activated memory T cells produce ligands that can activate NF-κB-dependent inflammatory activation of the endothelium: identification of novel therapeutic targets

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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-κB-inducing kinase (NIK)-dependent noncanonical NF-κB signalling. The ligands activating these pathways are well studied, but less is known about the cells producing ligands that can activate NF-κB signalling in EC.

Objectives: To study the effects of factors produced by activated memory T (Tm) cells on NF-κB 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% Tm supernatant (Tm sup) after which protein and RNA was harvested followed by analysis of NF-κB 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-κB signalling an inhibitor of IKKβ (iIKKβ) was used and to repress NIK-dependent NF-κB signalling an inhibitor of NIK (iNIK) was used.

Results: Stimulation with Tm sup led to activation of both canonical NF-κB signalling (increased levels of phosphorylated IκBα) and noncanonical NF-κB signalling (increased p100 to p52 processing). After stimulation with Tm 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 Tm sup stimulated EC was significantly reduced after treatment with iIKKβ and to a lesser, but still significant, extent after treatment with iNIK. Treatment with iIKKβ 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 iIKKβ or iNIK led to a significant reduction of CXCL5 in the culture supernatant of Tm 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 Tm cells produce factors that can cause NF-κB-dependent inflammatory activation of EC. Targeting canonical NF-κB signalling via iIKKβ or NIK-dependent NF-κB signalling reduces inflammatory activation of the endothelium and may be a potential novel therapeutic target.

Disclosure of Interests: None declared

Individual functions of the histone-acetyltransferases CBP and p300 in regulating the inflammatory response of rheumatoid arthritis synovial fibroblasts

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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 gagmeRs (12.5 nM), 24h after transfection, cells were stimulated with TNF-α (10 ng/ml), 24h. 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. 13.5% of overlapping genes 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 gagmeR-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), CXC3L1 (p<0.05), and CXCL10. Silencing of p300 reduced the expression of CCL2 and CXC3L1 (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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