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Original Article

Evaluation of IL-17 and IL-10 Production in Infectious Mononucleosis in EBV Patients

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Abstract

Background and Aims: Epstein–Barr virus is primarily the cause of acute infectious mononucleosis and can also cause lymphoma and autoimmune diseases. Th17 cells, which are a unique subset of ThCD4+ cells, direct the infection toward inflammation through production of inflammatory cytokine IL-17. In contrast, Treg Foxp3 cells inhibit inflammation through secretion of anti-inflammatory cytokine IL-10, leading to chronic infection.

Materials and Methods: As IL-17 and IL-10 play a key role in determining the acute and chronic state of the disease, in this study, by evaluating the levels of IL-17 and IL-10 and their ratio using qRT-PCR in 10 patients with acute infectious mononucleosis and 10 healthy individuals as negative control, we investigated the correlation between production of these immune factors and the disease. After collecting 5 ml blood samples from patients and healthy individuals, PBMC culture, RNA extraction, cDNA synthesis, qRT-PCR, and statistical analysis were performed.

Results and Conclusions: The results showed a significant increase in the level of IL-17 and a significant decrease in the level of IL-10 in the patients compared to healthy subjects and consequently an increased IL-17 to IL-10 ratio. Therefore, future treatment strategies might be established which are capable of preventing reactivation of the virus and development of tumors and autoimmune diseases.

Keywords: EBV, interleukin-10, interlukin-17, infectious mononucleosis.

Introduction

The Epstein-Barr virus (EBV) belongs to the Herpesviridae family. As its primary infection, 2 weeks after entering the body through oral secretion through deep kissing, it causes an infectious mononucleosis in the reticuloendothelial system. Although most prevalent in younger ages, this disease affects a wide age range from children to elderlies. At least 90% of the world's population has been infected with the virus, most of them with no known disease [1].

By contaminating B lymphocytes, EBV proliferates and activates these lymphocytes and leads to increased destruction of B lymphocytes and consequently extensive activation of cytotoxic T lymphocytes specific of EBV. As a result of scavenging the destructed B lymphocytes by cytotoxic T lymphocytes, clinical symptoms such as fever, swollen lymph nodes, pharyngitis, fatigue, hepatitis, etc. will be seen. Since the EBV finds many routes to evade the immune system and remains latent in undamaged B lymphocytes [2,3], under immunodeficiency it becomes active again, causing various types of malignancies including Burkitt lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, and also chronic autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis and the like [4–7].

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At the beginning of infection by EBV, Toll-Like receptors (TLRs) lead to activation of the innate and adaptive immune systems through detecting the pathogen [2][8]. The Th17 cells, which exist as a result of the interaction of two transcription factors RoR8t and stat3, are differentiated from Th cells. These are the principal cells in neutrophil recruitment to the site of inflammation and play a key role in interleukin 17 (IL-17) secretion, which in turn functions to connect innate and adaptive immunity [9–11]. Th17 cells have extensive flexibility, meaning that not only they promote inflammation through producing interleukin 17, but also diminish inflammation by producing interleukin 10 (IL-10) [4,9,12].

An example of this sort of flexibility can be seen in Crohn's inflammatory patients. On another note, Treg cells, the transcription factor of which is FoxP3, by secreting IL-10 at the center of immune response regulation, reduces Th1 differentiation and inflammation, but untimely production of IL-10 can disrupt the antiviral T responses and result in chronic infection, because IL-10 disrupts T cells function, and thus protects B cells and facilitates transmission of EBV. An instance is in patients with chronic active Epstein-Barr virus (CAEV) and systemic lupus erythematosus (SLE), where abnormally elevated levels of IL-10 are observed [13–16].

TLRs expressed on the surface of B lymphocytes are involved in innate immunity and TLR2 is a key factor affecting the ratio of Th17 to Treg. TLR2 and TLR also affect the number of TH17 and Treg cells[2]. It is worth noting that following exposure of TLRs to EBV, the cytokine environment, including IL-2 and TGFB, noticeably influences cells differentiation toward increasing Th17 or Treg such that small amounts of TGFB, in presence of inflam-matory cytokines (the most important of which is IL-6), induces Th17 proliferation and in larger amounts and absence of inflammatory cytokines promotes Treg proliferation.

Similarly, IL-2 causes Treg proliferation in small amounts while in larger amounts inhibits Treg proliferation and induces Th17 proliferation [17–19].

Methods

Sample collection. 10 patients with EBV infectious mononucleosis syndrome as well as 10 healthy individuals as the negative control group were selected. 5 ml peripheral blood samples were collected in EDTA tubes, and cells were cultured within 3 hours after collection. The entry and exit criteria included those diagnosed with infectious mononucleosis caused by Epstein-Barr virus.

These indivi-duals were positive for the heterophile antibody and also had a high titre of IgM-VCA. Patients who were infected with other factors causing infectious mononucleosis were excluded from the study.

Peripheral blood mononuclear cells culture. After sample collection, peripheral blood mononuclear cells were isolated using ficoll-paque density gradient media. The buffy coat layer was separated from whole blood and mononuclear cells were isolated by centrifugation, once at 400g for 20 min at 25˚C with accelerate and brake set at 3, and for a second time at 300g for 20 min at 4˚C with accelerate and brake set at 3.

