Articular pathology and Connective tissue turnover

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Overview

- Connective tissue turnover and matrix proteinases
- Articular pathology
  - Rheumatoid arthritis
  - Osteoarthritis

Learning objectives

- Define what is meant by matrix metalloproteinase and give some examples of their substrates
- Define what is meant by ADAMTS protease and understand that aggrecanases are important in the turnover of proteoglycan in articular cartilage
- Understand that cathepsin K is important protease in bone matrix turnover
- Define two abnormalities seen in the synovium of patients with rheumatoid arthritis
- Understand the importance of the inflammatory cytokine, tumour necrosis factor-α (TNF-α) in rheumatoid arthritis pathology.
- Define two abnormalities seen in the cartilage and two abnormalities seen in the bone in the osteoarthritic joint.

Connective tissue turnover - 1

- Cartilage and bone destruction develops when there is an imbalance between matrix synthesis and matrix degradation

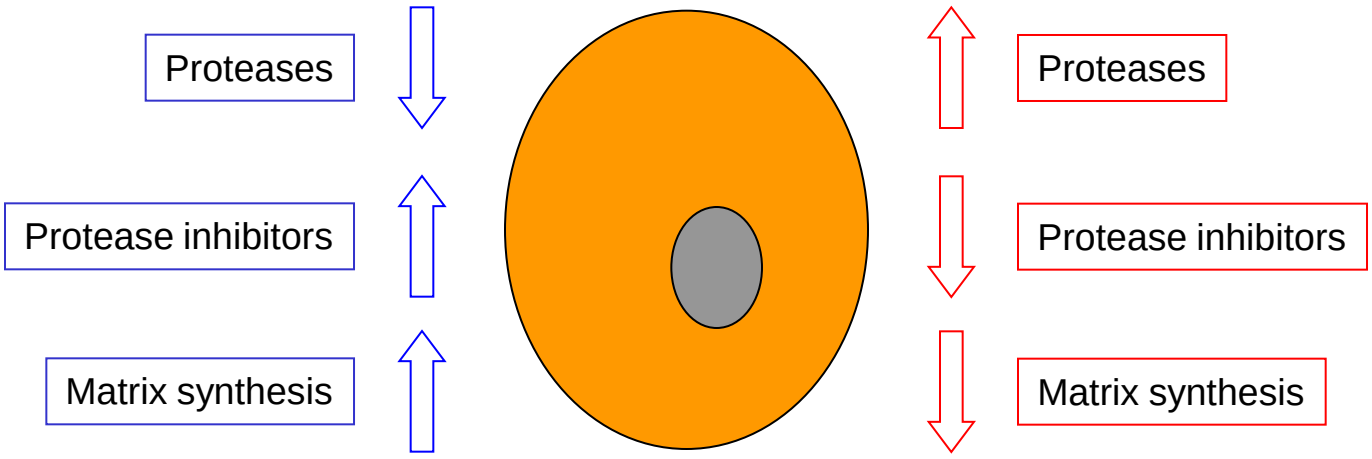
Chondrocyte in health maintains steady state so that:

MATRIX SYNTHESIS = MATRIX DEGRADATION

…thereby maintaining normal articular cartilage

Connective tissue turnover - 2

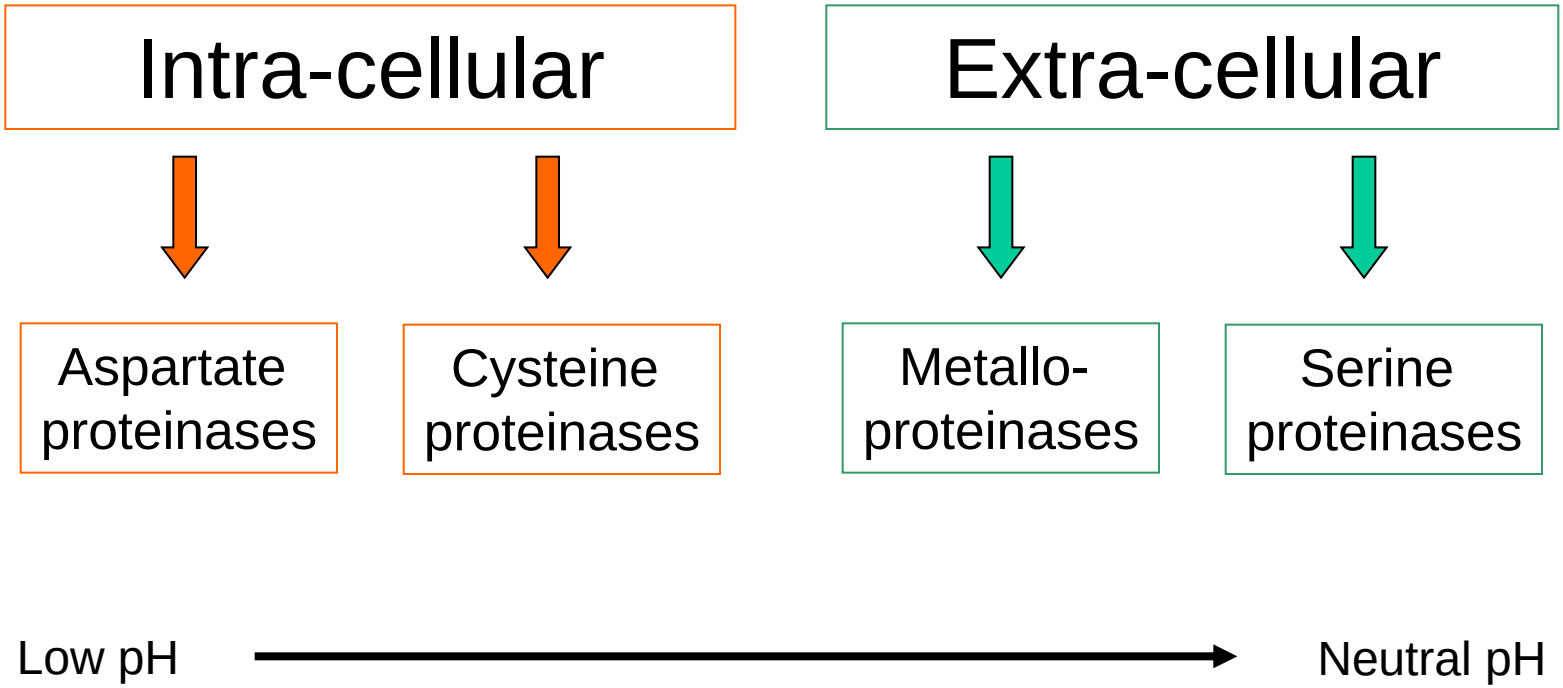
- Cartilage and bone destruction is mediated by proteinases that degrade collagen and proteoglycan



Connective tissue turnover - 3

- Source of proteinases depends on pathological process
  - Osteoarthritis = chondrocyte
  - Rheumatoid arthritis = synovial cells, inflammatory cells (extrinsic), chondrocytes
  - Infection = inflammatory cells (extrinsic), bacterial proteases (exogenous)

Proteins that degrade connective tissue matrix



Proteinases that degrade connective tissue matrix

Proteinase	Class	Inhibitors	Substrates
Collagenases MMP-1, -8, -13	Metallo	TIMPs, α_2-m	Fibrillar collagen I, II, III, VII, X gelatins
Stromelysins MMP-3, -10, -11	Metallo	TIMPs, α_2-m	Broad spectrum of activity, e.g. proteoglycan, fibronectin, laminin, some gelatins and collagens, link protein, vitronectin, etc.
Gelatinases MMP-2, -9	Metallo	TIMPs, α_2-m	Denatured collagens, collagens IV, V, VII, X, elastin, fibronectin, vitronectin
Cathepsin B	Cysteine	Cystatins, α_2-m	Proteoglycan, link protein, procollagen II, collagen II
Cathepsin L	Cysteine	Cystatins, α_2-m	Proteoglycan, elastin, link protein, collagen II
Cathepsin S	Cysteine	Cystatins, α_2-m	Proteoglycan, link protein, procollagen II, collagen II
Cathepsin H	Cysteine	Cystatins, α_2-m	Proteoglycan
Cathepsin D	Aspartate	α_2-m	Proteoglycan, gelatin
Plasmin	Serine	α_1-PI, α_1-AC, α_2-m, α_2 antiplasmin	Activates MMPs
Plasminogen activator (tissue)	Serine	Protease nexin 1, PA1-1, α_2-m	Activates plasminogen
Plasminogen activator (urokinase)	Serine	Protease nexin 1, PA1-1, α_2-m	Activates plasminogen

MMP = Matrix metalloproteinase; TIMP = Tissue inhibitors of metalloproteinase; α_2-m = α_2-macroglobulin; α_1-PI = α_1-antiproteinase inhibitor; α_1-AC = α_1- antichymotrypsin; PAI-1 = Plasminogen activator-1.

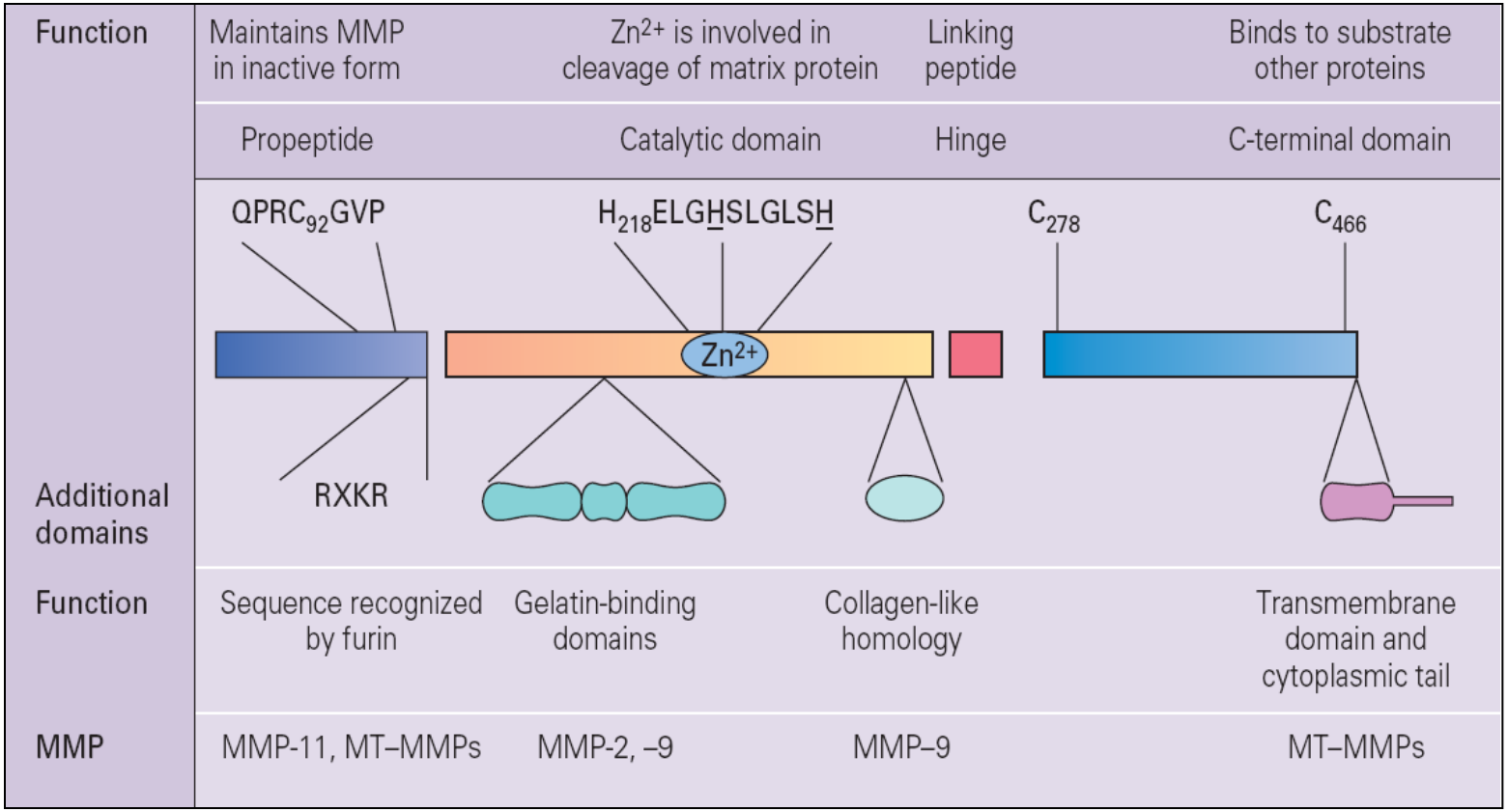
Connective tissue turnover - 4

- Important proteinases in connective tissue turnover
  - Matrix metalloproteinases (MMPs)
    - collagenases
  - ADAMTS -4 and -5
    - aggrecanases
  - Cathepsin K
    - bone matrix

Matrix metalloproteinases - 1

- Family of proteins
  - Catalytic centre contains zinc
  - Calcium-dependent endopeptidases

Domain structure of the matrix metalloproteinases (MMPs)



Matrix metalloproteinases - 2

- Family of proteins
  - Catalytic centre contains zinc hence ‘metallo’
  - Calcium-dependent endopeptidases
- Responsible for
  - Tissue remodelling
  - Degradation of extracellular matrix (ECM)

Role of MMPs in physiology and disease

Physiological processes	Pathological processes	
Angiogenesis	Arthritis	Multiple sclerosis
Apoptosis	Alzheimer's disease	Nephritis
Blastocyst implantation	Atherosclerosis	Neurological disease
Bone remodeling	Breakdown of blood–brain barrier	Osteoarthritis (OA)
Cervical dilation	Cancer	Periodontal disease
Embryonic development	Cardiovascular disease	Rheumatoid
Endometrial cycling	Central nervous system disorders	Skin ulceration
Hair follicle cycling	Corneal ulceration	Sorby's fundus disease
Immune response	Emphysema	Vascular disease
Inflammation	Fibrotic lung disease	
Nerve growth	Gastric ulcer	
Organ morphogenesis	Guillian-Barre disease	
Ovulation	Liver cirrhosis	
Postpartum uterine involution	Liver fibrosis	
Wound healing	Metastasis	

RP Verma and C. Hansch. Matrix metalloproteinases. Bioorg. Med. Chem. 2007, 15:2223-2268.

