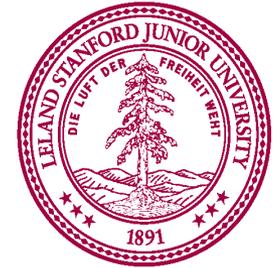


Inflammation in osteoarthritis: It's not just wear and tear....



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Division of Immunology and Rheumatology

Stanford University School of Medicine

What is OA?

- “Wear and Tear”
- “Worn out shock absorbers”
- An inevitable part of aging

- A disease of cartilage degeneration
- A non-inflammatory degenerative arthritis

Osteoarthritis (OA)



OA is a costly condition

- Annual costs per patient due to OA (from 2007 insurance and medicare claims)
 - \$18-20,000 per year
- Arthritis is a major risk factor for losing one's job
- Arthritis patients earned \$1590 less per year compared to those without (2003)

Kim-Le et al., *Pain Practice* 2012; Kotlarz et al., *Arthritis Rheum* 2009; Yelin et al. *Arthritis Rheum* 2007

OA: Risk Factors

- Why did this patient develop osteoarthritis?

Does anyone have “primary OA”?

Primary

- no history of joint trauma or other inciting factor
- family history of disease
- multiple joint involvement
- hand involvement (Heberden and Bouchard nodes)

Secondary

- history of joint injury
- previous inflammatory arthritis
- metabolic disease
- hemochromatosis
- diabetes
- obesity????

Risk Factors for OA: Joint Injury

- 5-fold higher risk of developing OA
- about 50% patients undergoing surgery for ACL tears or meniscal injuries develop OA
- almost 1 million arthroscopic meniscal procedures per year in US

Gelber A et al. *Ann. Int. Med* 2000; Englund M, Lohmander LS *Arthritis Rheum* 2004; Lohmander LS et al. *Arthritis Rheum* 2004

Risk Factors for OA: Obesity

- 2-3 fold increased risk of developing knee OA if overweight
- BMI > 30 kg/m² : 4 times greater risk
- Increased risk of hip replacement
- Weight loss associated with reduction in knee loads
- Weight loss (5 kg) associated with 50% reduction in risk of development of symptomatic knee OA

** Reported association between obesity and rates of OA in non-weight bearing joints of hand suggest systemic effects of obesity...

- Potential role for obesity as a systemic risk factor for OA

Other Risk Factors for OA

- Joint alignment
- Joint shape
- Subtle abnormalities
- Congenital dysplasias
- Joint laxity / hypermobility
- Endocrine diseases (Diabetes)
- Hemachromatosis (Iron deposition)

OA is not a single disease but a common endpoint

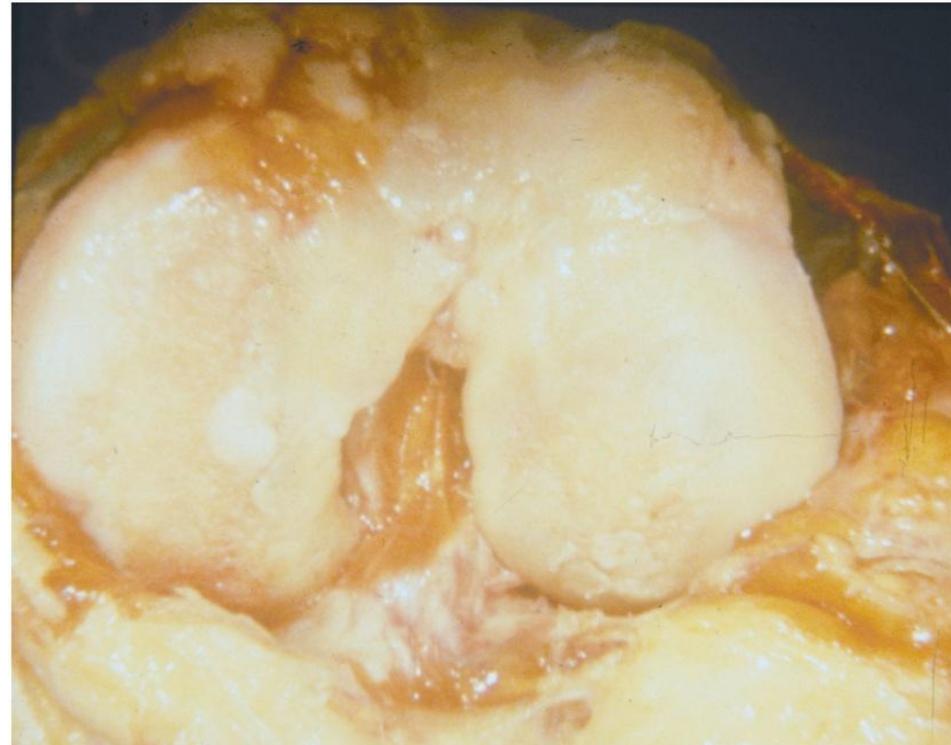
- OA is associated with multiple risk factors:
 - age
 - joint trauma
 - altered biomechanics
 - obesity
- Everyone's risk factors are different...
- OA is likely a clinical endpoint of numerous disorders leading to the eventual failure of one or more joints of the body

Current View of Osteoarthritis

Normal knee



OA knee



Photos courtesy of R.J. Anderson, MD

OA has traditionally been considered a “non-inflammatory arthritis”

- OA fluids and tissues frequently served as “controls” for RA studies
- Led to oversight that OA synovial fluid, when compared to “normal” fluid, is highly enriched for plasma proteins, complement components, and cytokines.

Commenting on “Protein Patterns in Synovial Fluid and Serum in Rheumatoid Arthritis and Osteoarthritis”

“This may indicate that the type of permeability change in the synovial tissue is similar in both diseases (OA and RA), although this change is much more marked in rheumatoid arthritis”

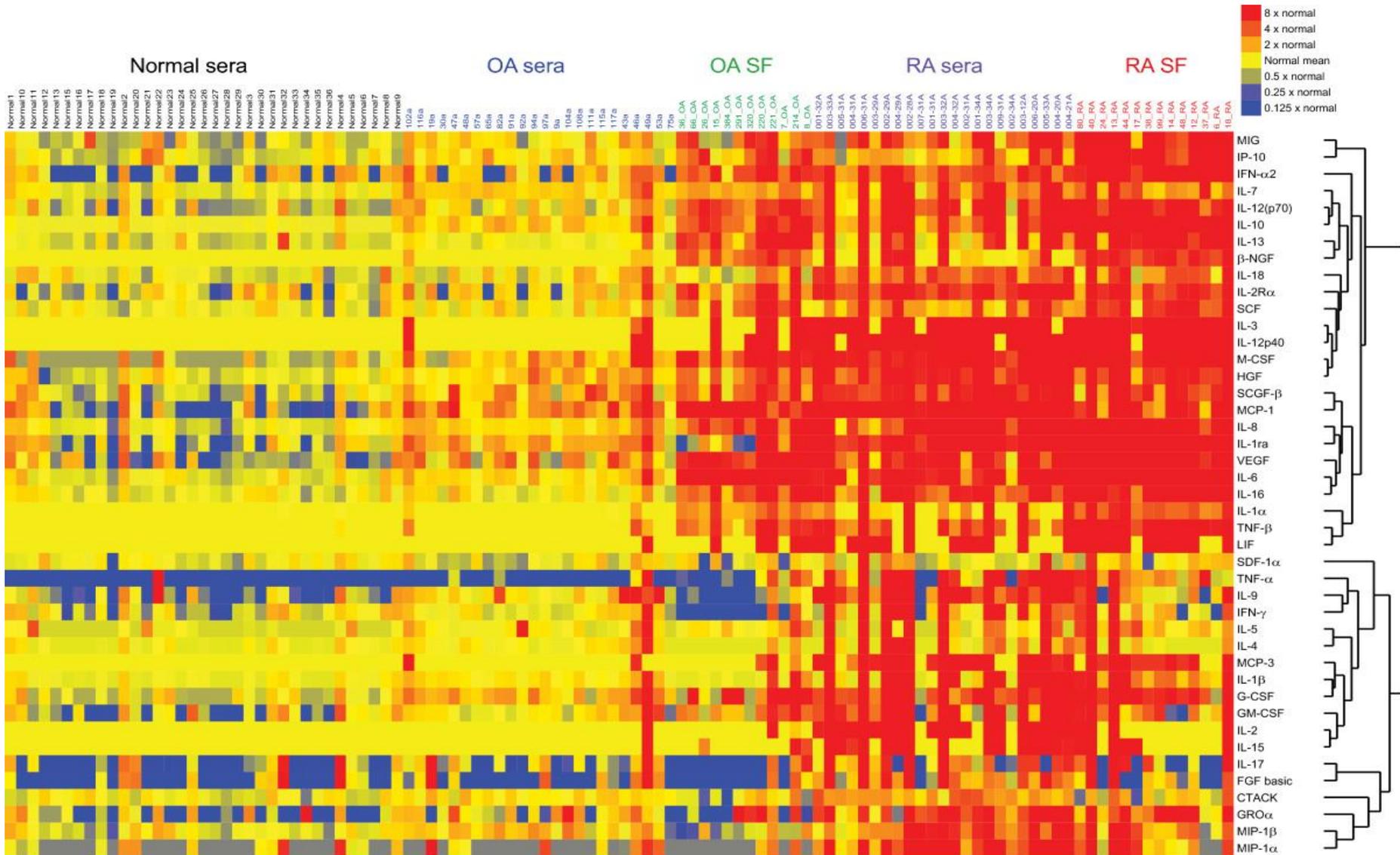
Nettelbladt *et al.*, Arthritis Rheum 1959

Shifting the paradigm: Understanding OA as an inflammatory disease

Epidemiologic observations supporting inflammation in OA

- Levels of serum C-reactive protein (CRP) are strongly associated with the presence and progression of knee OA.
- Positive correlation between serum CRP, histologic synovitis, and synovial fluid IL-6

Systemic and synovial inflammation in OA



There is Synovitis in OA!!!

Marked
Peripatellar
Synovitis
(white
arrows)



Extensive
bone marrow
lesion (small
black
arrows)/bone
cysts (bright
white
structures at
end of long
black arrow)

Gadolinium-enhanced magnetic resonance image (sagittal view) of a knee with multiple structural features typical of osteoarthritis. There are bone marrow lesions, cysts, and synovial thickening.

