heme oxygenase-1 (HO-1) pathway, which is able to counteract cellular stress *in vitro* and *in vivo*. The present study was aimed at identifying a possible regulatory effect of HO-1 on HMGB1 in OA synoviocytes.
Methods: Synovial tissue samples were obtained from 15 OA patients undergoing total knee joint replacement. Synoviocytes (fibroblasts and macrophage-like) were obtained by digestion with collagenase IA, cultured in third passage and treated with interleukin-1β (Il-1β, 100 U/ml) for 24 h. HO-1 was induced by treatment with cobalt protoporphyrin IX (CoPP, 10 μM). Matrix metalloproteinase (MMP) activity was determined by fluorometric procedures and HMGB1 release by ELISA. Protein expression was studied by Western blot.
Results: Basal expression of HMGB1 protein was reduced by HO-1 induction in OA synoviocytes. In addition, HMGB1 release into the medium was significantly decreased. Stimulation of synoviocytes with Il-1β resulted in an enhancement of HMGB1 cellular content and release. Our results indicate that both processes are down-regulated by HO-1 overexpression. In addition, HO-1 reduced RAGE expression in these cells. The effects of HO-1 induction were prevented when synoviocytes were transfected with a siRNA specific for human HO-1. In cells without HO-1 induction, HO-1 gene silencing resulted in the up-regulation of HMGB1 and RAGE. Regulation of HMGB1 by HO-1 was accompanied by the inhibition of MMP activity in synoviocytes stimulated with Il-1β.
Conclusions: Our data provide evidence that HO-1 can regulate HMGB1 in OA synoviocytes. Overall, HO-1 signaling appears to be an appropriate target for the development of novel therapies affecting articular disorders.

436 EFFECT OF EPIGALLOCATECHIN GALLATE ON THE INFLAMMATORY RESPONSE OF Il-1-EXPOSED SYNOVIAL FIBROBLASTS

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Purpose: Inflammation is increasingly recognized as contributing to the symptoms and progression of osteoarthritis (OA). Synovitis is a factor that likely contributes to dysregulation of chondrocyte function, favoring an imbalance between the catabolic and anabolic activities of the chondrocyte in remodeling the cartilage. In recent years, significant interest has emerged in the beneficial health effects attributed to the green tea polyphenols. Polyphenols in green tea are potent antioxidants, with the majority of the beneficial effects elicited by epigallocatechin-3-gallate (EGCG), one of the main constituent of green tea.
Among several inflammatory mediators, interleukin-1beta (Il-1) plays a pivotal role in the pathophysiology of OA. We therefore assessed the effect of EGCG on the production of interleukin-8 (Il-8) and interleukin-1 receptor antagonist (Il-1ra) in primary cultured synovial fibroblasts stimulated with Il-1.
Methods: Human synovial fibroblasts were stimulated for 24 hours with Il-1 (10 ng/ml) in the presence or absence of EGCG (0.1−5 μM).
The levels of Il-8 and Il-1ra were measured in cell supernatants by enzyme-linked immunoassay methods. The lack of cell cytotoxicity of EGCG was ensured using the colorimetric MTT assay.
Results: Treatment of synovial fibroblasts with EGCG resulted in a marked inhibition of Il-1-induced Il-8 production. EGCG also increased in a dose-dependent manner the release of Il-1ra by stimulated fibroblasts.
Conclusions: The present study shows that EGCG, at dose comparable with plasma concentration achieved by the consumption of two cups of tea, suppressed the inflammatory response of Il-1-exposed synovial fibroblasts. The suppressive effect of EGCG may be due to the interference with inflammatory signal transduction pathway or may be related to the inhibition of the release and the accumulation of reactive oxygen species. Our findings suggest that EGCG may be of potential therapeutic value in the treatment of OA.

