



Role of Th1/Th2 cells and related cytokines in autoimmune hepatitis

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ABSTRACT

Background/Aims: Dysregulation of T cell response is thought to play an important role in the immunopathogenesis of autoimmune hepatitis. However, no consensus has yet been reached regarding the implications of a distinct T cell subset in the pathogenesis of this progressive liver disease. Therefore, T-bet and GATA-3 expression was examined in patients with autoimmune hepatitis (AIH) and in healthy controls. Moreover, the profile of Th1 (IFN- γ) and Th2 (IL-4) cytokine gene expression was analyzed.

Materials and Methods: Levels of mRNA transcripts were measured in peripheral blood mononuclear cells (PBMCs) using a two-step reverse transcription quantitative real-time polymerase chain reaction with SYBR Green.

Results: T-bet and IFN- γ mRNA expression was significantly higher in AIH patients compared to healthy controls ($p < 0.05$), whereas no differences were observed for either GATA-3 or IL-4 mRNA expression ($p > 0.05$).

Conclusion: Alterations in the Th1/Th2 cell balance may be responsible for both disease progression and the resulting complications.

Keywords: Autoimmune hepatitis, gene expression, cytokines

INTRODUCTION

Autoimmune hepatitis (AIH) is an unresolved necro-inflammatory liver disease, and is more common in women than in men (1). The mechanisms underlying the onset of autoimmune hepatitis remain unclear. However, the main hypothesis postulates that disease susceptibility is affected by both genetic and environmental factors that lead to a loss of tolerance towards liver antigens (2).

The key process involves the failure of self-tolerance, although it is not completely understood, and perturbed homeostasis of T cells seems to be an important immune event in AIH (3). The significance of T cells in AIH is supported by different observations, namely (i) CD4+ and CD8+ T cell infiltration in the liver (4,5); (ii) the beneficial effects of immunosuppressive drugs in disease

treatment (3); (iii) the importance of T cell cytokines in maintenance and progression of the disease (6); and (iv) the increased frequency of T cells that are specific for liver autoantigens in patients with AIH (7).

To date, various T cell subpopulations with different markers have been reported. The two best characterized CD4+ Th cell populations are Th1 and Th2, which are involved in the development of cellular and humoral immune responses, respectively. Under specific conditions, these cells are derived from multipotent naive CD4+ T cells and produce different profiles of cytokines. For instance, IFN- γ is one of the main products secreted by Th1 cells, whereas Th2 cells primarily produce IL-4. In addition, the fate of Th1 and Th2 cells are regulated by the T-bet and GATA-3 transcription factors, respectively (8).

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Under normal physiological conditions, Th1 and Th2 cells, as well as their associated cytokines, can cross-inhibit each other. Therefore, an alteration in the balance of these subgroups can trigger an autoimmune response (9).

There are only few studies regarding the Th1/Th2 balance in AIH (10-13) but, at the same time, there is growing evidence supporting the involvement of both adaptive arms (cellular and humoral) of the immune system in the pathogenesis of AIH (14). Therefore, the specific aims of this study were to assess the expression levels of Th1 and Th2-restricted transcription factors and the prototype cytokines of Th1-type (IFN- γ) and Th2-type (IL-4) immune response.

MATERIALS AND METHODS

Study Group

Recruited patients for this study had been clinically diagnosed with AIH at the outpatient clinic of the Digestive Disease Research Center (DDRC). All patients were newly diagnosed cases with no prior treatment for AIH. Healthy controls had no history of AIH or other chronic and autoimmune diseases. Table 1 shows the demographic features for both patient and control groups. Written informed consent forms were signed by all participants before blood donation. PBMCs were separated from whole blood by Ficoll density-gradient centrifugation (Pharmacia, Uppsala, Sweden).

The study was conducted according to the Declaration of Helsinki principles and approved by the university ethical committee. Written informed consent was obtained from all subjects prior to enrollment.

RNA Isolation from PBMCs and cDNA Synthesis

Total RNA was extracted from PBMCs using a RibospinTM (GeneALL, Seoul, Korea) according to the manufacturer's instructions. The quality of the mRNA was checked by agarose gel electrophoresis and ethidium bromide staining. Furthermore, the purity and concentration of RNA in solution was determined with a NanoDrop instrument (Thermo Scientific, USA). In the next step, RNA was converted to cDNA (Fermentas, St. Leon-Rot, Germany) and stored frozen at -20°C until use.