Matrix metalloproteinases - 3

- Family of proteins
  - Catalytic centre contains zinc
  - Calcium-dependent endopeptidases
- Responsible for
  - Tissue remodelling
  - Degradation of extracellular matrix (ECM)
- Substrates are ECM components:
  - Collagen
  - Elastin
  - Gelatin (hydrolysed collagen)
  - Proteoglycans
  - Matrix glycoproteins

MMP classes

No.	MMP No.	Class	Enzyme
1	MMP-1	Collagenases	Collagenase-1
2	MMP-8		Neutrophil collagenase
3	MMP-13		Collagenase-3
4	MMP-18		Collagenase-4
5	MMP-2	Gelatinases	Gelatinase-A
6	MMP-9		Gelatinases-B
7	MMP-3	Stromelysins	Stromelysin-1
8	MMP-10		Stromelysin-2
9	MMP-11		Stromelysin-3
10	MMP-27		Homology to stromelysin-2 (51.6%)
11	MMP-7	Matrilysins	Matrilysin (PUMP)
12	MMP-26		Matrilysin-2
13	MMP-14	MT-MMP (membrane type)	MT1-MMP
14	MMP-15		MT2-MMP
15	MMP-16		MT3-MMP
16	MMP-17		MT4-MMP
17	MMP-24		MT5-MMP
18	MMP-25		MT6-MMP
19	MMP-12	Other enzymes	Macrophage metalloelastase
20	MMP-19		RASI 1
21	MMP-20		Enamelysin
22	MMP-21		MMP identified on chromosome 1
23	MMP-22		MMP identified on chromosome 1
24	MMP-23		From human ovary cDNA
25	MMP-28		Epilysin
26	MMP-29		Unnamed

RP Verma and C. Hansch. Matrix metalloproteinases. Bioorg. Med. Chem. 2007, 15:2223-2268.

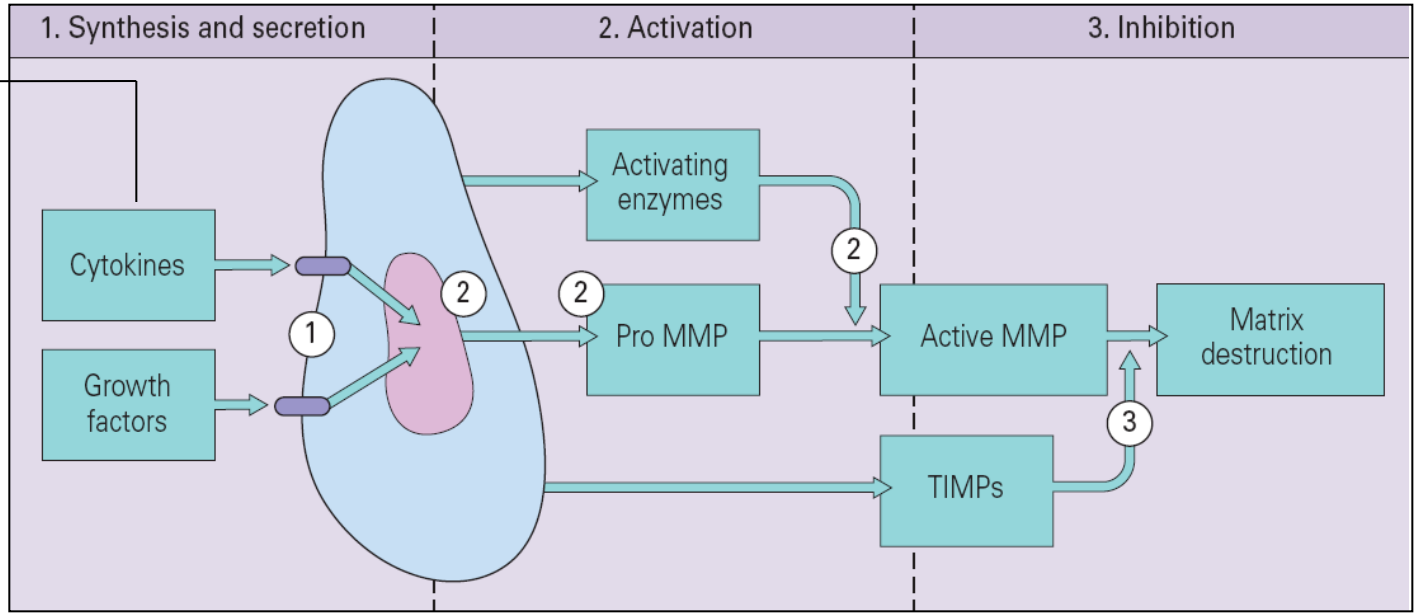
Examples of MMPs

- STROMELYSINS
  - Proteoglycans, Matrix glycoproteins (e.g. fibronectin, laminin)
  - Degraded collagen
- COLLAGENASES
  - Collagen
  - MMP-1, -8, -13 (= collagenase -1, -2, -3) cleave fibrillar collagen at a single site $\rightarrow$ stromelysins and gelatinases degrade the collagen fragments
- GELATINASES
  - Type IV collagen (basement membrane)
  - Elastin
  - Degraded collagen

Matrix metalloproteinases - 4

- Regulated by hormones, growth factors and cytokines

Regulation of MMPs



examples:

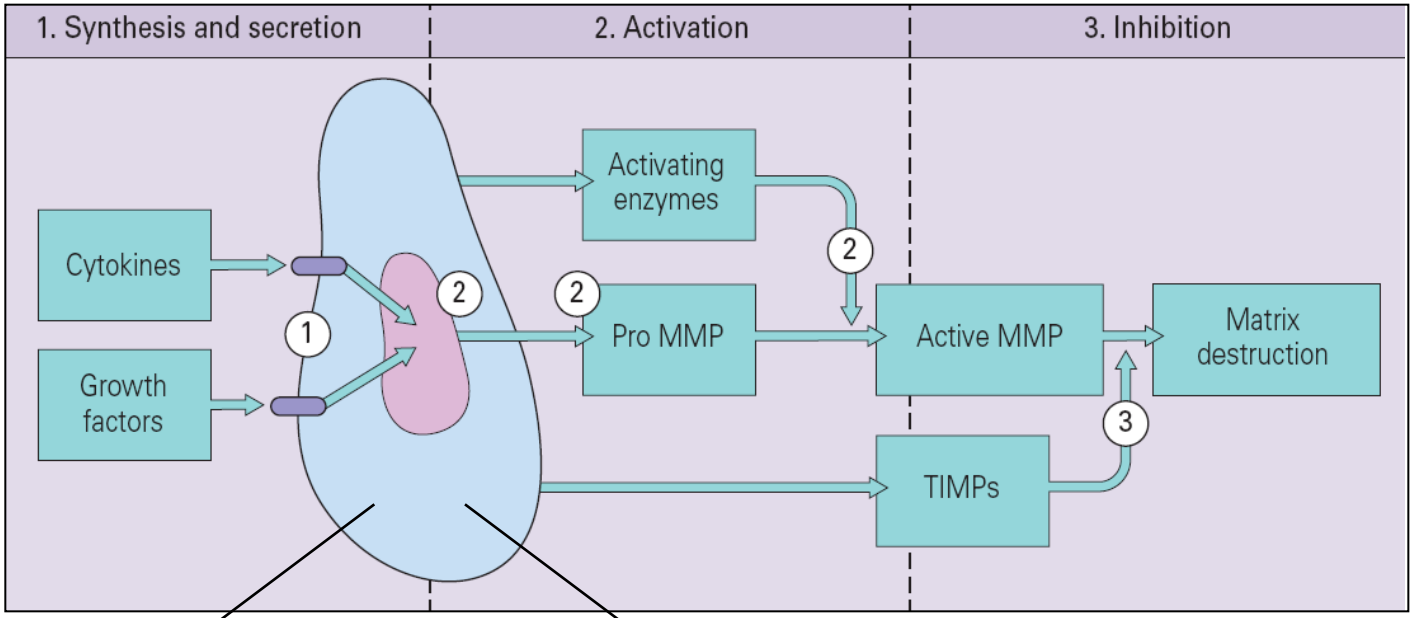
- In general the pro-inflammatory cytokines stimulate MMP synthesis and secretion e.g.

  → interleukin-1 and TNF-α stimulate collagenases1

Matrix metalloproteinases - 5

- Regulated by hormones, growth factors and cytokines
- Synthesised by many cell types

Source of MMPs



Examples:

Connective tissue cells:

- Synovial fibroblast
- Chondrocyte
- Osteoblast

Inflammatory cells:

- Macrophages
- Neutrophils

  Two MMPs are contained within neutrophil specific granules:

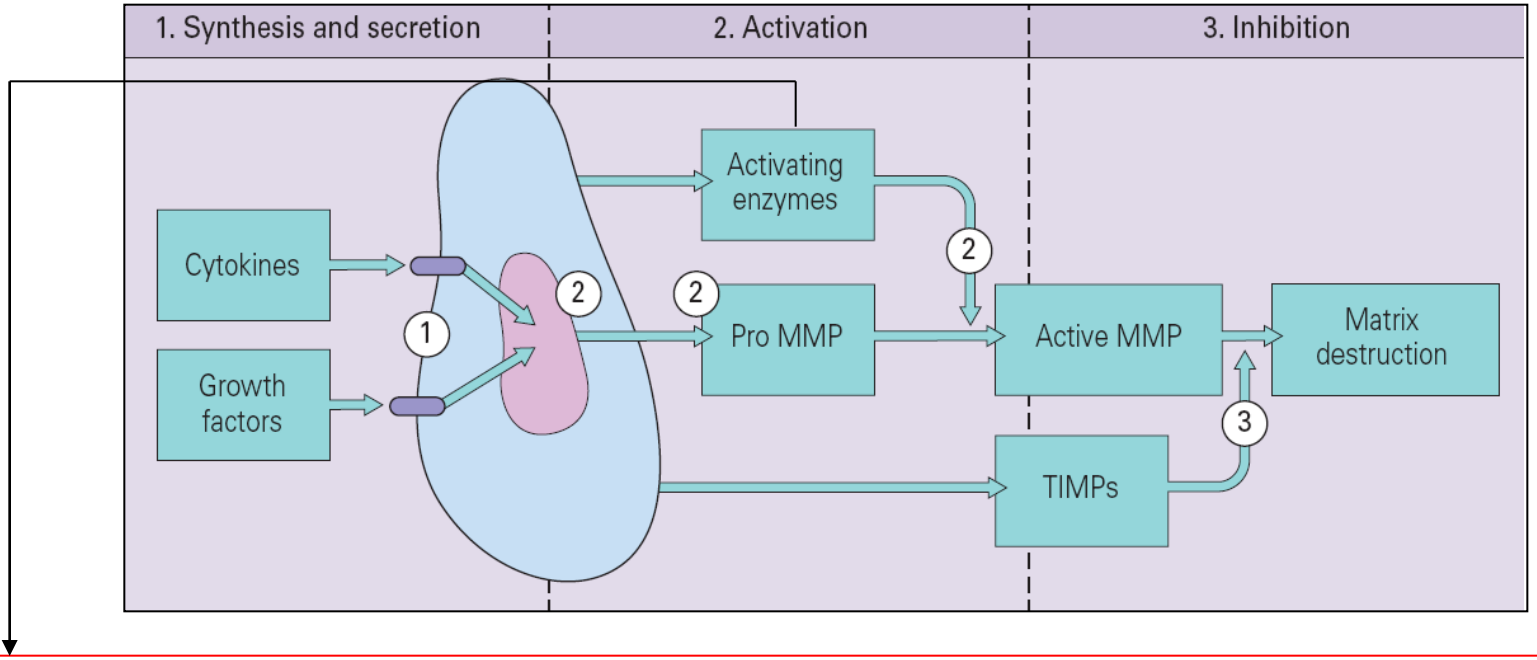
  → MMP-8 (collagenase -2, also termed neutrophil collagenase)