Synovitis

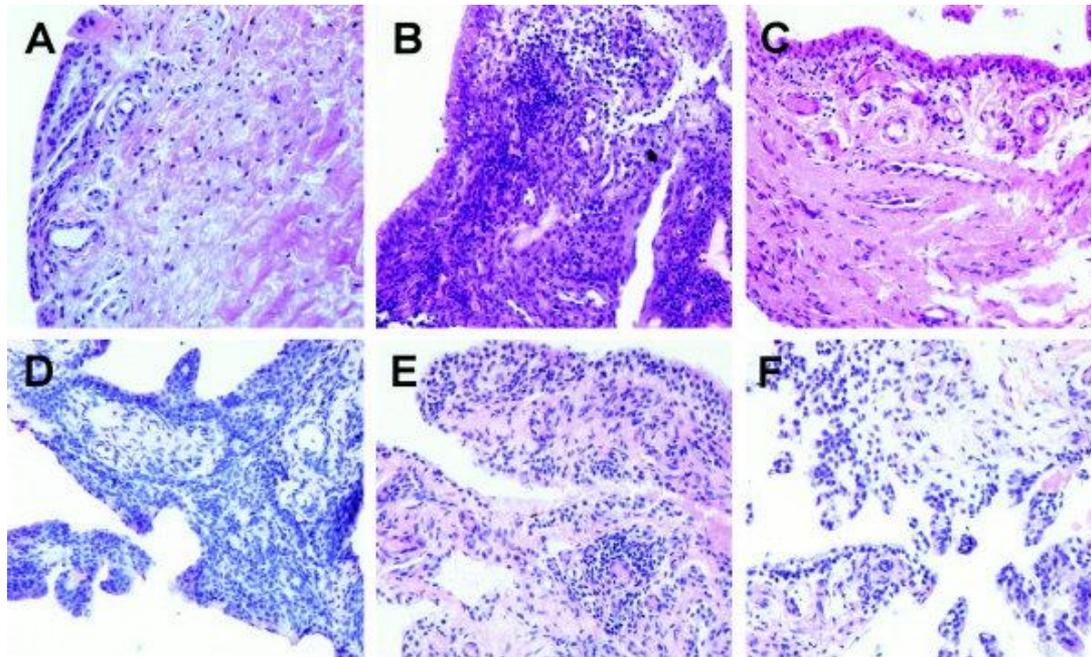
- Inflammation of the synovial membrane is characteristic of classical inflammatory arthritides.
- Presence of synovitis is increasingly recognized in a significant proportion of patients with primary OA
- Observational studies have strongly implicated joint synovitis in the pathogenesis of OA
- Historic studies described “post-traumatic” synovitis of similar histopathology to that in many cases of “primary OA”

Synovitis in OA

- Normal synovium is 2-3 cell layers thick with lack of inflammatory cells.
- In the setting of inflammation there is:
 - marked hyperplasia of the synovial lining cells
 - infiltration of inflammatory cells consisting primarily of macrophages but also a smaller but quantifiable number of T and B cells, mast cells and NK cells.
 - Degree of infiltration is highly heterogeneous

Bondeson, Arthritis Rheum 2010; Dean Arthritis Rheum 1993; Benito, Arthritis Rheum 2005; Skzeczynska-Moncznik, Arthritis Rheum 2009

Synovial-derived mediators drive inflammatory and destructive responses in osteoarthritis



OA synovium

RA synovium

- Inflammation is present in OA joints well before the development of significant radiographic change
- MRI studies demonstrate an association between the presence of synovitis and **OA pain** and **progression**



Synovitis in OA

Haywood, et al 2003:

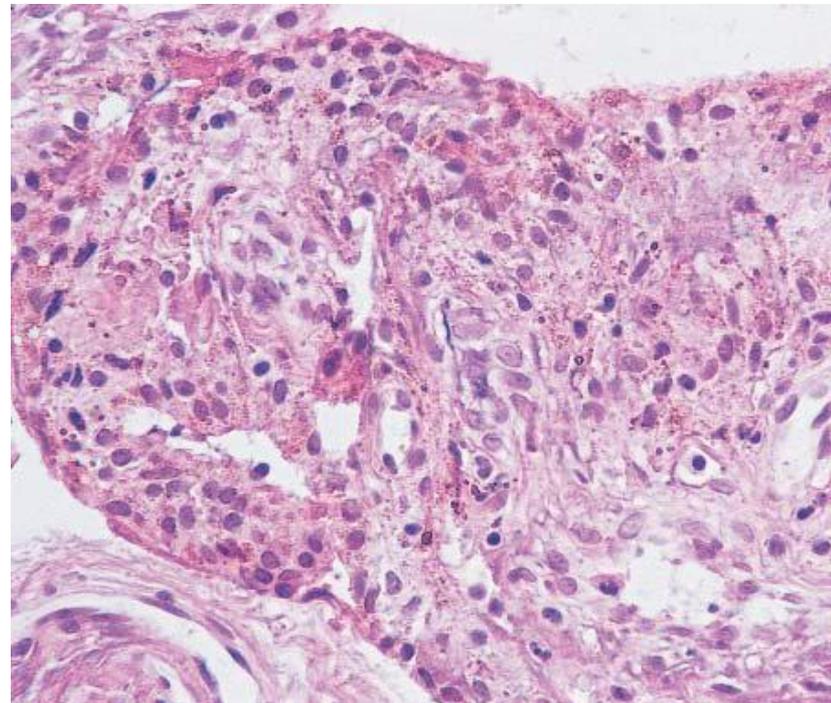
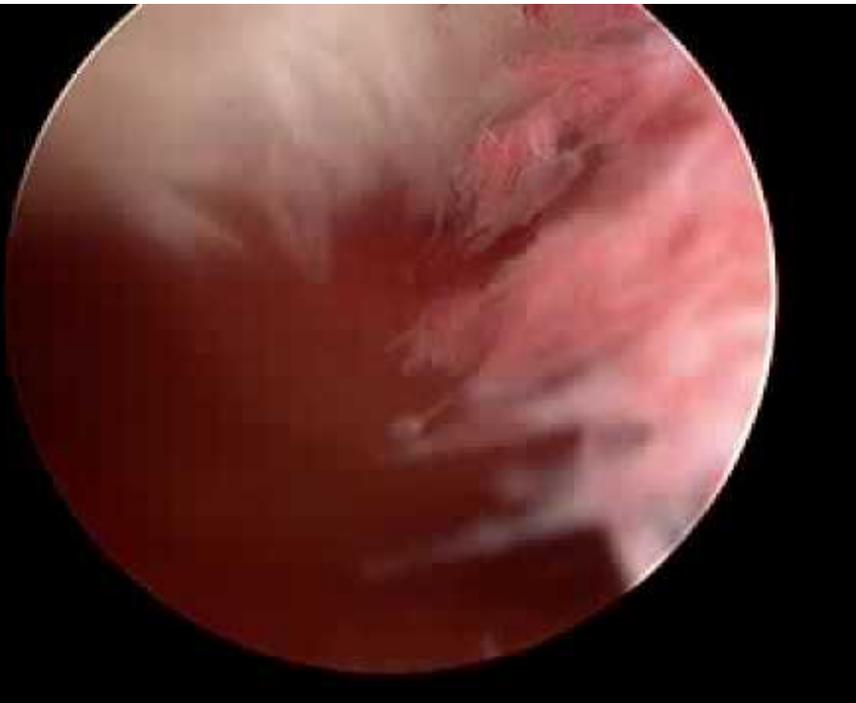
- 70 synovial tissues spanning a range of radiographic OA severity
- 
- Severe synovial inflammation observed in 31% of patients.
 - Including many subjects with minimal radiographic disease

Benito, et al 2005:

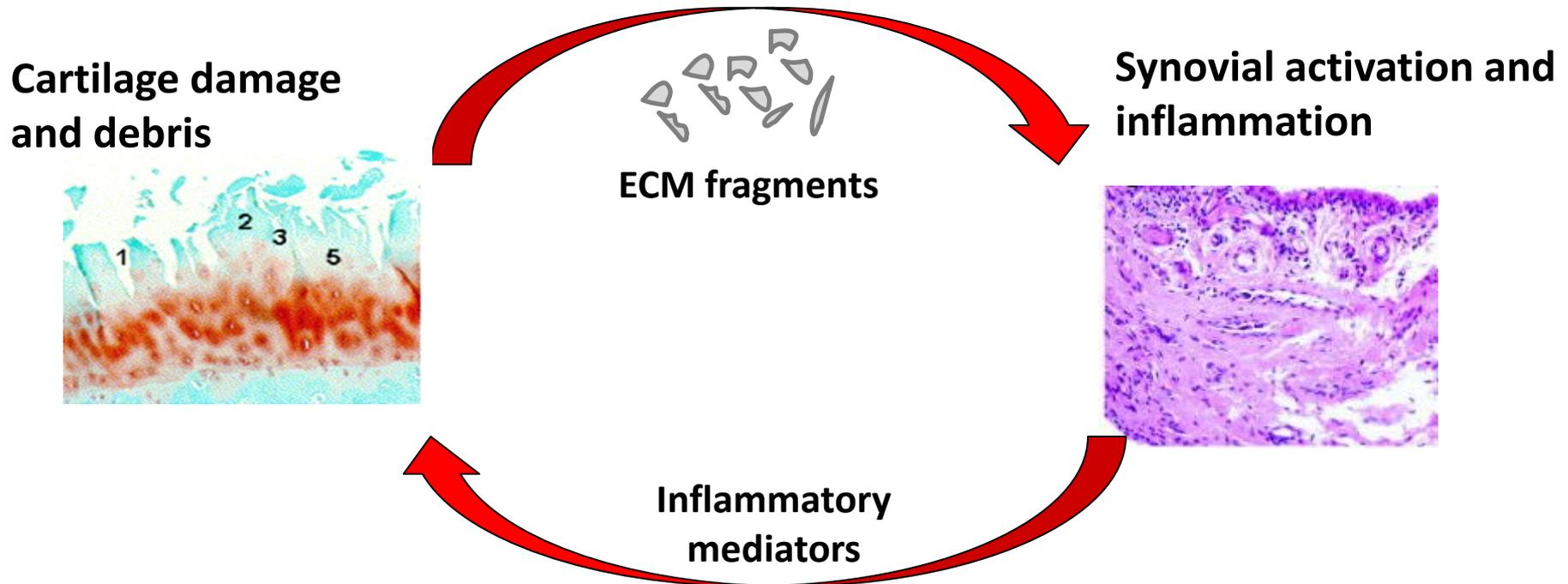
- Comparison of early and late OA demonstrated increased mononuclear infiltration/inflammatory mediators in **early OA** compared with late OA.

