Soluble CD14 in Synovial Fluid from Patients with Early Stage Osteoarthritis Augments Synoviocyte Responses to TLR-2 and TLR-4 Ligands


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Synovitis in OA

- Lower grade (severity) compared with RA (Krenn et al., Pathol Res Pract, 2002)
- Numbers of infiltrating macrophages and lymphocytes 2-3X normal (Pessler et al., ARD 2008)
- Prevalence increased compared with normal (Guermazi et al., ARD 2010)
  - no/equivocal radiographic OA: 22%
  - definite radiographic OA: 86%
- Related to:
  - pain and symptoms (Baker et al. ARD 2010, Hunter et al. OA & C 2011)
  - dysfunction (Sowers et al. JBJS 2011)
  - radiographic stage (Guermazi et al., ARD 2010)
  - rate of progression (Coughlin et al. Ann Rheum Dis 2010)

Meniscal Injury, Osteoarthritis, and Synovitis

- Meniscal tear: risk factor for development/progression
  - MOST study: OR 5.7 (95% CI 3.4-9.4) (Englund et al., A&R 2009)
- Meniscal degeneration and OA often coexist
- Meniscal injury may be a sign of early OA
- Cohort (n=33) of patients undergoing arthroscopic surgery for meniscal tears (Scanzello et al., A&R 2009, Poster #1065)
  - 80% had intra-operative evidence of cartilage softening/fissuring/fibrillation
  - enriched for early stage, pre-radiographic disease
  - 40% had synovitis, and synovitis was associated with symptoms
- Molecular basis for synovitis development in OA patients is unclear

Initiation of Inflammation: The Toll-like Receptors and Their Ligands

- Triggered by pathogen-associated molecular patterns (PAMPs) in microbial infection
- Also triggered by endogenous products (damage-associated molecular patterns, DAMPs) in sterile tissue injury

![Diagram of Toll-like receptors and their ligands](http://example.com/diagram.png)

Adapted from Scanzello et al., Curr Op Rheum 2008

DISCLOSURES

I have no financial or other relationship to disclose.
HYPOTHESIS

A TLR-2 or TLR-4 agonist activity in synovial fluid from patients with OA can lead to synoviocyte activation (production of inflammatory cytokines)

Early stage OA:
- Knee Injury and Arthritis Repository study
- Presenting for arthroscopic meniscal procedures with cartilage abnormalities documented intra-operatively

Advanced OA:
- Patient undergoing total knee replacement with full thickness cartilage loss

SF (25%) alone from early (n=23) or advanced (n=8) OA patients did not activate HEK-293 TLR transfectants

BUT

SF augmented responses to TLR-2 and TLR-4 ligands

#13, 19, 2, 42 are SF specimens from early OA patients

SF does not increase TLR expression

Early OA SF and LPS dose responses

Suggested the presence of a SF factor(s) that could modulate TLR responses

Methods

- Synovial fluid (SF) collected from patients at time of surgery
- Tested for ability to stimulate cytokine production in vitro

Primary Fibroblast-like synoviocytes (FLS)
- FLS cultures established from post-mortem donors without history of arthritic disease
- IL-8 and IL-6 measured in supernatants after 18 hours

• Control TLR-2 stimulus: Pam3CysK4, 100ng/ml
• Control TLR-4 stimulus: LPS, 100ng/ml

TLR transfected cell lines
- HEK-293 transfectants
  - TLR-2/CD14 or CD14
  - TLR-4/MD2 or TLR4
- IL-8 measured in supernatants after 18 hours

TLR-2

TLR-4

SF = 25% in media

LPS and Pam3CysK4 = 100ng/ml
SF sCD14 levels are comparable in early OA, advanced OA and RA, and higher than in serum

SF sCD14: Conclusions

- a TLR-2 and -4 co-receptor found in high concentrations in SF
  - evaluating the range of TLR ligand responses that SF may impact
- not necessarily disease or stage specific
  - suggests similarities at the level of innate activation of FLS between inflammatory and "non-inflammatory" arthritis
- sensitzes FLS responses in vitro to TLR-2 and TLR-4 ligands
- increasing sCD14 levels in the setting of arthritis (either OA or RA) may sensitize FLS to respond to inflammatory stimuli (i.e. TLR ligands) when they are produced within the joint

Potential impact of SF sCD14

- might relate to increased risk of septic arthritis in OA and RA
  - could lower the threshold for, or increase the severity of response to infectious agents via TLRs
- may contribute to association between OA and gout
  - may increase propensity to develop inflammation in the presence of uric acid crystals
- potential role in development of flares of inflammation/effusion in arthritic patients during periods of increased tissue injury or cellular stress (i.e. after a meniscal tear, during periods of increased matrix turnover)

Limitations

- use of post-mortem asymptomatic donors
  - SF from normal living donors difficult to obtain
- cannot test 100% SF in this in vitro system
  - limits ability to detect TLR modulating factors or agonists that might be in low concentrations
  - whether SF/sCS14 exerts augmenting effect in vivo yet to be determined

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