



EFFECT OF SAHA AND C-JUN N-TERMINAL KINASE INHIBITOR ON THE INDUCIBLE MRNA EXPRESSION OF INTERLEUKIN-17

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ABSTRACT

Interleukin-17 (IL-17A) is a critical cytokine for immune defence against extracellular bacterial and fungal infections. Excess production during chronic inflammation has been associated with many inflammatory and autoimmune disorders. On the other hand, the inducible expression of many cytokine genes is regulated by the receptor-activated intracellular signaling pathways, including the JNK pathway, and different epigenetic mechanisms, including histone deacetylation by HDACs. We investigated the comparative effect of the HDACs inhibitor Suberoylanilide Hydroxamic Acid (SAHA) and an inhibitor of the JNK signaling pathway in the regulation of the inducible IL-17 expression at the mRNA level in PBMCs from healthy donors. For the detection of IL-17 mRNA transcripts was used qRT-PCR. We detected significantly increased levels of IL-17mRNA under the stimulation of the PBMC cultures with lipopolysaccharide (LPS) or C3 binding glycoprotein (C3bpg) in the presence of an inhibitor of the JNK transduction pathway. The inhibition of the JNK signaling pathway leads to the upregulation of the expression of IL-17mRNA. SAHA did not demonstrate a significant effect on the IL-17mRNA transcription compared to the inhibitor of the JNK pathway. In conclusion, we suppose that the synthesis of IL-17 mRNA is regulated by both the JNK transduction pathway and HDAC activity, but with different effects.

Keywords: IL-17 gene expression, JNK inhibitor, HDAC,

INTRODUCTION:

The interleukin-17 family is a family of pro-inflammatory cytokines having a critical role in the immune defense against extracellular bacterial and fungal infections. The excess production during chronic inflammation has been associated with many inflammatory and autoimmune disorders, including rheumatoid arthritis, asthma, lupus, psoriasis, and multiple sclerosis. IL-17A cytokine is produced by CD4+ and CD8+ T cells, $\gamma\delta$ T cells, and various innate immune populations in response to IL-1 β and IL-23 [1]. Ultimately, this leads to the attraction and activation of neutrophils and macrophages to the site of inflammation, the production of antimicrobial peptides, and the improvement of barrier function [2]. The regulatory T cells and anti-inflammatory cytokines IL-10, TGF- β , and IL-35 control inflammation that is driven by IL-17, and dysregulated IL-17 production can lead to autoimmune diseases [3, 4]. JNK is a serine-threonine protein kinase which activates c-Jun, a part of the transcription factor AP-1 [5]. In the cells of the immune system, JNK is a crucial factor in the transcription control of inflammatory and anti-inflammatory cytokine genes such as TNF- α , IL-12p40, IL-10, and IL-23 that has been established in previous studies of our laboratory [6, 7]. On the other hand, the epigenetic mechanism of IL-17 gene expression regulation is not yet fully elucidated. In recent years, the potential clinical applications of epigenetic drugs, for example, in cancer therapy, are widening, and some of these are based on the regulation of gene expression by acetylation and deacetylation of histones [8]. These chromatin modifications are performed by two types of enzymes, named Histone Acetylases (HATs) and Histone Deacetylases (HDACs), which can regulate gene expression [9]. Histone deacetylase inhibitors (HDI) are a group of organic molecules that inhibit histone acetylation and the function of HDACs and hold the acetylation level of histones. Suberoylanilide hydroxamic acid (SAHA) is a selective HDI to the class I and class II HDAC enzymes. SAHA was FDA-

approved in 2006 for the treatment of cutaneous T-cell lymphoma as a drug named vorinostat [10]. SP600125 is a potent, cell-permeable, selective and reversible inhibitor of c-Jun N-terminal kinase (JNK). It inhibits, in a dose-dependent manner, the phosphorylation of JNK. The aim of the current study was to investigate the effect of the HDACs inhibitor Suberoylanilide Hydroxamic Acid (SAHA) and the JNK signaling pathway inhibitor in the regulation of the inducible IL-17 expression at the mRNA level in PBMCs from healthy donors.