  → MMP-9 (gelatinase B)

Matrix metalloproteinases - 6

- Regulated by hormones, growth factors and cytokines
- Synthesised by many cell types
- Synthesised in zymogen form

Activation of MMPs



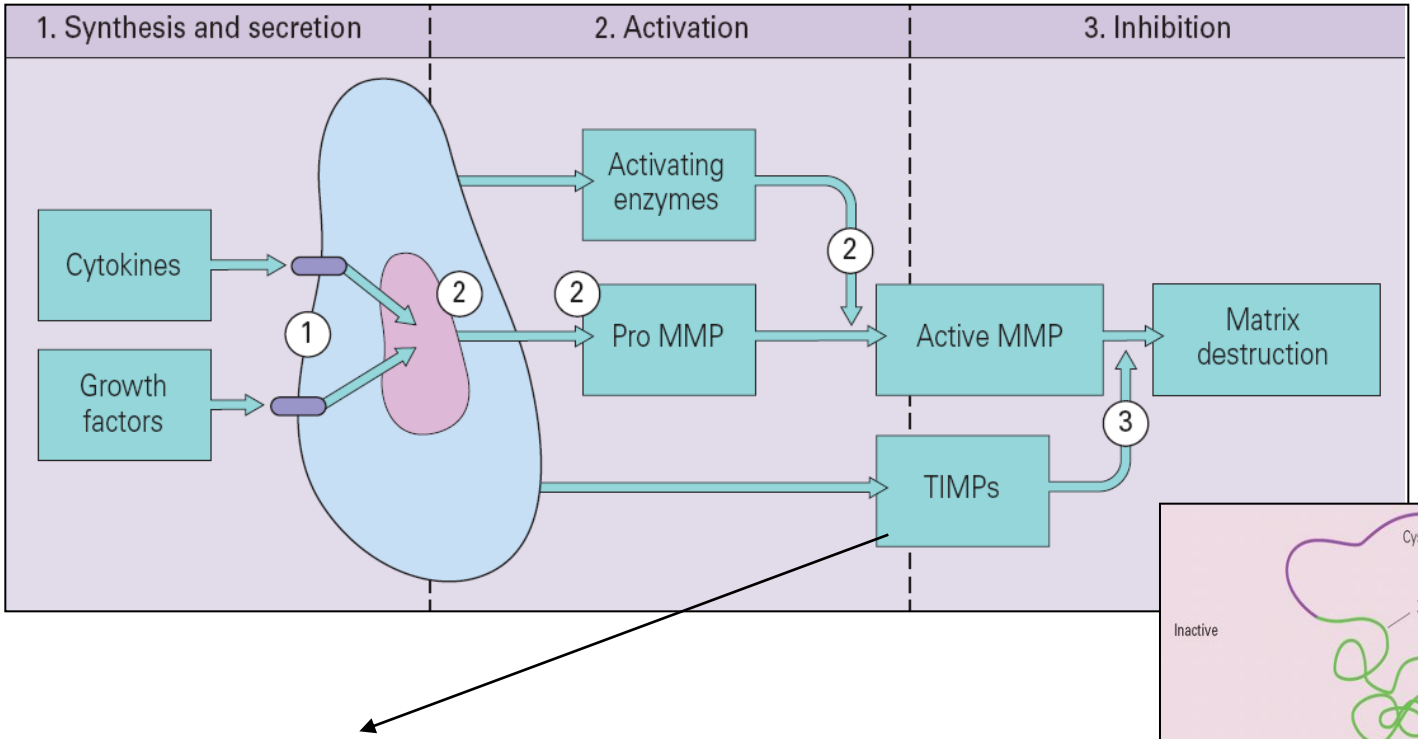
examples:

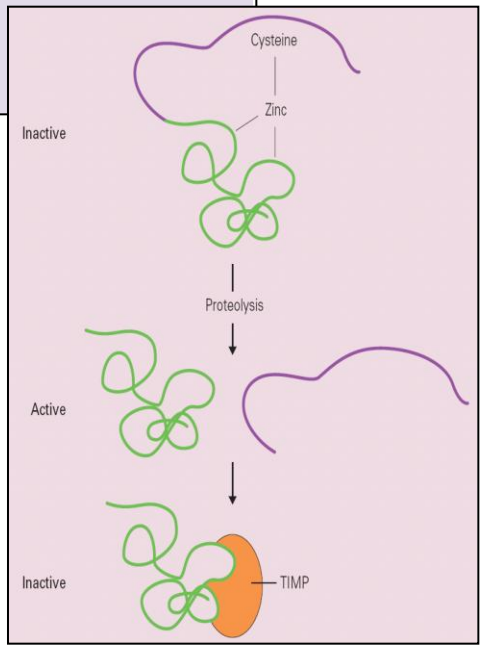
- FURIN – ubiquitous golgi proteinase
- Plasmin (protease produced from plasma zymogen plasminogen) activates pro-MMP-3
- Stromelysin-1 (MMP-3) activates pro-collagenases
- MMP14 (an MT-MMP) may initiate MMP activation cascades
- MMP-17 (MT4-MMP) activates ADAMTS-4

Matrix metalloproteinases - 7

- Regulated by hormones, growth factors and cytokines
- Synthesised by many cell types
- Synthesised in zymogen form
- Inhibition of MMPs

Inhibition of MMPs





TIMPS:
- Tissue inhibitors of matrix metalloproteinase
- Four identified: TIMP-1, -2, -3, -4
- upregulated by growth factors e.g. TGF-β

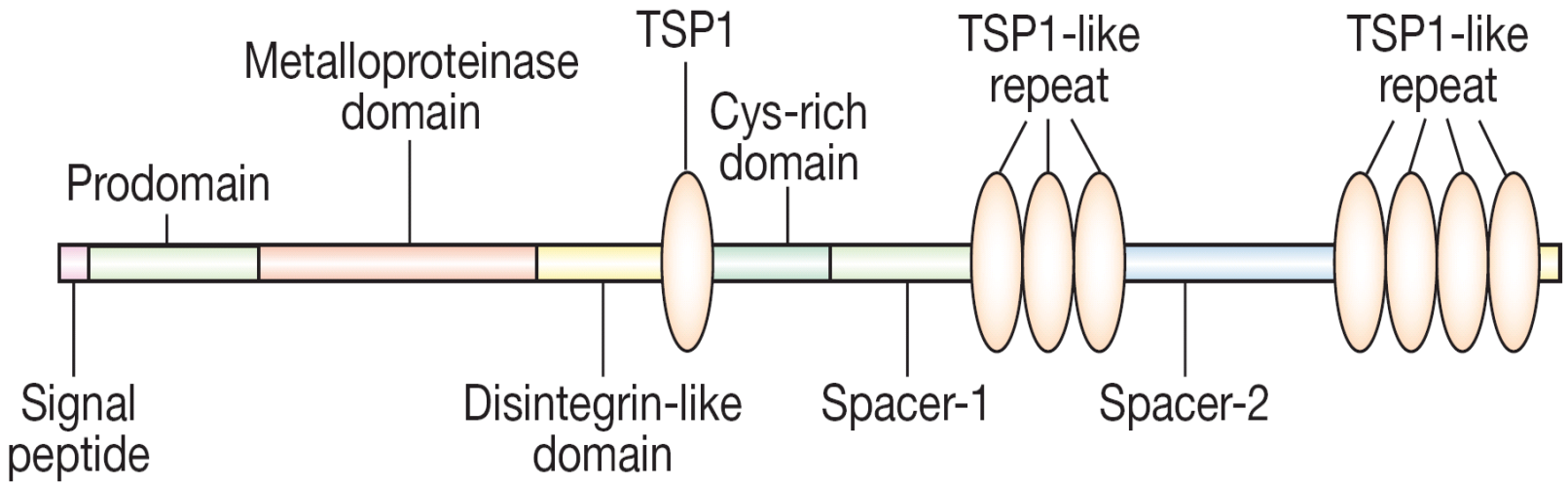
α2-macroglobulin
•natural inhibitor of MMPs (and all other proteinase classes)

Connective tissue turnover - 4

- Important proteinases in connective tissue turnover
  - Matrix metalloproteinases (MMPs)
    - collagenases
  - ADAMTS -4 and -5
    - aggrecanases
  - Cathepsin K

ADAMTS metalloproteinases - 1

- ADAMTS family of proteinases
  - Related to MMPs i.e. are metalloproteinases
  - ADAMTS = a disintegrin and metalloproteinase with thrombospondin motifs



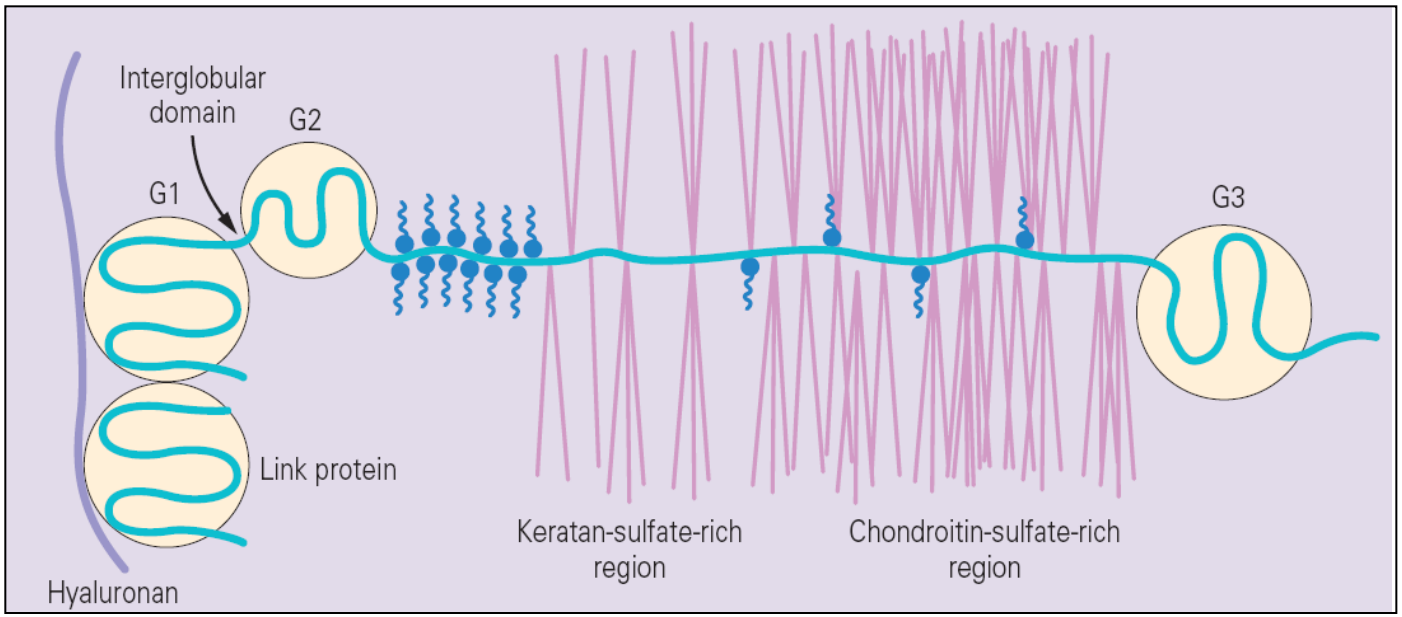
ADAMTS metalloproteinases - 2

- ADAMTS family of proteinases
  - Diverse functions but important to note that ADAMTS-4 and 5 are aggrecanases i.e. degrade cartilage proteoglycan

NAME:	SUBSTRATE:	INHIBITORS:
ADAMTS-4 (aggrecanase-1)	Aggrecan	TIMP-1, -2, -3 α2-macroglobulin
ADAMTS-5 (aggrecanase-2)	Aggrecan	TIMP-3 α2-macroglobulin

