Cholesterol Accumulation By Synovial Lining Macrophages Results In Ectopic Bone Formation During Experimental Osteoarthritis, Possibly By Activating Transforming Growth Factor-Beta

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Disclosures:

Introduction: Synovial macrophages have previously shown to play a significant role in the etiopathology of experimental collagenase-induced osteoarthritis (OA).[1] In addition to production of pro-inflammatory proteins such as S100A8/9[2] and IL-1[3] in early OA, synovial lining macrophages have also shown to promote transforming growth factor-beta (TGFβ) mediated osteophyte formation.[4, 5] In an inflammatory milieu such as OA, accumulated low density lipoprotein (LDL) is oxidized, resulting in high intra-articular levels of oxidized LDL (oxLDL).[6] OxLDL is taken up by macrophages via scavenger receptors, resulting in a more aggressive phenotype.[7] In the present study, we investigated whether LDL accumulation by either LDL-receptor deficiency (LDLr−/−) or a cholesterol-rich diet leads to increased oxLDL uptake by synovial macrophages and affects synovial activation and osteophyte formation. S100A8/9[2] and IL-1[3] in early OA, synovial lining macrophages have also shown to play a crucial role in promotion of transforming growth factor-beta (TGFβ) mediated osteophyte formation.[4, 5] In an inflammatory milieu such as OA, accumulated low density lipoprotein (LDL) is oxidized, resulting in high intra-articular levels of oxidized LDL (oxLDL).[6] OxLDL is taken up by macrophages via scavenger receptors, resulting in a more aggressive phenotype.[7] In the present study, we investigated whether LDL accumulation by either LDL-receptor deficiency (LDLr−/−) or a cholesterol-rich diet leads to increased oxLDL uptake by synovial macrophages and affects synovial activation and osteophyte formation.

Methods: LDLr−/− mice and their wild type (WT) controls received either a high cholesterol or control diet for 120 days. Experimental OA was induced by intra-articular injection of collagenase on day 84 and 86. Paraffin sections of OA knee joints were analyzed for cartilage destruction and osteophyte formation using the Pritzker score and image analysis, respectively. ApoB, the primary apolipoprotein component in (oxidized) LDL, and S100A8 were detected using immunohistochemistry and synovial wash-outs were tested for active TGFβ using a TGFβ reporter gene assay and gene expression. Murine bone marrow derived macrophages were stimulated with 50 μg/mL oxLDL, after which supernatant was functionally tested for active TGFβ presence.

Results: Mice receiving a cholesterol-rich diet not only develop increased serum LDL cholesterol levels, but also showed enhanced ApoB expression in synovial lining macrophages, which is a specific molecule present in (oxidized) LDL. In line with that, LDLr−/− mice, which already had systemically high basal levels of LDL, showed a much higher accumulation of ApoB in the synovial lining after receiving a cholesterol-rich diet. Although a cholesterol-rich diet did not enhance thickening of the synovium, S100A8 expression within macrophages was markedly increased, reflecting an elevated activation status. Even though no effect of LDL accumulation on cartilage destruction was found, both a cholesterol-rich diet and LDLr−/− strongly increased cartilage and bone formation in ligaments with a fold change of 6.7 and 6.1, respectively. Moreover, an increase in osteophyte size was found at the margins of the tibial plateau (figure a and b). To elucidate the mechanism, we finally studied the presence of active TGFβ, which is crucial in driving osteophyte formation, in synovial wash-outs and culture supernatant of oxLDL stimulated macrophages. Using a TGFβ reporter assay, synovial wash-outs of LDLr−/− mice (figure c) and stimulation of macrophages with oxLDL showed a significantly increased presence of functional TGFβ compared to controls (fold change of 1.4 and 2.9, respectively), while TGFβ gene expression was not altered.

Discussion: Synovial accumulation of LDL cholesterol leads to increased synovial activation and osteophyte formation in experimental OA. Enhanced levels of functional TGFβ are found in synovial wash-outs of LDLr−/− mice compared to WT mice and in vitro uptake of oxLDL by macrophages resulted in activation of TGFβ, rather than production. This would hint towards a possible mechanism in which high cholesterol levels aggravate OA pathology.

Significance: Our experimental data points towards a potential mechanism in which uptake of oxLDL by synovial lining macrophages results in activation of TGFβ and, therefore, provides a firm step forwards unraveling possible factors affecting etiopathology of OA and osteophyte formation in particular.

Acknowledgments:
References:
1 Blom AB, et al., 2007
2 van Lent PL, et al., 2012
3 Bondeson J, et al., 2006
4 Blom AB, et al., 2004
Osteophyte formation Tibia

** WT normal diet
*** WT cholesterol-rich diet
LDLr" normal diet
LDLr" cholesterol-rich diet

Lateral Medial Mean

Normal diet Cholesterol-rich diet

WT

LDLr"

CA GA-LUC wash-outs

Orthogonal ligament

Figure. Increased serum cholesterol levels enhanced osteophyte formation at the margins of the tibial plateau and increase active TGFβ presence. Osteophyte size was digitally measured at the margins of the tibial plateau (A). B shows representative microphotographs (stained with safranin O - fast green) of osteophytes at the lateral side of the tibial plateau. Presence of active TGFβ was found to be increased in LDLr deficient mice on a cholesterol-rich diet compared to WT mice on a cholesterol-rich diet by testing synovial wash-outs in a CAGA-Luc assay (C). *p<0.05; **p<0.01; ***p<0.001. n=10 mice per group.