Active JAK/STAT Signaling in Circulating Leucocytes Defines Distinct Immunologic Endotypes of Rheumatoid Arthritis

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Background: In rheumatoid arthritis (RA) stratification is considered an important step towards the development of patient-tailored therapeutic concepts. The fact that less than 50% of RA patients experience a substantial improvement in response to any single biologic therapy has brought up the idea that yet unidentified subtypes of RA (endotypes) might exist. This concept is in line with distinct microscopic patterns of synovitis found in biopsies of RA joints.[1] Furthermore, a subset of RA patients has leucocytes with interferon driven gene expression, whereas the majority of RA patients does not[2]. Interferons activate receptor associated Janus kinases leading to phosphorylation of STAT1 and STAT2. Other STAT family members are activated by cytokines such as IL-6 (STAT3) or IL-15 (STAT5). Therefore, the phosphorylation pattern of the different STAT molecules in circulating leucocytes might mirror the specific cytokine milieu of a given patient.

Objectives: To define endotypes of RA based on the phosphorylation patterns of the different STAT molecules in circulating leucocytes.

Methods: Cross-sectional study of 63 patients with established RA fulfilling the 2010 EULAR/ACR criteria (mean age: 64.5 ± 1.7 (SEM) years, female ratio: 0.79). Ten healthy subjects served as a control group. Flow cytometry was performed to detect the phosphorylated forms of STAT1-6 in Monocytes, Granulocytes, B cells, naïve-, effector-, and memory-T cells of the CD4+ and CD8+ lineage. All steps from blood draw to cell fixation were performed at 4°C to prevent auto-activation of leucocytes. The mean fluorescence intensity (MFI) of fluorochrome labeled antibodies against phosphorylated STATs in the different leucocyte populations was used for statistical analysis. MFIs were correlated with disease activity measured by the cDAI. MFIs of populations with elevated STAT phosphorylation not associated with disease activity were analyzed by unsupervised hierarchical clustering. The resulting groups were validated by principal component analysis. Finally, criteria for patient assignment to specific groups by MFI were generated by calculating ROC-curves.

Results: Pronounced e*x vivo* phosphorylation of STAT1-6 in any leucocyte population was detected in 20 of 63 (48%) RA patients but not in healthy subjects (n=10). Active STAT5 signaling in Monocytes,

naïve CD4+ T cells and CD4+ effector T cells was significantly associated with disease activity. Unsupervised hierarchical cluster analysis of RA patients based on pSTAT MFIs not associated with disease activity resulted in 3 groups: 1) Patients with active STAT1 and STAT3 signal in Monocytes and Granulocytes (n=14/63, 22%), 2) Patients with active STAT5 signal in naïve CD8+ T cells, CD8+ effector T cells and CD4+ memory T cells (n=16/63, 25%) and 3) Patients without active STAT signal in any leucocyte population (n=33/63, 52%). cDAI, CRP, ESR, current treatment, RF and ACPA status did not differ significantly between the groups. To test if the assignment to a group changed over time, we performed a second analysis of STAT phosphorylation after 3-6 months. Eighty percent of the patients tested (12/15) were re-assignment to their initial group.

Conclusions: We identified three distinct RA endotypes based on active STAT signal. Whether patients within different endotypes respond differently to a given therapy will be subject to further research.

References

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