Arthroscopy

Serial arthroscopies in symptomatic but pre-radiographic OA demonstrate synovitis and predict future development of cartilage loss (Ayrar, 2005)



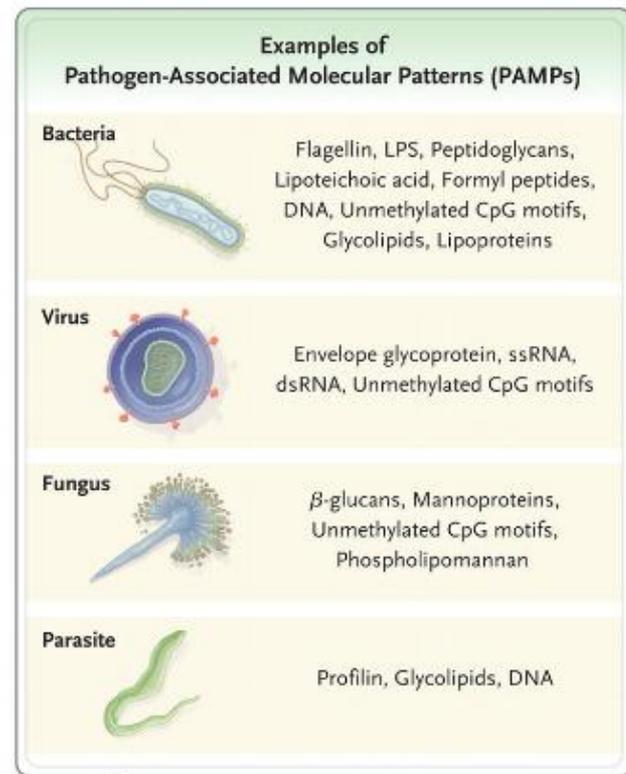
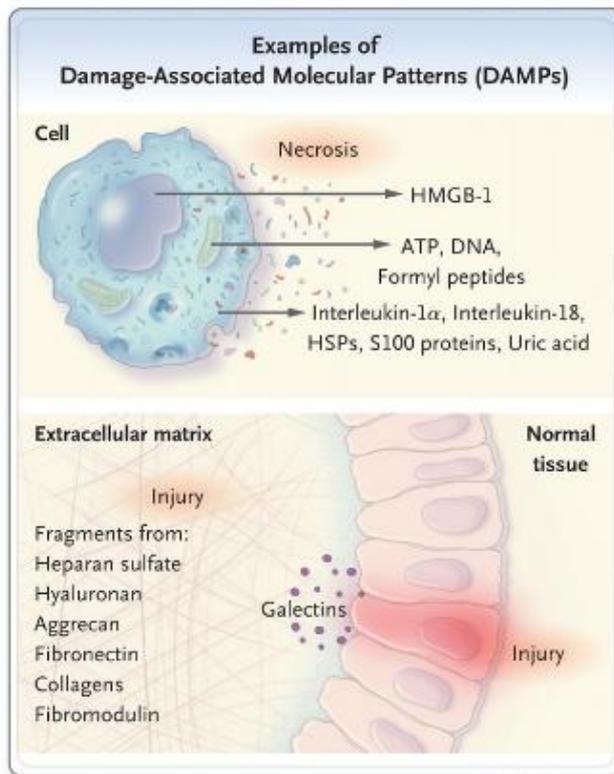
- Synovitis is likely a **secondary process** induced by innate immune activation following cartilage damage
- Synovium provides a critical link in the chain of initiation and propagation of OA.



Immune mechanisms in OA

- Unlike RA, OA does not appear to be associated with a robust adaptive immune response (i.e. antigen-specific T and/or B cell response)
- Innate immunity induced by invariable pattern-recognition receptors (PRRs) which respond to conserved patterns in nature
 - invading pathogens (bacteria, viruses, and fungi)
 - Endogenous damage/danger signals





Innate Immune Response Triggered by Interactions with Pattern Recognition Receptors:

- Toll-like receptors
- Receptor for advanced glycation end products
- Nucleotide-binding oligomerization domain-like receptors
- C-type lectin receptors
- Retinoic acid-inducible gene-1-like receptors

Examples of Proinflammatory Cytokines and Chemokines:

- Interleukin-1 β , Interleukin-6, Interleukin-8, TNF- α , Interferon- γ

DAMPs

- **Damage** associated molecular patterns
- **ENDOGENOUS** danger signal released at sites of tissue damage
 - Cellular necrosis
 - ECM breakdown products
 - Plasma proteins
- **Innate** immune adjuvants
- Implicated in host defense, wound healing, as well as multiple disease pathogenesis

Innate immunity in OA

Damage associated molecular patterns (DAMPs)

- Cartilage derived DAMPs
 - ECM breakdown products such as fibronectin, hyaluronan, tenascin C, Biglycan
- Alarmins such as S100 proteins, HSP
- Plasma proteins which become elevated in synovial fluid secondary to vascular exudation

Complement activation

- C5 activation by ECM breakdown production
- Link between coagulation and complement activation

Cellular mediators of innate immunity

- Many cell types within the joint possess PRRs capable of responding to DAMPs.
- Much of innate immune activation and cytokine production in OA is attributed to the action of synovial macrophage
- Primary and secondary role for fibroblast-like syoviocytes (FLS) and chondrocytes in responding to innate immune activation

Cartilage ECM-derived DAMPS

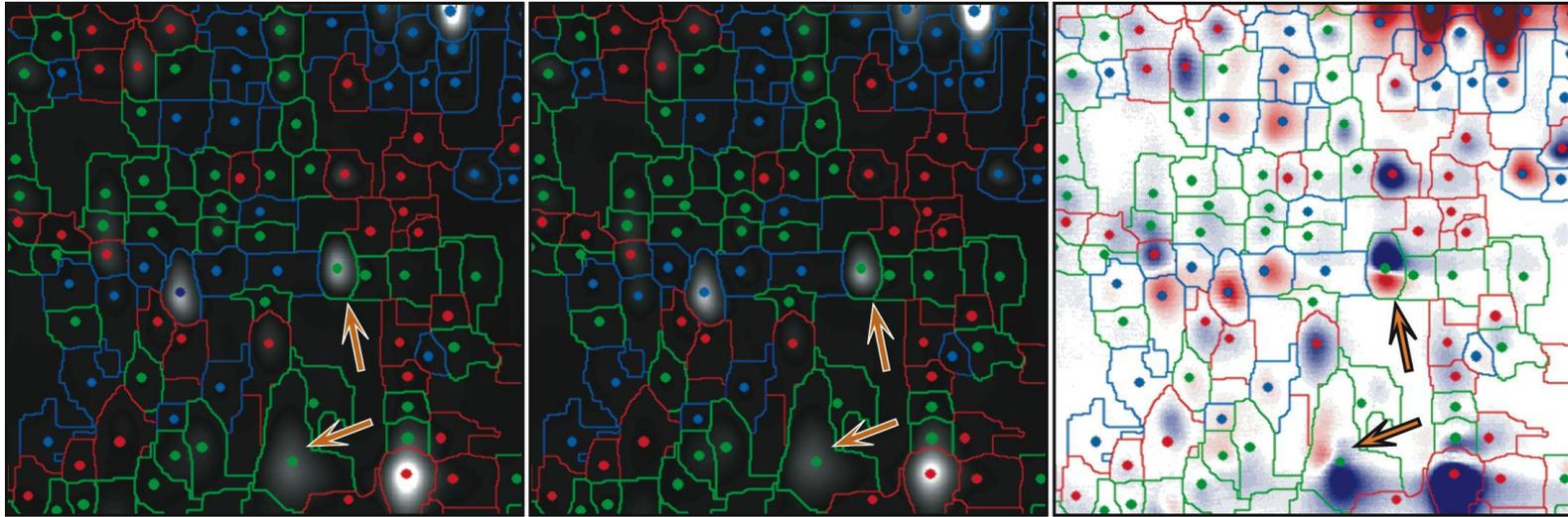
- Fibronectin fragments can induced the production of pro-inflammatory cytokines TNF α and IL-1 β as well as chondrolytic mediators MMP1 and MMP3.
- Hyaluronic acid stimulates chondrocyte activation via TLR2 receptor
- Numerous ECM products have been implicated in innate immune activation including Tenascin C, and Biglycan

Inflammatory Mediators in OA Synovial Fluid

Cy3

Cy5

Differential

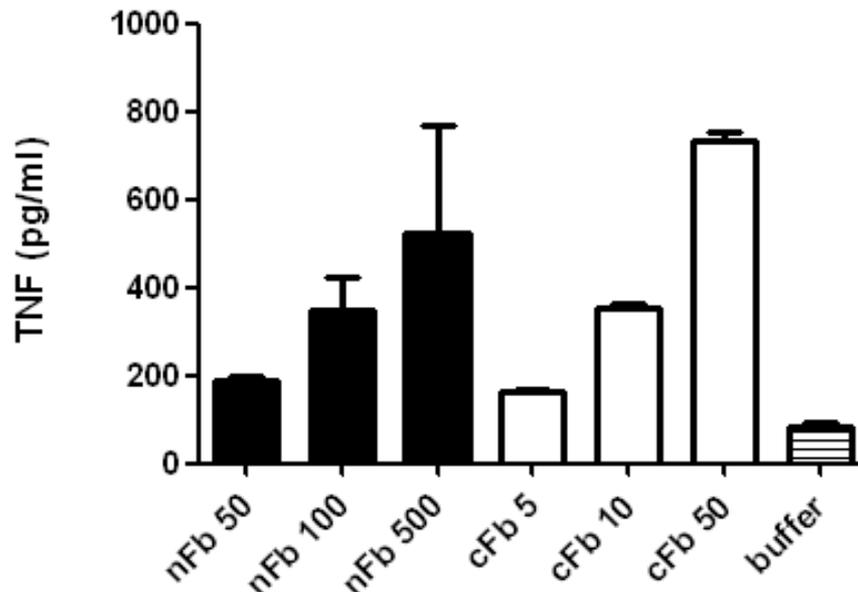


- 2-D DIGE: 10 healthy, 10 early OA, 10 late OA synovial fluids
- Mass spectrometry → 193 proteins differentially expressed in OA, $P < 0.05$
- Bioinformatic analysis → inflammatory mediators present:
 - Inflammation pathway
 - Immune cell receptors (including TLRs)
 - **Complement pathway**
 - **Plasma proteins/wound healing pathway**

