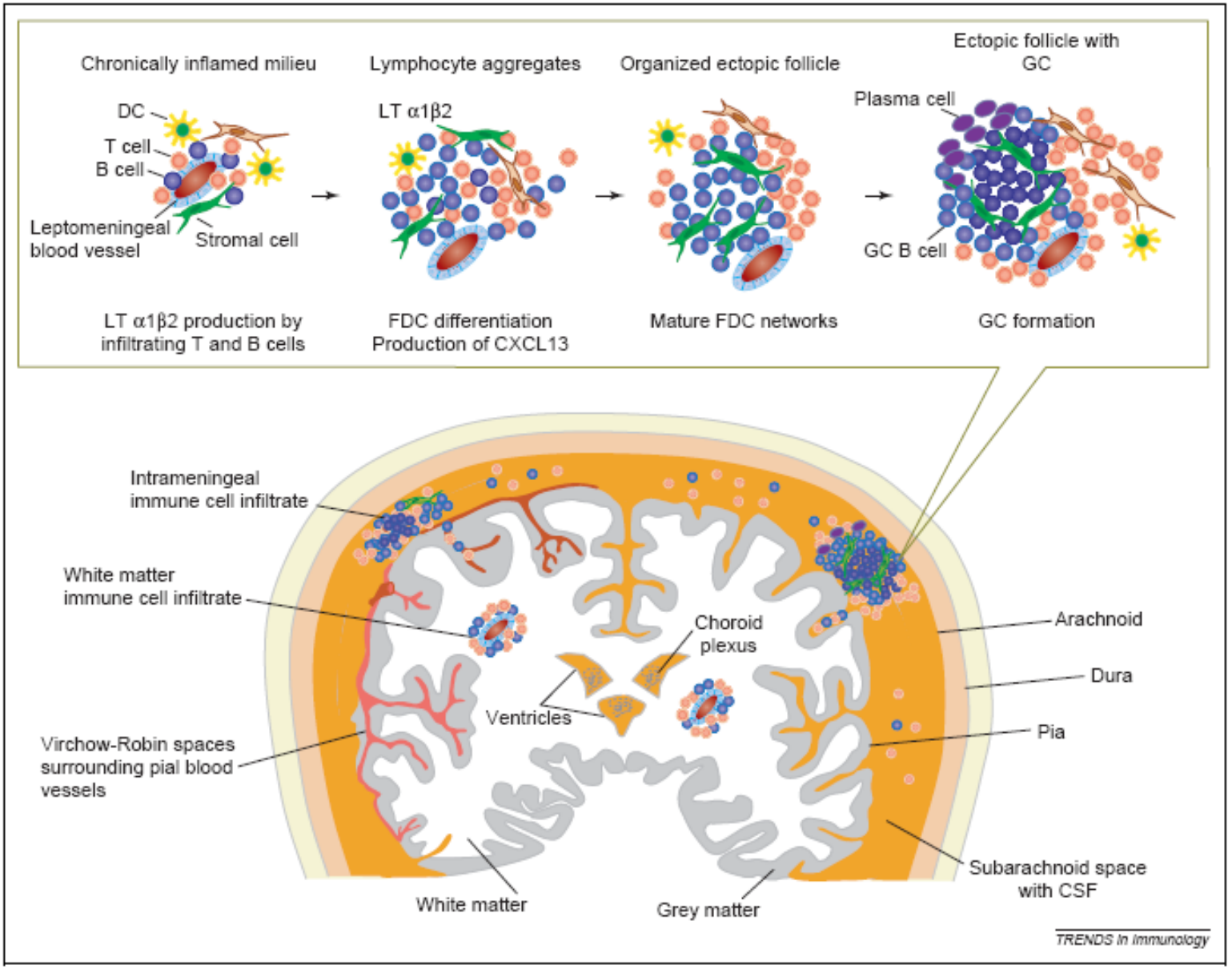
The blood-CSF route to the CNS



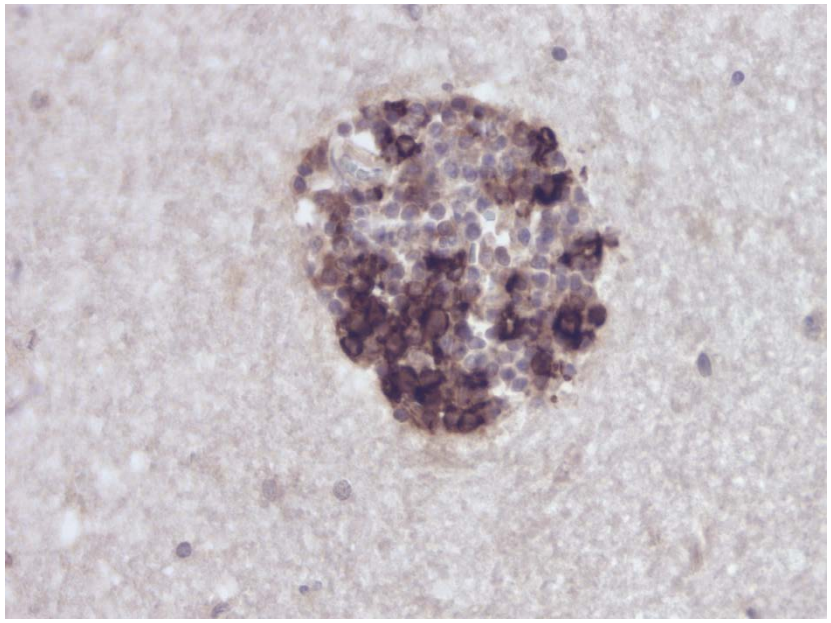
- Choroid plexus
- Subarachnoid space
- Pia
- Brain cortex

(Ransohoff 2003 Nat Rev Immunol)

