induction of DUSP-1 gene expression was mediated by EP2/EP4 receptors coupled to both the protein kinase A and p38 MAP kinase pathways. In the dorsal air-pouch mouse model of synovial inflammation, LPS-treatments provoked air pouch edema and significant leukocyte infiltration (predominantly neutrophils, CDw17 and monocytes, CD11c) after 6–24 h, with concomitant increases in TNF-α, IL-1β, P-1α, MPP-8, MMP-9, and MMP-13 levels in the exudates. Pre-treatment (30 min) with 1 μmol/L of PGE2 or PGE2 mimetics like forskolin/rolipram reduced LPS-induced leukocyte infiltration by 42±5% (mean±SD) while TNF-α, MIP-1α, MPP-9, and MMP-13 expression levels fell by 67 to 91% on average. PGE2-dependent suppression of induced leukocyte infiltration and MMP-9/TNF-α expression were abrogated in DUSP-1 null (−/−) mice.

**Conclusions:** We conclude that PGE2-dependent modulation of molecular and cellular components of the inflammatory/proliferative/catabolic response is mediated, at least in part, by DUSP-1. DUSP-1 may be a promising drug target for modulating MAPK-dependent proliferative responses in arthritis, infectious diseases and cancers.

**439 MITOCONDRIAL DYSFUNCTION ACTIVATES CYCLOOXYGENASE-2 EXPRESSION IN CULTURED NORMAL HUMAN SYNOVIOCYTES**


**Purpose:** Prostaglandin E2 (PGE2) plays a profound role in the pathogenetic processes of rheumatoid arthritis (RA). Recently, it has been reported that mitochondrial alterations may contribute to the progression of RA. In this study, we investigated the relationship between the dysfunction of mitochondrial respiratory chain (MRC) and the in vitro expression of COX-2 in cultured normal human synoviocytes.

**Methods:** Normal human synoviocytes were isolated from knee synovium obtained from necropsy from 9 adult cadavers (mean age 43 years). Commonly used inhibitors of the MRC were employed to induce mitochondrial dysfunction. Rotenone (1 and 10 μg/ml), 3-nitropipronic acid (0.5, 2 and 10 mM), Antimycin A (AA: 5, 10 and 20 μg/ml), Sodium azide (2, 10 and 25 mM) and Oligomycin (5, 10 and 25 μg/ml) were employed as inhibitor of the complex I, II, III, IV and V of MRC, respectively. Protein and mRNA COX-2 expression were analyzed by cytometry and real time PCR. PGE2 levels were evaluated by ELISA. As a positive control, COX-2 expression was induced by IL-1β (1 ng/ml).

**Results:** Firstly, only the exposure of synoviocytes to AA and oligomycin significantly increased COX-2 protein expression in a time- and dose dependent manner. The maximal response was observed at 6 h with a concentration of 20 μg/ml AA and 25 μg/ml oligomycin (15±3.3 and 28.2±10.8 respectively vs. basal 3.6±0.6, n=6). At the same time, the positive control, 1 ng/ml IL-1β, induced a COX-2 protein expression of 45±12.6. When the percentage of cells that expressed COX-2 mRNA was examined by real time RT-PCR the results obtained at 4 h of stimulation were consistent with those of protein expression (30- and 40-fold increase for 20 μg/ml AA and 25 μg/ml oligomycin, respectively, vs. basal 1). The positive control, 1 ng/ml IL-1β, induced a level of COX-2 mRNA expression of 787-fold increase. When the production of PGE2 at 24 h was assessed similar results were obtained (72±23 and 99±38 for AA and oligomycin, respectively vs. basal 23±1). Secondly, we tested if mitochondrial dysfunction induced by AA or oligomycin could modulate the response induced by IL-1β (1 ng/ml) on COX-2 protein expression. We found that pre-treatment of synoviocytes with either 5 μg/ml AA or 10 μg/ml oligomycin for 30 minutes increases significantly the expression of COX-2 induced by IL-1β (1 ng/ml) at protein levels. The values of COX-2 protein expression were 104.7±38.6 for AA + IL-1β and 96.4±24.4 for oligomycin + IL-1β vs. 45.0±12.6 for IL-1β (n=6, p<0.05).

**Conclusions:** These results showed that the dysfunction of mitochondrial respiratory activity induces an inflammatory response in synoviocytes contributing to the chronic inflammation of synovial tissue in RA and aging joint. These data may prove valuable for a better understanding of the participation of mitochondria in the pathogenesis of RA synovium.