Analysis by Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Analysis by qRT-PCR was conducted on the cDNA samples with 5 primer pairs using a SYBR Premix EX Taq II (Takara, Japan) on a Rotor Gene 6000 thermal cycler (Corbett Life Science, Australia).

PCR was performed in a total volume of 10 μL containing 5 μL of SYBR Premix, 1 μL of cDNA, 0.5 μL of forward and reverse primers, and 3 μL of double-distilled water. The am-

Table 1. Demographic features of the patients with autoimmune hepatitis and controls

Topics	Patients	Controls	Statistical significance
Number	18	18	NS
Sex (female/male)	4/14	5/13	NS
Age (years)	41.50 \pm 11.90	42.56 \pm 14.07	NS
NS: not significant			

Table 2. Primer sequences for real-time quantitative PCR

T-bet (141bp)	F: 5'CAAGTTTAAATCAGCACACAGAG3' R: 5'CAAGACCAGTCCACAAACATC3'
GATA-3 (90bp)	F: 5'GAGACAGAGCGAGCAACG3' R: 5'CTCGGGTACCTGGGTAG3'
IFN- γ (139bp)	F: 5'TCGGTAAGTACTGACTTGAATG3' R: 5'ATTACTGGGATGCTCTTC3'
IL-4 (195bp)	F: 5'AGAACACAAGTGAAGGAAAC3' R: 5'TCACAGGACAGGAATCAAG3'
β -actin (161bp)	F: 5'AGACGCAGGATGGCATGGG3' R: 5'GAGACCTCAACACCCAGCC3'
PCR: polymerase chain reaction; F: forward primer; R: reverse primer; bp: base pair	

plification took place in a two-step PCR, and thermal cycling conditions consisted of an initial denaturation step for 2 min at 95°C , followed by 38-45 cycles of 5 s at 95°C (denaturation) and a final step of 25 s at 62°C for T-bet, 20 s at 62°C for GATA-3, 45 s at 60°C for IFN- γ , 30 s at 62°C for IL-4, and 20 s at 63°C for β -actin.

Polymerase chain reaction primer pairs were designed using the OLIGO software (National Biosciences) and purchased from Gene Fanavaran (Tehran, Iran). The specific sequences of the primers used in this study are listed in Table 2.

Relative gene expressions were calculated by the ΔCt method. ΔCt values were determined by subtracting the Ct value of the endogenous control (β -actin gene) from that of the target gene. The β -actin gene was used for normalization, thus providing accurate and reliable data for qRT-PCR gene expression analyses. For all samples, levels of both endogenous control and target genes were determined. All statistical analyses were performed on ΔCt values. Higher ΔCt values represented lower mRNA levels, and vice versa.

Statistical Analysis

The independent samples t-test was used to determine whether the differences between the experimental and control groups were significant. P values lower than 0.05 were reported as statistically significant. The results were indicated as the mean \pm standard deviation (SD). All statistical analyses were conducted on ΔCt values. Higher ΔCt values represented lower mRNA levels, and vice versa.

RESULTS

Increased Expression of T-bet mRNA Transcripts in Patients with AIH

T-bet and GATA-3 gene expression in PBMCs was evaluated in AIH patients and healthy controls. As shown in Figure 1, T-bet mRNA expression was significantly higher ($p=0.000$) in the AIH group (7.29 ± 1.47) compared with the control group (16.81 ± 2.72). Analysis of GATA-3 mRNA levels revealed (Figure 2) no significant difference ($p=0.06$) between the AIH group (6.61 ± 1.30) and the control group (7.44 ± 1.23).

Dysregulated IFN- γ Expression in AIH Patients

To further evaluate possible changes in Th1 and Th2 subsets, the mRNA transcript levels of Th1/Th2-related cytokine genes were measured in isolated PBMCs of AIH patients and healthy controls.

The results showed (Figure 3) that PBMCs from AIH patients expressed increased levels of IFN- γ (9.57 ± 1.25) compared with those of healthy subjects (11.35 ± 1.60).