MATERIALS AND METHODS

The peripheral blood (10 ml) was taken from 14 healthy volunteers (8 males and 6 females, age: 30-50 years), nonsmokers. The informed consent was obtained from each participant. PMBC cultures were undergone stimulation with 30 µg/ml C3 binding glycoprotein (C3b_{gp}) isolated as described previously [11] or 1 µg/ml Lipopolysaccharide (LPS) from *Escherichia coli* serotype 026:B6 in a presence or not of JNK inhibitor or SAHA. The cells were incubated at 37°C for 6 h, after that, were centrifuged at 1800 rpm for 10 min, and the cell pellet was collected for total RNA isolation. An hour before the stimulation was added inhibitor of JNK kinase or an inhibitor of HDACs to some of the PBMC cultures. For the inhibition of c-jun N-terminal kinase, we used the selective anthracycline inhibitor SP600125, and for the inhibition of HDACs, we used the HDAC inhibitor SAHA. All culture reagents were obtained from Sigma Merck, Germany.

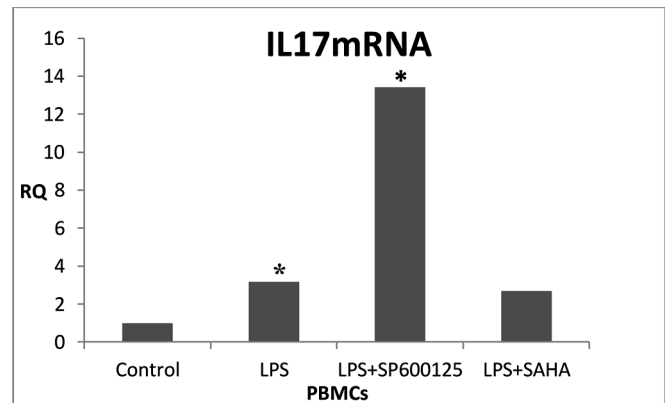
The extracted RNA was quantified by spectrophotometric analysis and applied for the synthesis of cDNA by a High-Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a 7500 Real Time PCR System.

The student's t-test was used to compare the data, and the results were considered statistically significant at $p < 0.05$. The data are expressed as a mean and standard error (SE).

RESULTS

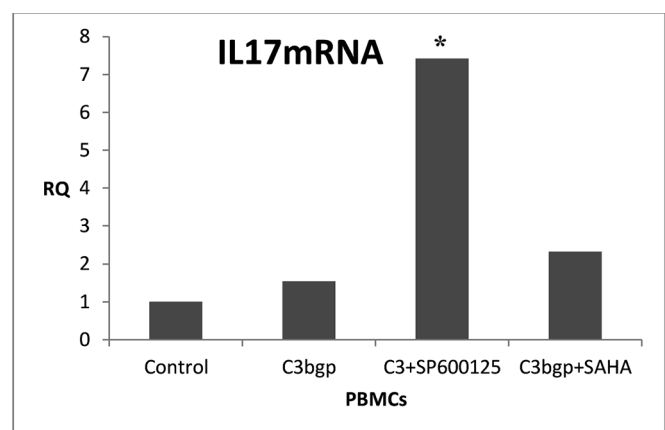
Figure 1 presents the results which show that the transcription of IL-17 mRNA is significantly upregulated upon stimulation with LPS with a mean level of $RQ 3.18 \pm 0.93$, $p=0.032$. In addition, a statistically significant increased level of IL-17 mRNA was observed in PBMCs of healthy donors cultured with LPS and pretreated with the SP600125 compared to cultures without this JNK inhibitor (13.41 ± 5.09 vs 3.18 ± 0.93 , $p=0.031$). Regarding the HDACs inhibitor - SAHA, we didn't find a significant effect on the quantities of transcript in the treated PBMCs cultures.

Fig. 1. IL-17 levels of mRNA transcription in PBMC of healthy donors after stimulation by LPS with or no inhibitor SP600125 and SAHA. The cells were harvested at 6 h after stimulation. The results are presented as mean $RQ = 2^{-\Delta\Delta Ct}$. * p -value <0.05



The results presented on Figure 2 showed that after the stimulation of PBMCs with C3b_{gp} statistically significantly increased level of IL-17 mRNA was measured in the cultures pretreated with JNK pathway inhibitor (SP600125) compared to the group stimulated with C3b_{gp} alone ($RQ 7.43 \pm 2.40$ vs. 1.54 ± 0.37 , $p=0.04$). Moreover, the level of IL-17 mRNA was slightly higher in PBMCs pretreated with SAHA than in the cultures treated only with C3b_{gp} ($RQ 2.32 \pm 0.87$ vs. 1.54 ± 0.37 , $p=0.5$).