A role for B-cells in cortical pathology

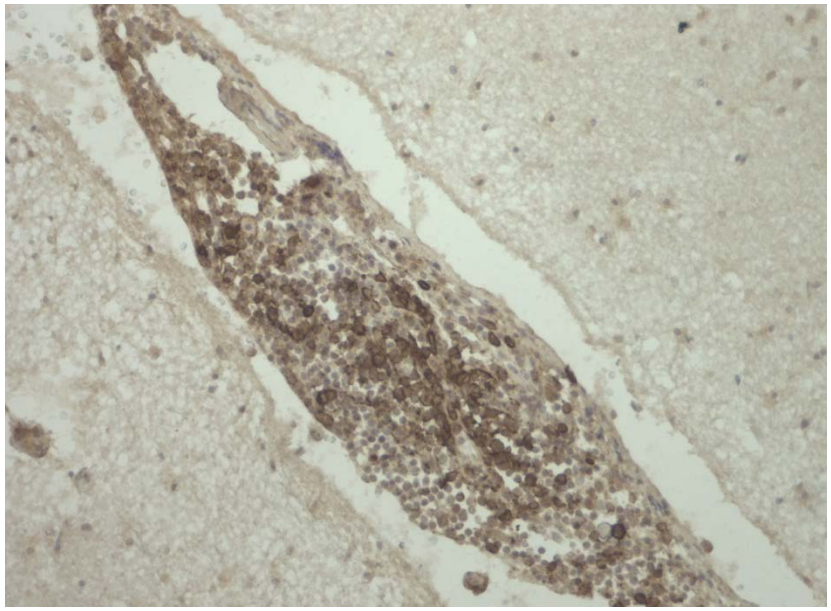


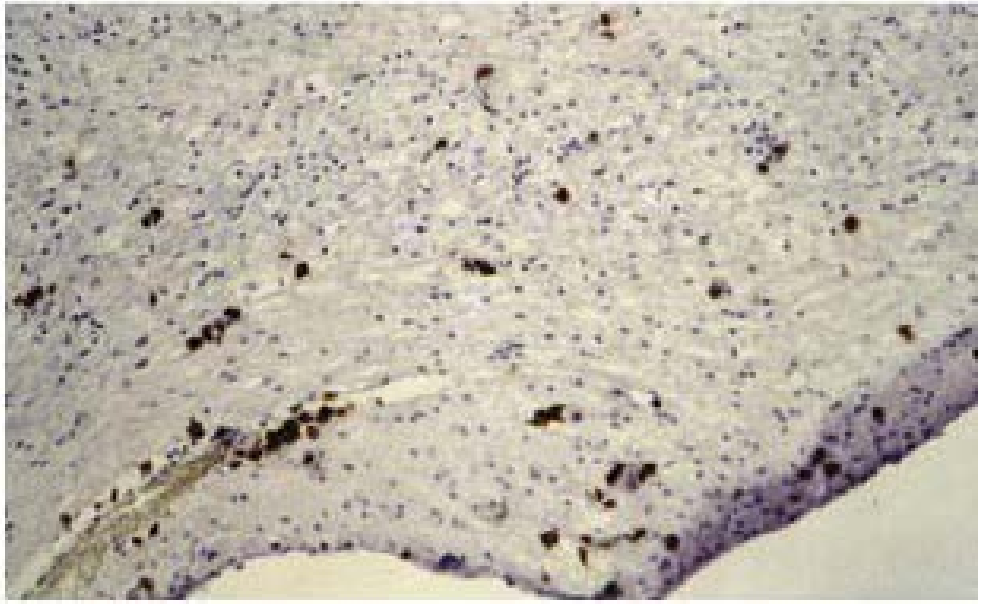
B-lymphocytes and plasma cells



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

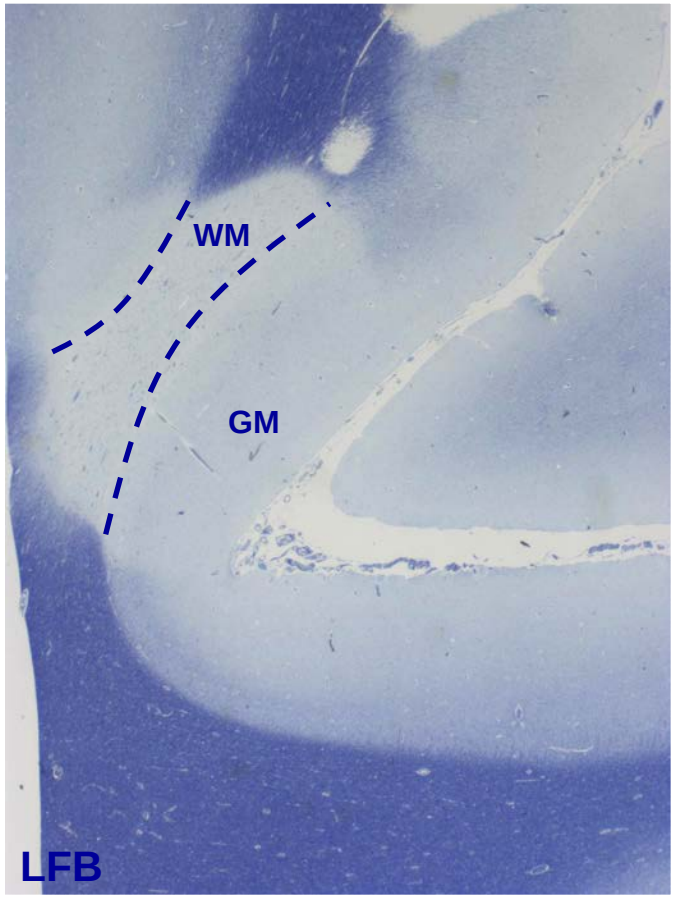


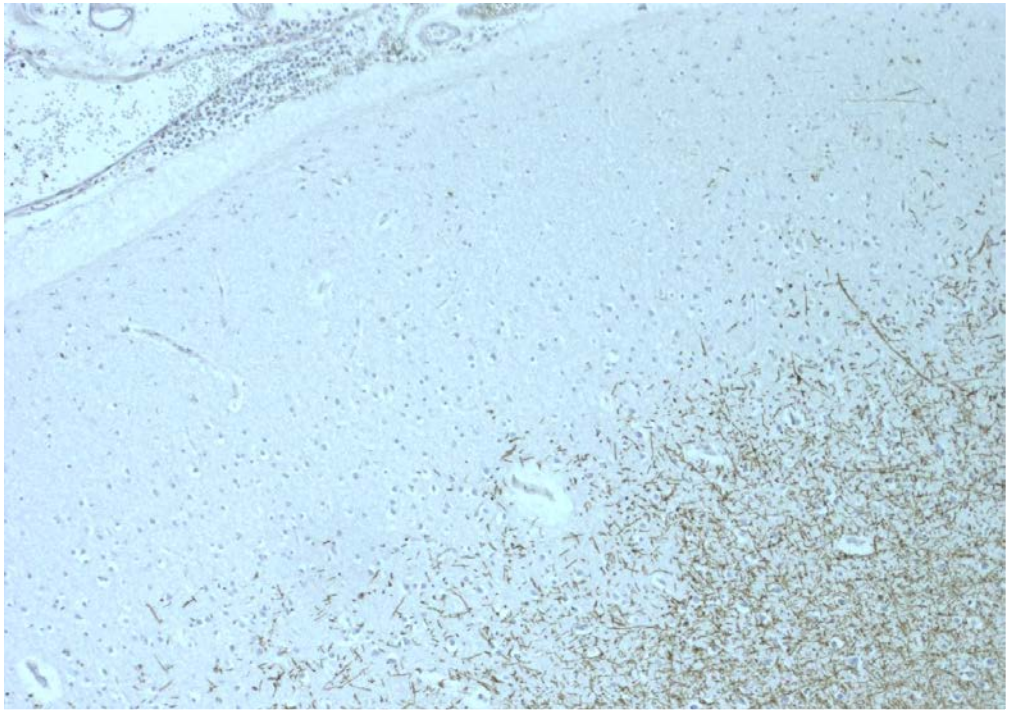


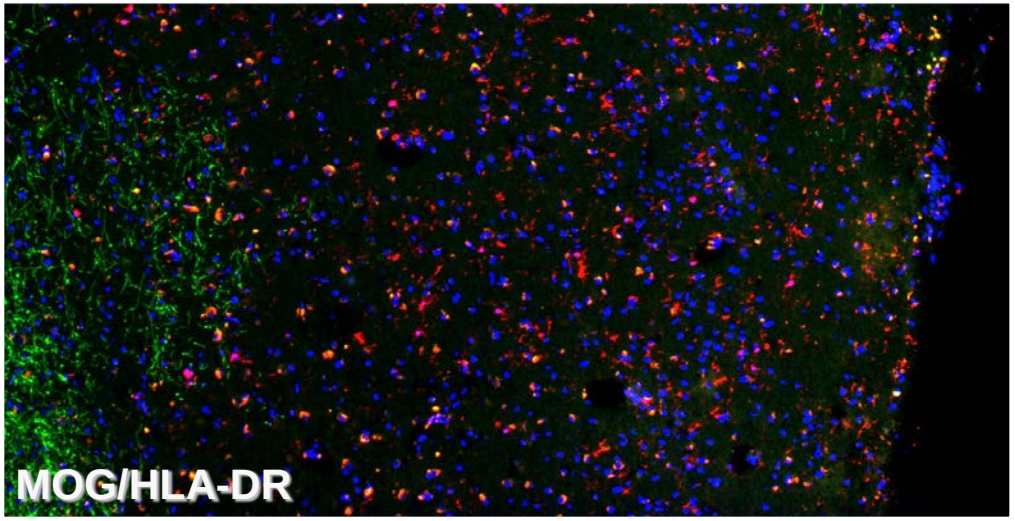
B cell involvement in MS

- 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

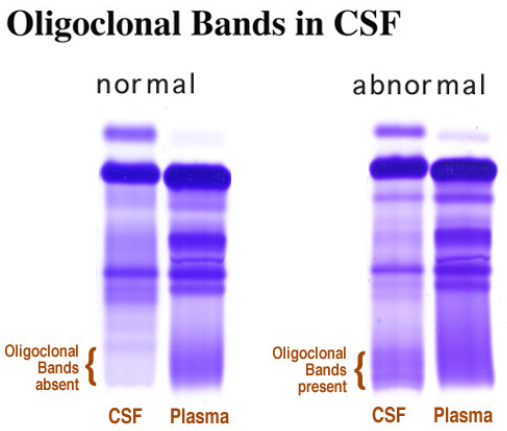
