inadequately responding to RA treatments. Lacking predictive biomarkers of response or personalised medicine approaches to guide use of targeted therapies in RA patients, EULAR and ACR guidelines [1,2] both recommend to loop over available ts- and bDMARDs as long as the patient's response is considered as inadequate. Meanwhile, for historical reasons and despite the similar clinical efficacy of these drugs, anti-TNFs have a large dominance in medical practice.

Objectives: Determine in what proportions the different mechanisms targeted by existing DMARDs families are dominant in RA patients synovial tissue.

Methods: Retrospective analysis of 7 private or public datasets of 300 rheumatoid arthritis patients, consisting in all cases of transcriptomic data from synovial biopsies. Three datasets come from the RheumaKit platform [3,4] (low-density microarrays or qPCR). Four other datasets are publicly available on Gene Expression Omnibus (GSE89408, GSE45867, GSE97165, GSE21537, produced on Illumina, KTH or Affymetrix platforms). Each mechanism of action is associated to a « drug target complex » (DTC), consisting of the genes coding for proteins directly targeted by the DMARDs of interest. For a given dataset, computations are made in four steps: 1) each patient is described by its different DTC values (an average of the expression values of the members of each DTC) 2) each patient is scored for each of its DTC value. Each of these DTCs are scored as a percentile of the corresponding DTC distribution over the full dataset from which a given patient's data is extracted. This gives, for a single patient, as many percentiles as defined DTCs. 3) for each individual patient, a ranking of DTC values is then operated. This allows to overcome the fact that absolute numerical values of two different DTCs should not be compared. 4) A summary statistic is then computed over each dataset separately, to conclude which DTC is dominant in which proportion of the cases among this dataset.

Results: This analysis exhibits different dominance patterns for RA-related mechanisms of action in individual patients. Statistically, no unique mechanism is shown dominant in a majority of patients; On the four public datasets, where all DTCs of interest are available, averaged dominance proportions across datasets are : IL1 15%, IL6 20%, TNF 11%, B Cells 20%, JAK 17%, T Cells 7%. About 10% of the patients exhibit equivalent dominance patterns between multiple DTCs. On all seven datasets, this analysis also outputs weak or moderate correlations between the dominance levels of multiple DTCs.

Conclusion: These results highlight large variability in metabolic patterns underlying RA; such observation is consistent with the similar efficacy observed for b or tsDMARDs when evaluated in clinical trials. Herein we show that TNF-dependent pathways is dominant in only a relatively small proportion of RA patients, whereas non-TNF-dependent pathways (e.g. IL-6 or JAK-dependent pathways) are more dominant. This work pleads for a more balanced use of available treatments. In addition, these results support further investigations towards precision medicine-oriented approaches, namely based on biomarkers from synovium, in the treatment of RA.

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THU0036

ACTIVATED MEMORY T CELLS PRODUCE LIGANDS THAT CAUSE NF-KB-DEPENDENT INFLAMMATORY ACTIVATION OF THE ENDOTHELIUM: IDENTIFICATION OF NOVEL THERAPEUTIC TARGETS

Kim Jeucken^{1,2}, Jan Piet van Hamburg^{1,2}, Sander W. Tas^{1,2}. ¹Amsterdam Rheumatology and Immunology Center, Amsterdam, Netherlands; ²Amsterdam UMC, Experimental Immunology, Amsterdam, Netherlands

Background: Endothelial cells (EC) are important contributors to inflammation via expression of inflammatory mediators, including cytokines, chemokines and adhesion molecules. Production of these inflammatory mediators can be induced via canonical and NF- κ B-inducing kinase (NIK)-dependent noncanonical NF- κ B signalling. The ligands activating these pathways are well studied, but less is known about the cells producing ligands that can activate NF- κ B signalling in EC.

Objectives: To study the effects of factors produced by activated memory T (T_m) cells on NF- κ B dependent inflammatory activation of EC.

Methods: CD4⁺CD45RO⁺ memory T cells were isolated from healthy PBMC using MACS sorting and cultured in presence of anti-CD3 and anti-CD28 for 72h, after which supernatant was harvested. Endothelial cells were stimulated for 72h with 50% T_m supernatant (T_m sup) after which protein and RNA was harvested followed by analysis of NF- κ B signalling and downstream expression of inflammatory mediators using qPCR and western blot. Culture supernatants were analysed by ELISA for various inflammatory mediators. To repress canonical NF- κ B signalling an inhibitor of IKK β (iIKK β) was used and to repress NIK-dependent NF- κ B signaling an inhibitor of NIK (iNIK) was used in the EC cultures.

