

Investigating interaction between IL-17A, IL-17E, TNF- α and rs3783605 and its effect on VCAM-1 promoter activity in the human umbilical vein endothelial cells

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ABSTRACT

The present study is created to determine the effect of rs3783605 and its interaction with IL-17A, IL-17E and TNF- α cytokines on the action of VCAM-1 gene promoter in human umbilical vein endothelial cells (HUVEC). Recent research shows that IL-17A and TNF- α cytokines have main effect on VCAM-1 expression, and IL-17E as a proinflammatory ligand for IL-17 receptor homolog IL-17Rh1 might also partake in this process. Two vectors with altered rs3783605 alleles are fabricated to express the GFP. Unlike the other two cytokines, HUVECs stimulated with TNF- α alone show an increase of expression. The stimulation with IL-17A in addition to TNF- α show an increase of expression in G allele vector, while the stimulation with IL-17E in addition to TNF- α led to an increased expression in the cells containing an allele. The stimulation by IL-17A in addition to IL-17E led to decrease of expression in the cells containing G allele and stimulation with three cytokines simultaneously showed a decrease of expression in G allele vector.

Keywords: SNP; promoter; VCAM1; HUVEC; IL-17E

INTRODUCTION

Vascular cell adhesion molecule 1 (VCAM-1) is a protein present on the surface of the endothelial cells, stromal bone marrow, skeletal muscle and some hematopoietic cells [1,2]. This adhesion molecule is a receptor for circulating both immune cells and neoplastic cells, and therefore has important role in immune responses and tumor metastases [1]. Some single nucleotide polymorphisms found in the VCAM-1 promoter are determined to be correlated with many human disorders [1].

On the other hand, VCAM-1 expression is affected by the inflammatory cytokines, TNF- α and IL-17A [2-7]. In the VCAM-1 promoter, many binding sites for transcription factor (including NF-kB, GATA, SP1, ETS, AP1 sites) have been reported to have regulatory roles (inhibitory or stimulatory) in the VCAM-1 expression [3]. These transcription factors are activated by different cytokine stimulations [4,6-10]. To determine the role of the ETS2 binding site polymorphism (rs3783605) and inflammatory cytokine effects on the regulation of cytokine-mediated VCAM-1 induction in human endothelial cells, we examine the consequences of interaction between IL-17A, IL-17E, TNF- α (involved

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in ETS factors stimulation) and rs3783605 on activity of VCAM-1 promoter in the human umbilical vein endothelial cells (HUVECs).

MATERIALS AND METHODS

Plasmid expression vectors

The expression vectors used in this research include vectors containing CMV promoter, promoter-less vector and vectors comprised of VCAM1 promoter with various alleles of rs3783605. The reporter gene of these vectors is the Green Fluorescent Protein (GFP); the details of which have been previously described [11].

Cell culture, transfection and treatments

HUVECs were obtained from Pasteur institute (Iran) and cultured in DMEM/Ham's F12 medium containing 10 % fetal bovine serum, 100 IU/ml penicillin and 100 μ g/ml streptomycin (Gibco co., USA) at 37 °C and 5 % CO₂ in 75 cm² flasks. Following three passages, about 7x10⁴ cells were seeded per well in 4 ml of growth medium of a 6-well plate for 24 h prior to transfection. Then, HUVECs were transfected by TurboFect transfection reagent (Thermo Scientific, USA), according to the manufacturer's protocol. After transfection for 16 hours, stimulation of cells occurred with TNF- α , IL-17A and IL-17E, and also with combination

of double cytokines including: TNF- α with IL-17A, TNF- α with IL-17E and IL-17A with IL-17E, as well as combination of triple cytokines including: TNF- α , IL-17E in addition to IL-17A for 7 h. In all treatments, the concentration of TNF- α used was 5 ng/ μ l, while 10 ng/ μ l of IL-17A and IL-17E was used. Unstimulated cells served as controls. After 7h, observation of fluorescence activity was followed by harvesting through trypsin-EDTA dissociation; then, the pellet of cells was prepared for subsequent RNA extraction.

