Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2020-eular.335

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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Disclosure of Interests: Sylvia Braselmann Shareholder of: Shareholder of Rigel Pharmaceuticals, Employee of: Employee of Rigel Pharmaceuticals, Ernest Tai Shareholder of: Rigel Pharmaceuticals, Employee of: Rigel Pharmaceuticals, Roy Frances Shareholder of: Rigel Pharmaceuticals, Employee of: Rigel Pharmaceuticals, Chi Young Shareholder of: Rigel Pharmaceuticals, Employee of:

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DOI: 10.1136/annrheumdis-2020-eular.3852

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.

Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2020-eular.2998

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

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