ADAMTS metalloproteinases - 3

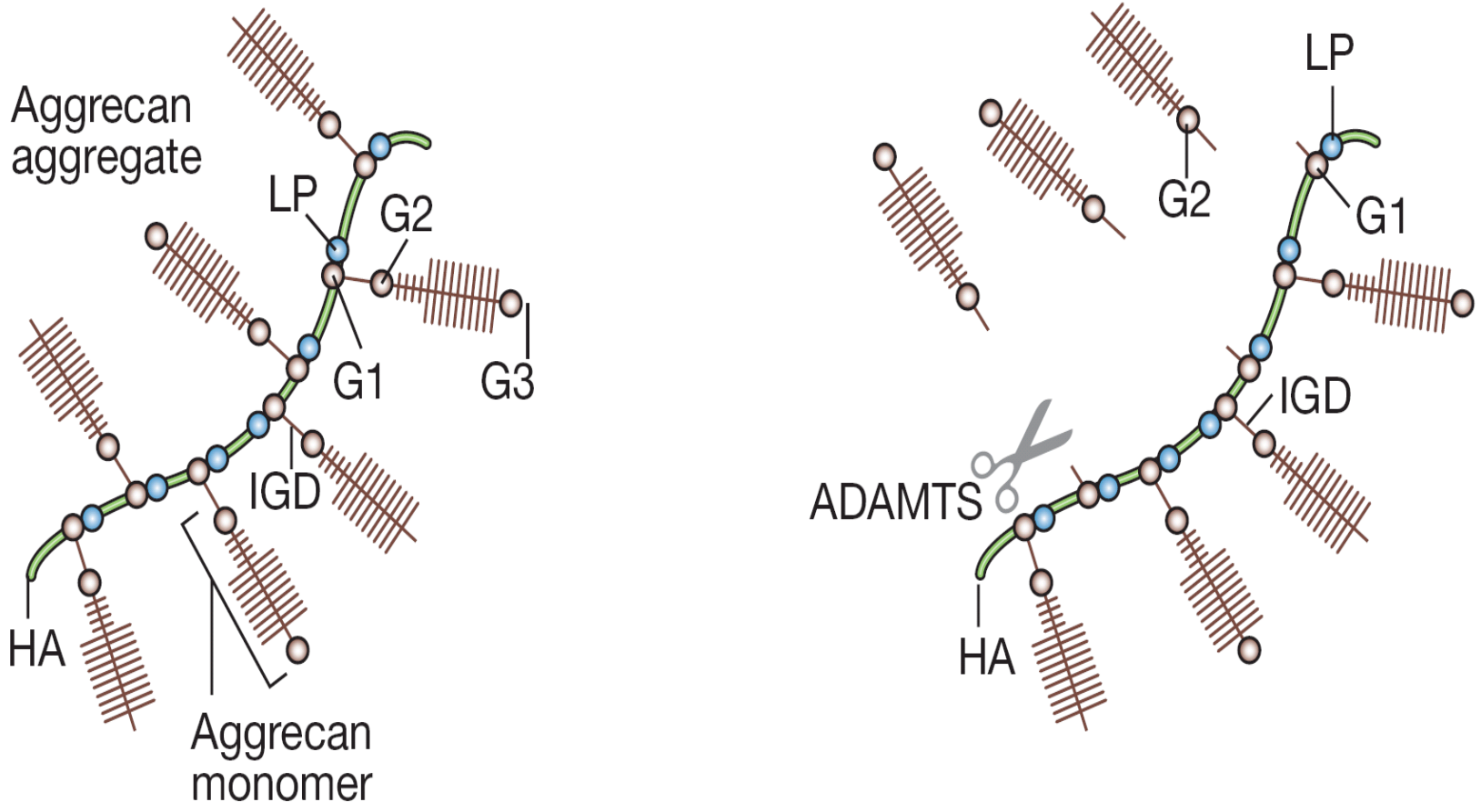
- The major proteoglycan in human cartilage is aggrecan



Link protein enables non-covalent interaction between aggrecan and hyaluronic acid

ADAMTS metalloproteinases - 4

- ADAMTS-mediated cleavage of aggrecan



Connective tissue turnover - 4

- Important proteinases in connective tissue turnover
  - Matrix metalloproteinases (MMPs)
    - collagenases
  - ADAMTS -4 and -5
    - aggrecanases
  - Cathepsin K

Cathepsin K

- Cysteine protease most active at acidic pH
  - lacunae of the osteoclast is acidic
- Important in turnover of matrix of long bone
  - Highly expressed by osteoclast
  - Active against helical type I collagen
  - Cathepsin K-deficient osteoclasts cannot degrade bone protein matrix
  - Human deficiency results in skeletal dysplasia (Pycnodysostosis)
  - Inhibition of cathepsin K under investigation as potential therapy for conditions associated with excessive bone turnover

Connective tissue turnover - summary

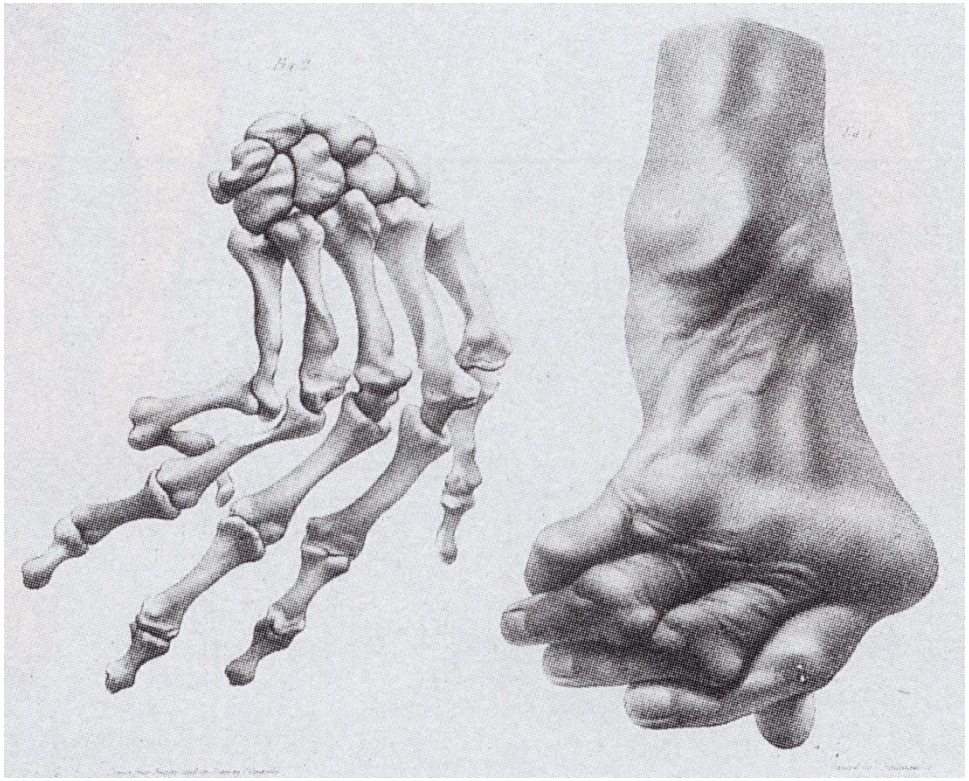
- Most of joint tissue is extra-cellular matrix and proteinases are key in ECM degradation
- Proteases are numerous and combine to form complex regulatory networks
- Important roles for:
  - MMP collagenases and cleavage of cartilage collagens
  - ADAMTS aggrecanases and cartilage proteoglycans
  - Cathepsin K in the acidic lacunae of osteoclasts and bone matrix turnover

Articular pathology

- Rheumatoid arthritis
- Osteoarthritis

Rheumatoid arthritis

Chronic autoimmune disease characterised by pain, stiffness and symmetrical synovitis (inflammation of the synovial membrane) of diarthrodial joints



Synovium and synovial fluid

Synovium:
Lining, 1-3 cells deep

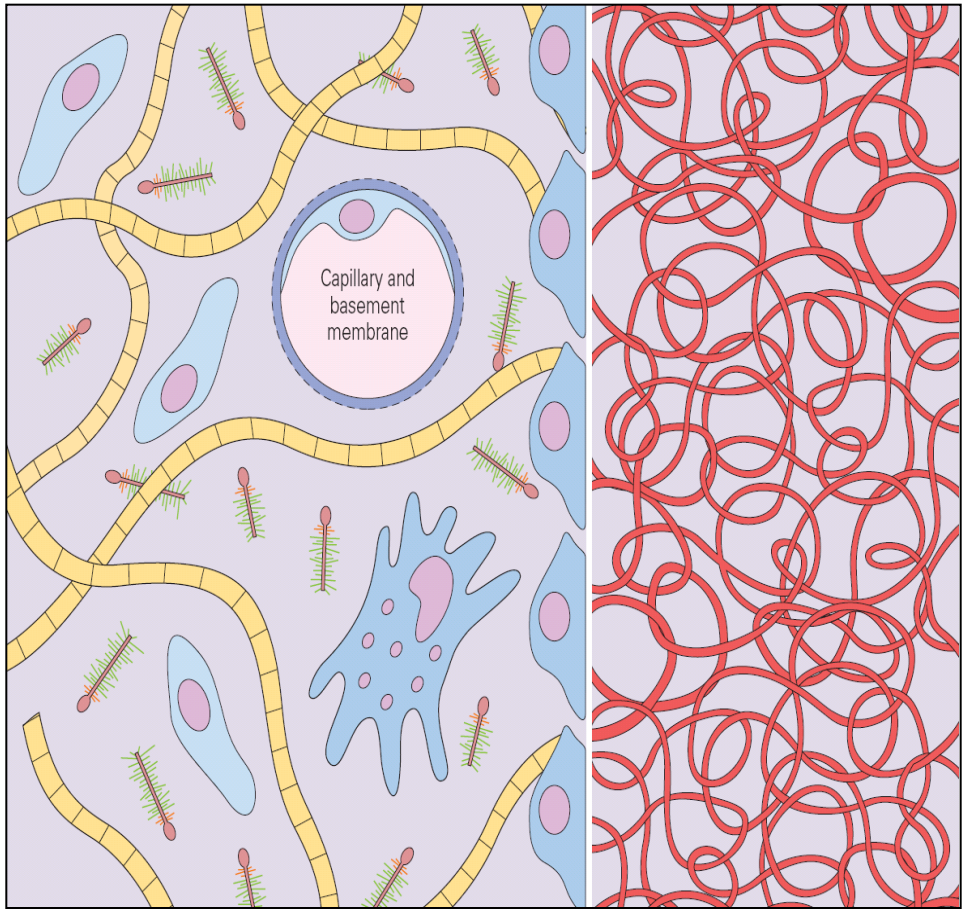
Type A synoviocytes:
macrophage-like
phagocytic

Type B synoviocytes:
fibroblast-like
produce hyaluronate

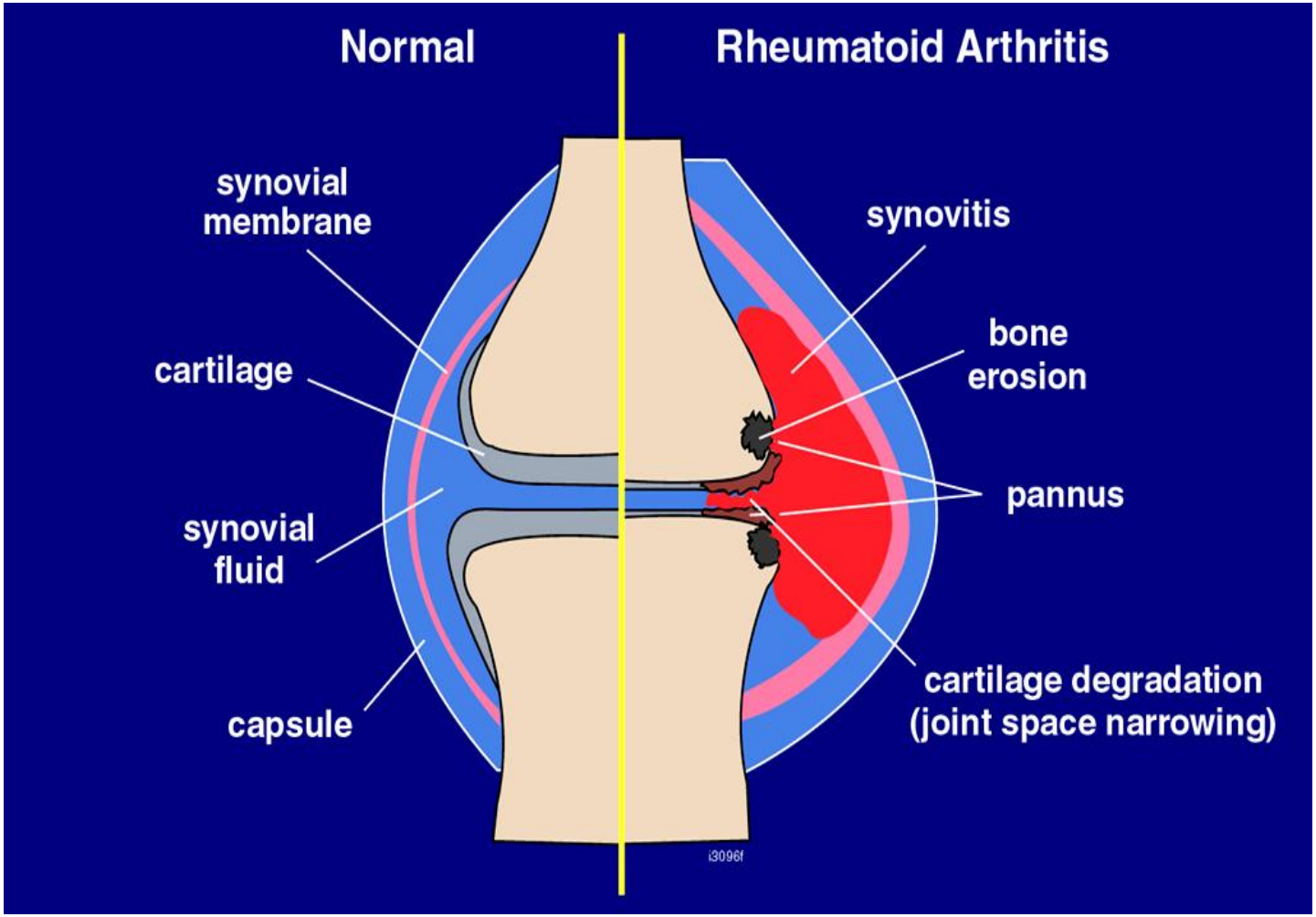
Collagen is type I

Synovial fluid:

Hyaluronate-rich
viscous fluid



Rheumatoid arthritis - pathology



Rheumatoid arthritis - pathogenesis

Synovial membrane in rheumatoid arthritis:

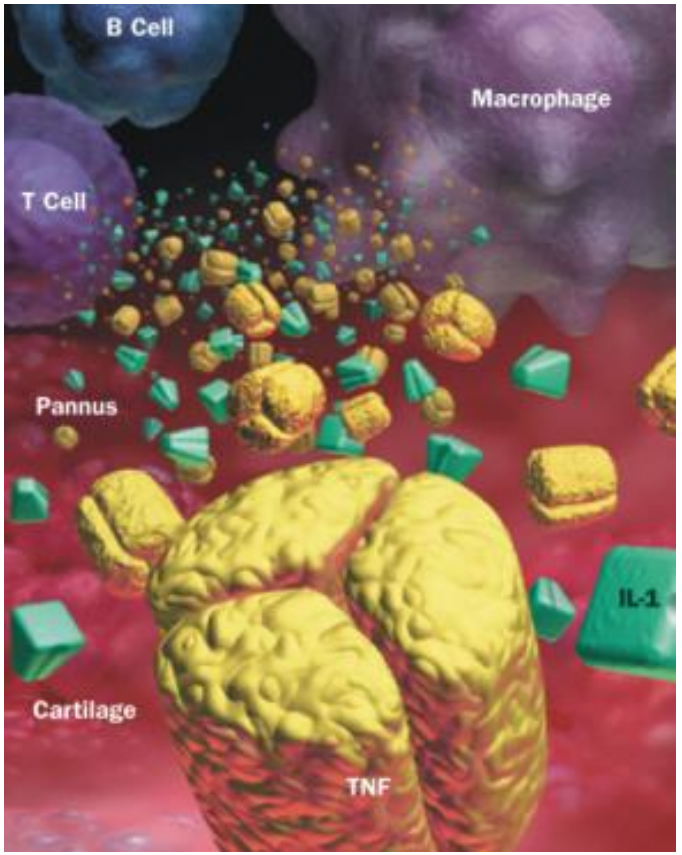
Proliferated mass of tissue (pannus)

- neovascularisation
- lymphangiogenesis

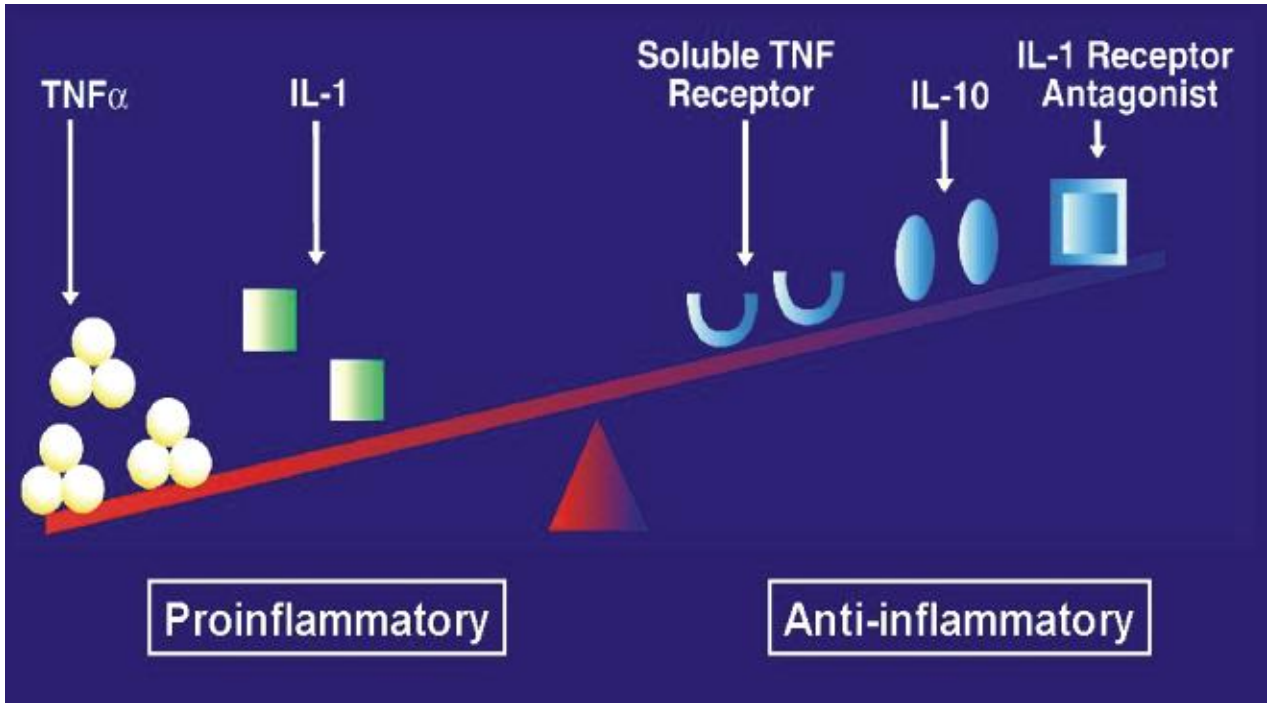
Contains inflammatory cells:

- activated B and T cells
- plasma cells
- mast cells
- activated macrophages

Recruitment, activation and effector functions of these cells controlled by cytokine network



Rheumatoid arthritis - pathogenesis



Cytokine imbalance in rheumatoid arthritis

Rheumatoid arthritis - pathogenesis

The cytokine TNF-α is the dominant pro-inflammatory cytokine in the rheumatoid joint:

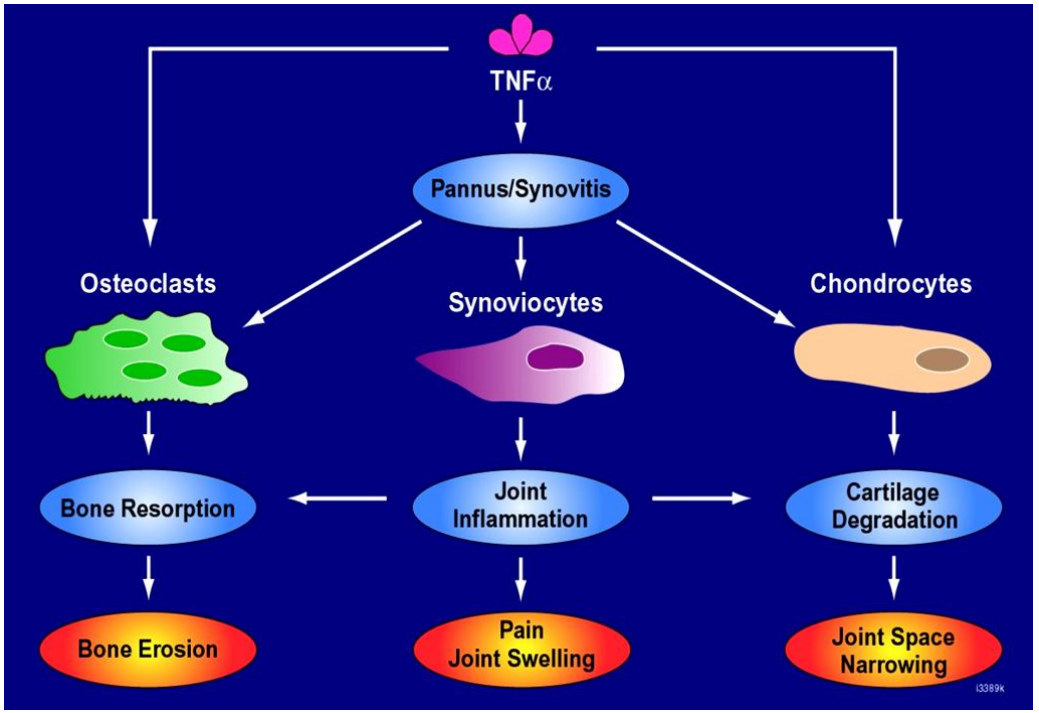
Tumour necrosis factor-α

Identified in 1975 as serum factor isolated from mice treated with endotoxin that induced necrosis in murine sarcoma

Studies in rheumatoid synovial cultures showed that TNF-α was dominant cytokine i.e. inhibition of TNF-α resulted in blockage of production of interleukin-1, interleukin-6, the chemokine interleukin-8 and GM-CSF

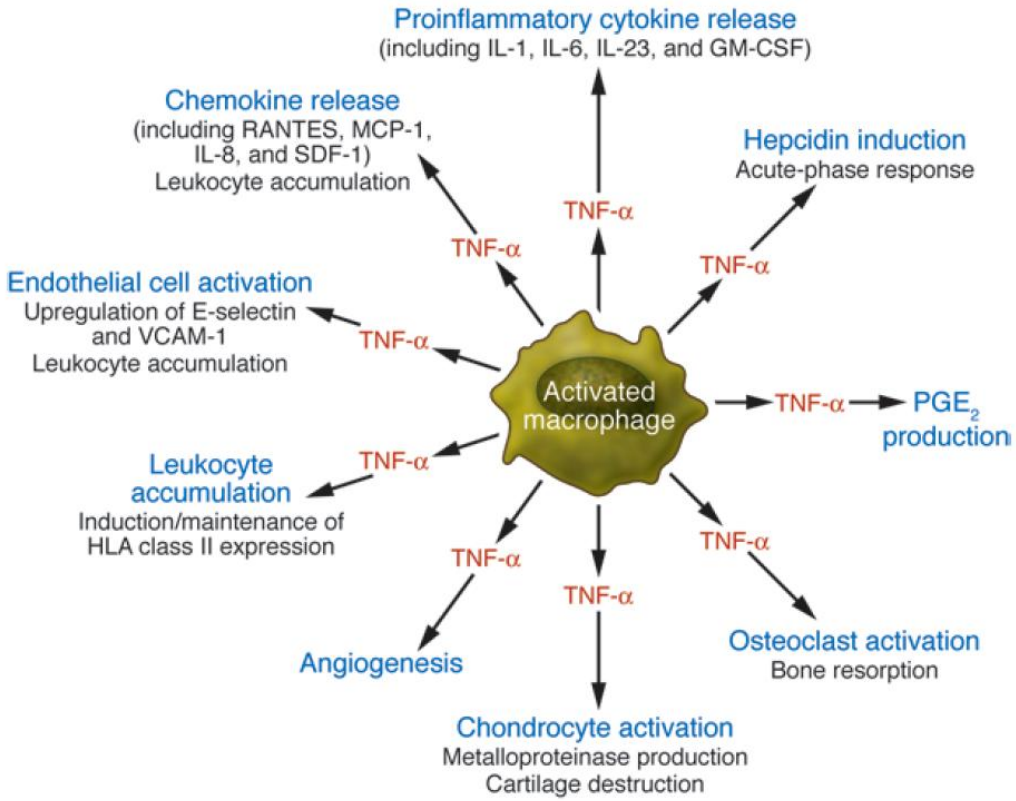
In rheumatoid arthritis TNF-α is mainly produced by activated macrophages in synovium

Pleiotropic cytokine



Rheumatoid arthritis - pathogenesis

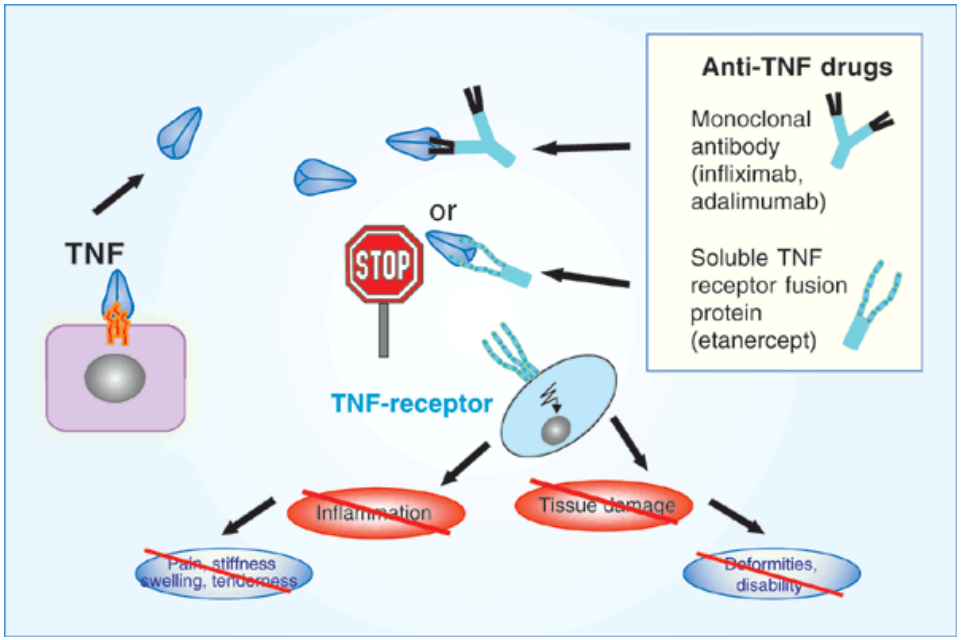
Actions of TNF-α relevant to pathogenesis of rheumatoid arthritis:

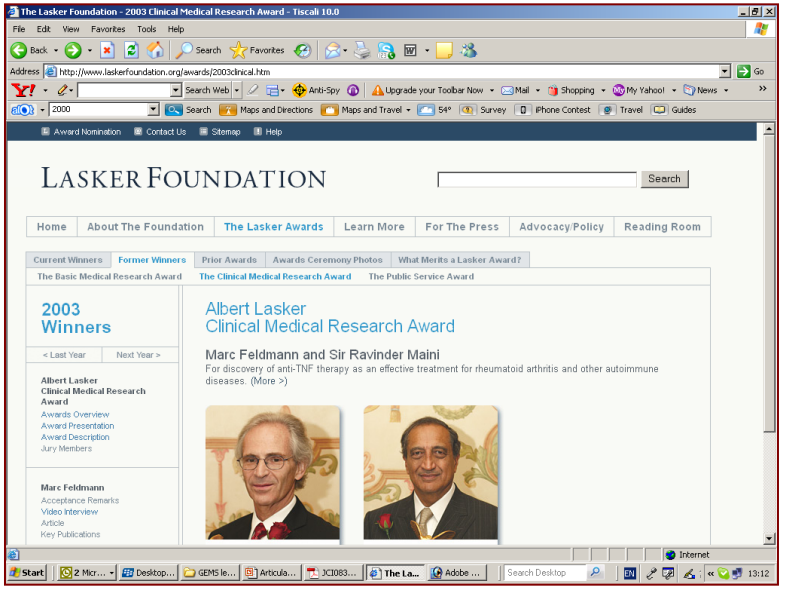


FM Brennan and IB McInnes. Evidence that cytokines play a role in rheumatoid arthritis. 2008. *Journal of Clinical Investigation*, 118:11. 3537-3545.

Rheumatoid arthritis - pathogenesis

Major role of TNF-α in pathogenesis of rheumatoid arthritis validated by major therapeutic success of drugs that specifically inhibit TNF-α:



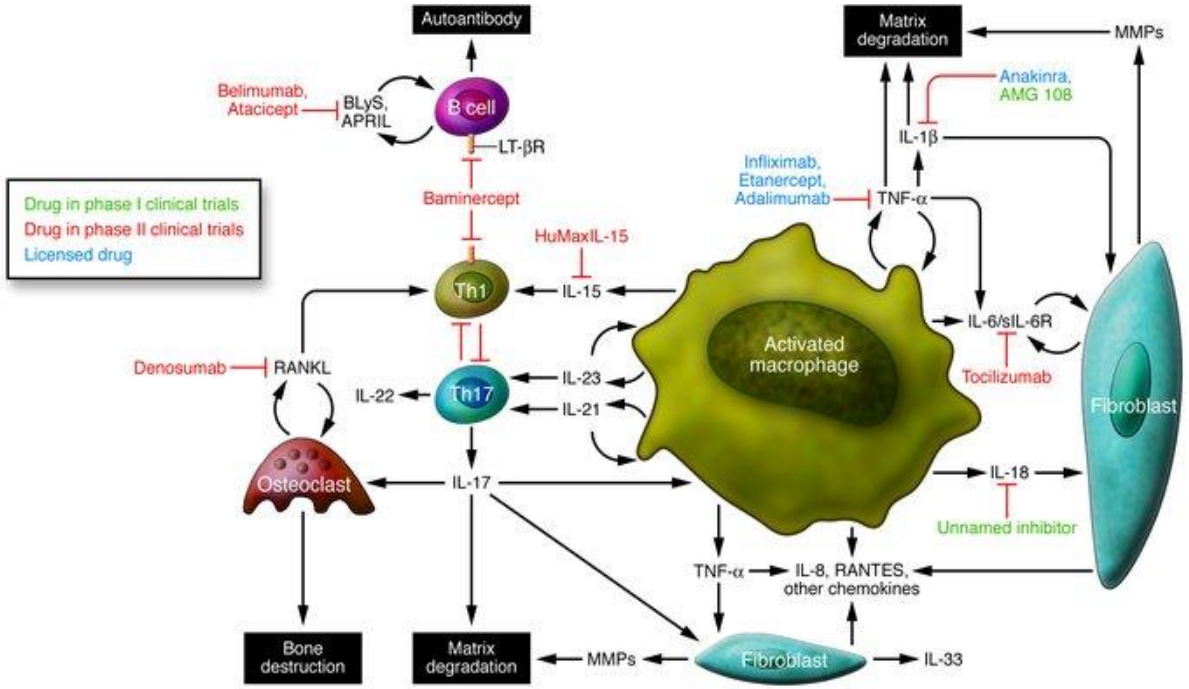


http://www.laskerfoundation.org/awards/2003clinical.htm

M. Feldmann and RN Maini. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. Nature Medicine 2003, 9:1245-1250.

Rheumatoid arthritis - pathogenesis

Many other cytokines under investigation as possible therapeutic targets in rheumatoid arthritis:



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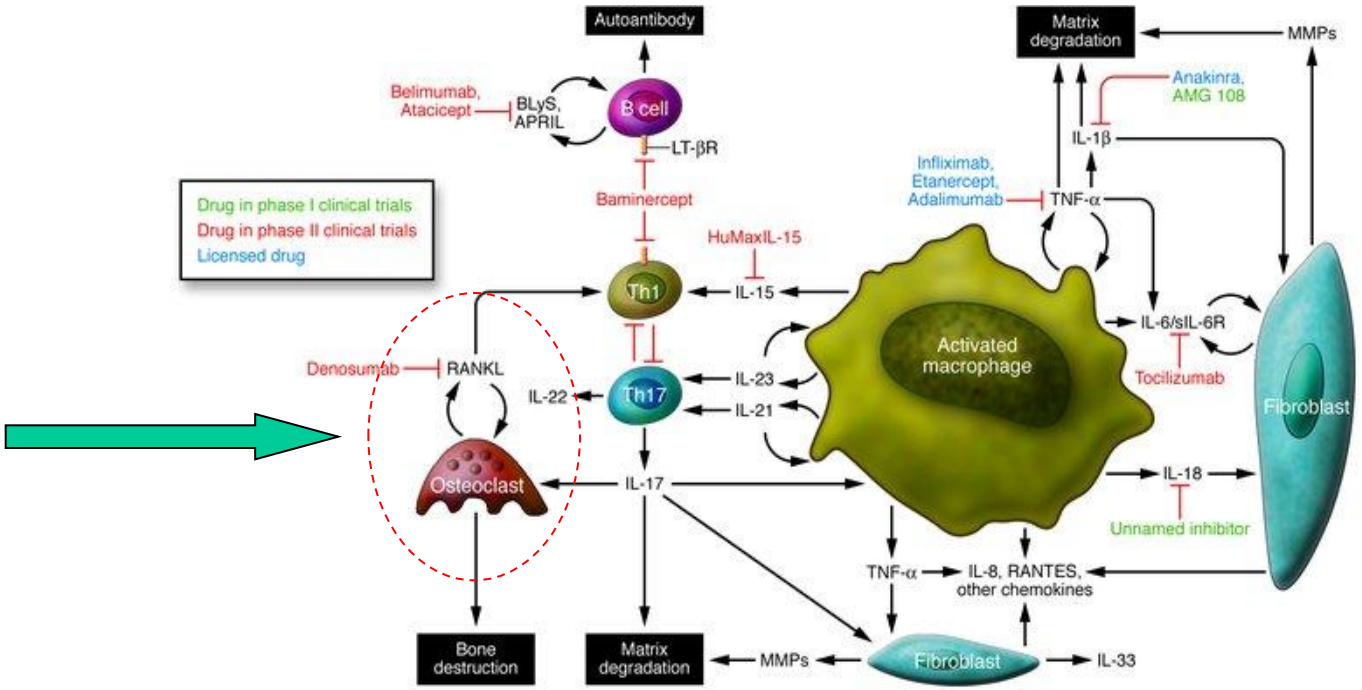
Rheumatoid arthritis - pathogenesis

Mechanisms of bone destruction in rheumatoid arthritis:



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Rheumatoid arthritis - pathogenesis

RANKL is important in bone destruction in rheumatoid arthritis:

- RANKL (receptor activator of nuclear factor κB ligand)
- Produced by T cells and synovial fibroblasts in rheumatoid arthritis
- Acts to stimulate osteoclast formation (osteoclastogenesis)
- Upregulated by:
  - INTERLEUKIN-1, TNF-α
  - INTERLEUKIN-17 – potent action on osteoclastogenesis via RANKL-RANK pathway
  - PTH-related PEPTIDE
- Binds to ligand on osteoclast precursors (RANK)
- Action antagonised by decoy receptor – osteoprotegerin (OPG)

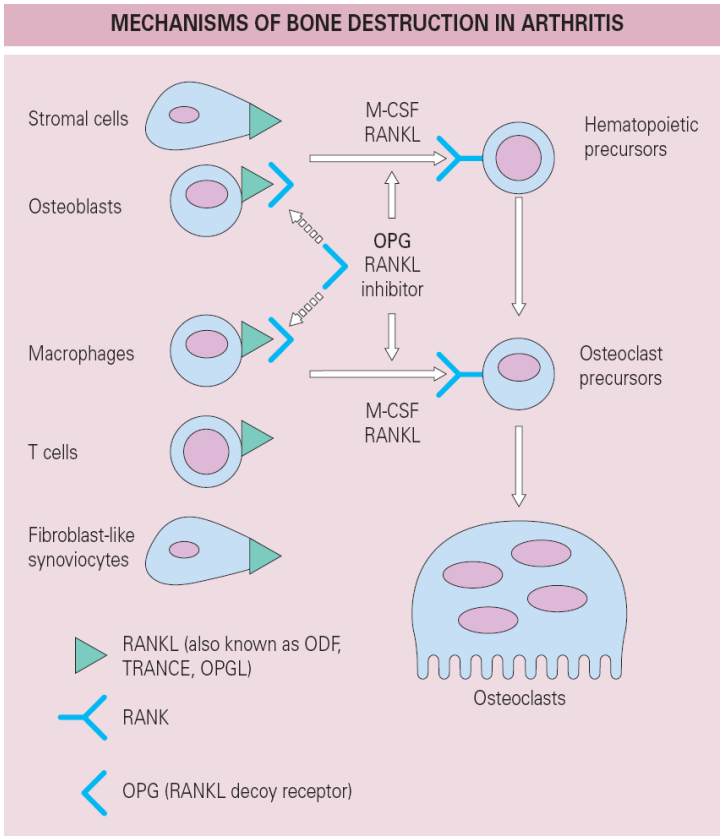
Rheumatoid arthritis - pathogenesis

Mechanisms of bone destruction in rheumatoid arthritis:

Denosumab

Denosumab is a fully human monoclonal antibody that specifically targets a ligand known as RANKL (that binds to a receptor known as RANK) which is a key mediator of osteoclast formation, function, and survival. Denosumab is being studied across a range of conditions including osteoporosis, treatment-induced bone loss, bone metastases, multiple myeloma and rheumatoid arthritis.

Amgen website 2009
http://www.amgen.com/science/pipe_denosumab.html



Articular pathology

- Rheumatoid arthritis
- Osteoarthritis

Osteoarthritis – pathology

- Irreversible loss of articular cartilage
- Normal weight-bearing properties of articular cartilage depend on intact collagen scaffold and high aggrecan content
- Collagen and aggrecan turnover is slow
  - half-life aggrecan ~ 3 – 4 years
  - half-life collagen ~ decades

Articular cartilage - normal

Avascular and aneural structure

Collagen - >90% is type II

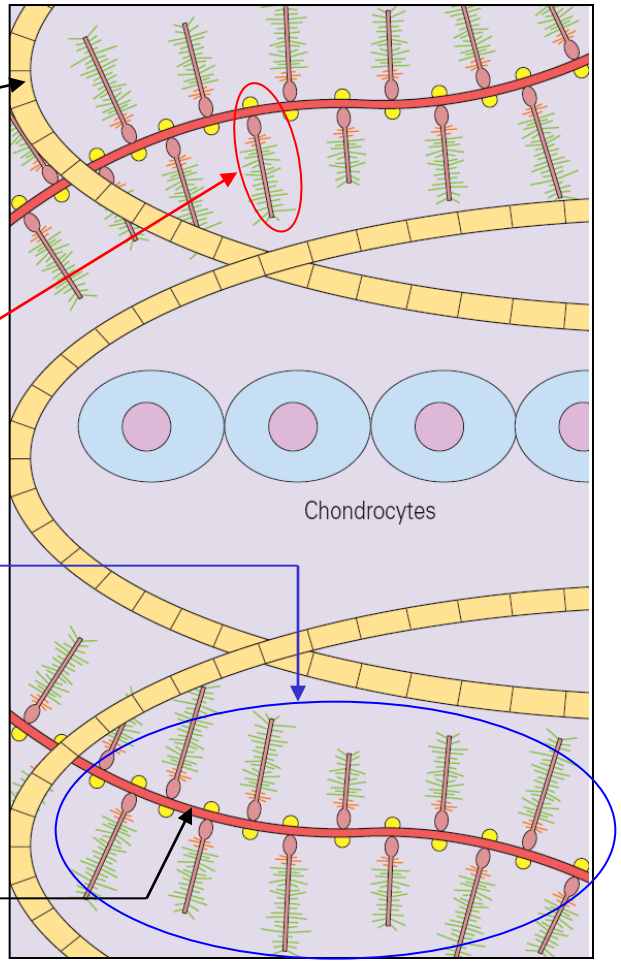
Chondrocytes

Proteoglycan monomers (aggrecan)

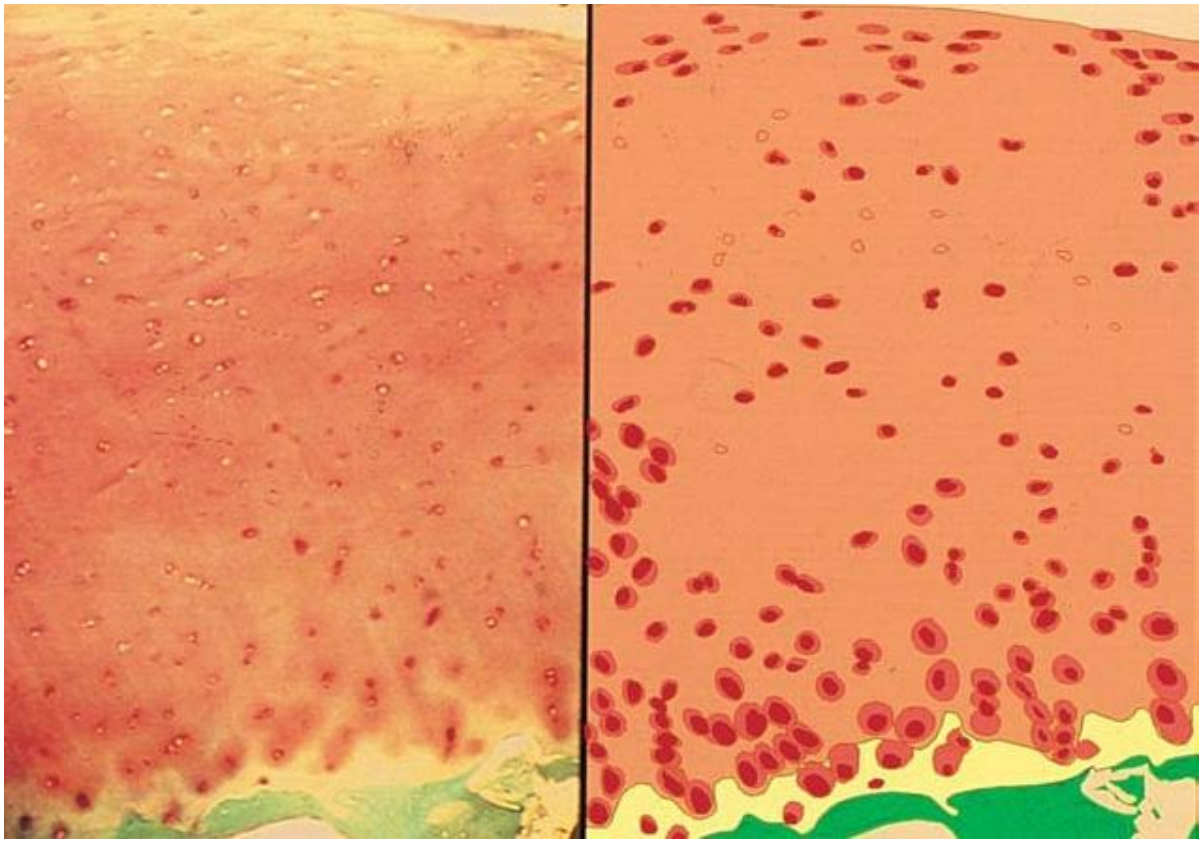
Molecular weight approx. 2 – 3 million kDa
100 chondroitin sulphate chains
60 keratan sulphate chains

Monomers are arranged into supramolecular aggregates consisting of central hyaluronic acid filament and non-covalently linked aggrecan

Negatively charged chemical groups of GAGs attract water into cartilage – 80% of wet weight is water



Articular cartilage - normal



cartilage surface

osteochondral junction

histology

schematic

Osteoarthritis – pathology

- Cartilage changes in osteoarthritis
  - reduced proteoglycan
  - increased cartilage hydration
  - reduced collagen

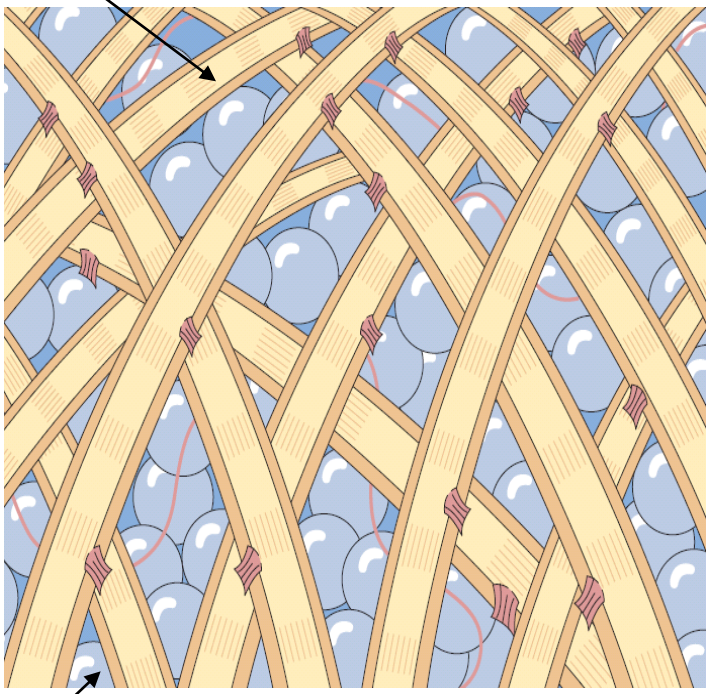
} chondromalacia

Chondromalacia = softening of cartilage because of increased water : proteoglycan ratio in cartilage matrix

Osteoarthritis – pathology

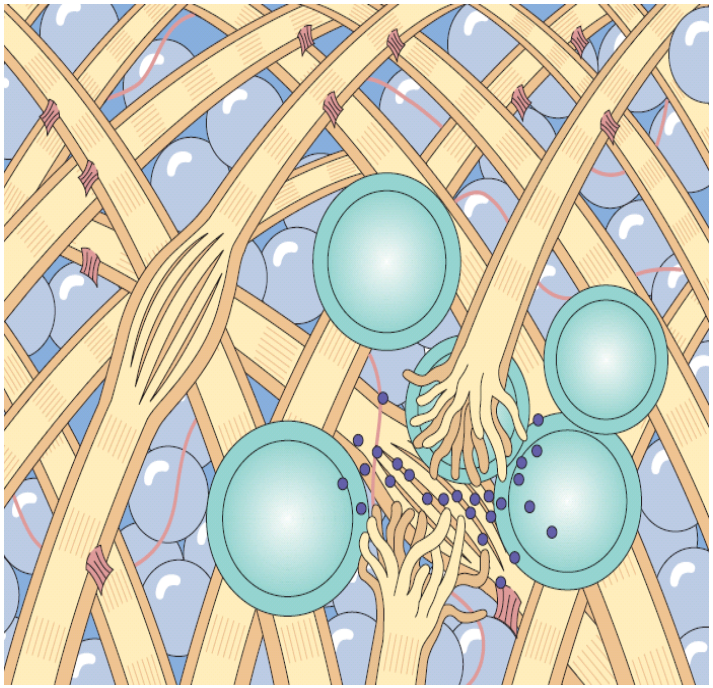
collagen

normal



aggrecan monomers

osteoarthritis



loss of aggrecan → swollen proteoglycan molecules

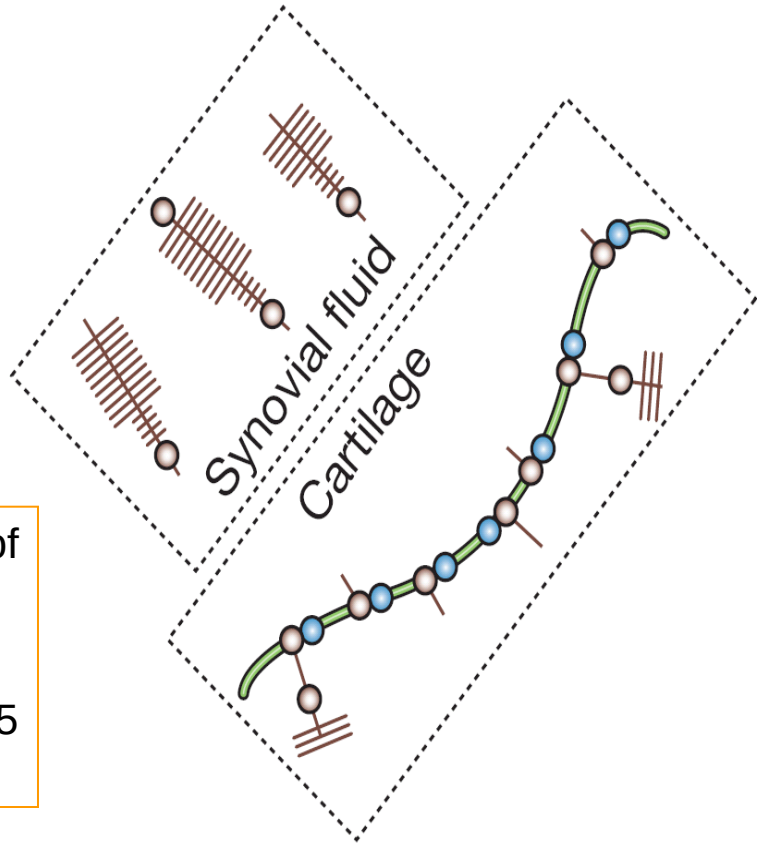
collagen degradation → damaged collagen network

Osteoarthritis - pathology

- Cleavage of aggrecan in osteoarthritis mediated by ADAMTS aggrecanases

- aggrecan fragments

present in osteoarthritis joint



• ADAMTS-4, -5 important in the cleavage of aggrecan *in vitro* and in animal models

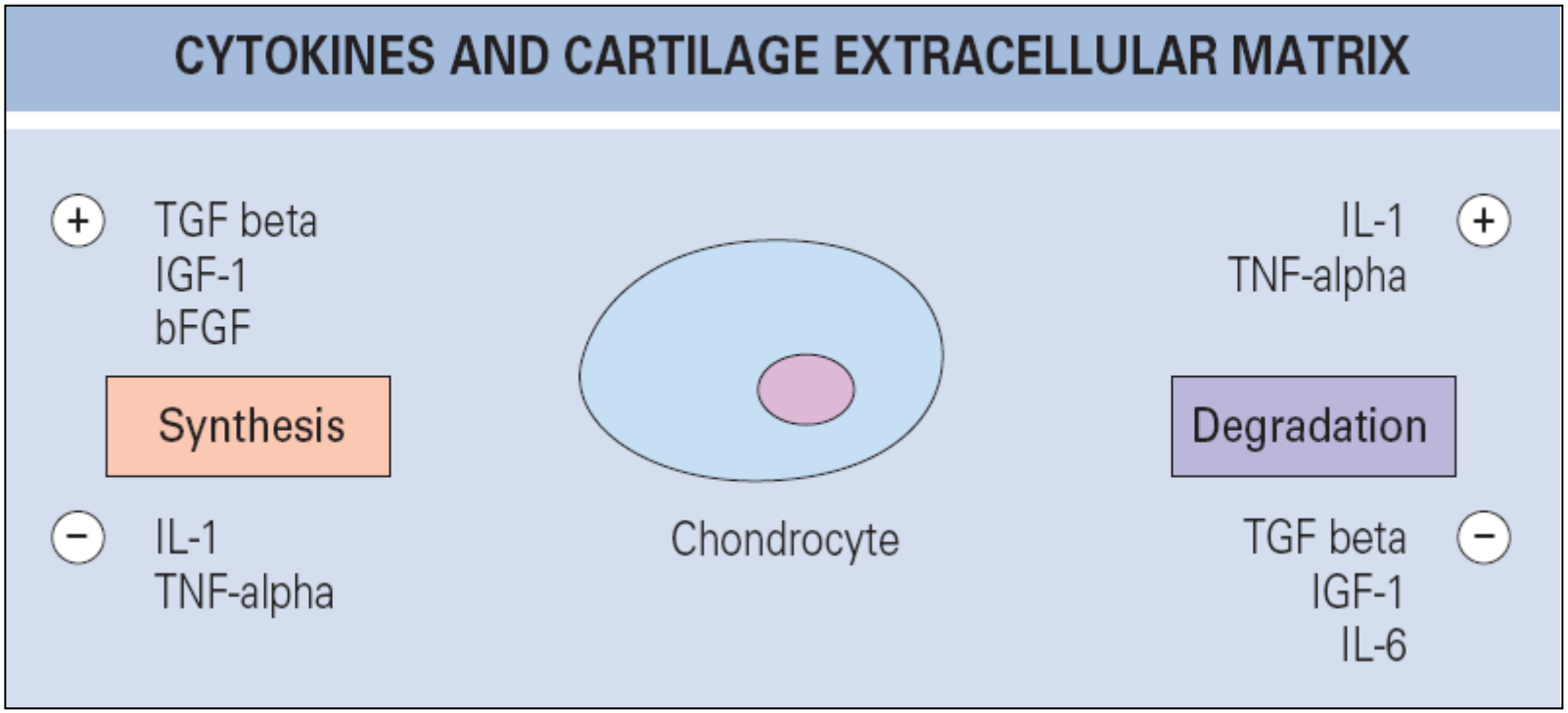
• ? Small molecule inhibitors of ADAMTS-4, -5 future therapies for osteoarthritis

Osteoarthritis – pathology

- Cartilage changes in osteoarthritis
  - reduced proteoglycan
  - increased cartilage hydration
  - reduced collagen
  - increased chondrocyte proliferation
    - intrinsic repair mechanism → matrix synthesis
    - abnormal mechanical stress leads to chondrocyte producing inflammatory mediators in osteoarthritic tissue e.g. growth factors and cytokines which may have important roles in the disease
  - focal areas of chondrocyte apoptosis

Osteoarthritis – pathology

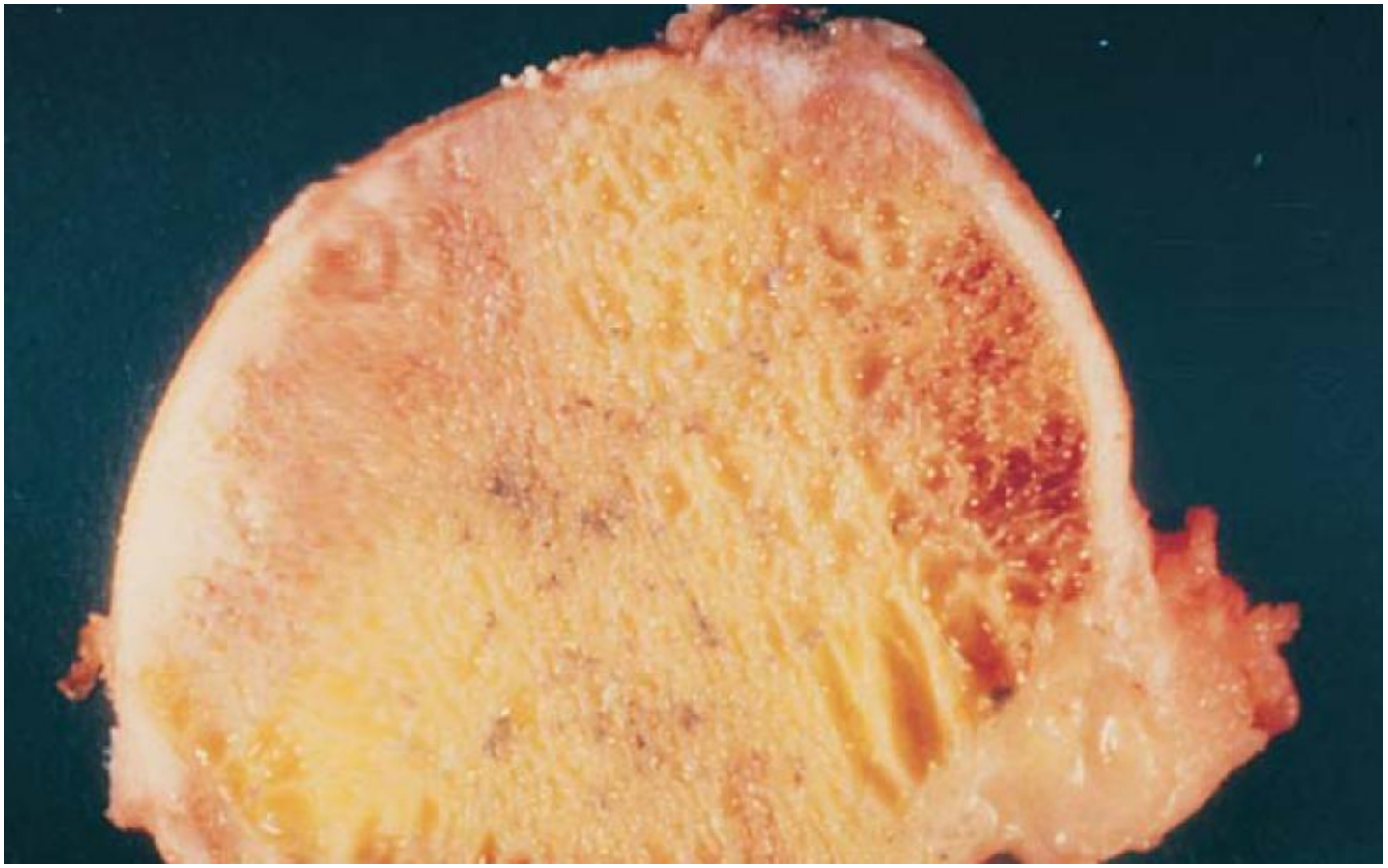
- Examples of cytokines influencing cartilage matrix synthesis and degradation



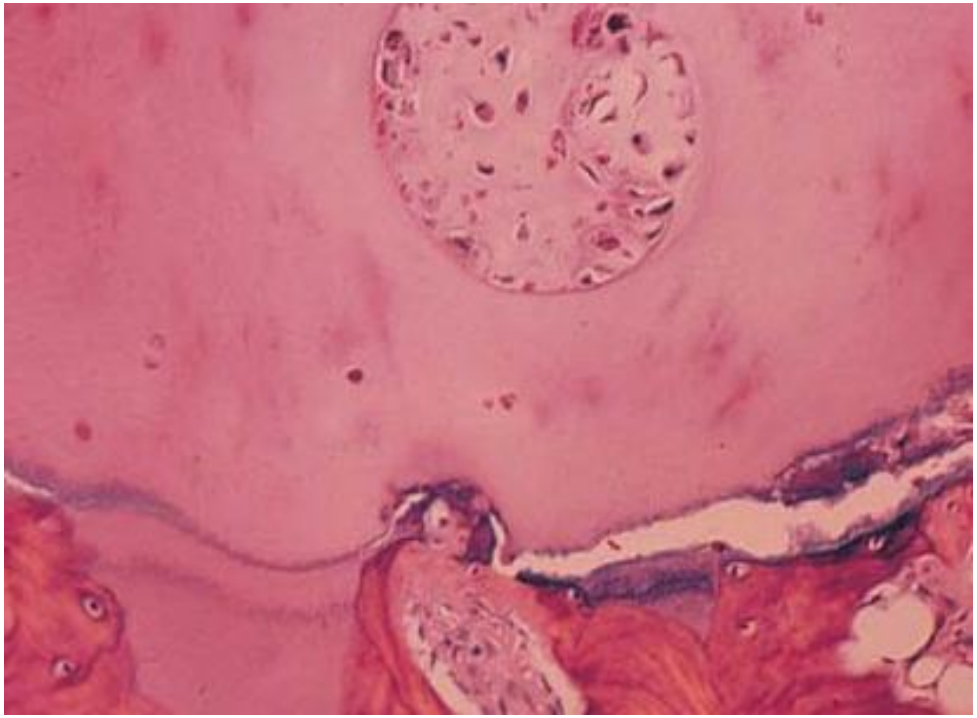
Osteoarthritis – pathology

- Bone changes in osteoarthritis
  - Changes in denuded sub-articular bone
    - Proliferation of superficial osteoblasts results in production of sclerotic bone
    - Focal stress on sclerotic bone can result in focal superficial necrosis in underlying bone and bone marrow

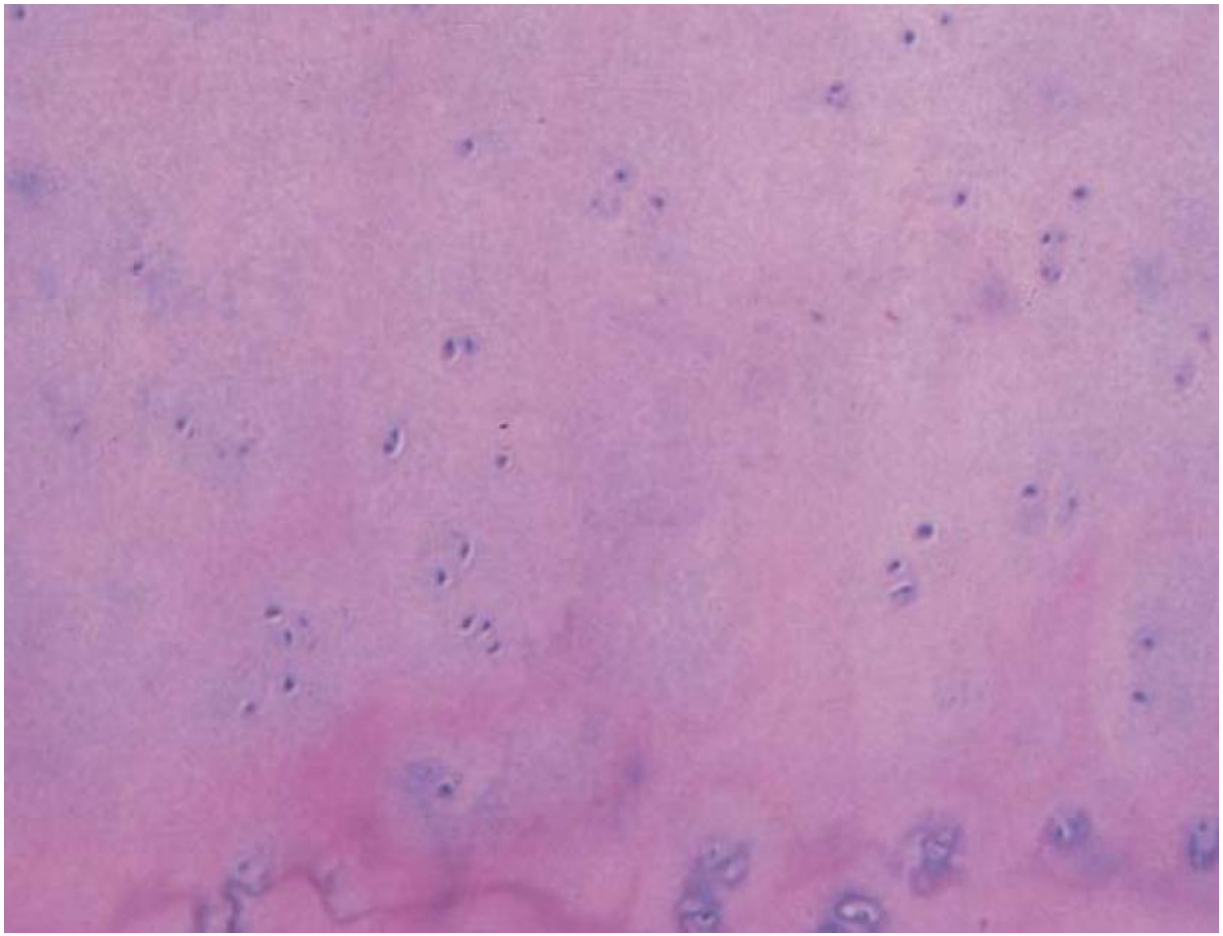
Examples of pathology in osteoarthritis



Loss of articular cartilage with underlying superficial bone necrosis (yellow area)



Areas of chondrocytes proliferation in cartilage (intrinsic repair)



Areas of cartilage with no chondrocytes due to focal cell necrosis

Osteoarthritis – pathology

- Unlike rheumatoid arthritis no disease modifying osteoarthritis drug (DMOAD)

Possible strategies:

- Prevent matrix degradation
  - ? aggrecanase inhibitors
  - ? anti-cytokine e.g. anti-interleukin-1 antibodies
- Stimulate matrix synthesis
  - ? stimulate chondrogenesis (bone morphogenetic protein-4)

Summary

- Connective tissue turnover is mediated by matrix proteinases through complex regulatory networks
- Rheumatoid arthritis and osteoarthritis represent the two major articular pathologies
- Rheumatoid is a disease of synovium whilst osteoarthritis is disease of articular cartilage
- Impressive advances in rheumatoid arthritis therapy have been made but this has not been achieved for osteoarthritis

Comment

- I hope that this presentation will evolve over time in a Darwinian fashion. The ‘environmental pressures’ driving this adaptation are (1) medical advances and (2) student feed-back. In the current economic climate there is not much research money to achieve (1) so (2) is critically important.

- Comments (polite) to: matthew.pickering@imperial.ac.uk