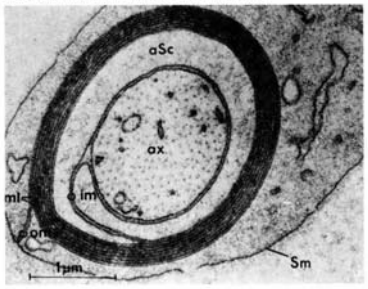
ZOO332H1S - Lecture 13 (AJE)

REMINDER ABOUT MYELINATION, AND CURRENT FLOW IN AXONS

INTRO TO INTEGRINS, MODELS OF DEMYELINATING DISEASE AND ROLE OF INTEGRINS IN MS



Immature axon and myelin in PNS

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Recall: Myelination

- Myelin interrupted at nodes of Ranvier (1 - 1.5mm spacing)
- Measurements made indicate CV for fibres > 11μm is 6 times axon diameter; fibres <11μm about 4.5 X
- Balance: thickness of myelin (increases R) and cross-sectional area of axon (decreases - causes increases in internal longitudinal R) - compromise: axon diameter 0.7 x overall fibre diameter
- Distance between nodes optimized

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cont. Myelination

• Classic experiments done by Ritchie and co-workers (mostly on rabbit nerves)

• Location of V-gated channels - **not** what you might expect!

or

•Na^+ channels conc'd in nodes of Ranvier; none paranodal

•K^+ channels conc'd under sheath (between nodes)

• V/C showed nodes displayed only inward currents and repol'n **NOT** by an increase of G_K+ - then what?

•Chronic demyelination by **diphtheria toxin** - Na^+ channels eventually populate demyelinated region and then get continuous conduction through the area, but poor substitute

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cont. Myelination

•Single Schwann cell makes myelin in one internode region (*ca*. 500 needed for single peripheral axon); oligodendrocyte can do several axons

•Formation of myelin by Schwann cells appears to be axon dependent-signaling; oligodendrocytes rely on astrocytes for signaling

•Myelin Basic Proteins - found in both; group of 7 related proteins (alternative splicing variants)

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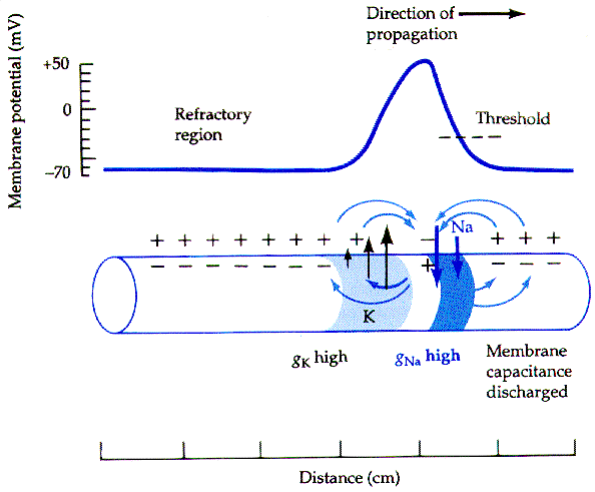
Current flow in an unmyelinated axon



NMW 5.4

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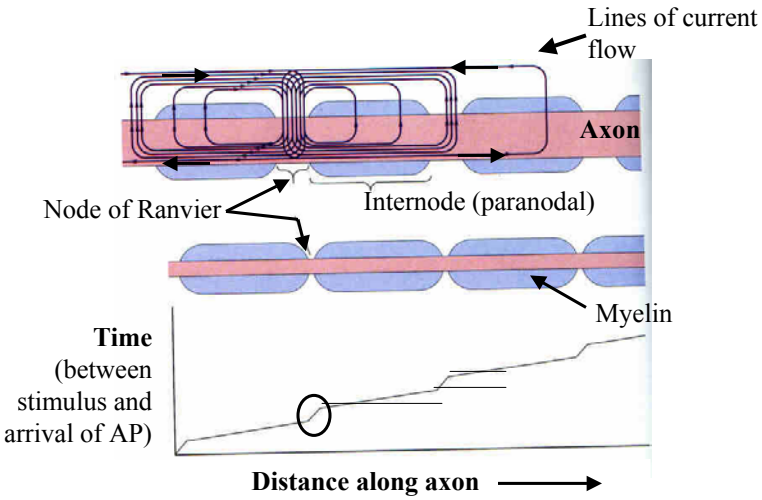
Action Potentials and current flow in myelinated nerves