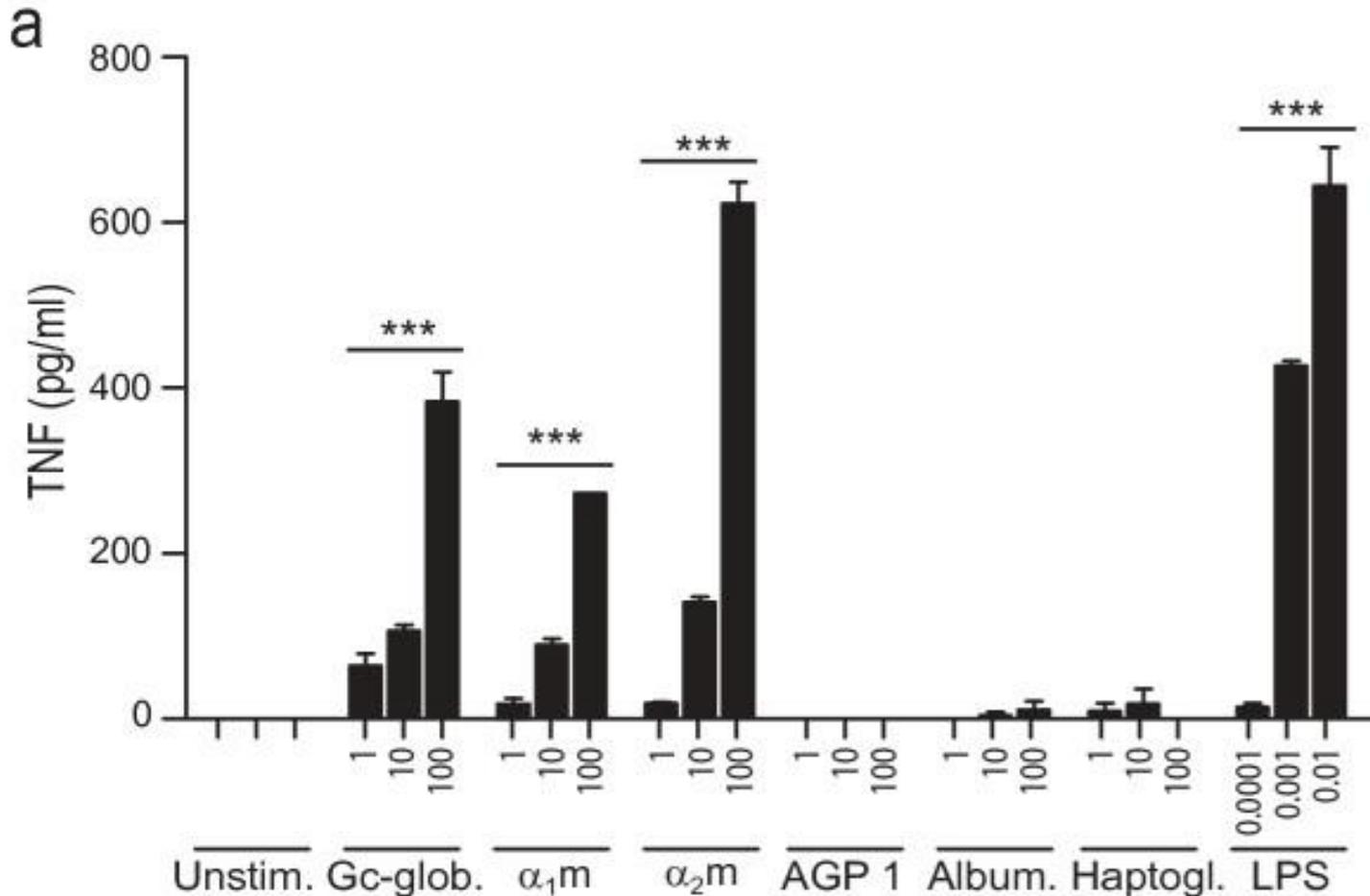
Plasma proteins as DAMPs

- Plasma proteins exudation occurs in the setting of inflammation and injury
- This makes extravascular plasma proteins an ideal danger signal

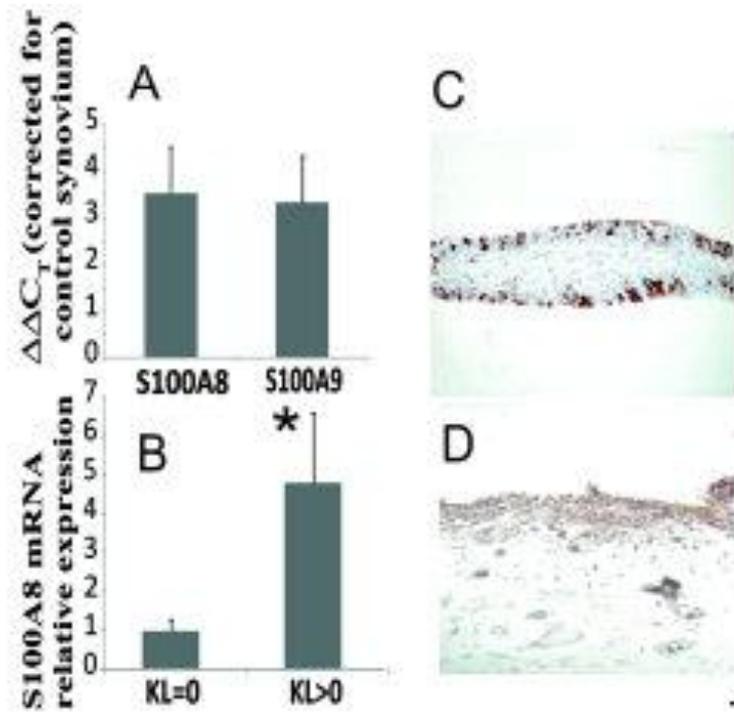
Fibrinogen activates TLR4-induced TNF production and citrullination increases potency 10-fold



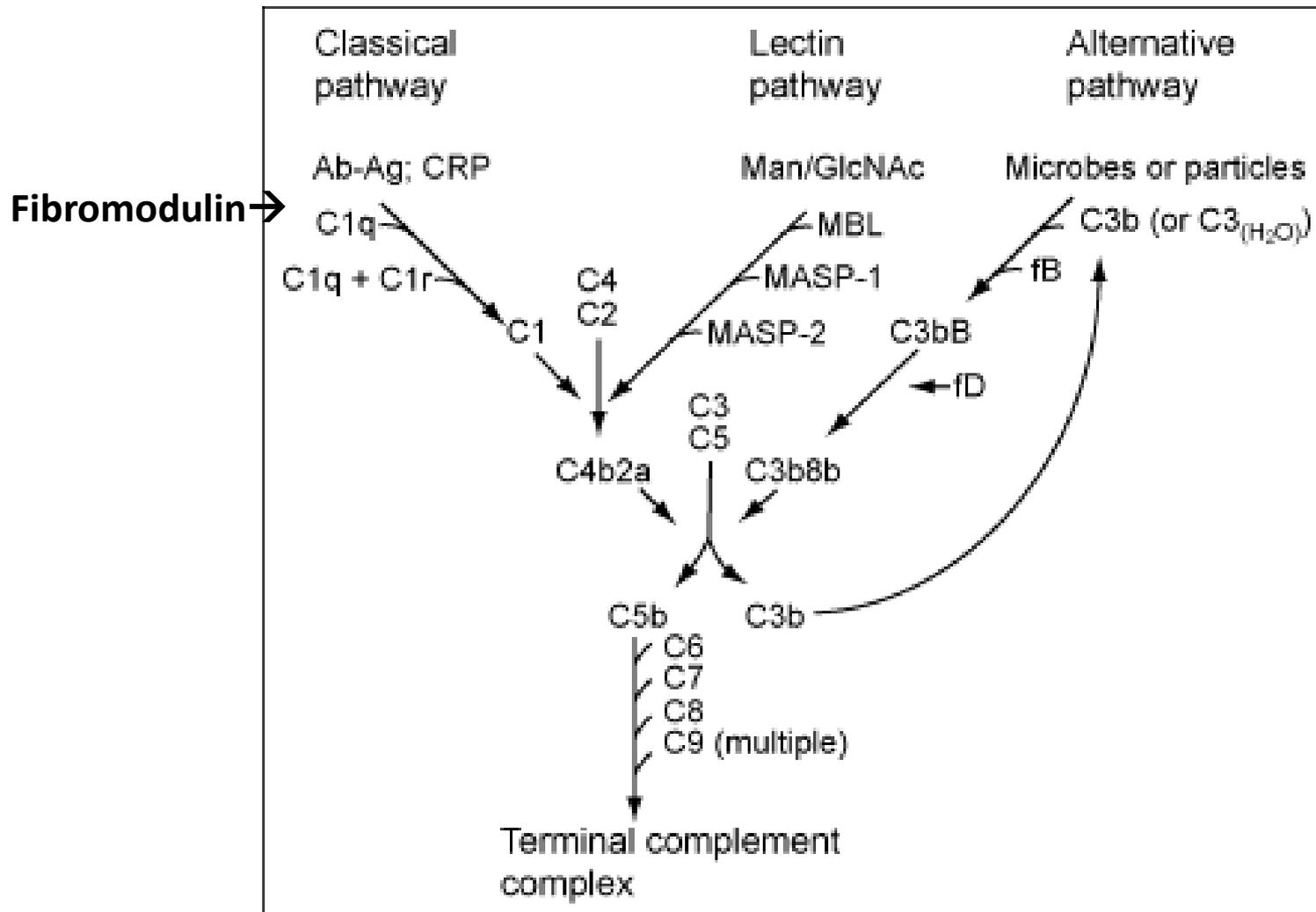
Plasma proteins detected in osteoarthritic synovial fluid are immunostimulatory



Alarmins S100A8/A9 in synovial activation and joint destruction during mouse and human OA

