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

Carla R Scanzello,1 Anjali Nair,1 Veero Kanda,2 Charles Bush-Joseph,2 Nikhil Verma,2 Mary K Crow,4 Katalin Mikecz,2 Tibor Glant,2 Greg T Spear,2 Alison Finnegan1 1Section of Rheumatology, Rush University Medical Center, Chicago, Illinois, USA; 2Department of Orthopedics, Rush University Medical Center, Chicago, Illinois, USA; 3Department of Immunology and Microbiology, Rush University Medical Center, Chicago, Illinois, USA; 4Division of Rheumatology, Hospital for Special Surgery, New York, New York, USA

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. Synoviocyte cell lines were established from patients and post-mortem tissue donors, and used between passages 4 and 8. Synoviocytes were stimulated with a TLR-4 stimulus (ultrapure 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 synoviocytes 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 synoviocytes. CD14, a cofactor for TLR-4 responses to LPS, is expressed on the cell-surface or as a soluble mediator (sCD14). As synoviocytes 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 synoviocytes to LPS. This effect appeared to be largely due to sCD14. As synoviocytes 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 synoviocytes 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.