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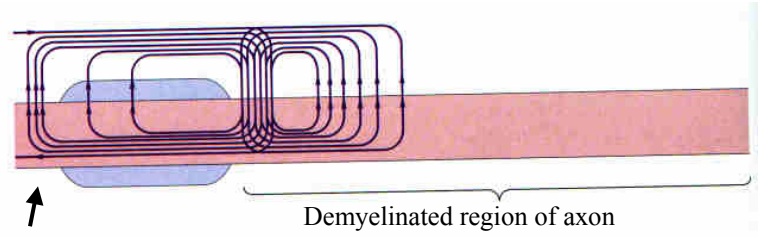
cont. APs and current flow in demyelinated nerves



Last intact
Node of
Ranvier

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Why myelin so important (other than CV)?

- Severe antigenicity of myelin basic proteins, used in mouse models of demyelinating diseases
- Human diseases: Multiple sclerosis and Guillain-Barre syndrome
- Genetic mouse models Shiverer (CNS) and Trembler (PNS) - Shiverer has deletion in gene encoding myelin basic protein; Trembler similar deficiency in producing myelin
- Experiments that show defect in Trembler is in Schwann cells, not signaling from axons

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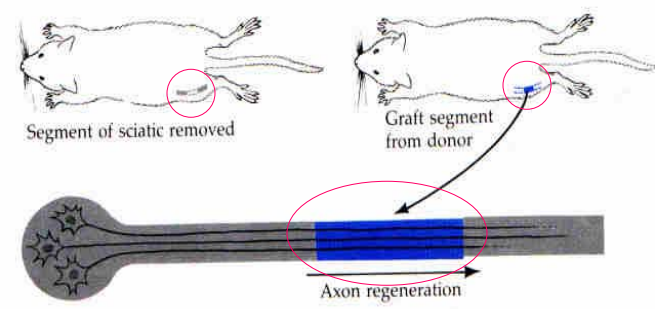
Trembler mutant mouse

- deficiency of myelin formation (PNS)
- Question: defect in signaling from axon or in Schwann cells ?



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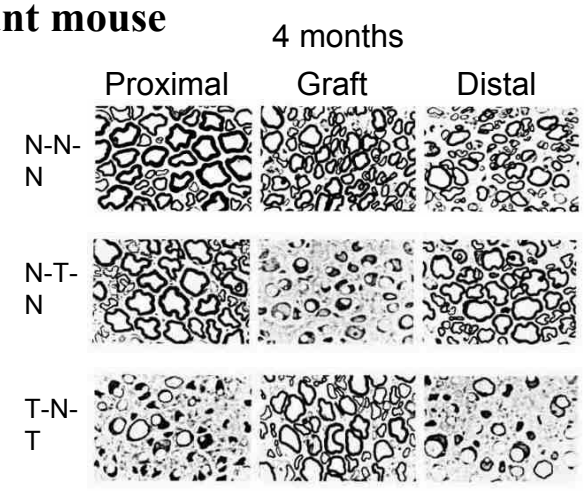
Trembler mutant mouse

- Question: defect in signaling from axon or in Schwann cells ?
- Complex and precise interactions - axons and satellite cells
- Therefore, defect resides in Schwann cells and not in axon



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Intro to models of demyelinating diseases

(1) Introduction to Integrins,
and
(2) Demyelination in MS and GBS

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Background: Potential roles of integrins in normal cell processes and demyelinating diseases

- are heterodimeric receptors (α and β subunits) - mediate cell-cell and cell-extracellular matrix interactions
- involved in embryogenesis, tissue remodeling, repair after injury
- developing NS: neurite outgrowth, synapse formation, neural crest cell migration, glial cells, and myelination
- very closely regulated; KO is lethal despite overlap of F'
- increasingly implicated in synaptic plasticity and memory formation, neural regeneration, and epileptogenesis (see next page for refs…)
- cytoskeleton mechanically linked to the ECM by integrins so that cytoskeletal stiffening increases in direct proportion to applied stress
- in immune system: generation of haematopoietic progenitors, maturation of T cells, lymphocyte recirculation and homing, platelet aggregation, cellular and humoral immune responses to foreign antigens

