Experimental Demyelination

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Neuroscience
Learning Objectives (*all knowledge based*):

- Be able to explain a variety of techniques giving rise to experimental demyelination

- Describe the symptoms of demyelination - both positive and negative

- To realize the functional importance of structural proteins in myelin, and their involvement in autoimmune models of demyelination

- To become familiar with some therapies for demyelination – in clinical use and experimental.
• Experimental demyelination

• Functional effects of demyelination are both +ve and –ve

• -ve – e.g. loss of sensation such as visual field defects and paralysis

• +ve – e.g. paraesthesias, neuromyotonia, tinnitus, pain

• Temperature dependent symptoms (e.g. ‘hot bath’)

• Inability of axons to carry trains of impulses
• Experimental demyelination

  Experimental acute demyelination

  • Diphtheria toxin (blocks protein synthesis) – used to produce demyelination of spinal root axons by intrathecal injection

  • Lysophosphatidyl choline (detergent) – used to produce demyelination in peripheral nerve

  • Ethidium bromide (dye that binds to DNA) – can be used to demyelinate dorsal column axons by intraspinal injection

  • X-rays
Recording transmembrane current using a tripole

Axon
Myelin

Local circuit current
Fig. 2. Thresholds (A) and membrane currents (B) recorded together at many positions along an undissected single fibre in a normal ventral root at 37°C. A, continuous line: thresholds to 10 μsec stimuli; dashed line: thresholds to 100 μsec stimuli. Note logarithmic scale. B, contour lines join points of equal membrane current density. Continuous lines: net inward current; dashed lines: net outward current. Contour interval: 1.5 nA.

Bostock, Sears and Sherratt (1983)
• animation
Mouse mutants of structural CAMs and other proteins
• Experimental demyelination

Mouse myelin mutants, PNS:

• Myelin protein P0, major structural component of peripheral myelin (and a CAM)
  
  – P0-null produces a dysmyelinating neuropathy, with partial loss of normal ion channel distributions in peripheral nerve. The sodium channel Na\textsubscript{v}1.8 (C-fibre channel) is found at large axon nodes. Disease associated with myelin loss and axonal degeneration
  
  – P0 exhibits haploinsufficiency, so with one functional gene there is a late-onset neuropathy that mimics CMT1B (Charcot-Marie-Tooth disease Type 1B).

• PMP22 expressed by Schwann cells (PMP22 is a homophilic CAM)
  
  – Two autosomal-dominant mouse mutants (Trembler and Trembler-J) are point mutants of PMP22
  
  – Major dysmyelination in PNS, CNS axons normal. PNS axons subject to a constant cycle of myelination and demyelination. Associated with ataxia and tremor.
  
  – Overexpression of PMP22 in transgenic animals mimics CMT1A. Disease includes progressive (motor) axonal degeneration.
• Experimental demyelination

P0-null

Na⁺ channels + Caspr
Misexpression of a C-fibre Na⁺ channel

Na⁺ channels + K⁺ channels
K⁺ channel distribution less circumscribed

Altered expression of ion channel isoforms at the node of Ranvier in P0-deficient myelin mutants
Jochen C. Ulzheimer, Elior Peles, S. Rock Levinson and Rudolf Martini, 2004
• Experimental demyelination
  
  Mouse myelin mutants, CNS:
  
  • Myelin proteolipid protein (PLP) in myelin forming oligodendrocytes. (PLP spans the membrane, suggested to act as a ‘zipper’)  
    - Natural point mutant jimpy mouse. Also transgenic mouse mutants, models of Pelizaeus-Merzbacher disease (PMD)  
    - Almost complete loss of central myelin and mature oligodendrocytes  
    - PLP appears to be an antigen in MS  
    - PLP can be used as an antigen in EAE

  • Myelin Basic Protein (MBP) - various null mutations  
    - Shiverer mouse. Defects in myelin formation, and are nearly devoid of CNS myelin in adulthood.  
    - Mice develop severe tremor early in post-natal development  
    - Expression of MBP dependent on protein tyrosine phosphatase SHP-1 (and other genes)  
    - MBP appears to be an antigen in MS  
    - MBP is a major structural component of CNS but not PNS myelin

  • 2′,3′-cyclic nucleotide 3-phosphodiesterase (CNP)-deficient.  
    - Lack of CNP from oligodendrocytes causes a severe degenerative disorder, myelinated axons lost from the cortex. Myelin sheaths seem to be preserved. Points to an oligo-axon interaction
• Experimental demyelination
  PLP knock-out

Klugmann et al 1997

Rosenbluth et al 1995
PLP-null, myelin deficits

Rosenbluth et al 2006 PLP-null, myelin deficits

Wide splits are evident in the outermost layers (asterisk). x 130,000

Interlamellar spaces appear dense and irregular in width
Models of MS

Experimental allergic encephalomyelitis (EAE)

- EAE and TMEV are most often used as models of MS

- Different models of EAE: e.g. induced in SWXJ mice by immunization with the 139-151 peptide of PLP. This induces a relapsing disease and ends with a chronic disability. Neurological disability is first correlated with inflammation and subsequently with axonal loss.

