Synovial Macrophages Promote TGF-β Activation After Intra-Articular Injections of Oxidized LDL in Naïve Murine Knee Joints, Preventing Production of Pro-Inflammatory Factors S100A8/9, Chemokines and Aggrecanase-Induced Neo-Epitopes

Wouter de Munter, Msc¹, Arjen B. Blom, PhD¹, Peter M. van der Kraan, PhD¹, Johannes Roth, PhD², Thomas Vogl, PhD², Wim B. van den Berg, PhD¹, Peter L. van Lent, PhD¹.
¹Radboud University Medical Center, Nijmegen, Netherlands, ²Institute of Immunology, Muenster, Germany.


Introduction: In previous studies we found that synovial macrophages regulate joint pathology during experimental osteoarthritis (OA). Recently, we found that high systemic levels of LDL aggravate joint pathology during experimental OA with synovitis. LDL in inflamed synovium is oxidized and taken-up by macrophages via scavenger receptor A and CD36, leading to an activated macrophage phenotype. In this study, we investigate whether direct injection of oxLDL into a normal murine knee joint induces joint pathology and elucidate the role of synovial macrophages in that process.

Methods: Knee joints of C57BL/6 mice were injected at five consecutive days with 1.2 mg/mL oxLDL, LDL, or an equal volume of vehicle (PBS). This same procedure was done in mice which were depleted of synovial macrophages by intra-articular injection of clodronate liposomes seven days prior to the (ox)LDL or vehicle injections. Joint pathology was investigated by immunohistochemistry and RNA expression and protein production by synovium were determined using RT-PCR and luminex, respectively. Active TGF-β was measured using a functional CAGA-luciferase assay. Data are depicted as mean ± standard deviation.

Results: LDL and oxLDL injection in naïve knee joints did not increase synovial thickening, or production of pro-inflammatory factors (IL-1β, IL-6 and S100A8/9) compared to vehicle injection. Levels of active TGF-β in synovial wash-outs was, however, significantly increased by 33% (from 84.7 ng/mL/g synovium ± 14.4 to 113.0 ng/mL/g synovium ± 33.3; p<0.05). Immunohistochemistry of total knee joints showed that oxLDL injection decreased formation of aggrecanase-induced neo-epitopes (NITEGE) compared with vehicle injections, especially in areas along the bone margins that are prone to develop osteophytes (from arbitrary score 1.19 ± 0.57 to 0.33 ± 0.30; p<0.05).

In contrast, repeated injections of oxLDL in macrophage-depleted knee joints led to a 3.1 fold increase of synovial thickening (due to cell influx), compared with injection of vehicle (p<0.01), while LDL injections did not alter synovial thickening. Protein levels of S100A8/A9, markers for inflammation, were significantly increased in synovial wash-outs of oxLDL injected joints, compared with LDL (fold increase 5.6; p<0.05) or vehicle (fold increase 8.3; p<0.01) injection. RNA levels of chemokines CCL2 (Mcp-1) and CCL3 (Mip-1α) were also significantly upregulated after oxLDL injections (6.7 fold and 4.6 fold, respectively; p<0.01). No raise in active TGF-β was measured in macrophage-depleted joints. NITEGE expression was markedly increased (fold increase 1.92) in the synovial-cartilage contact areas after oxLDL injection (p<0.05).
**Discussion:** Synovial macrophages promote anabolic effects after oxLDL injections in knee joints, supporting earlier studies which show increased ectopic bone formation during LDL-rich conditions in experimental osteoarthritis. In absence of synovial macrophages, however, oxLDL induces cell influx, production of pro-inflammatory mediators and aggrecanase activity.

**Significance:** It is known that LDL cholesterol, and modifications thereof, can affect immunological processes. Since the metabolic syndrome is associated with OA, it is important to understand the effects of serum cholesterol on OA pathology. Unraveling the etiopathology of OA could lead to new insights into disease development and new therapeutic possibilities.

*ORS 2015 Annual Meeting*

*Poster No: 0418*