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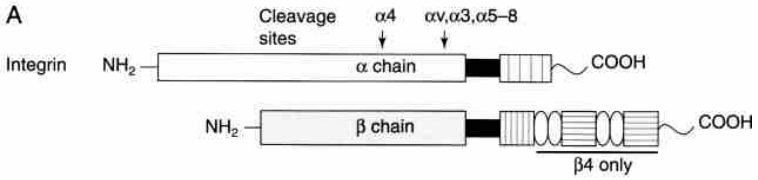
cont. Integrins - characteristics

- all integrins are transmembrane heterodimers made of one alpha and one beta subunit
- 16 different alpha and 8 different beta subunits available for combination
- ligand specificity is determined by particular combination of alpha and beta subunit
- integrins are essential for effective antigen presentation *in vitro*
- MABs against specific integrins or ligands (*in vivo*) attenuate EAE and EAN



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Selected References on Role of Integrins in the Nervous System

• Integrin-mediated short-term memory in Drosophila. MS Grotewiel *et al*. (1998). Nature.

• Making memories stick: cell-adhesion molecules in synaptic plasticity. DL Benson *et al*. (2000), TCB.

• Rapid neuromodulatory actions of integrin ligands. Wildering *et al*.(2002). J Neurosci.

• The role of cell adhesion molecules in synaptic plasticity. S Murase and EM Schuman (1999). Curr Opinion in Cell Biology.

• Time-dependent reversal of long-term potentiation by an integrin antagonist. Staubli *et al*. (1998). J Neurosci.

• Adult neuronal regeneration induced by transgenic integrin expression. ML Condic (2001). J Neurosci.

• Modulation of calcium current in arteriolar smooth muscle by αvβ3 and α5β1 integrin ligands. X Wu *et al*.(1998). JCB.

• Evidence for a functional interaction between integrins and G protein-activated inward rectifier K^+ channels. JC McPhee *et al*.(1998). JBC.

• Integrin signalling: a new Cas(t) of characters enters the stage. GM O'Neill *et al*.(2000). TCB

Next Slides:
Some selected data and commentary to illustrate role of integrins in nervous system function

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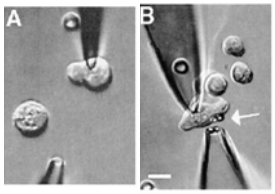
Effects of soluble cRGD* peptide on I_{Ba2+} recorded from arteriolar smooth muscle cells

Hypothesis: that L-type, voltage-gated Ca^{2+} channel was involved in the vasoactive responses of arterioles since this channel is known to be a major pathway for calcium entry into vascular SMCs (smooth muscle cells); and that activation of integrins are involved in modulating activity of L-type voltage-gated Ca^{2+} channel.

A,B : methods of application of integrins to smooth muscle cells (picospritzer or beads)

Record from L-type Ca^{2+} channels (P/C)



* tripeptide recognition site on ECM that a large group of integins bind.

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X Wu *et al*.(1998). JCB

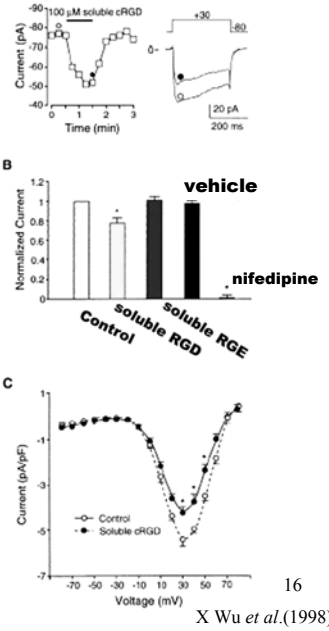
Effects of soluble cRGD* peptide on I_{Ba2+} recorded from arteriolar smooth muscle cells

A,B,C :