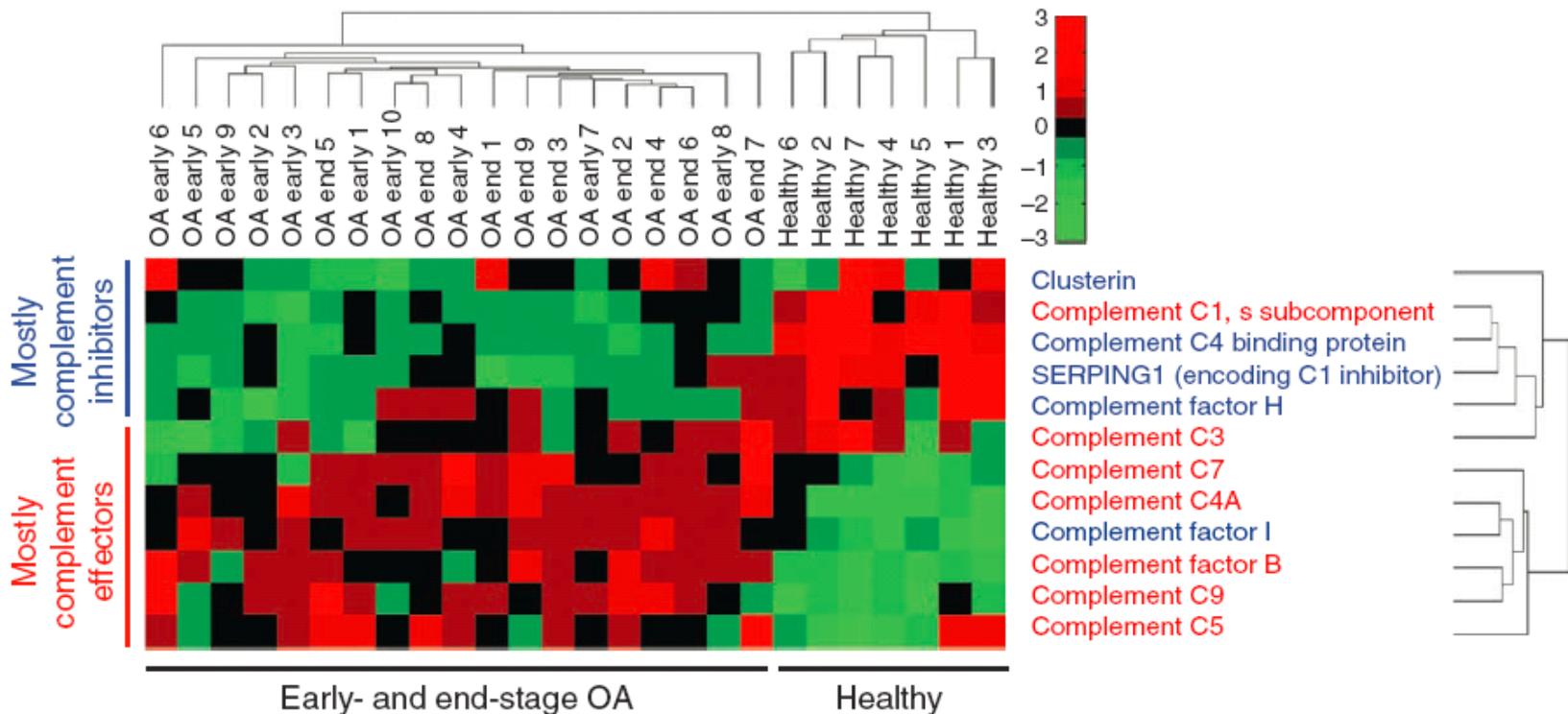


Central role for complement in OA

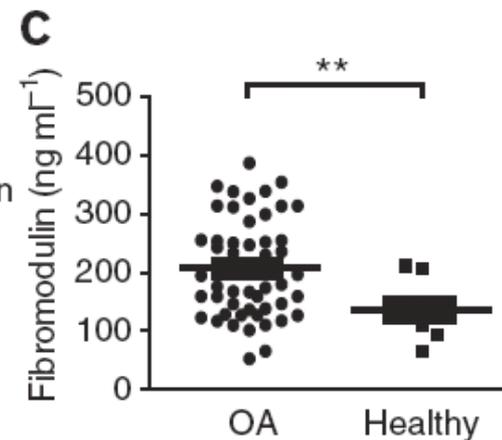
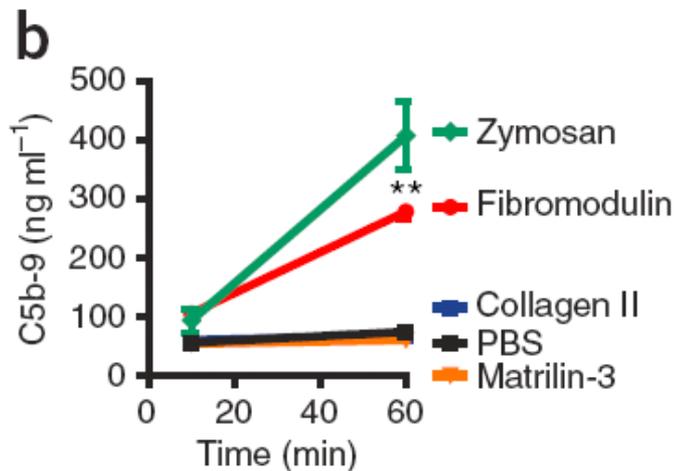
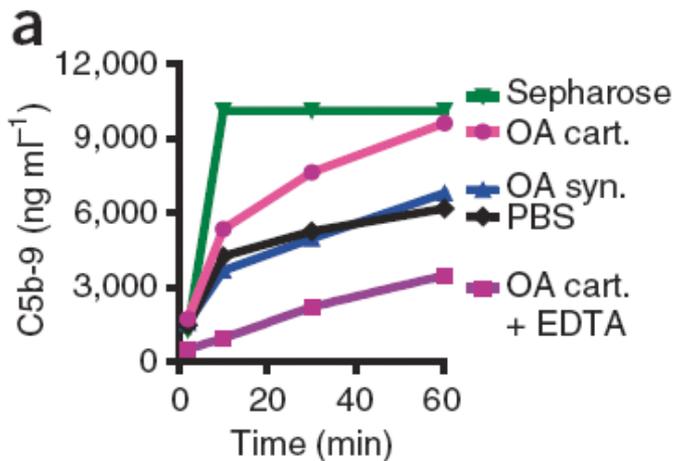


Complement in OA

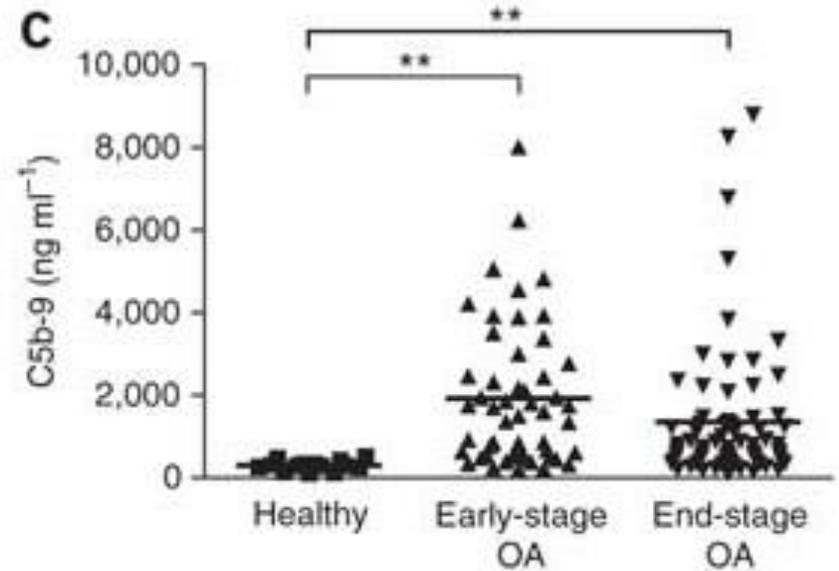
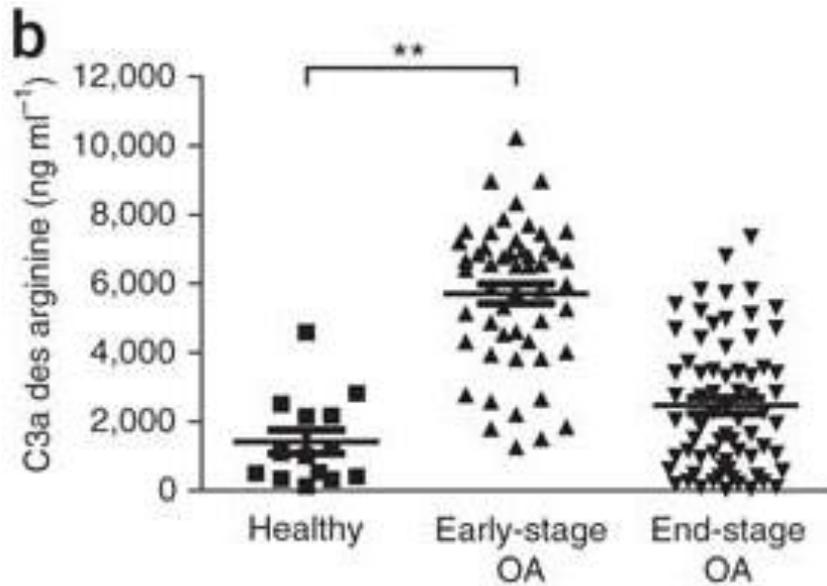
- Proteomic and transcriptomic analysis demonstrates
 - upregulation of complement effector genes
 - downregulation of complement inhibitors in OA synovium



