Cholesterol accumulation in synovial lining macrophages results in ectopic bone formation during experimental osteoarthritis

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Disclosure

We have no competing interests

Osteoarthritis (OA)

Not only a disease of the cartilage, also synovial involvement.

Synovial macrophages and OA

• Essential for cartilage destruction during experimental OA.1

• Important players in driving inflammatory and destructive responses in OA.2

• Crucial for osteophyte formation (ectopic bone formation at cartilage margins) and enthesophyte formation (ectopic bone in tendons or ligaments).3

Modified LDL and macrophages

• In an inflammatory milieu, LDL can be modified.2

• Increased LDL levels will therefore result in enhanced oxLDL levels during inflammatory processes.3

• OxLDL is taken up by macrophages via SR-A and CD36.4,5

• OxLDL uptake can change the phenotype of macrophages.4,5

Hypothesis

Objective: Cholesterol-rich diet

LDL receptor deficiency

Experimental OA model

Aggravation OA pathology
Experimental design

Collagenase injection: 
- Arthritis damage
- Cartilage instability
- Ligament damage
- Osteophyte formation and synovial activation

LDLr deficiency and/or a cholesterol-rich diet leads to increased serum cholesterol levels

**p<0.05 compared to WT normal**
***p<0.001 compared to all other groups

LDLr deficiency or a cholesterol-rich diet does not affect cartilage damage

Synovial lining cells take up ApoB during cholesterol-rich conditions, suggesting oxLDL accumulation

LDLr deficiency or a cholesterol-rich diet does not affect synovial thickening

A cholesterol-rich diet enhances synovial S100A8 expression in WT mice (synovial activation)

LDLr deficiency or a cholesterol-rich diet does not affect cartilage damage

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S100A8 staining synovial lining

Introduction - Experimental Design - Results - Conclusions
**Introduction**

LDLr deficiency or a cholesterol-rich diet does not affect cartilage damage

**Results**

LDLr deficiency and a cholesterol-rich diet increase enthesophyte formation

**Discussion**

Ectopic bone formation due to growth factors

Growth factors capable of ectopic bone formation

- TGF-β is secreted by many cell types, including macrophages, in a latent form.
- TGF-β is activated by proteinases.  
  - Active TGF-β can be detected using a Luciferase reporter gene assay, which detects active TGF-β signaling (CAGA box in PAI-1 promoter).  
- Also Bone Morphogenetic Proteins (BMP) 2, 4 and 7 have shown to induce ectopic bone formation.  
  - BMP signaling can be detected using a BMP Responsive Element (BRE) Luciferase reporter gene assay.  

LDLr/− mice on a cholesterol-rich diet have increased levels of active TGF-β in synovial washouts compared to WT mice on a cholesterol-rich diet

**Experimental Design**

<table>
<thead>
<tr>
<th>LDLr deficiency or a cholesterol-rich diet does not affect cartilage damage</th>
<th>LDLr deficiency and a cholesterol-rich diet increase enthesophyte formation</th>
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<tbody>
<tr>
<td>WT normal</td>
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<tr>
<td>LDLr−/− cholesterol diet</td>
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</tbody>
</table>

**Figure Legends**

**Figure 1**

Cartilage damage

**Figure 2**

Ectopic Bone Formation

**Figure 3**

Osteophyte Formation

**Figure 4**

LDLR-/- cholesterol diet

**Figure 5**

Enthesophyte Formation
**Conclusions**

LDL cholesterol accumulation during experimental OA aggravates pathology by activation of synovial resident cells and anabolic pathways.

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**In vitro design**

- M-CSF  
- Differentiation into macrophages (4 days)  
- Control  
- 50 μg/mL oxLDL (24 hours)  
- RNA expression growth factors and functional Luc-assay

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**OxLDL-stimulation of macrophages increases anabolic processes by activation of TGFβ, rather than production of TGFβ and BMP**

- Gene expression Growth factors  
- Active TGF-β  
- Active BMP

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**Conclusions**

- Increased serum LDL levels result in enhanced ApoB accumulation in synovium → oxLDL accumulation in LDLr deficient mice.
- OxLDL accumulation results in increased activation of synovial macrophages in WT mice.
- OxLDL accumulation during experimental OA results in ectopic bone formation in the murine knee joint.
- Cholesterol-rich models show higher levels of active TGF-β in synovial washouts than models with less cholesterol accumulation.
- OxLDL accumulation leads to activation of TGF-β and, to a lesser extent, activation of BMP.