**440 ELEVATED LEVELS OF INFLAMMATORY MEDIATOR PROSTAGLANDIN E2 (PGE2) IN EX-VIVO CULTURED PERIPHERAL BLOOD LEUKOCYTES (PBL) OF OSTEOARTHRITIS (OA) PATIENTS**


**Purpose:** OA is a degenerative joint disease causing loss of joint function, pain and physical disability. The articular joint’s diseases tissues (bone, cartilage, synovium) are sites of production of cytokines (e.g., IL-1β, TNF-α) and inflammatory mediators (e.g., prostaglandins, nitric oxide). We hypothesize that circulating blood cells, exposed to inflammatory mediators as they perfuse the OA joint, may act as sensors reflecting OA disease activity and/or burden. In the current study we explored whether PBL from OA patients are primed to produce increased inflammatory mediators compared to PBL from healthy controls.

**Methods:** We recruited 56 patients with knee OA and 8 age-matched healthy controls. QRT-PCR was performed using Applied Biosystems. PGE2 levels were measured by ELISA (Cayman) from stored plasma samples.

**Results:** OA patients produced moderately higher levels of PGE2 than healthy controls in unstimulated plasma at baseline (p=0.081). However, when whole blood from both OA and controls was cultured (24 h) ex vivo (100 and 94 pg/ml respectively), PGE2 in controls did not change, while levels in OA patients increased 300% over baseline (p<0.01). The increased PGE2 production at 24 h ex vivo suggested that OA PBLs may be primed or activated in vivo. We therefore examined mRNA expression of PBL COX-2, IL-1β and TNFα. Each of these transcripts were elevated in OA patients (p<0.02) compared to controls. PBL levels of IL-1β in OA correlated with TNFα levels (r=0.43, p=0.003); increased COX-2 expression correlated weakly with TNFα expression (r=0.213, p<0.003). When stratifying these inflammatory mediator levels in OA patients by NSAID use, we observed higher levels of both baseline PGE2 (p<0.04) and IL-1β mRNA (p=0.04) in NSAID users compared with non-users. Finally, we asked whether evidence of PBL activation correlated with radiographic findings. PBL PGE2 production significantly correlated with semi-quantitative subchondral sclerosis scores (r=0.37, p<0.013) and negatively correlated with osteophyte scores (r=0.268, p<0.05). PGE2 levels trended to correlate with increasing KL scores (p=0.1). Relative expression levels of IL-1β but not TNFα moderately correlated (r=0.263, p<0.05) with WOMAC pain score and not with any other x-ray findings. (p=0.4).

**Conclusions:** OA PBL produce higher levels of PGE2 than do age-matched controls. PGE2 production is associated with increased PBL expression of mRNA for COX-2, IL-1β and TNFα. These data indicate that PBL are activated by exposure to inflammatory stimuli as they circulate through the diseased synovium and bone in patients with OA. We propose that activated PBL can serve as biomarkers for disease activity in OA patients and at risk for disease progression. Whether the activation of PBL confers a risk of endothelial injury and vascular disease over time merits additional evaluation.

**441 COMMON GAMMA-CHAIN CYTOKINES IN PATIENTS WITH EARLY AND END-STAGE OSTEOARTHRITIS**

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**Purpose:** Innate immune system activation has been implicated in osteoarthritis (OA) pathogenesis, but much of what we know about the synovial inflammatory response in this prevalent joint disease is derived from studies of end-stage patients. In this study, we sought to better characterize cytokine production in patients with early signs of knee OA, focusing on the common gamma chain cytokines IL-15 and IL-21, important mediators linking the innate and adaptive immune response.

**Methods:** Synovial membrane (SM) and fluid (SF) specimens were collected from patients with degenerative meniscal tears and early cartilage degeneration undergoing arthroscopic procedures (early OA) and patients undergoing total knee replacement for end-stage OA. Quantitative real-time PCR was used to compare expression of SM cytokines and cell lineage-specific markers. SF cytokine and matrix-metalloproteinase (MMP-1 and MMP-3) levels (proteases implicated in cartilage extracellular matrix remodeling) were quantified by ELISA. Transcript and protein levels were compared in early and end-stage specimens, using the
Mann-Whitney t-test. Correlations between cytokines, proteases, and cell-lineage markers were detected using Spearman’s correlation test.