Cartilage breakdown products activate classical complement cascade

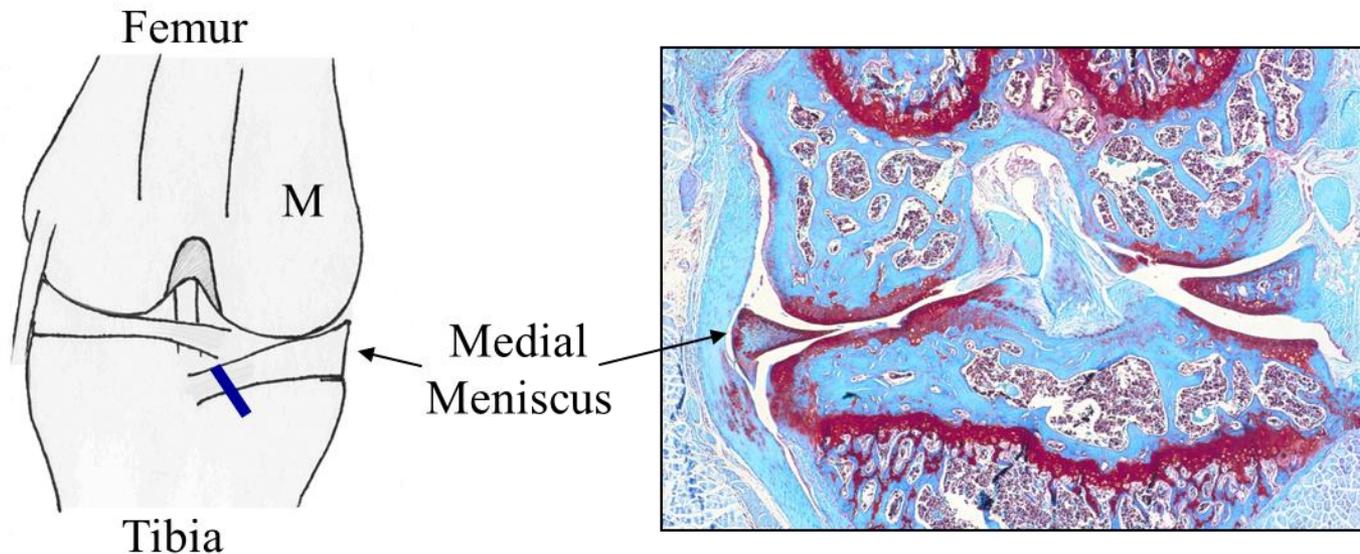


Increased complement activation products in early and late OA



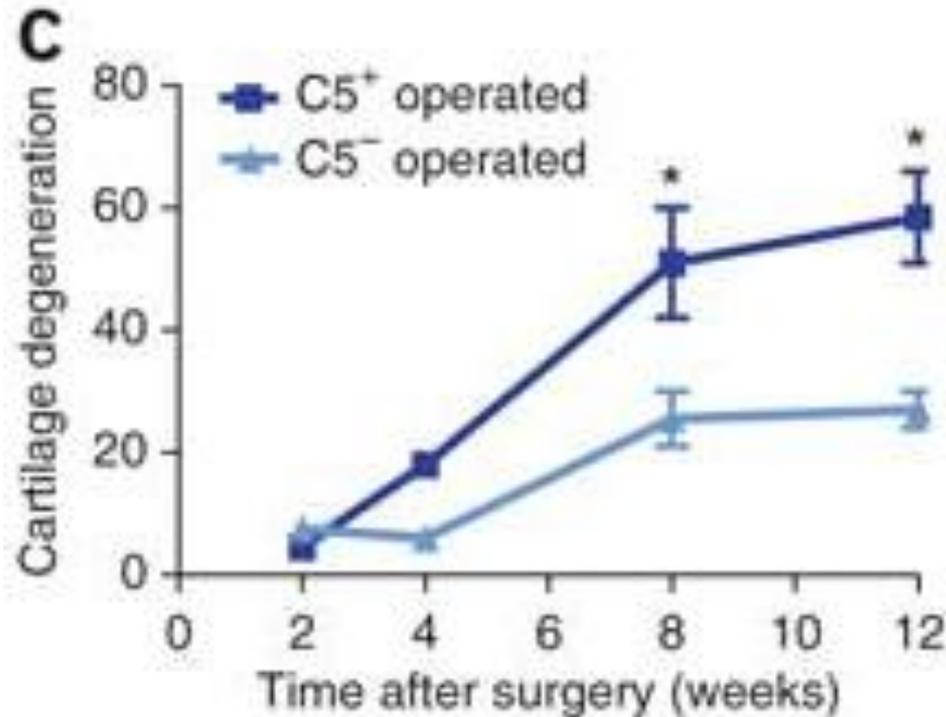
Surgical mouse models of OA:

- Medial meniscectomy
- Destabilization of the medial meniscus (DMM)



OA Score: sum of the depth X width of cartilage degeneration across condyles

Central Role for Complement in murine OA



- Similar observations with C6 -/- and opposite effect with CD59a -/-

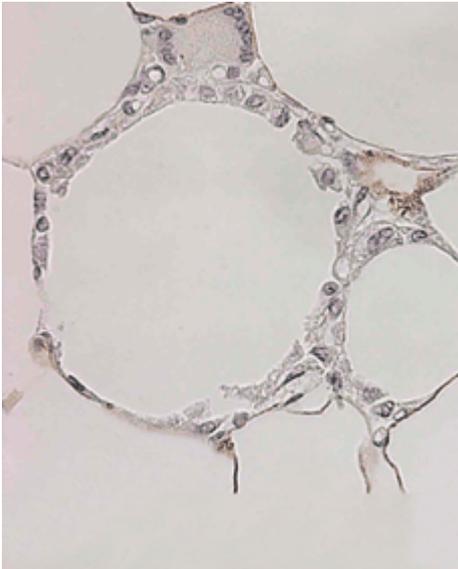
Soluble inflammatory factors in OA:

Cytokines

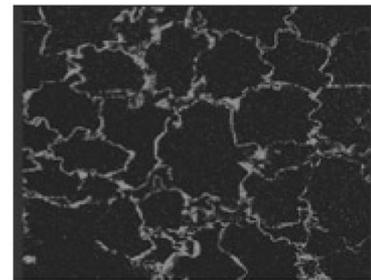
- Many cytokines induced by innate immune activation
- IL-1 β and TNF α activate NF- κ B and AP1 transcription factors expression of PGE2, NO, and MMPs 1, 9, 13
- IL-15 is elevated in early OA synovial fluid and correlates with synovial fluid MMP1/3 and histologic synovitis
- Role of IL-6 controversial

Role of Fat in OA

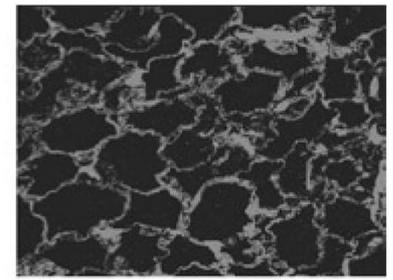
- Adipose tissue produces is an immunmologically a active tissue
- Adipose tissue can become infiltrated with inflammatory macrophage



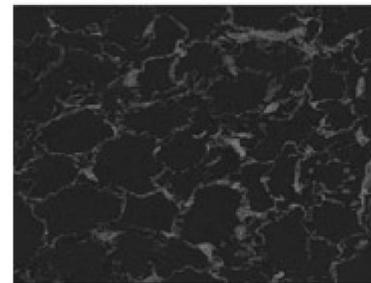
Cinti 2012
Weisberg 2003



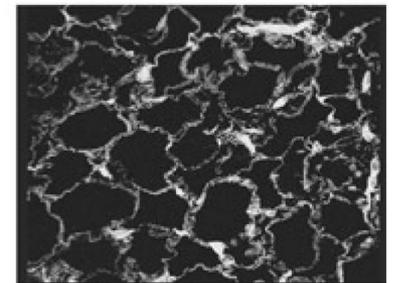
TLR 2



TLR 4



Adiponectin



Co-localization

Vitseva 2008

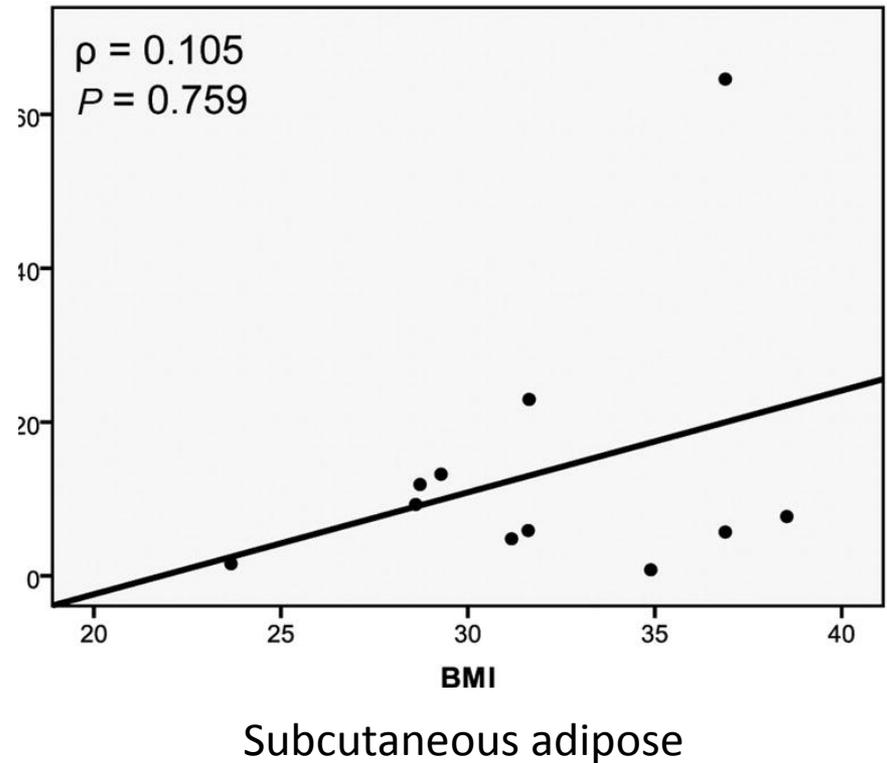
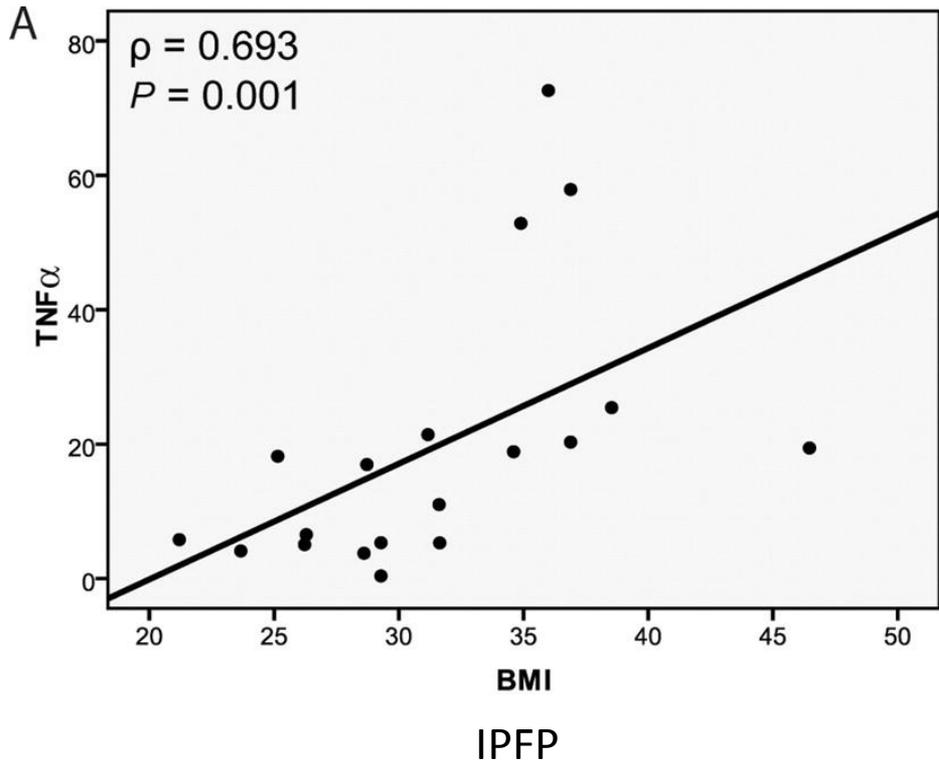
Adipokines

- **Adipokines:** soluble mediators which influence inflammation
- Leptin, Adiponectin, Visfatin, Resistin, ?IL-6, chemerin
- Variable results suggesting protection or pathogenicity of adipokines in OA
- Obesity may lead to low-grade systemic inflammatory state that can promote joint tissue destruction in OA

Infrapatellar fat pad as a local mediator of inflammation in OA.

- IPFP contain not only adipocytes but also increased numbers of macrophages and lymphocytes
- Capable of spontaneous and induced production of inflammatory cytokines, adipokines, and MMPs
- Capable of neuropeptide mediated neuro-inflammation and potential mediation of inflammatory pain

Infrapatellar fat pad as a local mediator of inflammation in OA.