  IL-7R

  IL-2 R alpha

  CD58

3. CSF immunological abnormalities

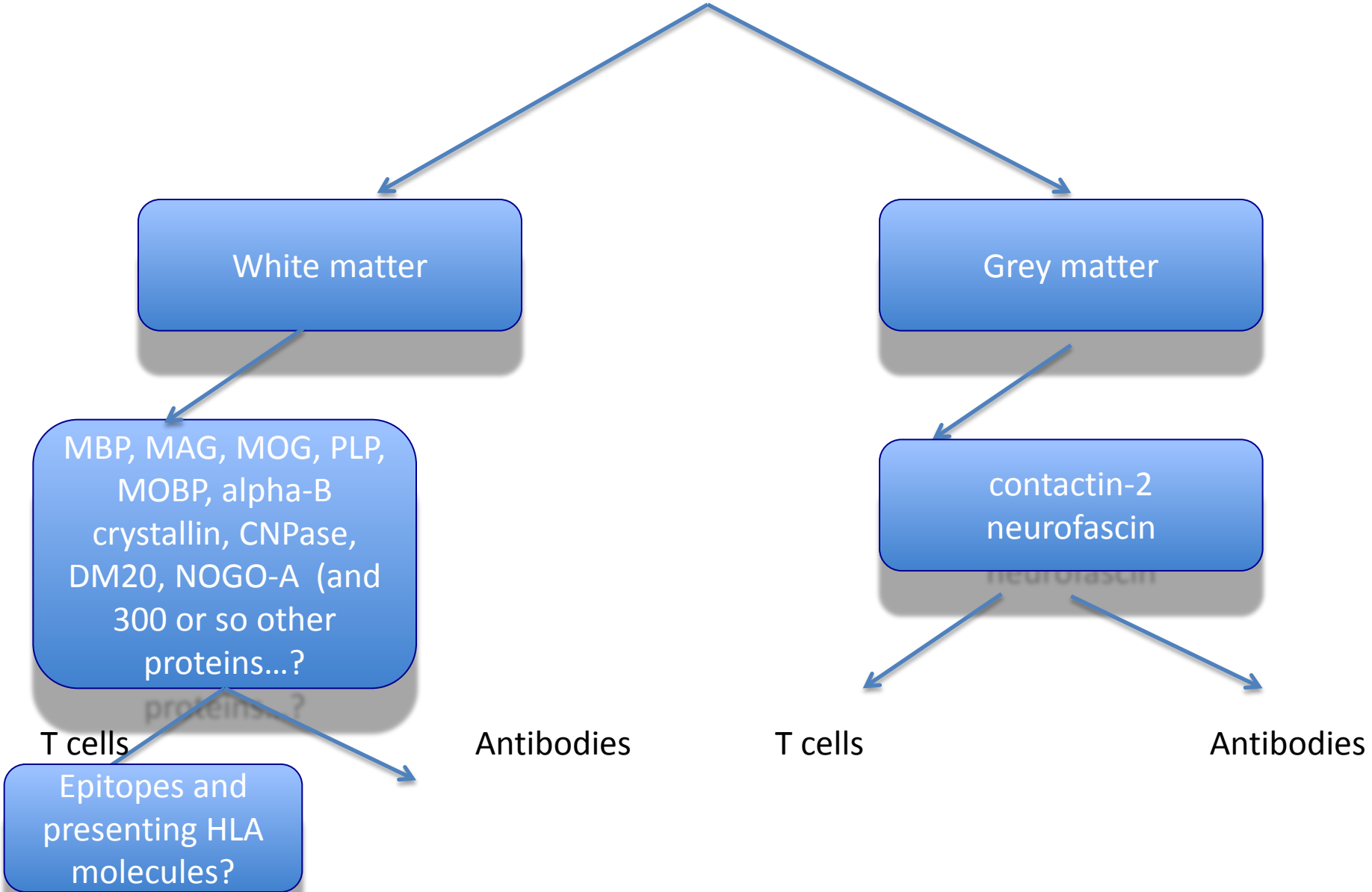
- Leukocyte counts counts can be normal but are often mildly increased above the normal of ≈3000 leukocytes/mL
- In MS 80% of total CSF cells are T cells (45% in blood), mainly memory T cells (up to 30% of CSF cells during inflammation), 5% are monocytes, ≤ 1% B cells, plasma cells
- There can be a minor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



