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SOLUBLE CD14 IN SYNOVIAL FLUID FROM PATIENTS WITH OA AND MENISCAL INJURY MODULATES THE RESPONSE OF SYNOVIAL FIBROBLASTS TO LPS

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Background and objectives It has been hypothesised that inflammation is triggered in the osteoarthritic (OA) joint via stimulation of pattern-recognition receptors such as the toll-like receptors (TLRs) by products of tissue degradation. We tested whether a TLR-4 stimulating factor was present in synovial fluid (SF) from patients with meniscal injury with or without OA.

Materials and methods SF was obtained from patients undergoing arthroscopic surgery for meniscal tears, with or without concomitant OA, and tested for lipopolysaccharide (LPS) contamination using the LAL assay (Pierce Chemicals). HEK293 cells transfected with either TLR-4, or TLR-4 + MD2, were used to screen SF for the ability to induce IL-8 production. Synovial cell lines were established from patients and post-mortem tissue donors, and used between passages 4 and 8. Synovial cells were stimulated with a TLR-4 stimulus (ultra-pure LPS 100 ng/ml, Invivogen Inc.), SF alone, or SF + LPS for 6 or 18 h. IL-8 in stimulated culture supernatants was measured by ELISA. In blocking experiments, SF was pre-incubated with anti-CD14 (clone MEM-18), or immunoadsorbed with anti-CD14 bound to protein-G coupled beads, prior to using as stimuli. sCD14 levels in SFs were measured by ELISA.

Results Only 2 of 17 SFs stimulated IL-8 production from HEK transfectants. Moreover, SF inhibited the LAL assay. Therefore, we tested whether SF would inhibit IL-8 production by synovial cells in response to a TLR-4 ligand (LPS). Instead, the addition of SF (0.09–25%) to LPS prior to stimulation resulted in significant augmentation (> 100 fold) of IL-8 production by synovial cells. CD14, a cofactor for TLR-4 responses to LPS, is expressed on the cell-surface or as a soluble mediator (sCD14). As synovial cells did not express surface CD14 by flow cytometry, levels of sCD14 in SFs were measured and found at 1–6 microgram/ml. Removal of sCD14 from SF with anti-CD14 coupled protein G beads, or preincubation of SF with anti-CD14, abolished the ability of SF to augment IL-8 in response to LPS.

Conclusions In vitro, SF augments the response of synovial cells to LPS. This effect appeared to be largely due to sCD14. As synovial cells are expected to be in contact with SF in vivo, these results suggest that SF sCD14 in the setting of OA and meniscal injury can sensitise synovial cells to respond to inflammatory stimuli such as TLR-4 ligands. sCD14 levels in various arthritic states, and the effect of SF on other TLRs, is currently being explored.