(A) time course of changes in I_{Ba}, test every 15 sec;

(B) normalized peak current at 1 min after application;

(C) summary I-V curve (relation) before or 60 sec after applic'n RGD peptide



Conclusion: integrins are linked through intracellular signalling pathways to the L-type Ca^{2+} channel and thereby alter control of Ca^{2+} influx in vasc sm muscle

*tripeptide recognition site on ECM

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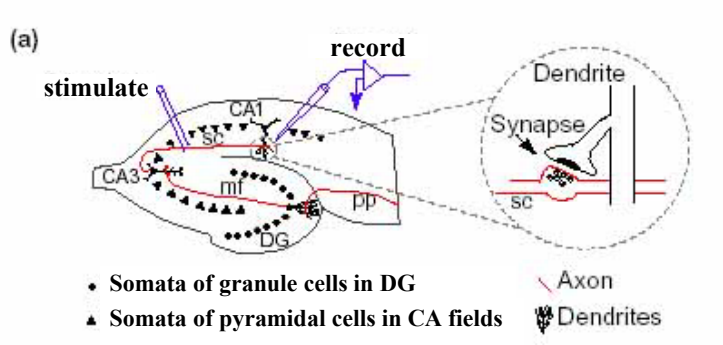
X Wu *et al*.(1998). JCB

Cell-adhesion molecules and integrins in synaptic plasticity



• Hippocampal slice – dominant model for studying LTP and role of CAMs in synaptic plasticity

• subregions, major cell types, axon pathways, and typical experimental set-up for inducing LTP

• major input area (PP); relay to CA3; relay to CA1; each area can record LTP postsynaptic

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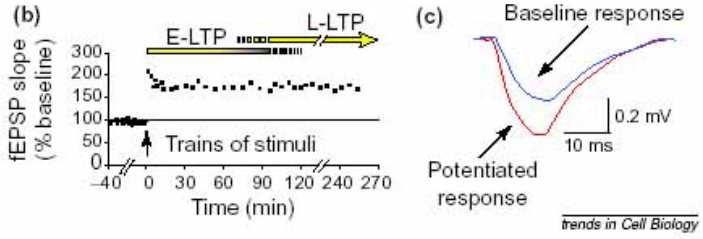
DL Benson *et al*. (2000), TCB

Cont…Cell-adhesion molecules in synaptic plasticity



• field (f) EPSPs in CA1 recorded; early and longer lasting LTP; baseline response established then multiple, widely spaced trains of high-frequency electrical stimulation to Shaffer collaterals

• potentiation seen as immediate and sustained increase in the magnitude of the CA1 synaptic response (E-LTP 1-2 hours; L-LTP when induced hours to days)

• note increase in response amplitude of CAM-potentiated response (taken at 2 h) showing increase in synaptic strength during LTP in comparison with baseline

• integrin-mediated adhesion appears to play a role in early stabilization of E-LTP but little or no role in its induction

DL Benson *et al*. (2000), TCB

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Cont…Cell-adhesion molecules and integrin in synaptic plasticity

• Integrin antagonist applied by local ejection to recording site in CA1.

• Window of time when application of antagonist was effective at causing steady decay of LTP relative to a within-slice control site.

• Ranged from 10 minutes before and up to 10 min after induction; if antagonist was applied >25 min after induction of LTP then no detectible effect on potentiation.

• Suggestion that time period after induction when prep was sensitive to antagonist corresponds to the time during which LTP is susceptible to reversal (and newly formed memories are vulnerable to various disruptive treatments).

• Results suggest that integrin activation and signalling occurring over several minutes after LTP induction are necessary for stabilizing synaptic potentiation and by inference may be required for the conversion of new memories into a not readily disrupted state.

