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Increased Expression of Aryl Hydrocarbon Receptor in Peripheral Blood Mononuclear Cells of Patients with Autoimmune Hepatitis

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ABSTRACT

BACKGROUND

Previous studies have indicated an elevated level of serum Interleukin (IL)-22 in patients with autoimmune hepatitis (AIH). However, there are no experimental data on the master transcription factor (aryl hydrocarbon receptor) that plays an important role in the development of T helper type 22 (Th22) cells as major producers of IL-22. The aim of the present study was to examine the expression of aryl hydrocarbon receptor in patients with AIH and in normal controls.

METHODS

Levels of mRNA transcripts were measured in the peripheral blood mononuclear cells of 18 patients with AIH and compared with 18 normal controls by a quantitative real-time polymerase chain reaction.

RESULTS

mRNA expression of aryl hydrocarbon receptor was significantly higher in patients with AIH compared with the healthy control group ($P = 0.006$).

CONCLUSION

Th22 cells may play an important role in the pathogenesis of AIH.

KEYWORDS:
Autoimmune hepatitis, Autoimmunity, Th22 lymphocyte

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INTRODUCTION

Autoimmune hepatitis (AIH) is an inflammatory liver disorder and its etiology remains unknown. Nonetheless, as the name of the disease implies, the immune system appears to play an important role in disease pathogenesis.[1] To date, the precise, underlying immune mechanism(s) that are involved in initiating abnormal immune response toward hepatocytes remain unknown. However, several hypotheses have been suggested for the exaggerated or inappropriate response of the immune system to normal liver constituents. Perturbations of T cells homeostasis appears to be an important immunologic event in AIH and several observations indicate the importance of CD4+ T helper (Th) cells and related cytokines in disease progression and maintanence.[2] Th22 is a recently recognized CD4+ subpopulation of Th cells, which seems to be involved

in the pathogenesis of several inflammatory and autoimmune diseases.[3] It produces different cytokines such as interleukin (IL)-22, IL-13, and tumor necrosis factor alpha (TNF-α), of which IL-22 is the dominant functional cytokine. Moreover, such helper celss exploit distinct developmental signaling pathways and aryl hydrocarbon receptor (AhR) has been reported to be a critical transcription factor of them.[4] This characteristic provides a valuable molecular tool for distinguishing Th22 subpopulation from other important sources of IL-22 such as Th17 cells. Because Th17 development is largely dependent on RORγ (retinoic acid-related orphan receptors γ) transcription factor but not AhR.[5]

Therefore, the specific aim of the present study was to measure the mRNA expression levels of Th22-restricted transcription factor in patients with AIH.

In this study, we evaluated the expression of AhR in the peripheral blood mononuclear cells (PBMCs) of patients with autoimmune hepatitis.

MATERIALS AND METHODS

Blood sampling and processing

Inclusion criteria were the diagnosis of AIH confirmed by a certified hepatologist and a signed written informed consent form before enrolling in the study. Exclusion criteria were exposure to a hepatotoxic drug or herbal medication, receiving immunosuppressive therapy, presence of chronic inflammatory and autoimmune diseases except AIH.

18 adult patients who referred to the outpatient clinic of the Digestive Disease Research Center (DDRC) affiliated to Tehran University of Medical Sciences between November 2014 and December 2016 were included in this study. Their diseases were newly diagnosed with no prior treatment for AIH. The diagnosis of the disease was made according to the diagnostic criteria defined by International Autoimmune Hepatitis Group Report.[6]

Baseline factors that were assessed in this study included sex, age, liver enzymes [alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT)], and immunoglobulin G (IgG) levels. The control subjects were selected from healthy population referred to health centers for routine examinations. They did not have a history of chronic inflammatory autoimmune or infectious diseases and were matched to the patients by sex and age.

5 mL venous blood was taken from the patients and controls in heparinized tubes. PBMCs were separated from the whole blood by Ficoll density-gradient centrifugation (Pharmacia, Uppsala, Sweden). Plasma samples were also prepared, aliquoted into 1.5 mL microcentrifuge tubes and stored at −20°C until analysis.

All the participants signed written informed consent before blood donation. The study was conducted according to the 1975 declaration of Helsinki and approved by the Ethics Committee at Tehran University of Medical Sciences through reference no. IR.TUMS.REC.1395. 2855.

RNA isolation from PBMCs and cDNA synthesis

Total RNA were extracted from PBMCs using RibospinTM (GeneALL, Seoul, Korea) according to the manufacturer's instructions and then converted to cDNA (Fermentas, Germany). All cDNA samples were stored frozen at -20ºC until use.

Reverse transcription-quantitative polymerase chain reaction (qRT-PCR) analysis

qRT-PCR was conducted on the cDNA samples with two primer-pairs using SYBR Premix EX Taq II (Takara, Japan) on a Rotor Gene 6000 (Corbett Life Science, Australia) thermal cycler.

The PCR was performed in a total volume of 10 µL containing 5-µL SYBR Premix, 1-µL cDNA, 0.5-µL of forward and reverse primers, and 3-µL double distilled water. The amplification took place in a two-step PCR and thermal cycling conditions consisted of an initial denaturation step for 2 min at 95ºC followed by 40 and 42 cycles of 5 s at 95°C (denaturation) and final step of 45s at 64ºC for AhR and 20s at 63ºC for β-actin.