In addition, no significant statistical difference ($p=0.28$) was observed (Figure 4) between the IL-4 transcript levels measured in samples of patients (13.15 ± 2.35) and those of healthy control subjects (13.94 ± 1.90).

DISCUSSION

Autoimmune hepatitis is a rare but important chronic liver disease that can affect different components of the immune system. The exact mechanisms that trigger the human immune system to attack and destroy its own liver cells are unknown. Nonetheless, T-cells appear to be of fundamental importance in orchestrating the complex cascade of events in AIH (14).

Although the exact identity of T cells that contribute to pathogenesis of AIH remains unknown, possible alterations in the Th1 (cellular immunity) and Th2 (humoral immunity) balance may play a role in liver cell injury. Thus, the first step of this study was to investigate the changes in the expression profile of Th1/Th2-specific transcription factors in PBMCs of AIH patients and healthy subjects.

Statistical analysis showed a significantly increased level of T-bet in patients versus healthy subjects ($p<0.05$). In contrast, there were no differences between the two groups regarding GATA-3 mRNA expression. These findings are in agreement with previous animal and human studies, indicating a role for T-bet in the pathogenesis of AIH (15,16).

In another part of the current study, IFN- γ and IL-4 mRNA levels were analyzed in PBMCs of patients and healthy subjects. Our results revealed considerable differences in the IFN- γ mRNA transcript levels of AIH patients and controls ($p=0.001$). Conversely, no significant differences were observed between the two groups with respect to IL-4 expression levels (0.28).

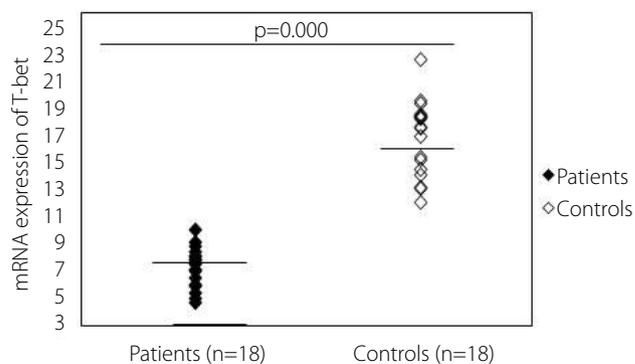


Figure 1. T-bet mRNA expression levels in AIH patients and control subjects. β -actin was used as the internal control. Please note that a higher Δ Ct value corresponds to a comparably lower expression level

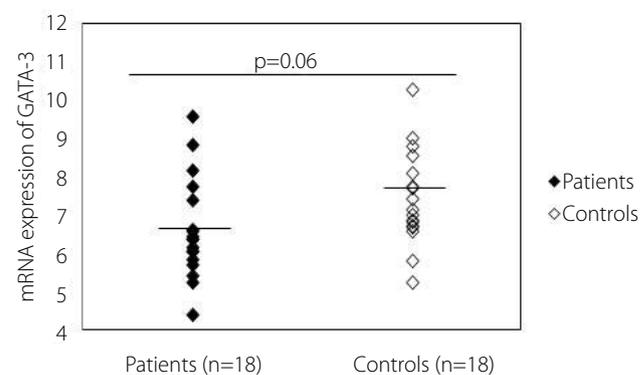


Figure 2. GATA-3 mRNA expression levels in AIH patients and control subjects. β -actin was used as the internal control. Please note that a higher Δ Ct value corresponds to a comparably lower expression level

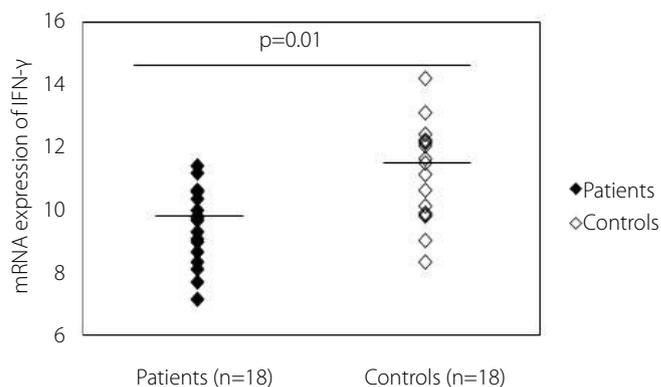


Figure 3. IFN- γ mRNA expression levels in AIH patients and control subjects. β -actin was used as the internal control. Please note that a higher Δ Ct value corresponds to a comparably lower expression level