Results: Stimulation with T_m sup led to activation of both canonical NF- κ B signalling (increased levels of phosphorylated I κ B α) and noncanonical NF- κ B signalling (increased p100 to p52 processing). After stimulation with T_m sup EC had increased mRNA levels of all tested inflammatory mediators compared to non-treated cells. Gene expression of chemokines, cytokines, and growth factors (CXCL1, CXCL5, IL6, IL8 and GM-CSF) in T_m sup stimulated EC was significantly reduced after treatment with iIKK β and to a lesser, but still significant, extent after treatment with iNIK. Treatment with iIKK β also led to a reduction in mRNA levels of the adhesion molecules VCAM-1 and ICAM-1, while this effect was less pronounced after iNIK treatment. Of note, treatment with either IKK β or iNIK led to a significant reduction of CXCL5 in the culture supernatant of T_m sup stimulated EC.

Conclusion: This study provides new insights into the cellular interactions leading to production of inflammatory mediators by EC. Our findings demonstrate that activated T_m cells produce factors that can cause NF- κ B-dependent inflammatory activation of EC. Targeting canonical NF- κ B signaling via IKK β or NIK-dependent NF- κ B signaling reduces inflammatory activation of the endothelium and may be a potential novel therapeutic target.

Disclosure of Interests:

None declared

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THU0037

ASSOCIATION BETWEEN PRO-INFLAMMATORY CYTOKINE POLYMORPHISMS AND JUVENILE IDIOPATHIC ARTHRITIS

Jae Hyun Jung, Jae-Hoon Kim, Sung Jae Choi, Gwan Gyu Song. Korea University College of Medicine, Seoul, Korea, Rep. of (South Korea)

Background: Juvenile idiopathic arthritis (JIA) is the most common chronic arthritis of childhood and has a variety of subtypes, but the etiology is unclear. Proinflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α play a major role in the development of JIA. Single nucleotide polymorphisms (SNPs) may be associated with susceptibility, phenotype, progression, and treatment of JIA, and the relevance of more than 20 SNPs and JIA has been studied.

Objectives: We investigated whether IL-1, IL-6, and TNF- α polymorphisms were associated with susceptibility to JIA.

Methods: A meta-analysis was conducted on the associations between IL-1 α -899 C/T, IL-1 β -511 C/T, IL-6-174 G/C, and TNF- α -308 G/A and -238 G/A polymorphisms, and JIA. A literature search was performed using PubMed and Embase. The strength of the association between these cytokine polymorphisms and risk of JIA was estimated using the crude odds ratio (OR) and 95% confidential interval (CI). We performed meta-analyses using 1) allelic contrast, 2) recessive, 3) dominant, and 4) additive models. The heterogeneity between studies was assessed with the Cochran Q test, and the heterogeneity among studies was tested with the *I*² statistic.

Results: A total of 27 studies included 4,678 JIA cases and 7,634 control subjects. There were 23 studies on Caucasians, 3 on Asians, and 1 on Africans. Seven studies were on IL-1 α -899 C/T, 5 were on IL-1 β -511 C/T, 6 were on IL-6-174 G/C, 19 were on TNF- α -308 G/A, and 14 were on TNF- α -238 G/A polymorphisms. Meta-analysis of IL-1 α -899 C/T, IL-1 β -511 C/T, IL-6-174 G/C, and TNF- α -308 G/A and -238 G/A polymorphisms revealed no significant associations between JIA and minor alleles (OR 0.99, 95% CI 0.87–1.12, P = 0.84; OR 1.10, 95% CI 0.97–1.25, P = 0.15; OR 1.04, 95% CI 0.91–1.19, P = 0.57; OR 1.09, 95% CI 0.87–1.37, P = 0.46; OR 0.85, 95% CI 0.63–1.13, P = 0.26). Subgroup analysis was conducted on the relationship between JIA and proinflammatory cytokines based on ethnicity. In Caucasians, there was no significant association between IL-1 α -899 C/T, IL-6-174 G/C, and TNF- α -308 G/A and -238 G/A polymorphisms and JIA; however, there were significant relationships between JIA and recessive (TT vs. CT + CC) and additive (TT vs. CC) models of IL-1 β -511 C/T polymorphisms (OR 1.48, 95% CI 1.09–2.00, P = 0.01; OR 1.46, 95% CI 1.05–2.03, P = 0.02).

Conclusion: IL-1 α -899 C/T, IL-1 β -511 C/T, IL-6-174 G/C, and TNF- α -308 G/A and -238 G/A polymorphisms were not significantly associated with overall JIA susceptibility in all ethnicities. However, differences according to ethnicity were observed, and the TT genotype of IL-1 β -511 C/T was associated with a high prevalence of JIA in Caucasians.