PCR primer pairs were designed using OLIGO software (National Biosciences) and purchased from Gene Fanavaran (Tehran, Iran). The specific primer sequences used for this study were as follows:

AhR: Forward 5′-CAAATCCTTCCAAGCGGCATA-3′
AhR: Reverse 5′-CGCTGAGCCTAAGAACTGAAAG-3′
β-actin: Forward 5′-AGACGCAGGATGGCATGGG-3′
β-actin: Reverse 5′-GAGACCTTCAACACCCCAGCC-3′

ΔCt method was used for relative quantification of gene expression. ΔCt values were determined by subtracting the threshold cycle (Ct) value for the housekeeping gene β-actin from the Ct value for the gene of

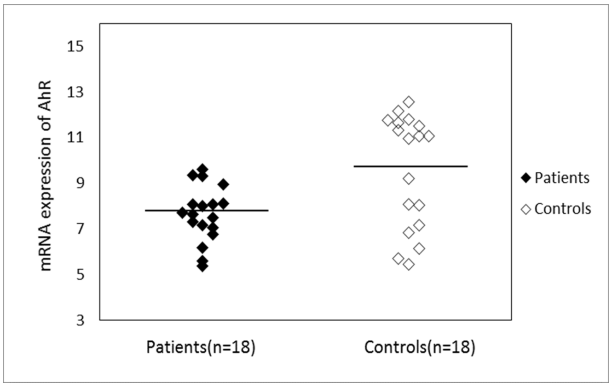

Fig.1: Detection of aryl hydrocarbon receptor (AhR) mRNA expression in peripheral blood mononuclear cells from patients with autoimmune hepatitis and healthy persons by reverse transcription - quantitative polymerase chain reaction. AhR mRNA levels were significantly higher in patients than normal controls. Please note that a higher delta cycle threshold (ΔCt) value corresponds to a lower expression level.

AhR. Higher ΔCt values represent lower mRNA levels and vice versa.

Statistical analysis

Data analysis was done using SPSS software, version 11.0 (SPSS, Inc., Chicago, IL, USA). Independent samples t test was used to compare the means of experimental group and control group. *P* values less than 0.05 were considered as statistically significant. The results were indicated as mean ± standard deviation (SD). All statistical analyses were carried out on ΔCt values.

RESULTS

The study group consisted of 18 patients with AIH (4 men, 14 women) and 18 healthy controls (5 men, 13 women). All the patients were newly diagnosed and did not take any treatment for their AIH. The mean age of the patients and controls were 41.50 ± 11.90 and 42.56 ± 14.07 years, respectively and no significant differences existed between them with respect to age and sex ($p > 0.05$).

The expression level of the transcription factor AhR that allows discrimination between Th22 cells and other T helper cell lineages was evaluated in the patients and normal subjects. As indicated in figure 1 greater expression of AhR (7.65 ± 1.21) was found in the PBMCs of patients with AIH as compared with healthy subjects (9.58 ± 2.48, $p = 0.006$).

DISCUSSION

The chronic inflammatory condition of the liver in AIH is related closely to different components of the immune system especially T cells. T cells are highly heterogeneous cell population in terms of their phenotypes and their functions. Although these cells play critical roles in host defense, their aberrant responses play a major role in promoting inflammatory processes that are implicated in the pathogenesis of different autoimmune diseases including AIH.[7]

Th22 cells are a novel Th cell lineage, which originate from naive CD4+ T cells in response to TNF-α and IL-6 and their expansion seems to be regulated by a transcription factor called AhR. These cells, as their name indicate are the most important source of IL-22 and IL-22 dependent mechanism(s) play a major role in their effector functions. IL-22 is a proinflammatory cytokine and stimulates the production of various proinflammatory cytokines. It acts as a double-edged sword in inflammatory responses. On the one hand, it is able to increase the pro- inflammatory defense mechanisms. On the other hand, it plays an inflammatory role in autoimmune diseases.[8]

Therefore, much attention has been devoted to resolving and understanding paradoxes about the role of IL-22 in the immune system and liver diseases. Since there is a wide variation in the cellular sources of IL-22,[9] assessment of Th22 specific transcription factor improves our understanding of the cellular sources of IL-22 and could help explain the mechanism and pathophysiology of diseases.

The results of the present study indicates a significant increase in AhR mRNA level in PBMCs of the patients compared with normal controls, suggesting a potential role of this cytokine in the disease pathogenesis.

To our knowledge, this is the first report in the literature on AhR expression pattern in patients with AIH. However, increased expression of this transcription factor has been observed in other inflammatory disorders such as multiple sclerosis, rheumatoid arthritis, allergic asthma, and lung inflammation.[10,11] The results of this study points to the possible importance of Th22 cells in AIH, while the precise mechanism underlying AhR upregulation remains to be determined. Analysis of AhR expression pattern in AIH is important because hepatocytes express high levels of AhR, which participate in the regulation of nutrient metabolism and xenobiotic detoxification.[11]

There is evidence that inappropriate activation of AhR signaling pathway contributes to hepatic fibrosis.[11] Overexpression of AhR in liver can also induce the transcription of several genes including Col1α1 (collagen, type I, alpha 1), α-SMA (alpha smooth muscle actin) and IL1β, which are important in liver fibrogenic and proinflammatory responses.[12] These findings show the probable importance of AhR in AIH and represent at the same time one of the main limitations of this study, because changes in the expression of AhR were not evaluated in the liver of the patients with AIH. Therefore, more studies are needed to explain the exact mechanism linking AhR and immunological processes within the liver in AIH.

In conclusion, the present study indicates a marked change in the mRNA expression of AhR in patients with AIH, which suggests a potential relationship between Th22 cells, and the development of the disease. How these immune cells impact the disease progression requires further investigations. Additional studies on the roles of Th22 cells and their cytokines can also help in the improvement of new therapeutic strategies for the treatment of liver inflammatory diseases, such as AIH.

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ETHICAL APPROVAL

There is nothing to be declared.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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