Cell counting was performed by trypan blue dye and neobar counting procedure. Then the cells were resuspended and cultured in 96-well micro-plate. The culture media was RPMI with 10% serum and 1% ampicillin. The cells were then incubated at 37˚C and 5% CO2.

Total RNA extraction and cDNA synthesis. The total RNA of the cultured cells was extracted by manual method using Trizol.

Throughout the procedure, chloroform, isopropanol, and ethanol were used and RNase free water was finally added. RNA concentration was measured using Nanodrop spectrophotometer.

Using a commercial kit (Bio FACT, South Korea), we performed CDNA synthesis and act according to the manufacturer's protocol.

Using a Nanodrop spectrophotometer, cDNA concentration was measured. cDNA was kept at -20˚C for future use.

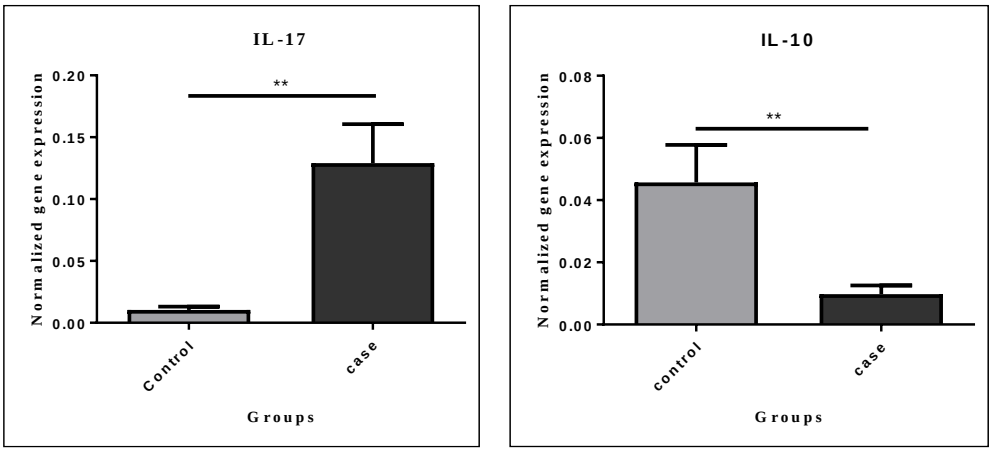

Fig. 1. The relative expression of Interlukin-10 and Interlukin-17

Qualitative RT-PCR. Initially, gene sequences for the desired interleukins were acquired from NCBI website. After determining the protected fragments, primers were designed and then evaluated using Primer-Blast. GAPDH was selected as a housekeeping gene.
The freeze-dried primers that had been synthesized by the commercial manufacturer, were diluted by sterile deionized water according to the manufacturer instructions.
The relative expression level of IL-17 and IL-10 were measured using SYBR Green Master Mix and StepOne Real-Time PCR System and ΔΔCt was used for analysis.

Statistical analysis. Statistical analysis of the data was performed with the one-way ANOVA and Student's t test using GraphPad Prism. Data are represented as mean ± standard deviation. Differences were considered significant when $P<0.05$.

Results

The results of qRT-PCR analysis on PBMCs showed that IL-17 gene expression is increased in infectious mononucleosis patients (0.1291± 0.09957) compared to the control group (0.01035±0.008456), indicating a statistically significant difference (p=0.0014).
The findings, however, indicates a decreased level of IL-10 expression in the patients (0.009778±0.008841) in comparison with the control (0.04571±0.03817), a difference which was also statistically significant (p=0.0095).
As a consequent of the above findings, the ratio of IL-17 to IL-10 is found to be increased by approximately 60 fold in patients compared with the control group.

Discussion

The results obtained in this study after measurement of two interleukins of 17 and 10 by qRT-PCR in 10 patients with infectious mononucleosis and comparison to 10 negative control (healthy) individuals after statistical analysis, indicates an increase in the pro-inflammatory interleukin 17 and the anti-inflammatory interleukin 10, and an increase in the ratio of Th17 to Treg in the acute phase of the disease in patients (positive IGM, VCA) compared with healthy subjects.
These result is consistent with those of Wingate et al. who reported reduced number of Treg cells in the peripheral blood of patients with infectious mononucleosis.
These results are also consistent with the results of Rahal et al. and Ohta et al. In their studies, they revealed a significant increase in Th17 in the peripheral blood of patients with acute phase associated with a significant increase in Treg cells during the recovery period.
They also reported increased expression of TLR2, TLR and an increase in the ratio of

TH17 to Treg in the PBMC of patients with acute infectious mononucleosis [2,19–21].

Based on the results of recent studies and the present one, it can be said that due to increased TLR2 and TLR in patients with acute infectious Mononucleosis, an increase in the number of Th17 cells and the level of inflammatory factors, the most important being interleukin 17, and also a decrease in Treg cellls and anti-inflammatory factors such as interleukin 10 is observed in the acute phase of the disease.

Therefore, we conclude that the level of interleukin 17, interleukin 10 and the ratio of Th17 to Treg cells plays an important role in the pathogenesis of infectious mononucleosis [2,15,20].

Conflict of interest

The authors report no conflicts of interest.

Acknowledgment

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