Figure 1. OR and 95% CI of individual studies and pooled data for the association between minor alleles of TNF- α -308 G/A and JIA in all study subjects.

Abstract THU0037 – Figure 1

Figure 2. OR and 95% CI of individual studies and pooled data for the association between the IL-1 β -511 C/T polymorphisms and JIA in Caucasian subjects. (A) recessive and (B) additive models.

Abstract THU0037 – Figure 2

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THU0038 INDIVIDUAL FUNCTIONS OF THE HISTONE-ACETYLTRANSFERASES CBP AND P300 IN REGULATING THE INFLAMMATORY RESPONSE OF RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

Marcel Gabathuler¹, Monika Krosel^{1,2}, Christoph Kolling³, Matija Tomsic², Oliver Distler¹, Caroline Ospelt¹, Kerstin Klein¹. ¹Center of Experimental Rheumatology, Department of Rheumatology, University Hospital Zurich, Zurich, Switzerland; ²University Medical Centre Ljubljana, Department of Rheumatology, Ljubljana, Slovenia; ³Schulthess Clinic, Zurich, Switzerland

Background: The two coactivators of transcription cAMP-response element binding protein (CREB) binding protein (CBP) and p300 are close homologues. They are widely accepted as redundant proteins and unique functions have not been investigated in depth. Both proteins contain a histone acetyltransferase (HAT) activity for writing of cell type specific activating H3K27 histone acetylation marks, and a bromodomain, which functions as a reader of acetylated lysine residues on histone tails. Inhibitors targeting the bromodomains are in drug development for inflammatory and malignant diseases.

Objectives: To analyze individual functions of CBP and p300 in regulating the inflammatory response of rheumatoid arthritis (RA) synovial fibroblasts (SF).

Methods: SF were isolated from knee, shoulder and hand joints of RA patients undergoing joint replacement surgery. The expression of CBP and p300 was silenced by transfection of antisense LNA gapmeRs (12.5 nM). 24h after transfection, cells were stimulated with TNF- α (10 ng/ml, 24h). The levels of H3K27ac in SF were analyzed by Western blotting (n=7). Transcriptomes were determined by RNA-seq (Illumina NovaSeq 6000, n=6). Pathway enrichment analysis of RNAseq data was performed using DAVID bioinformatic resources (fold change > 1.5, FDR < 0.05, top 3000 genes included). Changes in the mRNA expression of potential target genes were confirmed by quantitative Real-time PCR (n=12-14).

Results: Silencing of p300 reduced the levels of H3K27ac by 30% in unstimulated SF, and by 61.4% ($p < 0.05$) in presence of TNF- α , whereas silencing of CBP reduced H3K27ac by 43.5% only in presence of TNF- α . In line with the changes in the H3K27ac, silencing of p300 affected the expression of 6026 and 5138 genes in unstimulated and stimulated SF, respectively. In contrast, only 285 and 1911 genes were affected by CBP silencing in unstimulated and stimulated SF, respectively. 184 genes were affected by both, CBP and p300, in unstimulated SF, with 9.2% of genes being regulated in opposite directions. 13.5% of overlapping genes affected by CBP and p300 were regulated in opposite directions in TNF- α -stimulated SF. Principal component (PC) analysis of RNAseq data separated TNF- α -stimulated from unstimulated SF (PC1) and p300 gapmeR-transfected SF (PC2) from controls and CBP gapmeR-transfected SF, which clustered together. The top pathways regulated by CBP were 'cell cycle' and 'focal adhesion' in unstimulated cells and 'DNA replication' and 'cell cycle' after stimulation with TNF- α . Top pathways regulated by p300 in presence and absence of TNF- α were 'proteasome', 'spliceosome' and 'focal adhesion'. The expression of 16 chemokines and cytokines was changed in RNAseq data (fold change > 1.5, $p < 0.05$) by either silencing of CBP or p300. Whereas silencing of CBP reduced the expression of all of them, silencing of p300 had pro- and anti-inflammatory effects. We further confirmed expression changes in cytokine and chemokine expression by Real-time PCR. Silencing of CBP reduced the expression of IL6, CCL2 ($p < 0.01$), CXCL31 ($p < 0.05$), and CXCL10. Silencing of p300 reduced the expression of CCL2 and CXCL31 ($p < 0.001$) but increased the expression of IL8 ($p < 0.001$) and CXCL2 ($p < 0.05$).

Conclusion: Our results suggest that p300 is the major writer for H3K27ac marks in SF. We have identified overlapping and distinct functions for CBP and p300 in SF. CBP inhibition has anti-inflammatory effects. In contrast, p300 inhibition has pro- and anti-inflammatory functions.

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