Real-time PCR

For total RNA extraction, we used Ribospin kit (GeneAll Biotechnology Co., South Korea) and cDNA Synthesis Kit (Enzymomics, South Korea) for cDNA synthesis, according to the manufacturer protocol. For quantitative PCR (qPCR), Real QPCR 2X master mix with green dye without ROX (Ampliqon A/S, Denmark) was utilized. In this research, the gene of interest was EGFP, while GAPDH—the housekeeping gene—acted as a control.

Statistical analysis

Data was analyzed as previous reported by Relative Expression Software Tool for Rotor-Gene (REST-RG, version 3) [11].

RESULTS

Fluorescent microscopy results

Images were captured by fluorescence microscopy. Bright green fluorescence is observed in the HUVECs that were exposed to transfection by expression vector containing CMV promoter. There is no fluorescent signal in cells transfected with the promoter-less vector. Qualitative evaluation about the presence of fluorescent signal is performed for every treatment. Their pictures are not presented here.

Effects of interaction between IL-17A, IL-17E, TNF- α and -420A SNP on EGFP expression

Our results show that stimulation of HUVECs with 10 ng/ml IL-17A and 10 ng/ μ l IL-17E for 7 h decreases VCAM-1 expression compared with unstimulated cells but stimulation by 5 ng/ml TNF- α alone increases the gene expression. The HUVECs stimulated with 10 ng/ml IL-17A in addition to 5 ng/ml TNF- α demonstrate a decreased VCAM-1 expression, while stimulation with 10 ng/ml IL-17E in addition to 5 ng/ml TNF- α leads to an increased VCAM-1 expression in the cells. The HUVECs stimulated with 10 ng/ml IL-17A in addition to 10 ng/ml IL-17E lead to an increase of VCAM-1 expression.

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The HUVECs stimulated with 10 ng/ml IL-17A and IL-17E in addition to 5 ng/ml TNF- α exhibit a decrease of VCAM-1 expression. The results of stimulation with TNF- α and IL-17A both alone and combination with stimulation with TNF- α in addition to IL-17E are significant, while the rest are insignificant. The fold expression level of these treatments are shown in Figure 1.

Effects of interaction between IL-17A, IL-17E, TNF- α and -420G SNP on EGFP expression

Our results indicate that stimulation of HUVECs with 10 ng/ml IL-17A, 10 ng/ μ l IL-17E and 5 ng/ml TNF- α alone for 7 h significantly decreases VCAM-1 expression compared to unstimulated cells. The

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HUVECs stimulated with 10 ng/ml IL-17A in addition to 5 ng/ml TNF- α show an increase of VCAM-1 expression, and the stimulation with 10 ng/ml IL-17E in addition to 5 ng/ml TNF- α leads to a decrease VCAM-1 expression in the cells. The HUVECs stimulated with 10 ng/ml IL-17A and IL-17E in addition to 5 ng/ml TNF- α show a significant decrease of VCAM-1 expression. The results of these treatments are summarized in Figure 2.

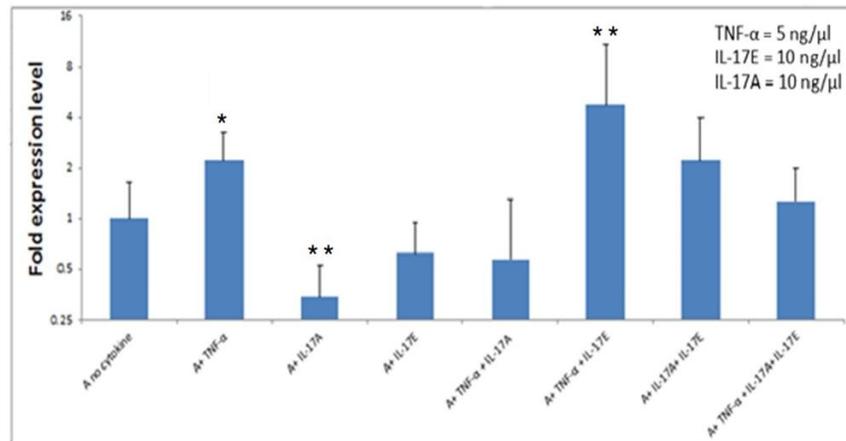


Figure 1. Effects of interaction between IL-17A, IL-17E, TNF- α and -420A SNP on the EGFP expression. Statistically significant effects include: (TNF- α treatment alone) P- Value = 0.045, (IL-17A treatment alone) P-Value = 0.004, (TNF- α + IL-17E) P-Value = 0.010.

