inhibited when mIL-1Ra was overexpressed using the RGD adenoviruses for infection compared to the wild type adenovirus.

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THU0049 PROTEIN KINASE INHIBITION ENHANCES STEADY STATE MRNA LEVELS ENCODING IL -16 BUT REDUCES IL-18 ENCODING MESSAGE LEVELS IN FIBROBLASTS

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Background In previous studies we have shown that synovial fibroblasts from rheumatoid arthritis (RA) patients transcribe elevated levels of IL-16 encoding mRNA. In addition, in RA synovial fluid high concentrations of IL-16 were detected. The chemotactic activity to CD4⁺ cells was associated with IL-16. In osteoarthritis synovial fibroblasts, IL-16 was not found.

Objectives Therefore we initiated experiments to study the activation pathway regulating IL-16 transcription in synovial versus skin fibroblasts.

Methods OA synovial fibroblasts (n = 3), skin fibroblasts (n = 6) and neonatal preputial fibroblasts (n = 3) were expanded (less than 4 passages). Cells were incubated in complete medium (10% FCS) supplemented with MAS-7, ocaidaic acid (ODA), ionomycin, cAMP, forskolin, staurosporin, phorbol ester, H7 and an unrelated peptide as mock control. Untreated cells served as controls. After 6 to 48 h of incubation, mRNA was extracted from experimental samples and controls. The mRNA yield was determined by spectrophotometry and, cDNA was generated from 1 µg RNA using the oligo (dT) priming procedure. Steady state transcript amounts encoding IL-16 or IL-18 were determined by real time quantitative RT/PCR (LightCycler, Roche) using the SYBR green method. The PCR products were visualised by EtBr fluorescence after gel electrophoresis. Quantitative GAPDH RT/PCR served as internal standard.

Results mRNA encoding IL-16 and IL-18 was detected in all fibroblasts analysed. Incubation of the fibroblasts for 24 to 48 h with staurosporin or forskolin enhanced IL-16 steady state mRNA levels two to three fold. Incubation with ODA or H7 reduced IL-16 mRNA three-fold and ten-fold, respectively. PMA reduced IL-16 signals to about 70%. Interestingly, IL-18 signals were not reduced in ODA treated fibroblasts whereas H7 reduced IL-18 signals about ten-fold. Staurosporin and forskolin did not enhance IL-18 encoding signals but reduced the IL-18 signals two to three fold. IL-18 encoding mRNA amounts were elevated in ionomycin treated cells. All other components did not show considerable activation or reduction of the IL-16 or IL-18 RT/PCR signals. In addition, there was no remarkable difference in IL-16 or IL-18 transcriptional responses to all agents analysed so far.

Conclusion Staurosporin is a broad protein kinase inhibitor and activated IL-16 transcript levels whereas phorbol ester PMA activates protein kinase C and reduced IL-16 mRNA levels. We conclude that IL-16 transcription is regulated by kinase/phosphatase signal transduction pathways. In addition, a cAMP dependent enhancement of IL-16 encoding steady state mRNA levels was

observed. By contrast, IL-18 RT/PCR signals are reduced upon kinase inhibition but not activated after PMA incubation. Raising intracellular calcium by ionomycin induced IL-18 encoding mRNA. Therefore the IL-16 and IL-18 gene activities are regulated differently. Since in RA synovial fibroblasts IL-16 mRNA is elevated when compared to OA fibroblasts and blocking kinase activity activated IL-16 mRNA levels in OA synovial or skin fibroblasts, we hypothesise that a reduced kinase activity may be associated with enhanced levels of IL-16 message in RA synovial fibroblasts.

THU0050 NOVEL DESCRIPTION OF FUNCTIONAL CHEMOKINE RECEPTORS ON FIBROBLAST LIKE SYNOVIOCYTES

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Background The relevance of chemokines (CK) in rheumatoid arthritis (RA) has been mainly focused on their chemotactic properties to direct selective migration of leukocytes. However, demonstration of chemokine receptors (CKR) on various non-haematopoietic cells is expanding the knowledge of their roles in biology.

Objectives To explore the presence of CKR and their functional status on cultured fibroblast-like-synoviocytes (FLS) obtained from synovial tissues of RA and osteoarthritis (OA) patients.

Methods

Results Flow cytometry analysis with monoclonal antibodies detected expression of CCR2 (MCP-1,2,3 R) on 28 ± 11% FLS, and yielded a wide range of expression of CCR5 (MIP-1a, -1b, RANTES R), CXCR4 (SDF-1a R) and CXCR3 (IP-10, Mig R) on FLS (8–18%) both from RA and OA. CXCR4 mRNA and, to a lower extent, CCR2 mRNA, were detected by RNase protection assays on RA FLS. To assess the functional ability of these CKR in response to putative ligands we performed assays of CKR engagement that typically lead to pertussis toxin-sensitive PLC activation. After stimulation with CK, FURAred-loaded FLS were screened for changes in cytosolic calcium concentration by measuring related fluorescence changes in a FACScan. SDF-1a induced a significant cytosolic calcium influx, as did positive control stimulation with calcium ionophore A23187. A consistent effect from IP-10 or MCP-1 was not detected.

Assays of synoviocyte chemotaxis using two-compartment trans wells showed significant increments in FLS transmigration induced by SDF-1a (224 ± 134%, p < 0,05) and IP-10 (252 ± 31%, p < 0,05) compared to MCP-1, RANTES, MIP-1b or unstimulated FLS (100%). This enhanced migration could be inhibited in the presence of pertussis toxin (57–77% inhibition).

Conclusion This is, to our knowledge, the first description of the presence of CKR on FLS, notably CXCR4. Our data demonstrate that these receptors are functional as it is shown by CK promotion of both calcium mobilization and cell migration. As occurs with leukocytes, CKR could guide synoviocytes through the tissue, likely following a hierarchy of chemotactic signals and therefore, CK might enhance invasive properties of pannus FLS into the cartilage and bone.