These data agree with findings of earlier studies showing high levels of IFN- γ expression in AIH patients (13,17). A prior report also indicated a simultaneous presence of IFN- γ - and TNF- α -producing cells in the inflammatory cell infiltrates of liver biopsies from patients suffering from autoimmune liver diseases. Moreover, the frequency of these cells was positively correlated with the intense infiltration of inflammatory cells and transaminase levels (18).

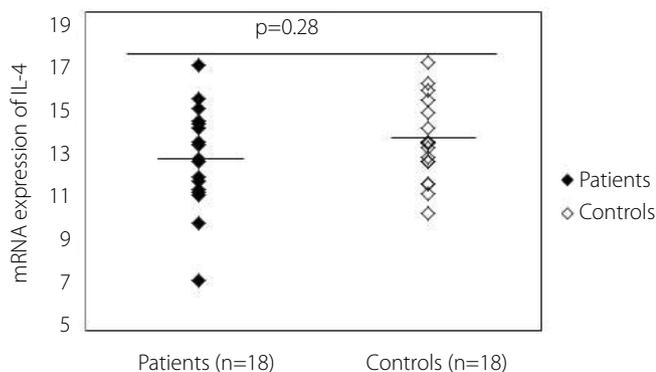


Figure 4. mRNA expression levels for IL-4 in patients and control subjects. β -actin was used as the internal control. Please note that a higher Δ Ct value corresponds to a comparably lower expression level

In the present study, we also explored whether there are distinct differences in IL-4 mRNA levels between AIH patients and controls. One of the main reasons for IL-4 mRNA quantification was to accumulate evidence of a role of Th2-biased cytokine responses in AIH. However, there is much controversy regarding the role that these cytokines play within the context of autoimmune liver disorders (19-21). These opposing views can be attributed to multiple factors including differences in the genetic backgrounds. In this context, cytokine genetics is of special interest because it has an important effect on immune and inflammatory responses.

It has been revealed that cytokine gene polymorphisms can affect cytokine levels and the proportion of Th1 and Th2 cells, which in turn can lead to the development of autoimmune diseases, including AIH. For instance, Li et al. indicated a significant association between Th1 cytokine polymorphisms (such as TNF- α) and the risk of AIH (22). In contrast, Th2 cytokine polymorphisms, such as the IL-10 gene, have not been implicated in the risk of developing AIH (23).

Another important factor that may affect Th1/Th2 polarization is treatment and several studies have revealed the influence of different therapeutic strategies on the immune response of AIH patients. For instance, Ferreyra et al. indicated that the immunosuppressive therapy of AIH patients resulted in decreased IFN- γ expression in the liver (24). The antagonizing effects of the immunosuppressive therapy on Th1 response are consistent with the observations of the present study, which recruited newly-diagnosed patients prior to treatment, and suggests an essential role for Th1 response in disease induction.

Nonetheless, we believe that the outcomes of this disorder will depend on complex interactions between different immune cell subsets and factors. Among them, regulatory T cells are particularly important because they participate in the maintenance of immune self-tolerance and homeostasis (25). Moreover, different studies have found a relationship between the

impaired number and function of Treg cells and AIH (26,27). It has also been shown that restoration of Treg cell function in subjects with AIH is paralleled by an increase in the expression of Th1/Th2/Th17 transcription factors (28). Therefore, further studies are required to clarify the function of other T cell subpopulations and related cytokines in disease pathogenesis.

In conclusion, it appears that an increased Th1 response is important in the pathogenesis of AIH. The data from this experimental study only suggest a possible association between AIH and immune Th1/Th2 cell balance, which in theory could be a hypothesis for the pathogenesis. Therefore, more studies need to be conducted to elucidate the mechanisms of action of these cells in the onset and development of this disease.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Tehran University of Medical Sciences.

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

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