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Immune-Mediated Diseases of the NS

background

CNS - Multiple sclerosis (MS)
PNS - Guillain-Barre syndrome (GBS)

• Major cause of transient and permanent neurological disability in adults

• Aetiology and pathogenesis not understood although appears to involve infiltration of T cells; macrophage-mediated demyelination; variable axon loss

• Transmembrane adhesion molecules (**integrins**) appear to be involved

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Experimental models of MS and GBS

Two animal models most used:

1. Experimental autoimmune encephalomyelitis (EAE)- CNS
2. Experimental autoimmune neuritis (EAN) - PNS

- EAE induced either by **sensitization with central or peripheral myelin antigen or by** intravenous injection of myelin specific **CD4+ T cells** that have been activated in vitro (adoptive-transfer EAE)

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cont. EAE and EAN

Two step process to elicit disease:

1. activation of T cells
2. transendothelial migration (effector phase of immune response in EAE) - breakdown of BBB critical to process

Amplification by chemokines and cytokines (recruit other T cells and macrophages), further infiltration, *etc*.

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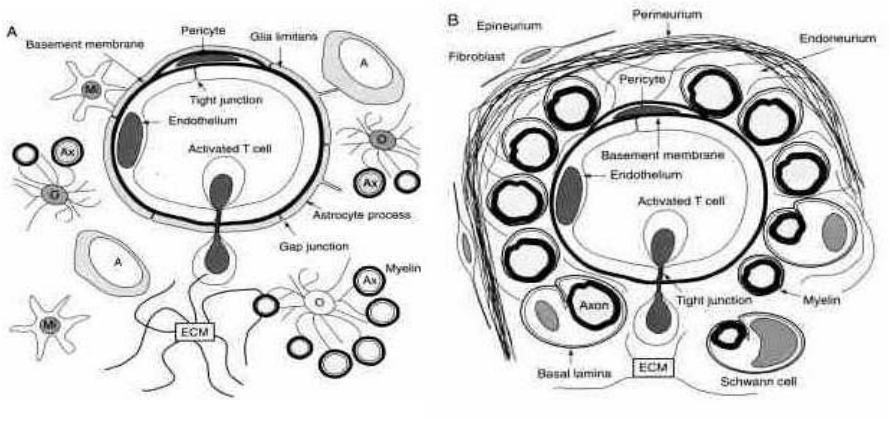
Getting in…TEM

CNS **PNS**



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cont. Aetiology and involvement of integrins

Indirect evidence of integrin involvement:

- **upregulation** of integrin ligands **ICAM-1** and **VCAM-1**
- redistribution of **PECAM-1** at endothelial tight junctions of lesion associated blood vessels
- presence of variety of integrins on mononuclear cells
- characteristics parallel clinical disease and infiltration to critical nervous system sites

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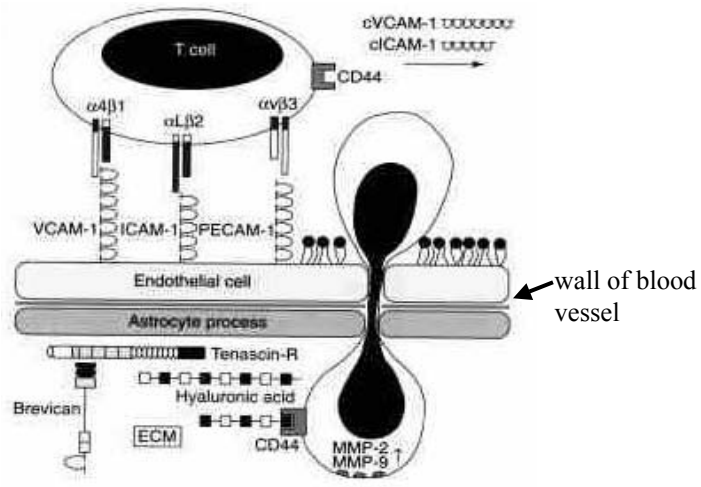
Adhesion molecules shown to be involved



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cont. Aetiology and involvement of integrins (1)

- after TEM: shift in protein expression/altered activation state, downregulate α4β1 integrin
- upregulate matrix metalloproteinases (degrade ECM molecules) on T cells
- integrins in target tissue (normally there) seems to be NB in effector stage

Ⓝ upregulation of integrins on glia; "modulated" on blood vessels

Ⓝ integrin promoters (for transcription) contain NFκ-B motifs or AP-1 binding sites (for TGFβ) - this links integrins to immune system

- enhance EAE by CNS overexpression of TGFβ

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cont. Aetiology and involvement of integrins(2)

Pathogenesis really only partially understood; MS and GBS (and other inflammatory neuropathies) clinically more complex than exp'al models
Exact phenotype and specificity of immune cells still not known
Possible therapies include antibody targeting of TEM of T cells governed by $\alpha 4\beta 1$ integrin (clinical trials in MS patients in UK)

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cont. Aetiology and involvement of integrins(3)

Conclusions: Integrins are key molecules for antigen presentation, TEM, effector phase (inflammation and demyelination), and in repair phase; coincident data on integrins for experimental models and in human counterparts (MS and GBS);
Implications: targeting specific integrins involved in TEM of T cells

- specific integrins altered in human pathologies
- regulatory mechanisms of integrins, emerging but complex
- new strategies will emerge

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Breadth of integrins effects

Lower diagram only:

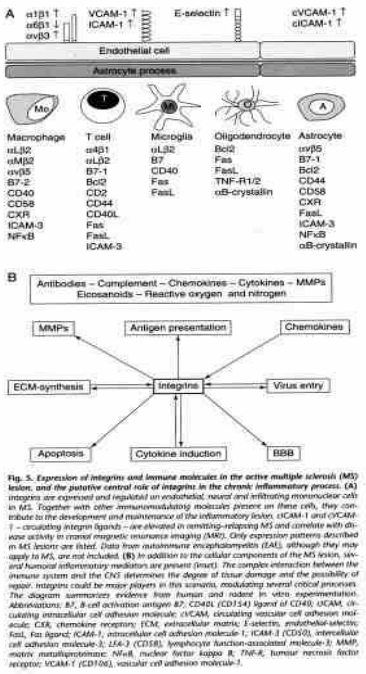

Fig. 5. ***Expression of integrins and immune molecules in the active multiple sclerosis (MS) lesion, and the putative central role of integrins in the chronic inflammatory process.*** *(**A**) Integrins are expressed and regulated on endothelial, neural and infiltrating mononuclear cells in MS. Together with other immunomodulatory molecules present on these cells, they contribute to the development and maintenance of the inflammatory lesion. sICAM-1 and sVCAM-1 – circulating integrin ligands – are elevated in remitting-relapsing MS and correlate with disease activity in cranial magnetic resonance imaging (MRI). Only expression patterns described in MS lesions are listed. Data from autoimmune encephalomyelitis (EAE), although they may apply to MS, are not included. (**B**) In addition to the cellular components of the MS lesion, several humoral inflammatory mediators are present (inset). The complex interaction between the immune system and the CNS determines the degree of tissue damage and the possibility of repair. Integrins could be major players in this scenario, modulating several critical processes. The diagram summarizes evidence from human and rodent in vitro experimentation. Abbreviations: B7, B-cell activation antigen B7; CD40L (CD154) ligand of CD40; sICAM, circulating intracellular cell adhesion molecule; sVCAM, circulating vascular cell adhesion molecule; CXR, chemokine receptors; ECM, extracellular matrix; E-selectin, endothelial-selectin; FasL, Fas ligand; ICAM-1, intracellular cell adhesion molecule-1; ICAM-3 (CD50), intercellular cell adhesion molecule-3; LFA-2 (CD58), lymphocyte function-associated molecule-3; MMP, matrix metalloproteinase; NFκB, nuclear factor kappa B; TNF-R, tumour necrosis factor receptor; VCAM-1 (CD106), vascular cell adhesion molecule-1.*

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Abbreviations and References

(Intercellular Cell Adhesion Molecule; Vascular CAM; Platelet/Endothelial CAM; ExtraCellular Matrix; NFk-B=nuclear factor kappa B, a central transcription regulator in the immune system; TGF-beta=transforming growth factor beta, a multifunctional cytokine involved in tissue repair; TransEndothelial Migration; Myelin Basic Protein)

See Rm 019 for copies of refs:

1. Archelos, JJ *et al*. (1999). The role of integrins in immune-mediated disease of the nervous system. TINS: **22**, 30-38.

2. Bellen, HJ *et al*. (1998). Neurexin IV, caspr and paranodin - novel members of the neruexin family… TINS: **21**, 444-449.

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