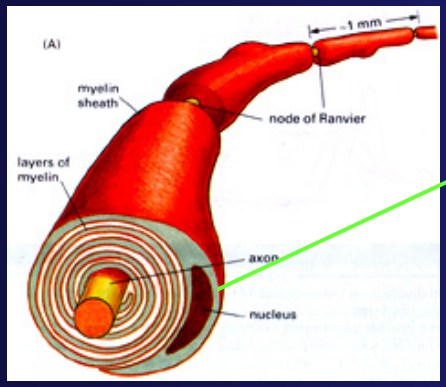
4. Subtle abnormalities of blood T cell functions

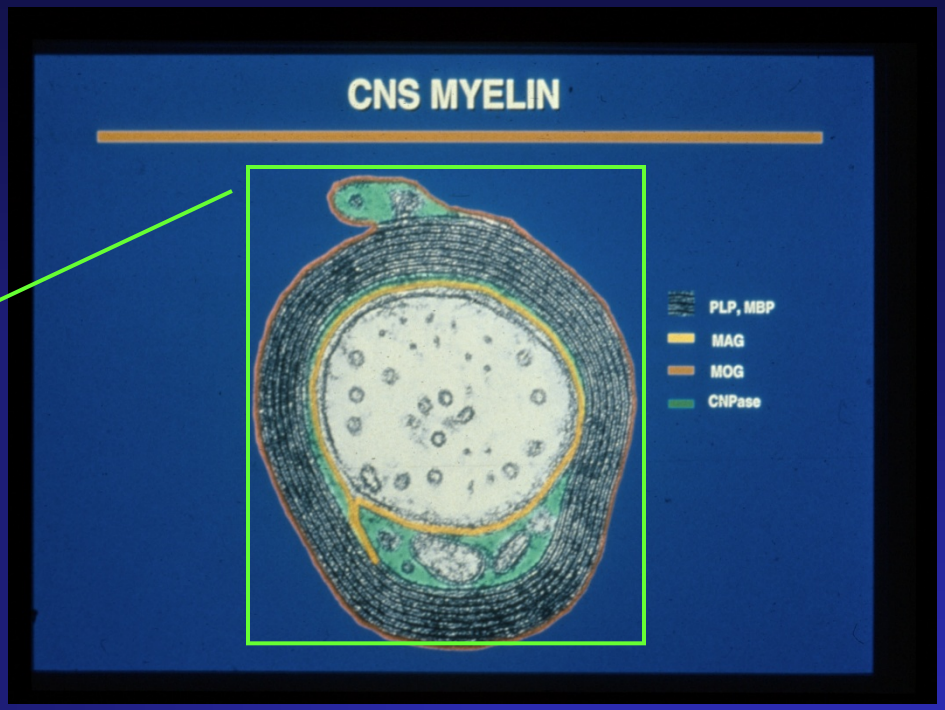
- Slightly increased frequency and reduced requirements for activation of T cells responding to myelin antigens
- Prevalence of T helper 1 cytokine secretion by *myelin* antigen-specific T cells
- Likely role of Th17 cells in driving inflammation
- Reduced activity of (CD4+/CD25+) regulatory T cells

CNS antigens – but which?



Candidate target myelin antigens in MS



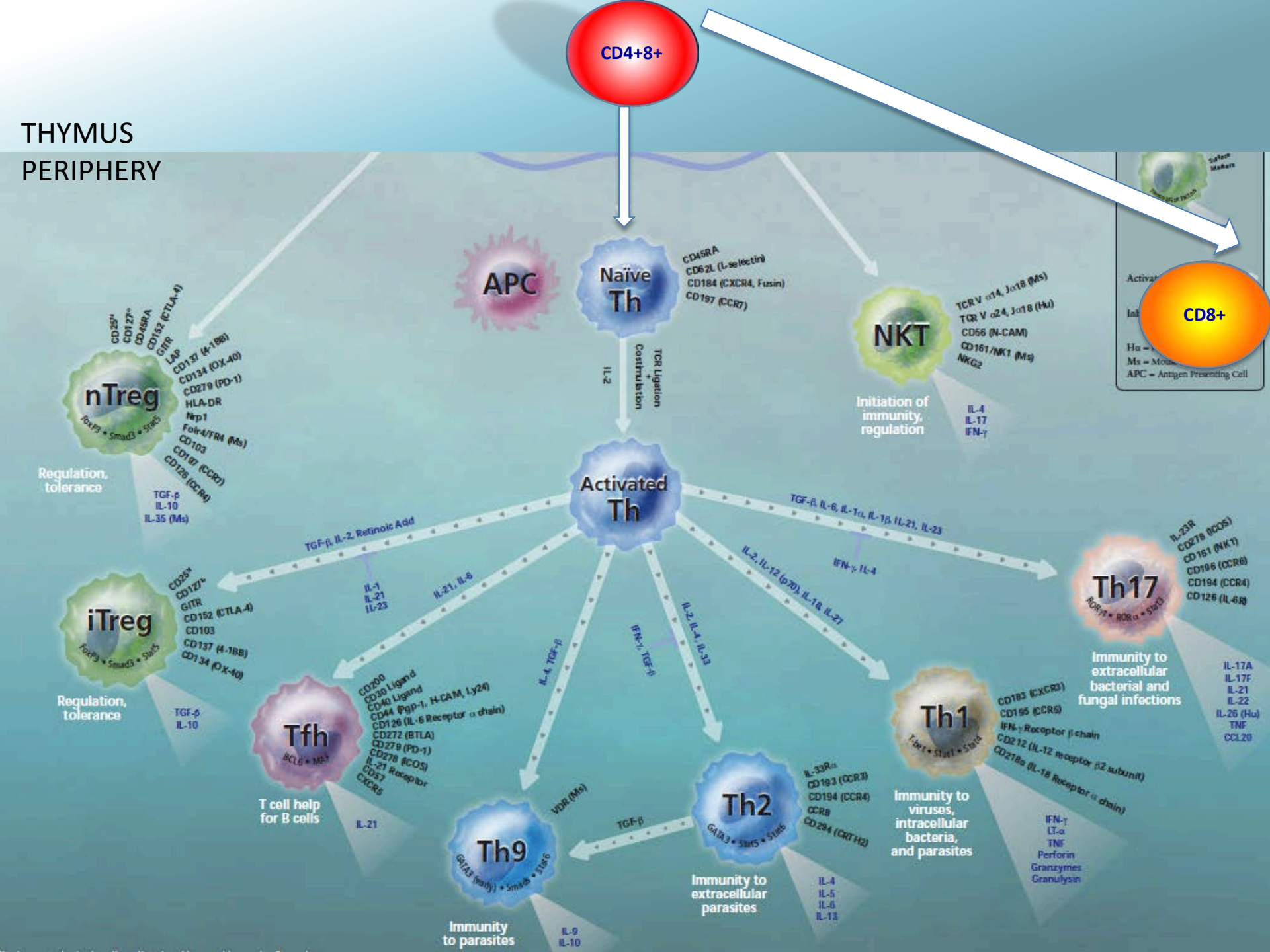


Myelin basic protein (MBP; 170 aa, several isoforms)

