Purpose: Monocytes play a pivotal role in controlling tissue inflammation and repair. They infiltrate the tissues and develop into two main subsets of macrophages in response to local signals: inflammatory (M1-) and tissue-repair (M2-) macrophages (Mφ). Here we investigated whether M1 and M2φ differentially modulate chondrogenesis of mesenchymal stem/stromal cells (MSC).
Methods: Monocytes isolated from buffy-coats were cultured for 5 days with GM-CSF or MCSF to induce respectively M1- or M2φ-polarization. MSC were isolated from the bone marrow of a total of 9 patients and expanded for two passages. MSC or skin fibroblasts (as control cells) and polarized macrophages were cultured in collagen scaffolds up to 3 weeks alone or after being mixed (at the ratio 1:1). In order to determine the reached cell number, selected constructs were generated by combining carboxyfluorescein succinimidyl ester (CFSE) labeled MSC with macrophages. To investigate the role of soluble factors in modulating MSC differentiation, conditioned media harvested from M1φ and M2φ were supplemented during the chondrogenic culture of MSC. Resulting tissues and/or isolate cells were assessed histologically (glycosaminoglycan, GAG), biochemically (GAG contents), cytofluorimetrically (percentage of cells expressing or not CD45, and CFSE) and by RT-PCR (Sox9 and collagen-II)
Results: Coculture of MSC/M2φ in scaffolds resulted in statistically significant 1.9-fold higher GAG content than what would be expected (defined as *chondro-induction*). Chondro-induction was lower (1.3±0.4) and less reproducible when coculture was performed with M1φ and did not occur with skin fibroblasts. GAG contents of constructs generated by solely macrophages were undetectable. Histological analyses of constructs confirmed the biochemical results. In the coculture there was no modulation of the chondrogenic genes. As compared to monocultures, in co-culture MSC and M2φ numbers decreased less markedly (at day 7, MSC were 84% and 42% of the initial number, M2φ were 26% of the initial number and undetectable, respectively for co-cultured and monocultured cells).
Conclusions: We have demonstrated that coculture MSC/Mφ2 results in synergistic cartilage tissue formation, which is not mediated by soluble factors alone. Further studies are envisioned to investigate whether Mφ2 modulate the survival of specific MSC sub-populations. Finally, in vivo studies are necessary to assess the clinical relevance of our findings in the context of cartilage repair.

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POSSIBLE CHONDROPROTECTIVE EFFECT OF CANAKINUMAB: AN IN VITRO STUDY ON HUMAN OSTEOARTHRITIC CHONDROCYTES

S. Cheleschi †, N.A. Pascarelli †, O.M. Lucherini †, E. Crainz ‡, S. Tenti †, M. Galeazzi †, G. Collodel §, A. Fioravanti †, L. Cantarini †. † *Dept. of Med., Surgery and NeuroSci., Rheumatology Unit, Siena, Italy;* ‡ *Orthopaedics and Traumatology Clinic, Univ. Hosp. of Siena, Siena, Italy;* § *Dept. of Molecular and Dev.al Med., Siena, Italy*

Purpose: Canakinumab is a human IgGκ monoclonal antibody targeting Interleukin (IL)-1β; its action involves the neutralization of IL-1β signaling, resulting in suppression of inflammation in patients with autoimmune disorders. IL-1β plays an important role also in the destruction of cartilage and it is the most important cytokine in the pathogenesis of osteoarthritis (OA). In response to IL-1β, chondrocytes secrete other proinflammatory cytokines, neutral metalloproteinases (MMPs), nitric oxide (NO); furthermore IL-1β inhibits chondrocytes proliferation and induces apoptosis. The aim of our study was to evaluate the possible *in vitro* effect of canakinumab on cultures of human OA chondrocytes cultivated in the presence or absence of tumor necrosis factor (TNF)-α.
Methods: Human articular cartilage was obtained from the femoral heads of 5 patients (range 63-71 years) with OA according to ACR criteria undergoing surgery for total hip prostheses. The chondrocytes were isolated from the articular cartilage using sequential enzymatic digestion. The primary cultures so obtained were seeded in 6-well plates until confluence. Cells were then incubated with two concentrations (1μg/ml and 10μg/ml) of canakinumab alone or in combination with TNF-α (10 ng/ml) for 48h. In these conditions we evaluated cell viability, release of proteoglycans (PG) and NO in culture medium, inducible nitric oxide synthase (iNOS) and MMP-1, 3, 13 expression, the percentage of apoptosis and necrosis. After 24h we performed IL-1β dosage (ELISA). Finally, we used a transmission electron microscope (TEM) for morphological assessment. Data are expressed as the mean ± standard deviation of triplicate values for each experiment. Statistical analysis was performed using an analysis of variance followed by Bonferroni multiple comparison tests.
Results: Canakinumab alone at the two concentrations studied hasn't cytotoxic effect, while TNF-α caused a significant decrease of the percentage of viable cell ($P < 0.001$). Both concentrations of canakinumab tested significantly restored the cell viability in TNF-α stimulated chondrocytes. As reported in Fig 1A, canakinumab alone at the two concentrations studied didn't modify significantly IL-1β levels in the culture medium, while TNF-α produced a significant increase of IL-1β. Both concentrations of canakinumab tested significantly reduced IL-1β production (Fig 1A). No significant modification of PG levels was observed in the chondrocyte cultures treated with canakinumab alone. The presence of TNF-α determined a significant decrease ($P < 0.001$) in PG levels and canakinumab significantly increased the PG levels in cells cultured with TNF-α in a dose-dependent manner. Incubation of chondrocytes with canakinumab alone didn't affect nitrite release. The presence of TNF-α induced a significant increase ($P < 0.001$) in NO production. However, after co-incubation with TNF-α and canakinumab, at different concentrations, the levels of NO decreased significantly (1μg/ml, $P < 0.01$; 10μg/ml, $P < 0.01$). The data of the NO levels were confirmed by the immunocytochemistry assay for iNOS. TNF-α stimulated chondrocytes displayed a significant increase of MMP-1, 3, 13 gene expression; a significant reduction was shown when cells were co-treated with TNF-α and canakinumab (Fig 1B). Our experiments confirmed the pro-apoptotic effect of TNF-α and demonstrated a protective effect of canakinumab at two concentrations examined. The results concerning biochemical data were further confirmed by the morphological findings obtained by TEM.
Conclusions: It is generally accepted that IL-1β and TNF-α are the pivotal cytokines involved in OA physiopathology. Hence, the neutralization of these cytokines appears to be a logical development for OA therapy. In the present study we showed, for the first time, that canakinumab counteracts the negative effect of TNF-α on OA chondrocyte cultures, probably inhibiting IL-1β signaling.

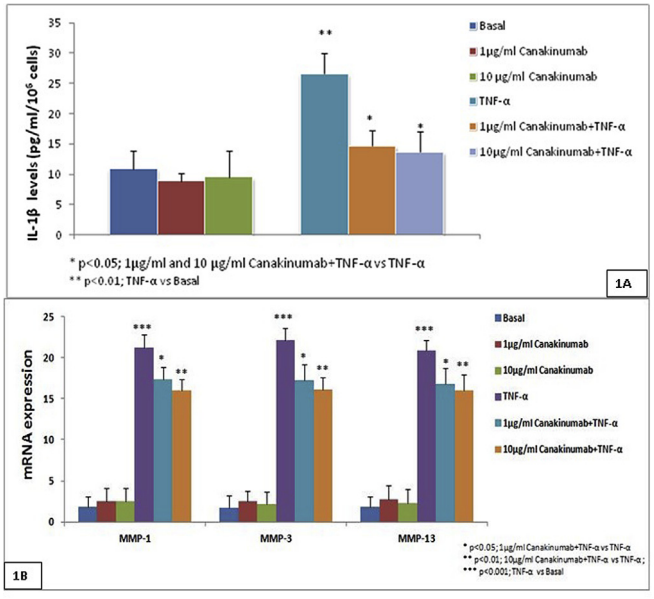

Figure 1. **A)** IL-1β levels in the culture medium; **B)**MMP-1, 3, 13 gene expression.

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BRAZILIN BLOCKS THE INDUCTION OF NFKB1/P50 IN HUMAN CHONDROCYTES AND SYNOVIOCYTES

D. Bieder †, S.M. Walzer †, R. Windhager †, H. Viernstein ‡, S. Toegel †,‡. † *Karl Chiari Lab for Orthopaedic Biology, Dept. of Orthopaedics, Med. Univ. of Vienna, Vienna, Australia;* ‡ *Dept. of Pharmaceutical Technology and Biopharmaceutics, Univ. of Vienna, Vienna, Australia*

Purpose: Brazilin is one major constituent of Caesalpinia sappan and is known to possess anti-bacterial, anti-oxidative, and anti-inflammatory activities. Osteoarthritis (OA) creates a disequilibrium between the catabolic and anabolic activities of chondrocytes in remodeling the