A: Allele A, IL-17E: Interleukin E, IL-17A: Interleukin A, TNF- α : Tumor necrosis factor α .

*Adjusted-P \leq 0.05. **Adjusted-P \leq 0.01. Values are represented as mean \pm SD (n = 3).

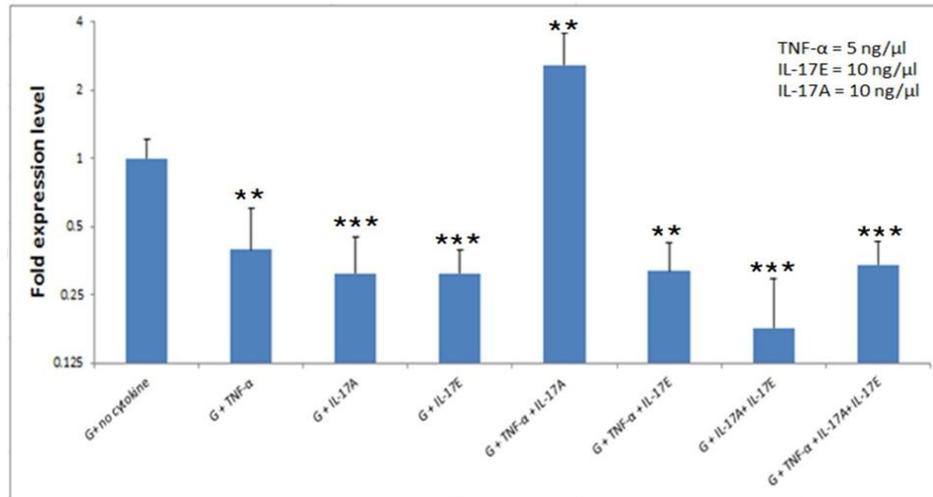


Figure 2. Effects of interaction between IL-17A, IL-17E, TNF- α and -420G SNP on the EGFP expression. Statistically significant effects: (TNF- α) P- Value = 0.003, (IL-17A) P-Value = 0.001, IL-17E P-Value = 0.001, (TNF- α + IL-17A) P-Value = 0.002, (TNF- α + IL-17E) P-Value = 0.007, (IL-17A + IL-17E) P-Value = 0.001, (TNF- α + IL-17A+ IL-17E) P-Value = 0.001.

G: allele G. ** Adjusted-P \leq 0.01. *** Adjusted P \leq 0.001. Values are represented as mean \pm SD (n = 3).

In this study, we investigate the consequence of interaction between IL-17A, IL-17E, TNF- α and rs3783605 on the activity of VCAM-1 promoter in the human umbilical vein endothelial cells (HUVECs).

It has been reported that rs3783605 is the candidate binding site for transcription factor ETS2 [1]. According to the literature, there is a 2-fold increase in transcriptional activity of the G allele compared with the A allele of this SNP [11]. Likely, this polymorphism affects the interaction among transcription factors (including ETS2) with VCAM-1 promoter, along with interactions between transcription

factors themselves that can influence VCAM-1 gene expression.

There are many transcription factor binding sites (including GATA, NFkB, AP1, OCT, ETS and SP1 binding sites) in the VCAM-1 promoter that are involved in cell cycle control [2,3]. The activity pattern of the VCAM-1 promoter regulates the pattern of its expression, all of which depends on the outcome of interaction between silencer elements and cytokine dependent enhancers [2].

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Some transcription factors activate VCAM-1 gene expression, including GATA, NFkB, AP1 and ETS factors. [3,12,13] Stimulation with the respective cytokines can result in activation of multiple signaling pathways within the cell and ultimately lead to

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activation of specific transcription factors (Figure 3). All three cytokines can lead to activation of NF-Kb, AP1 and ETS factors [4,5,11,14-18].

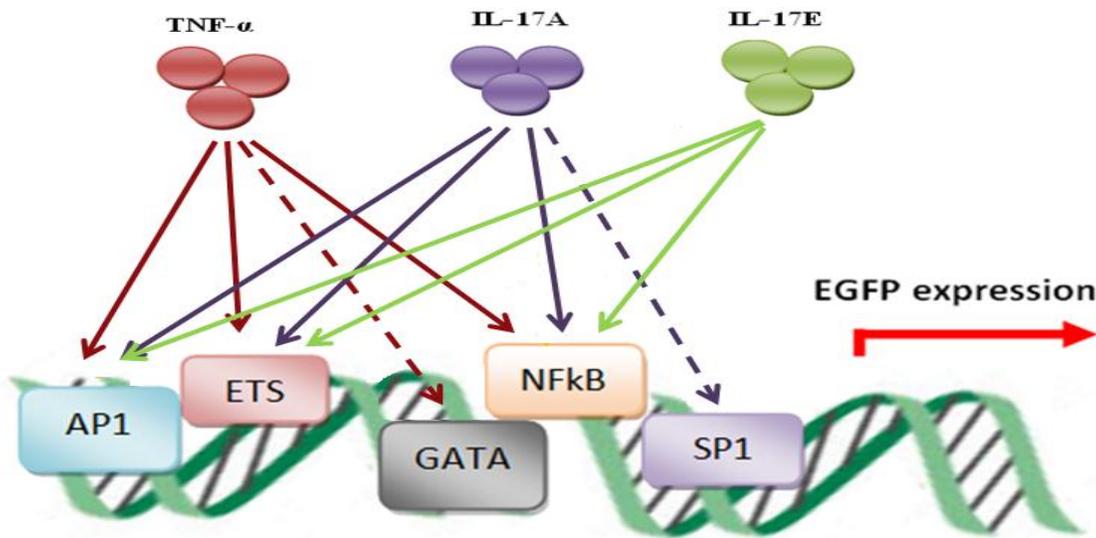


Figure 3. The effects of TNF- α , IL-17A and IL-17E cytokines in the activation of transcription factors involved in VCAM-1 gene expression [4,5,11,14-18].

DISCUSSION

In this study, we investigate the effect of interaction between TNF- α , IL-17A and IL-17E cytokines and VCAM-1 promoter

for 7 hours. We observed that the amount of target gene expression under the stimulation with IL-17A and TNF- α would reach its peak approximately after 7 h [14] The results can be interpreted in three states of stimulation with one, two and three

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cytokines that can affect the pattern of VCAM-1 promoter activity.

As mentioned in the results, cytokine stimulation by IL-17A and IL-17E lead to a decrease in gene expression and stimulation by TNF- α increases the gene expression. In this study, it is revealed that the upstream region of -500 bp (which include the AP1, ETS and OCT binding sites) has a crucial role in determining the activation pattern of VCAM-1 gene expression. On the other hand, the type of genetic allele is influential in gene expression after stimulation by cytokines.

The outcomes of stimulation by combination of two cytokines (double cytokine) on the pattern of VCAM-1 promoter activity are different in two alleles. It is likely that rs3783605 is involved in the interaction of transcription factors with binding sites on the promoter, as well as interaction between transcription factors and, therefore, can affect the transcriptional activity. Thus, the two promoters will have different patterns of response and affect the pathogenesis of VCAM-1 associated disorders.

As noted in the results, likely because of interaction between different transcription factors and their interaction with promoter, combined stimulation of three cytokines

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(triple cytokine interaction) can lead to a decrease of VCAM-1 expression in G allele. Therefore, future studies with VCAM-1 promoter should provide basic scientific information about the effects of interaction between different cytokines and their effects on the VCAM-1 expression. Data from these studies can be useful in the treatment of various diseases in the future.

CONCLUSION

As the results showed, simultaneous stimulation with different cytokines yields different results from VCAM-1 gene expression. This suggests that even an increase in gene expression by a cytokine, such as TNF- α , can lead to a decrease in gene expression by stimulation simultaneously with another cytokines. On the other hand, the interaction between IL-17A, IL-17E, TNF- α cytokines and rs3783605 can lead to different results based on two alleles of this polymorphism. Data from this study can be useful in the treatment of diseases in the future.

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