Proteolipid protein (PLP; 272 aa, two isoforms)

Myelin oligodendroglia glycoprotein (MOG; target of T- and B cell response)

A few others


CD4+8+
THYMUS
PERIPHERY
APC
Naïve Th
CD45RA
CD62L (L-selectin)
CD184 (CXCR4, Fusin)
CD197 (CCR7)
IL-2
TCR Ligation
Costimulation
NKT
TCR V α14, Jα18 (Ms)
TCR V α24, Jα18 (Hu)
CD56 (N-CAM)
CD161/NK1 (Ms)
NKG2
Initiation of immunity, regulation
IL-4
IL-17
IFN-γ
CD8+
Activated Th
nTreg
FoxP3 • Smad3 • Stat5
CD25hi
CD127lo
CD45RA
CD152 (CTLA-4)
GITR
LAP
CD137 (4-1BB)
CD134 (OX-40)
CD279 (PD-1)
HLA-DR
Nrp1
Folr4/FR4 (Ms)
CD103
CD197 (CCR7)
CD126 (CCR6)
Regulation, tolerance
TGF-β
IL-10
IL-35 (Ms)
TGF-β, IL-2, Retinoic Acid
iTreg
FoxP3 • Smad3 • Stat5
CD25hi
CD127lo
GITR
CD152 (CTLA-4)
CD103
CD137 (4-1BB)
CD134 (OX-40)
Regulation, tolerance
TGF-β
IL-10
IL-1
IL-21
IL-23
IL-21, IL-6
Tfh
BCL6 • Maf
CD200
CD30 Ligand
CD40 Ligand
CD44 (Pgp-1, H-CAM, Ly24)
CD126 (IL-6 Receptor α chain)
CD272 (BTLA)
CD279 (PD-1)
CD278 (ICOS)
IL-21 Receptor
CD57
CXCR5
T cell help for B cells
IL-21
IL-4, TGF-β
IFN-γ, TGF-β
IL-2, IL-4, IL-33
Th9
GATA3 (early) • Smad4 • Stat6
VDR (Ms)
TGF-β
Immunity to parasites
IL-9
IL-10
Th2
GATA3 • Stat5 • Stat6
IL-33Rα
CD193 (CCR3)
CD194 (CCR4)
CCR8
CD294 (CRTH2)
Immunity to extracellular parasites
IL-4
IL-5
IL-6
IL-13
IL-2, IL-12 (p70), IL-18, IL-27
IFN-γ, IL-4
Th1
T-bet • Stat1 • Stat4
CD183 (CXCR3)
CD195 (CCR5)
IFN-γ Receptor β chain
CD212 (IL-12 receptor β2 subunit)
CD218a (IL-18 Receptor α chain)
Immunity to viruses, intracellular bacteria, and parasites
IFN-γ
LT-α
TNF
Perforin
Granzymes
Granulysin
TGF-β, IL-6, IL-1α, IL-1β, IL-21, IL-23
Th17
RORγt • RORα • Stat3
IL-23R
CD278 (ICOS)
CD161 (NK1)
CD196 (CCR6)
CD194 (CCR4)
CD126 (IL-6R)
Immunity to extracellular bacterial and fungal infections
IL-17A
IL-17F
IL-21
IL-22
IL-26 (Hu)
TNF
CCL20

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 $CD4^+$ T cells 3- to 10-fold in chronically inflamed MS plaques
- there is specific enrichment of highly differentiated $CD8^+$ rather than $CD4^+$ 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 $CD4^+$ T cells

MS as a disease of regulatory T cell dysfunction?

Loss of Functional Suppression by CD4$^+$CD25$^+$ 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$^+$ T_{Reg} 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 α-chain are excluded from the analysis

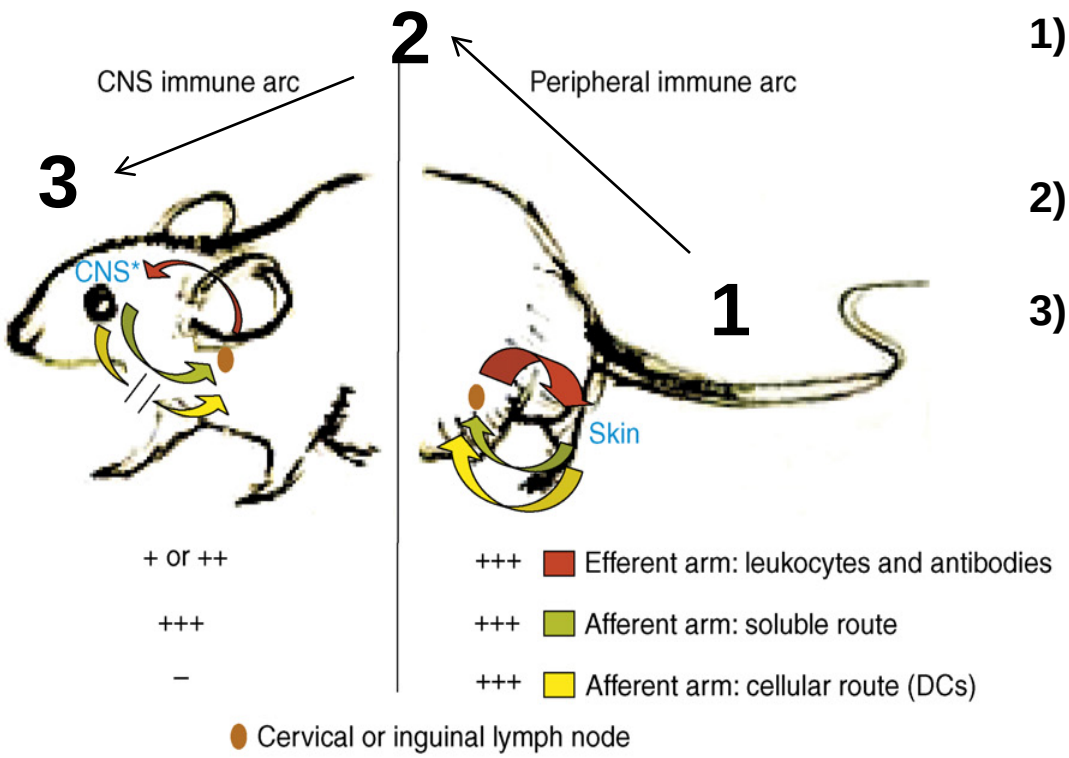
Laure Michel,[1,2] Laureline Berthelot,[1] Ségolène Pettré,[1] Sandrine Wiertlewski,[2,3] Fabienne Lefrère,[3] Cécile Braudeau,[1] Sophie Brouard,[1] Jean-Paul Soulillou,[1] and David-Axel Laplaud[1,2,3]

"…we conclude that CD4+CD25highCD127low Tregs from MS patients and healthy individuals exhibit similar suppressive functions.
J Clin Invest 118, 3411

5. Animal models of autoimmune disease

Experimental autoimmune encephalomyelitis (EAE):

- Induced by peripheral immunisation with myelin protein antigens
- Mediated by CD4+ myelin-specific T cells



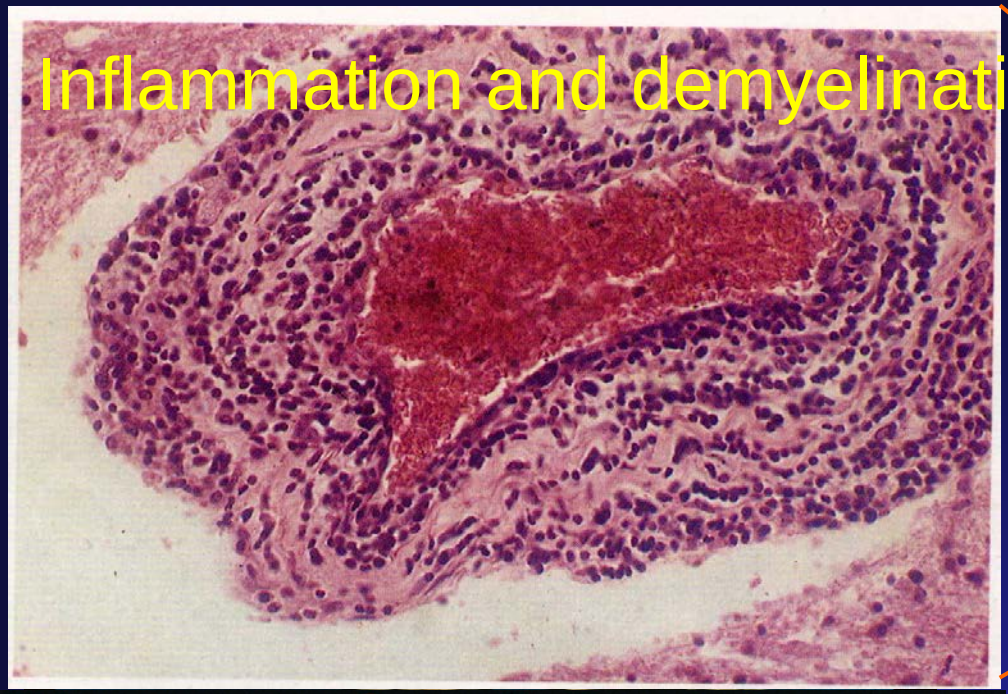
1) **Autoreactive myelin-specific effector T cells are primed in peripheral lymph nodes and**
2) **Migrate into uninflamed CNS to initiate tissue inflammation.**
3) **Antigen re-encounter in CNS propagates inflammation and damage**

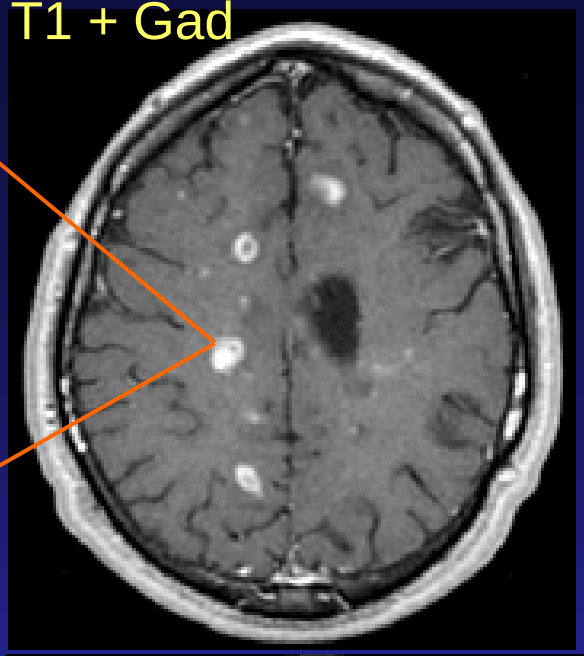
6. Response to immuno-suppressive and -modulatory therapies

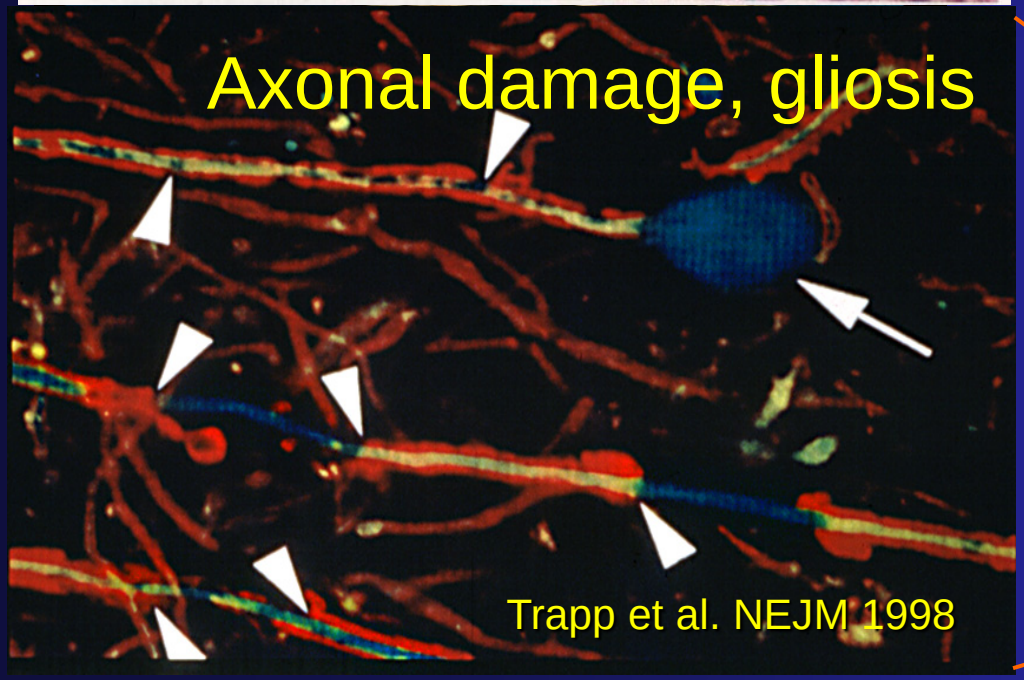
MS acute relapses are improved by high-dose corticosteroid administration

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)

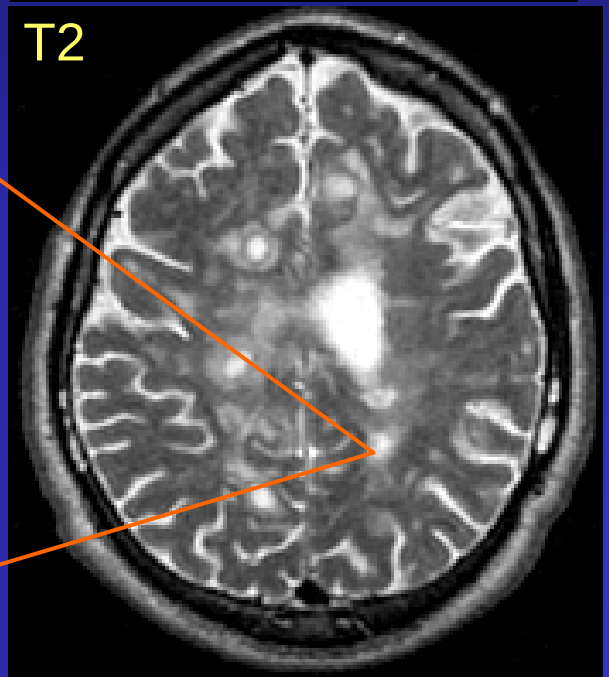
MRI


Inflammation and demyelination


T1 + Gad


Axonal damage, gliosis
Trapp et al. NEJM 1998

2


T2

Mechanisms of neuronal loss in MS

INDIRECT

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

DIRECT (mostly immune-mediated)

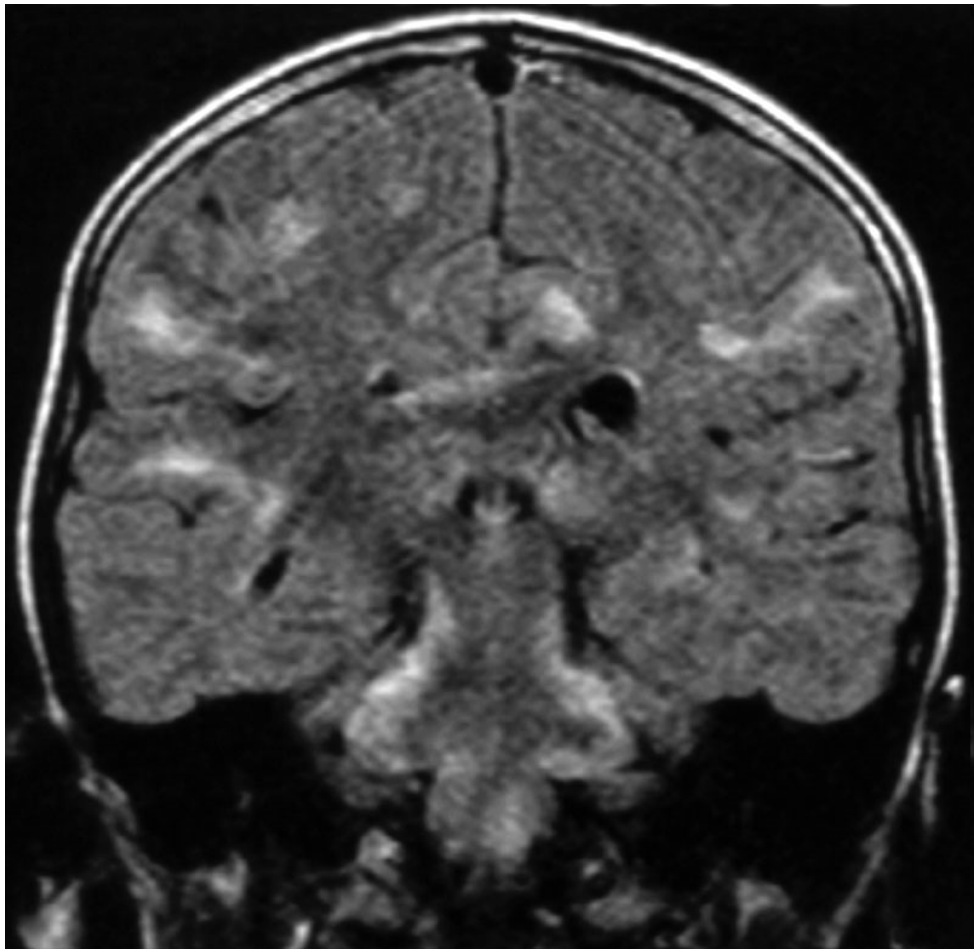
- direct effect of inflammatory mediators produced by immune cells on neuronal perikarya in grey matter lesions

- direct effect of low level release of inflammatory mediators by **activated microglia** in normal appearing tissues

Acute disseminated encephalomyelitis (ADEM)

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

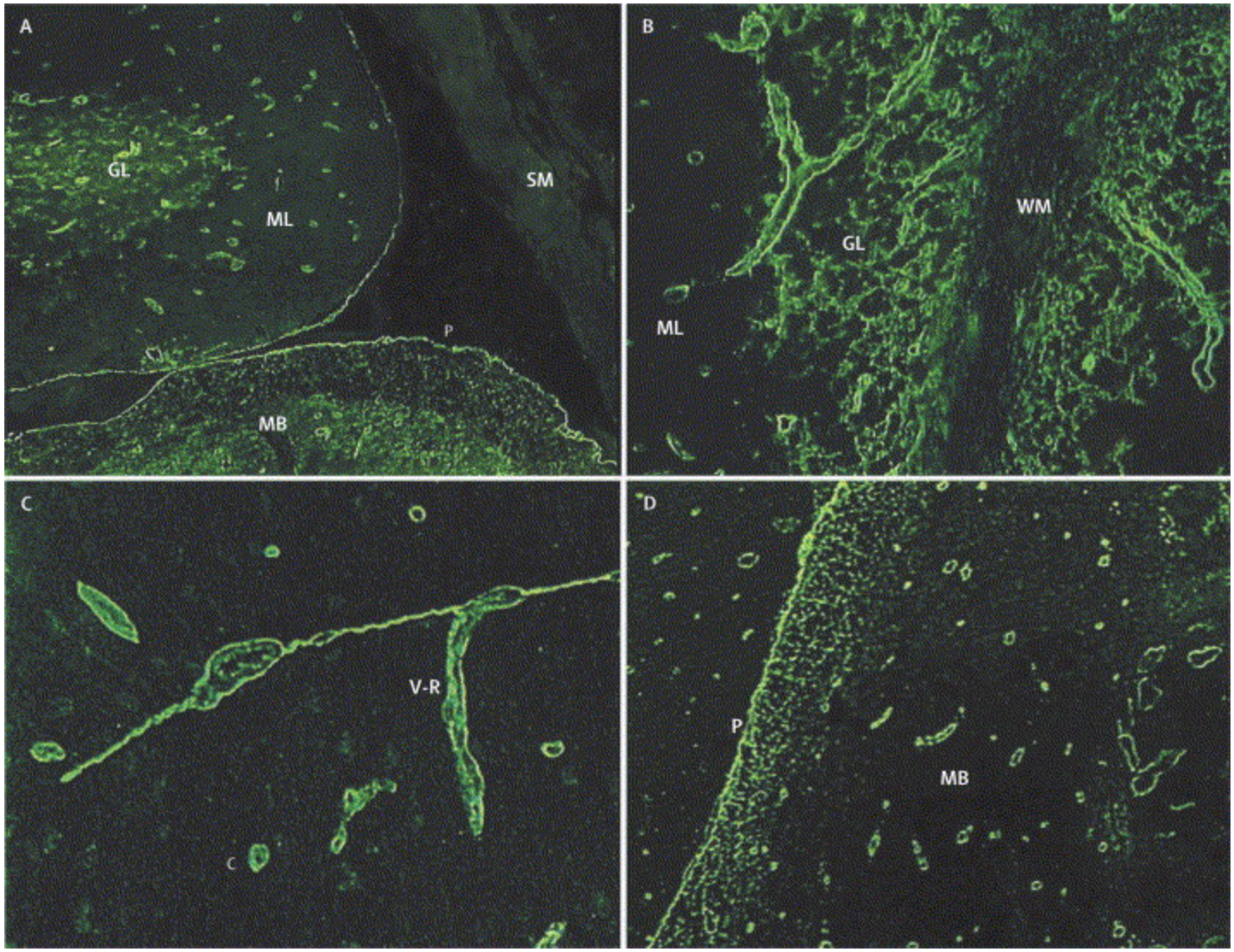
Coronal FLAIR sequence of a boy aged 8 showing multiple areas of high signal within the white matter, typical of ADEM