EAE induced by immunization with myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide in Biozzi mice

Optic nerve sections stained for myelin basic protein

Craner et al 2003
• Experimental demyelination

Freund’s Complete Adjuvant with either spinal cord homogenate (SCH)

Clinical Score

0 Normal
1 Limp tail (1) Remission
2 Impaired righting reflex
3 Partial paralysis
4 Hindlimb paralysis
5 Moribund

Day 0
Day 7

Clinical Score
Weight (g) - bars

Time (days)

Clinical score - line

Acute AP
Relapse RL1
Relapse RL2
Relapse RL3
Chronic CEAE

WL Weight Loss
RM1 Remission
RM2 Remission
RM3 Remission
RM4 Remission
Threshold of paralysis
• Experimental demyelination

**TMEV**

• Theiler's murine encephalomyelitis virus (TMEV) infection produces inflammatory demyelination. The severity of the condition depends on mouse strain.

• Non-pathogenic variant of the virus can be engineered to express a naturally occurring Haemophilus influenzae-encoded molecular mimic (HI574-586) of an immunodominant self-myelin proteolipid protein epitope (PLP139-151)(Croxford et al 2005).
Multiple Sclerosis
• Effects of demyelination

- In MS, the common form of the disease etiology is biphasic.
- A relapsing-remitting disease with prominent inflammatory involvement is eventually replaced with a progressive phase with loss of axons and neurones.
- Once it is not possible for the CNS to compensate for loss of axons, the relapsing-remitting form becomes secondary-progressive.
- Some of the characteristics of the disease process can be mimicked in animal models.
• Effects of demyelination

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• Effects of demyelination

What could be the process that leads to loss of axons and neuronal death?

One possibility is that NO release is key, (known to be released during the inflammatory process).

The combination of exposure to a NO donor and axonal activity causes irreversible damage to dorsal root axons and leads to degeneration.

Ken Smith
Treatments for demyelinating diseases
• **Therapy**

  Considering present therapies for MS (NB, MS is not the only demyelinating disease):

  • Present therapy for MS includes immune modulator drugs ie IFN-β-1a and 1b and random polymer.

  • Experimental immunomodulatory ‘drugs’ that prevent white cell movement across brain endothelia by blocking cell adhesion such as natalizumab and alemtuzumab (important toxic side effects are known)

  • Natalizumab is a monoclonal antibody that attaches to α4β1-integrin – which is a surface molecule found on lymphocytes.

  • Blocking the binding with VCAM-1 prevents movement of lymphocytes into the brain and immune response in the CNS.
• Therapy

Glial transplantation

• Schwann cell transplantation – causes remyelination in experimental demyelinating disease, but this is inhibited by astrocytes. However, there is evidence that transplants can improve function.

• Olfactory ensheathing cell transplantation – can promote axonal regeneration, remyelination and functional recovery

• Utzschneider et al 1994 transplantation of glial cells in Taiep rat – microtubular defect – deficient incorporation of PLP
Transplantation of glial cells from WT litter-mates enhances action potential conduction of amyelinated spinal cord axons in the myelin-deficient rat.

Utzschneider et al 1994

**Fig. 4.** Single-cell recording of impulse conduction through the transplant region. (A) Schematic showing placement of two stimulating electrodes in the transplant region and one intracellular recording electrode ($R_{\text{intracellular}}$) in the attached dorsal root ganglion (DRG). (B) Intracellularly recorded action potential of a dorsal root ganglion neuron stimulated at two different sites of the transplant region. Interstimulus distance is 2.0 mm and interstimulus latency shift is 0.77 msec, resulting in a dorsal column conduction velocity of 2.5 m/sec for this axon. (C) Aggregate data from dorsal column axons within the transplant region ($\bullet; n = 67$), nontransplanted md ($\circ; n = 258$), and nontransplanted control rats ($\bullet; n = 95$). C.V., conduction velocity.
• Therapy

Olfactory ensheathing cells

But is it OECs or endogenous/exogenous Schwann cells that actually remyelinate?

e.g. Raisman G and Li Y
Nat Rev Neurosci. 2007 Apr;8(4):312-9

Li Y, Li D and Raisman G
Glia 2007 Feb;55 (3):312-316
Summary

• Experimental demyelination causes conduction failure by increasing the effective axonal capacity and revealing periaxonal/internodal membrane that express kinetically fast K\textsuperscript+ channels

• Peripheral or central demyelination results from knock-out of myelin structural proteins (including CAMs)

• In neuronal disease the functional effects of demyelination are thought to be overcome by changes in the distribution of Na\textsuperscript+ channels along denuded axons and by remyelination. In MS eventually axons degenerate and neurones die, by a mechanism that might involve NO.

• Animal models of central demyelination use inoculation with a myelin component as antigen. In man immunomodulation strategies are used to slow the progression of MS.

• Glial or Olfactory ensheathing cell transplant may provide future therapies
References

Drugs used in MS eg:

e.g. R.A. Rudick (1999) Arch Neurol 56: 1079-1084
e.g. K.P. Johnson (2007) Natalizumab treatment for relapsing multiple sclerosis
  The Neurologist 13(4) 182-187

Models of demyelinating disease eg:


Gial transplantation eg: