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AB0058 CELL-TYPE SPECIFIC REGULATION OF IL-1R SIGNALING BY R835, A DUAL IRAK1/4 INHIBITOR

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Background: Interleukin-1 beta (IL-1b) is a key mediator of the inflammatory response and is known to exacerbate damage during chronic disease and acute tissue injury. Through association with the adaptor protein Myd88, interleukin receptor associated kinases (IRAK)1 and 4 initiate signaling downstream of IL-1Rs resulting in the activation of the NFkB and MAPK pathways and the production of proinflammatory cytokines (1). IL-1Rs are broadly expressed across cell types and little is known about differences in signaling between cell types and the role of IRAK1 and IRAK4 kinase activity.

Objectives: We have identified a potent and selective IRAK1/4 inhibitor, R835, that substantially suppressed the elevation of LPS (TLR4 agonist)-induced serum cytokines in healthy human volunteers in a recent phase 1 study. The aim of this study was to evaluate the effect of R835 on IL-1R signaling in primary human fibroblasts and endothelial cells.

Methods: Human dermal fibroblasts, lung fibroblasts or endothelial cells were stimulated with IL-1b and the effect of R835 on the signaling pathway was evaluated by western blotting. Human dermal fibroblasts were stimulated with different amounts of IL-1b to evaluate both the signaling pathways activated and the cytokines produced. The ability of R835 to inhibit cytokine production induced by high or low amounts of IL-1b in dermal fibroblasts was assessed.

Results: In human endothelial cells, inhibition of IRAK1/4 kinases with R835 resulted in a block of IL-1b-induced IRAK4 phosphorylation, IRAK1 degradation and downstream NFkB, p38 and JNK activation. In contrast, in both human primary dermal and lung fibroblasts stimulated with IL-1b, we observed potent inhibition of IRAK4 phosphorylation, IRAK1 degradation, and downstream JNK phosphorylation, but no inhibition of NFkB pathway proteins and only weak inhibition of p38. Upon titration of IL-1b we observed that dermal fibroblasts produced IL-8 and GRO in response to low levels of IL-1b (20pg/ml), and produced additional cytokines including G-CSF and GM-CSF with higher levels of IL-1b (400pg/ml). In the presence of low levels of IL-1b (20pg/ml), we observed a weak activation of NFkB pathway proteins and p38, compared to a very robust NFkB, p38 and additional JNK activation in the presence of higher levels of IL-1b (400pg/ml). Consistent with these results, in dermal fibroblasts, R835 showed little to no inhibition of IL-8 and GRO induced by low levels of IL-1b, but potently inhibited G-CSF and GM-CSF induced by high levels of IL-1b where JNK was activated.

Conclusion: This study has elucidated signaling differences between cell types downstream of the IL-1R. In endothelial cells, as in myeloid cells, the kinase activity of IRAK1 and IRAK4 is required for the activation of all downstream signaling. Unexpectedly, in human fibroblasts, IRAK1/4 kinase activity appears to primarily regulate the JNK pathway, and not the NFkB pathway. Concomitant with that, only the cytokines induced by the additional activation of JNK in fibroblasts are regulated by a dual IRAK1/4 inhibitor. Clinically, an IRAK1/4 inhibitor may show select inhibition of IL-1b-induced cytokines depending on the tissue and cell type involved in inflammation.

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AB0059 CLINICAL SIGNIFICANCE OF CIRCULATING MYELOID-DERIVED SUPPRESSOR CELLS IN PATIENTS WITH ANKYLOSING SPONDYLITIS

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Background: Myeloid-derived suppressor cells (MDSCs) represent heterogeneous population of immature myeloid cells with immunosuppressive functions. The important role of MDSCs is indicated for cancer, but their role in autoimmune pathology is currently controversial. Considering the clinical heterogeneity of ankylosing spondylitis (AS) and involvement of innate immunity in AS pathophysiology the investigation of the MDSC role in AS is of great interest.

Objectives: The aim of our study is to investigate the number of MDSC subsets in AS patients with different clinical manifestations, activity, disease duration, and treatment options and to evaluate the ability of MDSCs to mediate immunosuppressive function in AS patients.

Methods: The study included 34 patients with AS. Ankylosing Spondylitis Disease Activity Score (ASDAS) was used to assess disease activity and high activity was determined as ASDAS $\geq$ 2.1. The frequencies of monocytic (M-MDSC) (HLADR-CD14 +), granulocytic (G-MDSC) (lin-HLADR-CD33+ CD66 +) and early-stage (eMDSC) (lin-HLADR-CD33 + CD66-) MDSCs and biomarkers of MDSC functional activity including of Arg-1, IDO, PDL1 were determined in the peripheral blood by flow cytometry.

Results: We found significant elevation in the frequency of both M-MDSC and G-MDSC in the total group of patients compared to healthy controls (HC) (P=0.00006 and P=0.008 respectively), while eMDSCs did not differ from HC. Analysis of MDSCs populations in patient subgroups showed expansion of G-MDSCs in patients with axial plus peripheral damages (P=0.004), while M-MDSCs were elevated regardless of the presence (P=0.002) or absence (P=0.001) of peripheral manifestations. Moreover, the percentage of M-MDSCs was positively correlated with ASDAS in patients with axial disease only (R=0.8; P=0.03). Patients with low activity of disease demonstrated significant elevation of only M-MDSCs compared with HC (P=0.001). Patients who had high activity of disease had increase in both M-MDSCs and G-MDSCs (P=0.008 and P=0.005 respectively). By comparing the frequency of MDSCs in patient groups with different AS duration we showed increase in percentage of both M-MDSCs and G-MDSCs in patients with relatively short duration of disease (< Me=11.5 years) (P=0.002 and P=0.005 respectively) and elevation in M-MDSCs only in patients with longer AS duration (P=0.0003). Compared with patients receiving conventional therapy (NSAIDs, csDMARDs), patients who received biological agents (TNF α inhibitors) had lower disease activity but despite this showed elevated frequencies of M-MDSCs and PMN-MDSCs, comparable to patients receiving conventional therapy. Of note, M-MDSCs in AS patients had increased expression of PDL-1 and IDO (P=0.04 and P=0.02 respectively) and similar to HC expression of Arg-1. The expression of Arg-1, IDO, PDL1 in patients G-MDSCs did not differ from HC.

Conclusion: The data obtained indicate that both M-MDSCs and G-MDSCs are elevated in AS patients. However, the increase of G-MDSCs is associated with peripheral manifestations of AS, high activity, longer duration, and the percentage of M-MDSCs was positively correlated with activity in patients with axial disease only. The unchanged expression of Arg-1, PDL-1 and IDO in G-MDSCs and enhanced expression of PDL-1 and IDO in M-MDSCs suggest MDSCs capacity to mediate immunosuppressive function in AS patients.

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AB0060 STING AND PROINFLAMMATORY CYTOKINES IN SYNOVIAL FLUID OF PATIENTS WITH DIFFERENT ARTHRITIDES

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Background: STING (stimulator of interferon genes) is a cytosolic protein that is found in endoplasmic reticulum (ER) membrane, mitochondria and mitochondria-associated membranes. Although it is well established that STING plays an important role in innate immune responses, its potential involvement in rheumatic disease processes remains to be clarified (1).

Objectives: The aims of this study were to evaluate the levels of STING and its relationship with local inflammation in the synovial fluid (SF) of patients with psoriatic arthritis (PsA), rheumatoid arthritis (RA), gout, calcium pyrophosphate (CPP) crystal-induced arthritis (CPP-IA), osteoarthritis (OA) and OA with CPP crystals (OA+CPP).

Methods: SF was collected from the knees of 60 untreated patients: 10 with PsA, 10 with RA, 10 with gout, 10 with CPP-IA, 10 with OA and 10 with OA+CPP. SF was examined under optical light microscopy. White cell count (WBC) and the polymorphonuclear cell (PMN) percentage were determined in SF according to standard procedures. SF IL-8, IL-6, IL-1 β and extra- and intra-cellular STING levels were assayed by ELISA.

Results: The levels of WBC were higher in SFs from gouty patients ($27.7 \pm 20.56 \times 10^3/\text{mm}^3$), while OA and OA+CPP patients showed the lowest WBC count ($0.34 \pm 0.3 \times 10^3/\text{mm}^3$, $0.3 \pm 0.28 \times 10^3/\text{mm}^3$). SFs from inflammatory arthritis contained elevated percentages of PMN (gout: $85.5 \pm 10.86\%$, CPP-IA: $84 \pm 11.31\%$, RA: $80.33 \pm 8.14\%$, PsA: $42.6 \pm 35.97\%$). Extracellular STING was determined in OA ($440 \pm 413.31 \text{ pg/ml}$), OA+CPP ($225 \pm 205.06 \text{ pg/ml}$) and CPP-IA ($475 \pm 7.07 \text{ pg/ml}$) SF, while was not detectable in RA, PsA and gout. Intracellular STING levels were similar and the highest in SFs from gout ($96.4 \pm 35.44 \text{ pg/ml}$) and RA ($90.64 \pm 23.13 \text{ pg/ml}$), while remained under detection limit only in SFs from PsA. SF concentration of IL-6 was lower in OA ($354.87 \pm 377.56 \text{ pg/ml}$) and OA+CPP ($389.56 \pm 104.14 \text{ pg/ml}$) as compared with inflammatory arthritides (PsA: $3807.14 \pm 489.86 \text{ pg/ml}$; RA: $17354 \pm 2334.87 \text{ pg/ml}$; gout: $19359 \pm 84.85 \text{ pg/ml}$; CPP-IA: $20389.56 \pm 104.14 \text{ pg/ml}$). The patients with gout and RA had the highest levels of IL-8 ($2159.54 \pm 347.09 \text{ pg/ml}$; $2039.6 \pm 97.74 \text{ pg/ml}$) and IL-1 β ($35.93 \pm 20.46 \text{ pg/ml}$; $44.36 \pm 23.16 \text{ pg/ml}$), while OA showed the lowest concentrations (IL-8: $23.21 \pm 11.32 \text{ pg/ml}$; IL-1 β : $0.47 \pm 0.13 \text{ pg/ml}$). In the total group of patients, we found a negative correlation between extracellular STING and IL-8 ($r = -0.53$; $p = 0.004$) and IL-1 β ($r = -0.47$; $p = 0.012$). There was a positive correlation between intracellular STING and IL-8 ($r = 0.54$; $p = 0.017$), IL-1 β ($r = 0.77$; $p < 0.001$) and IL-6 ($r = 0.69$; $p = 0.009$).

Conclusion: This study is the first to define the presence of STING in SF of different arthritides. The high levels of extracellular STING in OA, OA+CPP and CPP-IA SFs may be due to the activation of factors that reduce its interaction with the ER. The effect of downregulating factors in PsA might explain the low concentration of intracellular STING in these patients.

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AB0061 INVOLVEMENT OF CELL-FREE DNA IN THE DIFFERENTIATION OF OSTEOCLASTS – IMPLICATIONS FOR CLINICAL RESEARCH

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Background: Cell-free DNA (cfDNA) is a molecule with undisputable immunogenic potential^[1]. Its role is well established in etiopathogenesis of systemic lupus erythematosus and many reports suggest it may play an important role in other rheumatic diseases^[2]. Patients suffering from rheumatic diseases are at higher risk of osteoporosis^[3], which may be a consequence of increased generation of osteoclasts (OC). Traditionally, increased formation of OC is attributed to changes in cytokine network^[4]. It is not yet known whether cfDNA can also act on mononuclear precursors and influence their differentiation into OC.

Objectives: The aim of this study was to assess role of cfDNA in differentiation of bone resorbing cells in rheumatic diseases. For this purpose, deoxyribonuclease I (DNase) was employed to eliminate cfDNA from sera of patients and healthy controls (HC) used for cell culture.

Methods: In order to evaluate effects of cfDNA on osteoclastogenesis, an *in vitro* experiment of peripheral blood monocytes (PBM) cultures was designed. Axial spondyloarthritis (AxSpA) was chosen as a model of rheumatic disease. Sera of

AxSpA patients (total n=13) and healthy controls (total n=13) were collected and subsequently used in cell cultures. PBM of 13 healthy donors were cultured in presence of 25 ng/ml macrophage colony-stimulating factor (M-CSF), 0.5 ng/ml receptor-activator of nuclear factor kappa B ligand (RANKL) and 10 % of pooled AxSpA or HC sera, respectively. Serum counterpart treated with DNase was used in parallel. After 14 days, cells were stained for tartrate-resistant acid phosphatase (TRAP) and counted. TRAP positive cells with 3 or more nuclei were recognized as OC.

Results: On average, differentiation of OC in presence of AxSpA sera led to higher osteoclastogenesis compared to DNase-treated counterpart (137 vs. 61; $p < 0.01$). In a similar way, treatment of HC sera with DNase greatly diminished osteoclastogenesis compared to untreated sera (17 vs. 111; $p < 0.05$). Overall, the effect of cfDNA depletion in HC sera regarding OC differentiation was more than 2.5x higher than the effect of cfDNA depletion in AxSpA sera ($p < 0.001$).

Conclusion: Results of our pilot study indicate possible involvement of cfDNA in osteoclastogenesis. Decrease in number of OC after cfDNA depletion in both AxSpA sera and HC sera suggests that cfDNA effect in osteoclastogenesis is non-specific with respect to healthy condition and may represent universal link between innate immunity and bone metabolism. Lower effect of cfDNA depletion in AxSpA sera on osteoclastogenesis is very possibly owing to presence of other inflammatory molecules, which influence osteoclastogenesis. Future research might identify cfDNA as useful therapeutic target to decrease osteoporosis risk not only in patients with rheumatic and autoimmune diseases, where pathological bone resorption is imminent, but also in general aging population.

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AB0062 CHARACTERIZATION OF IL-12 AND IL-23 REDUCTION BY TOFACITINIB IN MDCs

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Background: Psoriatic arthritis (PsA) is a chronic inflammatory auto-immune disease characterized by an excessive production of pathogenic mediators that cause inflammation of the skin, peripheral joints, entheses and the spine. Among these, interleukin (IL)-23, IL-12, the IL-17 family and TNF constitute key players in PsA pathogenesis.^{1,2} IL-23, consisting of IL23A (IL-23p19) and IL23B (IL-23p40) subunits, is predominantly produced by myeloid dendritic cells (mDCs). While the p19 subunit is unique to IL-23, the p40 subunit is shared with IL-12. Together, IL-12 and IL-23 play a crucial role in promoting the differentiation of naïve T lymphocytes into T helper (Th) interferon (IFN)- γ -producing Th1 or IL17-producing Th17 cells, respectively.³

Small-molecule inhibitors, such as the JAK/STAT inhibitor Tofacitinib, have recently shown promising therapeutic potential in PsA clinical trials.⁴ The inhibition of JAKs by Tofacitinib results in the direct suppression of multiple intracellular signaling pathways which constitute key hubs in the cytokine network.⁵ However, whether Tofacitinib is able directly target IL-12/IL-23 production by mDCs has not yet been documented. Suppression of these canonical inflammatory pathways would provide further evidence that Tofacitinib is an effective drug in halting both innate and adaptive immune responses.

Objectives: To evaluate the transcriptional and molecular events underlining IL-12 and IL-23 regulation by Tofacitinib in mDCs.

Methods: Peripheral blood mononuclear cells from healthy donors were isolated by Ficoll gradient. Monocytes and myeloid dendritic cells (mDCs) were isolated by using magnetic beads on autoMACS. Monocytes were cultured for 6 days in the presence of IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) to generate monocyte-derived dendritic cells (moDCs). MoDCs were harvested, washed and put to rest for 1 day prior to stimulation, while mDCs