Neuromyelitis optica (NMO) – a newly recognized CNS channelopathy

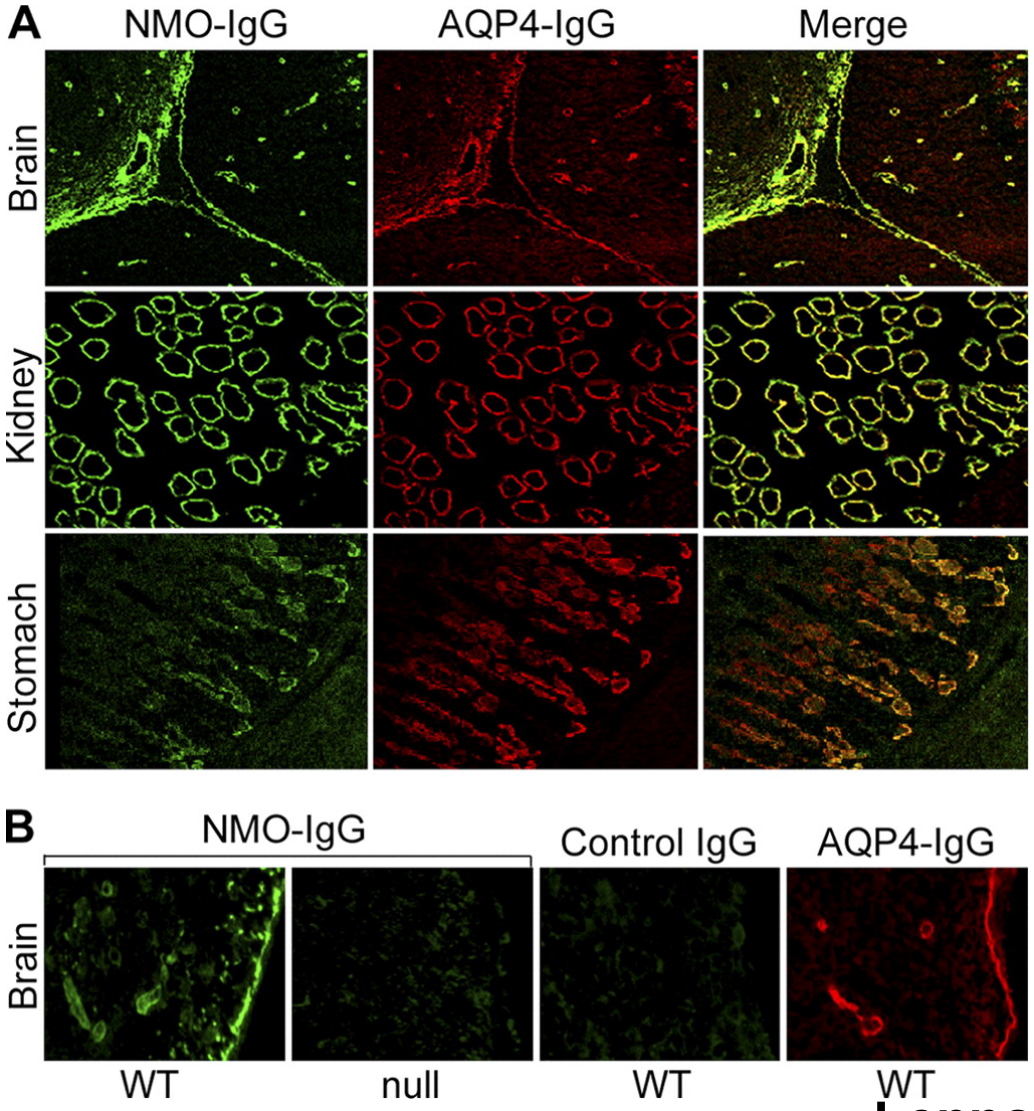
- NMO (Devic's disease) is a clinically defined severe CNS demyelinating syndrome characterized by optic neuritis and acute myelitis
- Characteristic immunopathology: IgG, IgM and complement deposited in a vasculogenic pattern suggest role for autoantibody
- IgG specific for NMO in serum of 73% of patients;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

- GBS: inflammatory + demyelinating disorder of the PNS
  - Variants (Miller-Fisher, CIDP, axonal)
- MS: inflammatory + demyelinating + degenerative disease of the CNS
  - Distinct from
    - ADEM
    - NMO

    Also inflammatory disorders of the CNS but have different evolution and underlying pathologies

Recommended reading

- Guillain-Barré syndrome. RAC Hughes, DR Cornblath, The Lancet. 2005. Volume 366, Pages 1653–1666
- Compston & Coles. Multiple Sclerosis. The Lancet. (2008)
- 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.
- Leake et al. Acute disseminated encephalomyelitis in childhood: epidemiologic, clinical and laboratory features. Pediatr Infect Dis J. 2004 Aug;23(8):756-64.
- Lennon VA, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet. 2004 Dec 11-17;364(9451):2106-12