

## Matrix Metalloproteinases in Demyelinating Disease

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## The extracellular matrix (ECM)

- Four major components:
  - Collagens
  - Proteoglycans
  - Noncollagenous glycoproteins
  - Elastin fibers in skin, aorta

## Extracellular matrix

- Fills space between cells
- Builds a scaffold for cells, providing structural integrity for some tissues
- Provides support for cells
  - Sustains cell adhesion
  - Proliferation
  - Differentiation
- Reservoir for growth factors

## Matrix metalloproteinases (MMPs)

- Zinc-dependent endopeptidases
- Digest extracellular matrix
- Secreted as zymogens
- Require processing by cleavage for activation

## MMPs

- Involved in physiological processes
  - Wound healing
  - Ovulation
  - Angiogenesis
- Involved in pathological processes
  - Tumor cell invasion
  - Inflammation (multiple sclerosis)

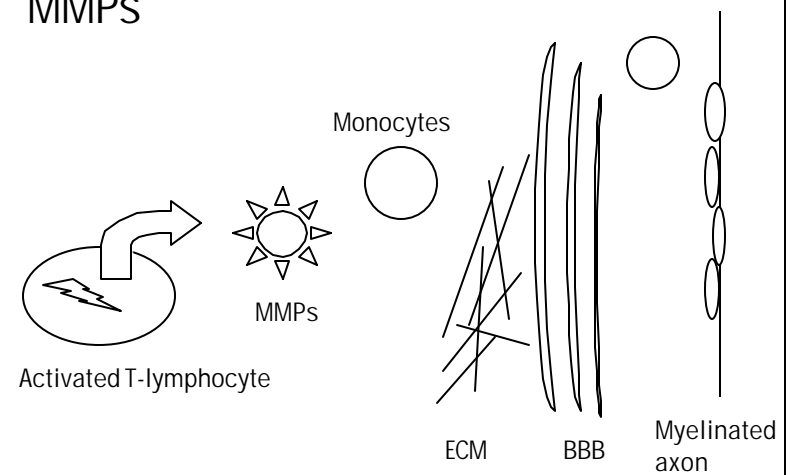
## MMPs

- Gene family with 20 members that comprise four major groups which differ in protein structure and substrate specificity

## MMPs

- Synthesized by resident glial cells
- Affect integrity of extracellular matrix
- May affect leukocyte entry into the central nervous system

## MMPs



## MMPs

- Major MMPs in brain:
  - Gelatinase A (MMP-2)
  - Stromelysin 1 (MMP-3)
  - Matrilysin (MMP-7)
  - Gelatinase B (MMP-9)
  - Membrane-type metalloproteinases

## Gelatinases (MMP-2 and MMP-9)

- Attack basal lamina surrounding blood vessels, altering permeability
- MMP-2 is normally found in CSF
- MMP-9 is induced during neuroinflammatory response

## MMP-9

- T-lymphocytes use MMP-9 to attack the capillary basal lamina, allowing crossing the BBB
- Experimental allergic encephalomyelitis causes increase MMP-9 in rodent brain
- Inhibitors of MMPs block the manifestations of the disease in animals

## MMPs in multiple sclerosis

- MMP-9 elevated in CSF multiple sclerosis (MS)\*
- MMP-9 increased before acute attack in MS

## Gelatinases in MS

- Increased in CSF of MS patients with gadolinium-enhancing lesions on MRI
- Treatment with high dose steroids return MMP levels to normal in MS

## MMPs and adhesion molecules

- MMPs mediate the destruction of myelin components
- Pro-inflammatory cytokine TNF-alpha involved in shedding of cell surface molecules

## MMPs and adhesion molecules

- Marimastat: broad spectrum MMP inhibitor
- Inhibited TNF-alpha mediated release of sVCAM-1 in human cerebral epithelial cells
- MMPs are actively involved in shedding of adhesion molecules at the BBB

## Gelatinases

- MMPs are toxic to tissues
- Controlled at several levels
  - Transcription
  - Activation
  - Inhibition
- Tissue inhibitors of metalloproteinases (TIMPs) are main inhibitors

## MMPs and the BBB

- MMP-2 (gelatinase A) intracerebral injection opens blood-brain barrier (BBB)
- Tumor necrosis factor- $\alpha$  injected into brain induces MMP-9 synthesis, causing delayed opening of BBB

## MMP and TIMPs and the blood brain barrier in stroke

- Adult rats with MCAO for 2 hours
- Treatment with synthetic MMP inhibitor
- BB-1011
- Effects on BBB and cerebral edema studied

## Cerebrospinal fluid analysis

- MMPs can be measured by zymography
- TIMPs can be measured by reverse zymography
- ELISA recently developed

## Devic's neuromyelitis optica

- Originally described in 1894, defined as:
  - Severe transverse myelitis
  - Acute unilateral or bilateral optic neuropathy
  - No clinical involvement beyond spinal cord or optic nerves
  - Monophasic or multiphasic illness

## MMPs and TIMPs in MS and Devic's

- MMP-9
  - Elevated in MS, but not Devic's or controls
- TIMP-1 and TIMP-2 similar in all three groups
- Conclusion: differences in MMP-9 support different pathological mechanisms

## MMP inhibitors

- KB-R7785 inhibits MMP-9 and MMP-2
- Mice with intraluminal middle cerebral artery occlusion
- Injection 30 minutes before occlusion decreased MMP-9 activity and infarct size
- Injection 1 and 4.5 hours after decreased infarct volume

## Results of inhibitor in stroke model

- Reduced BBB opening at 3 hours
  - Initial opening at 3 hours correlated with gelatinase A levels
- Reduced brain edema at 24 hours
- No effect on either at 48 hours
  - Delayed opening associated with elevated levels of gelatinase B failed to respond

## Other inhibitors

- Corticosteroids
- Aspirin (MMP-2)

## Acute transverse myelitis

Unknown levels of MMP and TIMP in CSF

Unknown effects of inhibitors

## Scar tissue in injured spinal cord

- Scar formation in spinal cord accompanied by remodeling of surrounding extracellular matrix
- MMP-2 and MMP-9 found transiently upregulated in the spinal cord wound
- In situ fluorescent zymography revealed gelatinase activity in the wound, spatially and temporally correlated with scar formation

## Spinal cord wound

- Interwoven pathways along which neurites were growing
- Corresponded to distribution of other molecules with antagonistic effects on axonal regrowth
- Neurite ingrowth may benefit from upregulation of MMPs

## Proposal

- Measure levels of MMPs in spinal fluid in:
  - Acute transverse myelitis
  - At 3-6 months
- Compare with results in MS, Devic's and CSF in infection