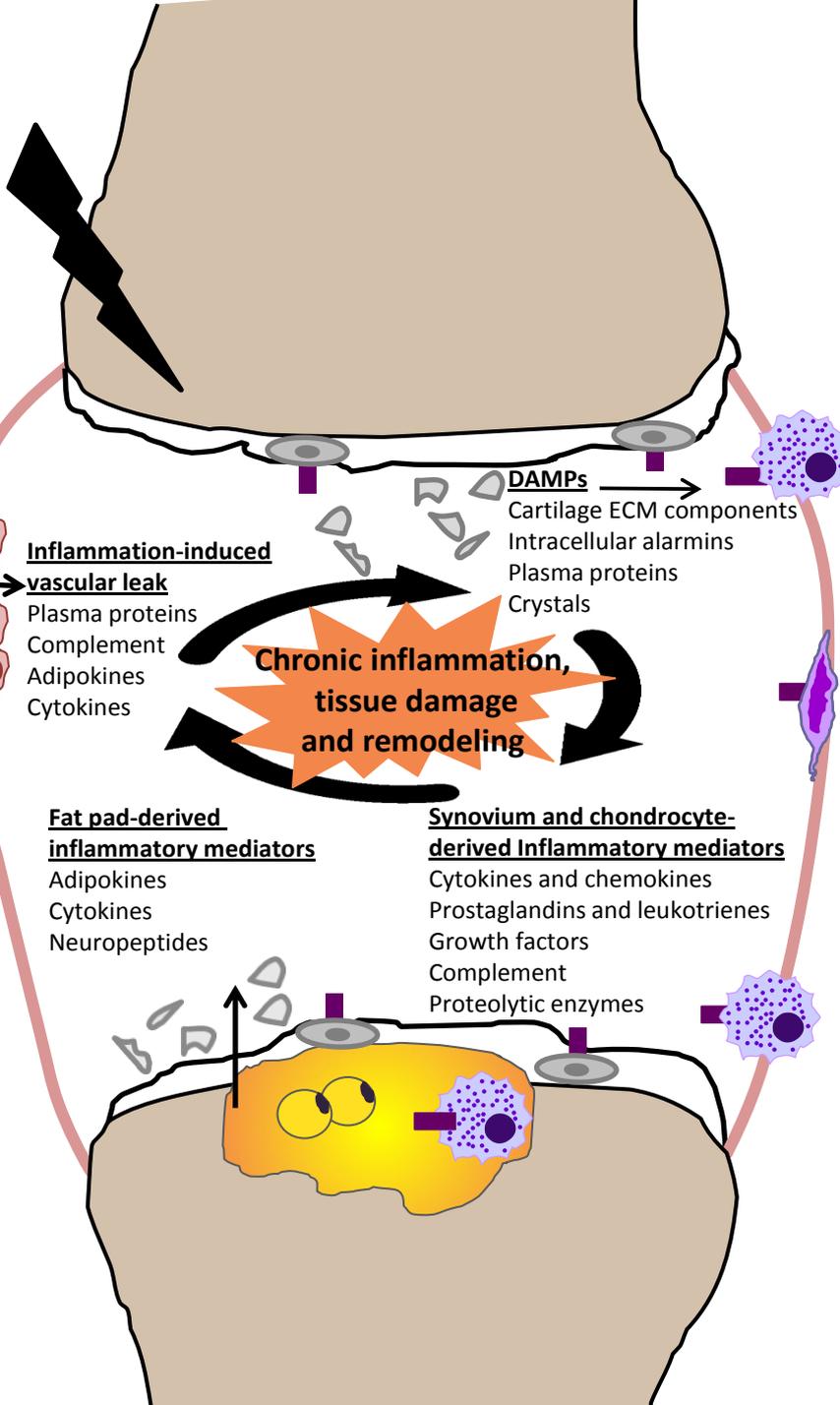
Prostaglandins, leukotrienes, and other lipid mediators

- The enzyme cyclooxygenase-2 (COX-2) is upregulated in inflamed joint tissues
- Overexpression of COX-2 is induced by pro-inflammatory mediators such as IL-1 β , TNF α , and IL-6, as well as TLR4 stimulation
- Arachadonic acid can also be converted to leukotrienes via lipoxygenase family of enzymes.
- LTB₄ and its metabolite LTC₄, are produced by OA synovium is a powerful leukocyte chemoattractant and stimulant of TNF α and IL-1 β

Crystals as DAMPs

- Crystals including uric acid, CPPD, BCP can all induce innate immune activation via NALP3 inflammasome
- Microscopic crystals (BCP, CPPD) are frequently observed in OA synovial tissue
 - Of 150 knees at autopsy, 93% demonstrated CPPD crystals in patients with severe OA, but only 24% of those with minimal or no OA.
 - Possible association of radiographic calcification with synovitis (though not observed in all studies)
- Recent data demonstrating association between synovial fluid uric acid levels and radiographic progression of OA

JOINT TRAUMA or OVERUSE →
Altered biomechanics,
instability, damage



**Angiogenesis,
Neovascularization**

**Inflammation-induced
vascular leak**
Plasma proteins
Complement
Adipokines
Cytokines

DAMPs →
Cartilage ECM components
Intracellular alarmins
Plasma proteins
Crystals

**Chronic inflammation,
tissue damage
and remodeling**

**Fat pad-derived
inflammatory mediators**
Adipokines
Cytokines
Neuropeptides

**Synovium and chondrocyte-
derived Inflammatory mediators**
Cytokines and chemokines
Prostaglandins and leukotrienes
Growth factors
Complement
Proteolytic enzymes

	Cartilage breakdown products
	Chondrocyte
	Macrophage
	Fibroblast-like synoviocyte (FLS)
	Pattern recognition receptor
	Adipocyte
	Infrapatellar fat pad
	Blood vessel

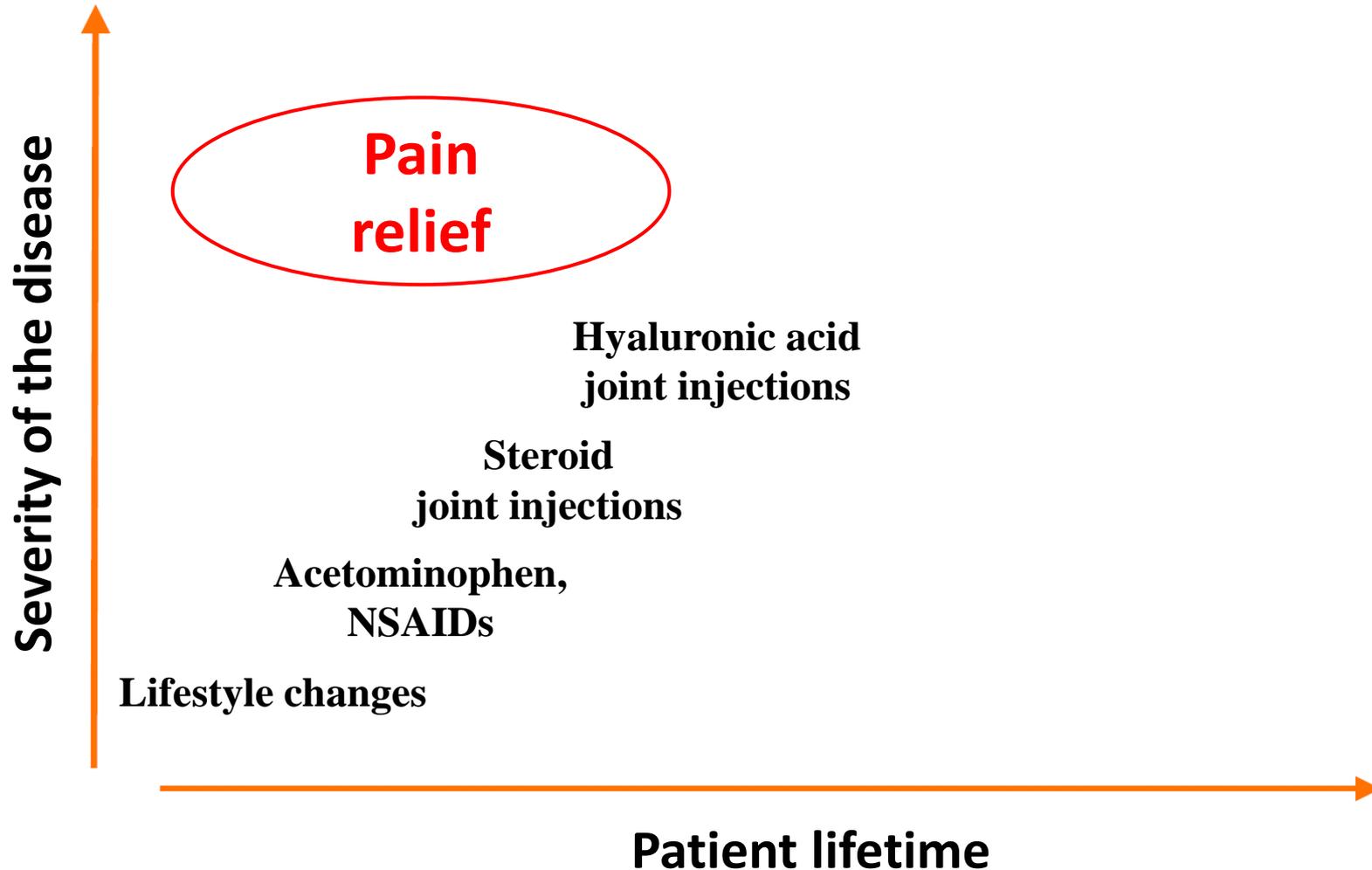
Biomechanics are still central

- Chronic damage from prior mechanical derangements produce ongoing low-grade damage
 - meniscal tear or extrusion, overuse, hypermobility, and/or anatomic misalignment
 - Thus, the proposed paradigm of chronic inflammation does not negate but rather expands on mechanical derangement as an inducing factor in OA pathogenesis.