437 EFFECT OF EPIGALLOCATECHIN GALLATE ON CALCIUM CRYSTAL-INDUCED CHEMOTACTIC FACTORS

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Purpose: Although osteoarthritis (OA) is defined as a cartilage disease, synovitis involving mononuclear cell infiltration and overexpression of proinflammatory mediators is common in early and late OA. Calcium crystals deposition is a factor that likely contributes to synovial membrane inflammation.
Polyphenols in green tea are potent antioxidants, with the majority of the beneficial effects elicited by epigallocatechin-3-gallate (EGCG), one of the main constituent of green tea. The aim of our study was to evaluate if EGCG may influence some inflammatory aspects of OA. To this aim we studied the effect of EGCG on chemotactic factors released by human fibroblasts stimulated with calcium crystals, regular features of the most severe forms of OA.
Methods: Human synovial fibroblasts were stimulated with pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals (0.01−0.1 mg/ml) in the presence or absence of EGCG (0.1−5 μM). Il-1beta (10 ng/ml) was used as a positive control.
CPPD and BCP crystals were synthesized by the methods of Cheng and McCarthy respectively. The levels of MCP-1 were measured in cell supernatants by enzyme-linked immunoassay methods. The chemotactic effect of culture supernatants was evaluated on chemotaxis chamber by the migration of fresh-isolated mononuclear blood cells. The lack of cell cytotoxicity of both EGCG and calcium crystals was ensured using the colorimetric MTT assay.
Results: EGCG inhibited MCP-1 release by stimulated fibroblasts in a dose-dependent manner. Supernatants of crystals-stimulated cells loose their ability to induce mononuclear cell migration when EGCG was added in the medium. EGCG inhibited both MCP-1 release and supernatants chemotactic activity of Il-1beta stimulated culture in a dose-dependent manner.
Conclusions: The present study shows that EGCG, at dose comparable with plasma concentration achieved by the consumption of two cups of tea, modify the inflammatory response of calcium crystal-exposed synovial fibroblasts. EGCG may interfere with inflammatory signal transduction pathway and may also inhibits the cellular generation, the release and the accumulation of reactive oxygen species. Our results suggest that EGCG might represents a good candidate for the prevention and treatment of OA.

438 MODULATION OF THE INFLAMMATORY AND CATABOLIC RESPONSE BY PROSTAGLANDIN E2 (PGE2) IS PARTIALLY DEPENDENT ON THE INDUCTION OF DUAL SPECIFICITY PHOSPHATASE 1 (DUSP-1) IN VITRO AND IN VIVO

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Purpose: Prostaglandin E2 (PGE2) is an eicosanoid with pleiotropic properties that binds G-protein coupled receptors, effecting changes in cell signalling through activation of protein kinases and phosphatases. We examined PGE2-dependent control of the dual specificity phosphatase-1 (DUSP-1) in arthritis-affected human synovial fibroblasts (HSF) in culture and in the dorsal air-pouch mouse model of synovial inflammation in vivo.
Methods:
- Cultured human synovial fibroblasts (HSF) were obtained by sequential enzymic digestion from osteoarthritis-affected synovial membranes.
- Western and Northern blot analyses were used to measure protein, phosphorylated protein and mRNA expression, respectively.
- Transient transfection assays were employed to express activated signaling molecules, shRNA constructs, and to analyze reporter luciferase activity.
- Wild type and DUSP-1 null mice were used in the dorsal air pouch studies. Analytes were measured by ELISA and RT-PCR. Cell infiltration measurements were assessed by flow cytometry.
- Statistical analyses included Student's T-test and ANOVA

Results: PGE2 induced a robust (7 fold) and rapid (10 min) increase in DUSP-1 mRNA in cultured HSF, reaching a zenith at 30−60 min followed by decay to control levels. A late phase of DUSP-1 mRNA expression was observed after 4 h and continued for another 20 h. In transient transfection assays using a DUSP-1 promoter-luciferase reporter construct or a luciferase reporter construct harbouring the DUSP-1 mRNA 3′-UTR region fused 3′ prime, PGE2 induced a modest (1.38) increase in promoter (transcriptional) activity while also stabilizing luciferase-DUSP-1 3′UTR mRNA chimeric transcripts (2.6 fold; post-transcriptional regulation). With respect to DUSP-1 protein, PGE2 induced a similar bi-phasic pattern as observed with DUSP-1 mRNA, appearing within 20−30 min, a plateau at 60 min, with protein levels declining to near control levels. A second phase of protein expression was observed from 4 h and DUSP-1 protein was still detectable after 24 h. In terms of measuring DUSP-1 phosphatase activity, PGE2 abrogated rhIl-1β induced phosphorylation of T183/Y185 of SAPK/JNK, a response abolished by knock down of DUSP-1 through targeting by over expression with either a shRNA expression construct or the DUSP-1 inhibitor sanquinarine sulfate. Using specific PGE2 receptor (EP) agonists and antagonists, we observed that the PGE2-dependent

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, MIP-1α, MMP-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, MMP-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 MITOCHONDRIAL DYSFUNCTION ACTIVATES CYCLOOXYGENASE-2 EXPRESSION IN CULTURED NORMAL HUMAN SYNOVIOCYTES

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Purpose: Prostaglandin E_2 (PGE_2) plays a profound role in the pathogenic processes of rheumatoid arthritis (RA). Recently, it has been reported that mitochondrial alterations may contribute to the progression of RA. In this study, we have 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-nitropropionic 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. PGE_2 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 (15.4±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 PGE_2 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

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Purpose: OA is a degenerative joint disease causing loss of joint function, pain and physical disability. The diarthrodial joint's diseased 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 hr ex vivo suggested that OA PBLs may be primed or activated in vivo. We therefore examined mRNA (QPCR) 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 ($r = 0.213$, $p < 0.0003$). 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 TNF-α. 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 and may predict patients 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