Fig. 2. IL-17 levels of mRNA transcription in PBMC of healthy donors after stimulation by C3b_{gp} with or no inhibitor SP600125 and SAHA. The cells were harvested at 6 h after stimulation. The results are presented as mean $RQ = 2^{-\Delta\Delta Ct}$. * p -value <0.05



DISCUSSION

The activation of leucocytes as a part of innate immunity is carried out mainly by pattern recognition receptors (PRRs), which are responsible for recognizing the common pathogen associated molecular patterns [12]. Lipopolysaccharide is an endotoxin, the main component of the outer membrane of Gram-negative bacteria, and it is a typical example of a ligand for particular PRRs, named toll-like receptor 4 (TLR-4) on many immune cells in PBMC

fraction. LPS stimulation of human mononuclear cells by TLR-4 activates several intracellular signaling pathways that include the I κ B kinase (IKK)–NF- κ B pathway and three well-defined mitogen-activated protein kinase (MAPK) signaling pathways: c-Jun N-terminal kinase (JNK), p38 MAPK and extracellular signal-regulated kinases (ERK) 1 and 2 [13,14]. These signaling pathways, in turn, activated the downstream transcription factors that include NF- κ B and activator protein-1 (AP-1), which translocate to the nucleus and induce pro-inflammatory cytokine expression [15]. c-Jun is a main unit of the AP-1 transcription factor family, which activation results in the expression of inflammatory genes in mononuclear cells [16]. This pathway activation explains our results for the upregulation of IL17 mRNA in PBMC stimulated with LPS. The selective JNK inhibitor implicated in the current study, SP600125 inhibits the phosphorylation of c-Jun and thus prevents the formation of AP-1[17]. The observation of significantly higher levels of IL17 mRNA after adding SP600125 in PBMC cultured with LPS shows that the transcription factor AP-1 is not the main regulator of this cytokine expression. Moreover, other intracellular signaling pathways interfering with TLR4 signaling induced the transcription of IL17 mRNA under the conditions of the blocked JNK signaling pathway. This trend was also maintained in cell cultures pretreated with the SP600125 inhibitor and stimulated with C3bpg, suspecting the same reason, and perhaps p38 MAPK and NF- κ B are highly involved in conditions of inhibited JNK regarding IL17 gene transcription regulation.

The present study also investigates the effect of the HDACs inhibitor Suberoylanilide Hydroxamic Acid (SAHA) in the regulation of the inducible IL17 gene transcription at the mRNA level in PBMCs from healthy donors. SAHA

inhibits the enzymes from class I (HDAC1, HDAC2, HDAC3) and HDAC6 from class II HDACs [18]. HDAC 1 has an enhancing effect on T-cell mediated immune response by the demonstration that mice with a T cell-specific deletion of HDAC1 revealed significantly decreased serum levels of inflammatory cytokines IL-17 and IL-6 [19]. Bing Yan et al. demonstrated that HDAC6 deficiency increases the production of IL-17 by V γ 4+ $\gamma\delta$ T cells in the spleen and lymph nodes in mice and suggested inhibition of HDAC6 activity in $\gamma\delta$ T cells promotes the expression of IL-17 in vitro[20]. These new findings partially overlap with our results obtained from cell cultures stimulated with C3bpg in the presence of SAHA. These PBMCs present higher levels of IL17mRNA than the cultures without the inhibitor of HDACs but without statistical significance. A better understanding of the molecular mechanisms of regulation of IL-17 gene expression would provide important insights regarding the practical approach in the treatment of autoimmune conditions mediated by this cytokine.

CONCLUSION

Our results indicate that the JNK transduction pathway is significantly involved in the regulation of IL-17 gene expression, following LPS and C3bpg stimulation, in contrast to HDAC activities. Inhibition of the JNK signaling pathway leads to the upregulation of IL-17 gene expression at the mRNA level.

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