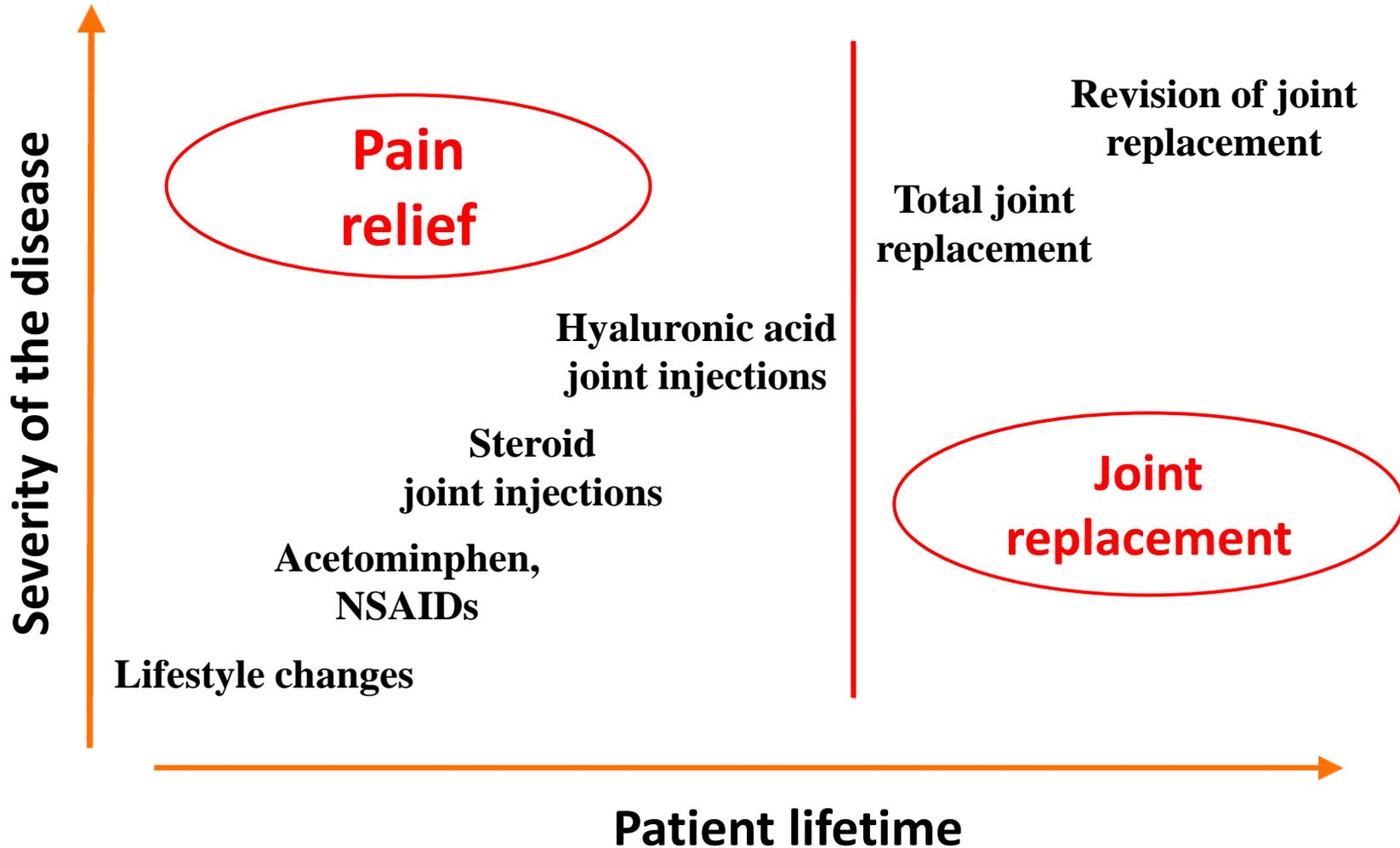
Synovial inflammation: a target for disease modification?

- Synovial inflammation in early OA suggests a window of opportunity in which disease-modifying interventions targeting inflammatory processes might be efficacious for the prevention and/or treatment of OA

Treatment of OA Today



Treatment of OA Today



Identification of “at risk population”

- Cartilage breakdown products in synovial fluid as well as microfissures in articular cartilage are present long before current MRI or arthroscopic visualization methods
- Early cartilage degradation may play a driving role in the development of OA inflammation
- Advances in imaging (high resolution and contrast-based MRI for synovitis and very early cartilage changes) may allow identification of those at risk for development of OA

Cartilage Breakdown

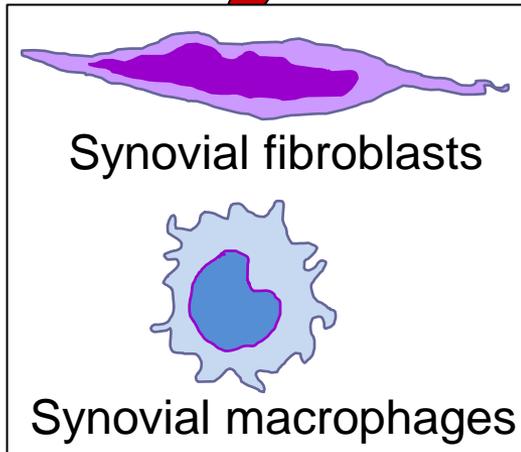
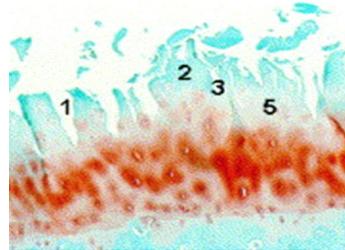
Plasma Exudation

→ DAMP release → Inflammation → OA

Joint trauma, instability, and/or overuse



Cartilage breakdown



- Inflammatory mediators
- Degradative enzymes



Cartilage Breakdown

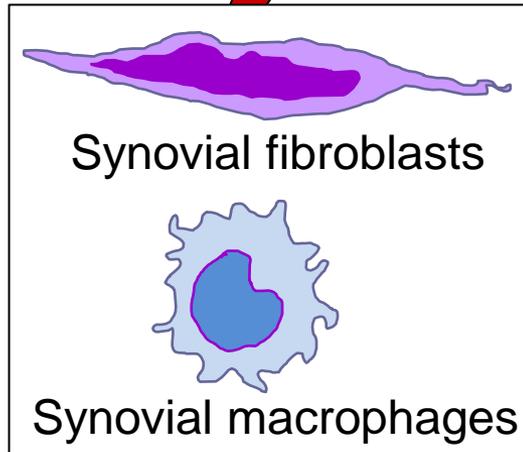
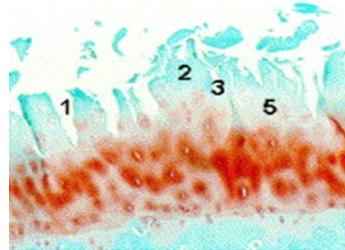
Plasma Exudation

→ DAMP release → Inflammation → OA

Joint trauma, instability, and/or overuse



Cartilage breakdown



**Anti-inflammatory
drugs**



- ~~Inflammatory mediators~~
- ~~Degradative enzymes~~



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Low Grade Inflammation:

Common mediator of chronic disease

Cardiovascular disease

- #1 killer in US
- Atherosclerotic plaque highly inflammatory site
- Closely linked with systemic inflammation

Non-alcoholic fatty liver disease (NAFLD/NASH)

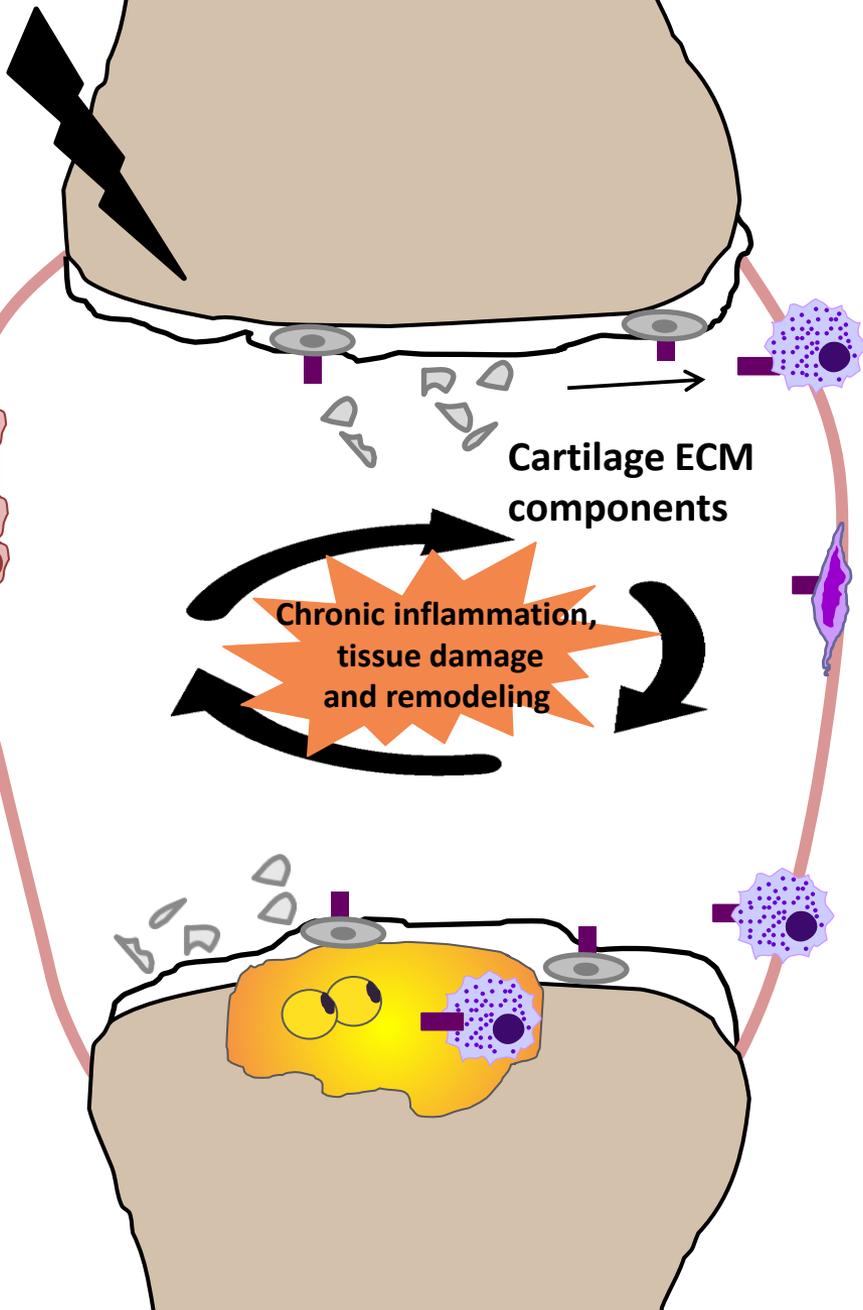
- Prevalence of NAFLD over 20% in US (and increasing)
- 20% of patients with NASH will develop cirrhosis
- Progression to chronic hepatic inflammation predict cirrhosis

Gout and hyperuricemia

- Leading cause of inflammatory arthritis in the world
- Hyperuricemia strongly associated with CV risk
- Role of uric acid in chronic inflammation largely unexplored

All potentially amenable to anti-inflammatory therapies

JOINT TRAUMA or OVERUSE →
Altered biomechanics,
instability, damage



-  Cartilage breakdown products
-  Chondrocyte
-  Macrophage
-  Fibroblast-like synoviocyte (FLS)
-  Pattern recognition receptor
-  Adipocyte
-  Infrapatellar fat pad
-  Blood vessel