**Results:** SM II-15 mRNA and SF protein levels were detectable in all early OA patients (18 SM and 11 SF), and in most end-stage patients as well (11/13 SM and 9/10 SF); furthermore protein levels were elevated in the early knee OA patients when compared to end-stage (p = 0.0004). In contrast, SM II-21 mRNA levels were detectable in 11/18 early OA patients and in all end-stage patients, while SF protein levels were measured in 7/10 end-stage and 5/9 early OA patients. II-21 mRNA and protein levels did not differ in the two patient groups. In early OA patients, II-15 transcript levels within the tissue were associated with CD8 transcript levels (r = 0.508, p = 0.04), and II-21 mRNA levels correlated with CD19 (r = 0.746, p = 0.0009) and CD56 (r = 0.742, p = 0.0015) expression, consistent with the known effects of these cytokines on specific lymphocyte subsets. II-15 transcript levels were associated with both II-21 mRNA (r = 0.445, ns trend p = 0.08) and protein levels (r = 0.783, p = 0.017), suggesting coordinate regulation of these two cytokines. Finally, increasing SF II-15 was positively associated with both total (r = 0.582, p = 0.023) and active (r = 0.576, p = 0.025) SF MMP-1 concentration.

**Conclusions:** The common-gamma-chain cytokines, II-15 and II-21, are produced within the joint in the majority of patients with end-stage knee OA, as well as in patients with degenerative meniscal tears and signs of early cartilage degeneration. Furthermore, II-15 levels are elevated in early knee OA, implicating innate immune system activation early in the disease process. The association of II-15 with MMP-1 implicates this cytokine in extracellular matrix remodeling in OA.

**442 ACTIVITY AND EXPRESSION OF HYALURONIDASES ASSOCIATED WITH HYALURONAN SYNTHASES EXPRESSION AND CHANGE OF MOLECULAR WEIGHT OF HYALURONAN IN THE JOINT FLUID**

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**Purpose:** Hyaluronidase (Hyal) and hyaluronic acid synthase (HAS) were cloned in human recently. HAS-1 and HAS-2 synthesize high molecular weight (2 × 10^6 Da) hyaluronic acid (HA), while HAS-3 synthesizes low molecular weight one (2 × 10^5 Da). It has been obscure why the molecular weight of HA in synovial fluid (SF) decreases in the patients with rheumatoid arthritis (RA). In order to study the relationship between the expression patterns of Hyals, HASs, Hyal activity and HA molecular weight in SF, we examined the expression of these enzymes in the articular tissue of patients with RA.

**Methods:** Synovial tissues/fluids were obtained from 58 patients with RA at various stage of inflammation, 27 patients with osteoarthritis and 6 with trauma. We studied Hyals (Hyal-1, -2, -3) and HASs (HAS-1, -2, -3) expression by using mRNA in situ hybridization analysis and immunohistochemistry. In addition, we investigated Hyal activity in SF samples by HA substrate gel enzyme zymography. Then we examined HA molecular weight in SF by HPLC.

**Results:** Hyals were detected in synoviocytes. The number of positive cells of Hyals was higher in RA active stage than in non-inflamed control patients. Utilizing Hyal enzyme zymography, the enzyme activity of Hyal in synovial fluid was higher in the active stage of RA than in the fibrotic one. There was negative correlation between Hyal activity and molecular weight of HA in SF (r = -0.55, p < 0.005) (Fig). The enzyme activity of Hyal in SF was positively correlated with the number of positive cells of Hyals mRNA in synovial tissues. HAS-1, -2 were detected in synoviocytes. On the other hand, HAS-3 was detected in synoviocytes and inflammatory cells. The number of positive cells of HAS-1 and HAS-2 was lower in RA advanced stage although that of HAS-3 positive cells reached maximum level in the active inflammatory stage. Moreover, the number of HAS-3 positive cells was negatively correlated with the HA molecular weight in synovial fluid (r = -0.51, p < 0.005) and positively correlated with histologically defined local inflammatory activity in RA (r = 0.563, p < 0.005).

**Conclusions:** The results suggest that HA may be actively synthesized by HAS-1 or HAS-2 in early stage of RA and become to smaller size by HAS-3 products and degeneration by Hyals in inflammatory stage of RA. The number and distribution of Hyals and HASs can be affected by synovial inflammation.