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IL-4 Regulate the Pro-inflammatory Cytokines in Rheumatoid Arthritis

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ABSTRACT

IL-4 is a cytokine that induces differentiation of naive helper T cells into Th2 cells. Once activated by IL-4, Th2 cells subsequently produce additional IL-4. Aim of present study is examine the effect of IL-4 on IL-17 production and proinflammatory cytokines and its effect in Rheumatoid Arthritis. In this study, the relationship between IL-4 and IL-17 were higher than other inflammatory factors. The expression of IL-17 and IL-4 as well as IFN-γ and IL-13 in sera of the paitents was measured by QRT-PCR and ELISA. The result of QRT-PCR analysis of IL-17 and IL-4 mRNA levels in the monocyte cells showed that IL-17 is increased significantly at the RA monocyte ($p<0.01$). Our Conclusion is that IL-4 can be involved in the production of IL-17 at especially the peak of RA. These results imply that the inhibition of IL-17 may decrease the expression of IL-1β and IL-6 production which will result in the aggravation of arthritis.

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INTRODUCTION

IL-4, a multifunctional pleiotropic cytokine discovered in the mid-1980s, remains a focus of attention and continues to spur vigorous research efforts. IL-4 is an anti-inflammatory cytokine which inhibits the production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6 and prostaglandin E2 (PGE2) [1, 2]. Also, IL-4 up-regulates the expression of anti-inflammatory mediators such as IL-1 receptor antagonist and IL-1 type II receptor .Functionally, IL-4 is best known for defining the so-called Th2 phenotype of lymphocytes and for regulating cell proliferation, apoptosis, and expression of numerous genes in various cell types, including lymphocytes, macrophages, and fibroblasts, as well as epithelial and endothelial cells [3, 4]. Interleukin 17 (IL-17) is a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to Interferon gamma [5-7]. IL-17 is produced by T helper cells and is induced by IL-23 which results in destructive tissue damage in delayed-type reactions. IL-17 functions as a proinflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. IL-6 and transforming growth factor (TGF)-β, IL-21 and IL-23 are important in the generation, expansion and maintenance of Th17 cells [8]. All of these Th17-associated cytokines are found in RA synovial tissue. In addition, IL-17 can synergize with IL-1β, a cytokine known to play an important role in the pathogenesis of RA. Rheumatoid arthritis (RA) is a systemic disease, characterized by chronic inflammation targeting the synovial membrane, cartilage, and bone. Synovial inflammation and joint damage occur in the course of RA [9]. The disease occurs in hands, wrists, feet, and other small joints, with recurrent and symmetrical distribution. In early stage, there appears swelling and pain in the joints; then, the joint experiences dysfunction [10]. Both B cells and T cells form aggregates in the synovium of joints and mediate the pathogenesis of RA and proinflammatory cytokines (such as IL-6 and IL-8), which are critically involved. Our objective was to examine the regulatory

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effect of IL-4 and IL-13 on IL-17 production, thus revealing the possible mechanism of how IL-4 can regulate the production of pro-inflammatory cytokines.

MATERIALS AND METHODS

Reagents:

β-actin, IL-17, IL-4 and IL-13 and IL-6,TGFβ primers were obtained from Sangon Company Primer express software (primer 5) was used to design primers from published cDNA sequences.

Patients and samples:

A total of 40 patients with RA enrolled in the Imam Reza Hospital of Tabriz Medical University were included in this study from November 2010 to September 2011. Among 40 patients, 28 women and 12 men ranged from 36 to 80 years old. Diagnoses were established according to the American College of Rheumatology (ACR) criteria [15]. Tweleve healthy volunteers were studied simultaneously as control, including eight women and four men ranged in age from 40 to 72 years old. This study was approved by the ethical committee of the Imam Reza Hospital of Tabriz Medical University. All individuals were informed about the consensus. Whole blood from healthy volunteers and patients were collected in EDTA-containing tubes. The blood samples were centrifuged at 1000 g 4 oC for 5 min, then the supernatant was collected and stored at 70 oC for measurement of cytokines, and sediment was used to separate PBMCs by standard Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density centrifugation. Monocytes were obtained from PBMC adhered to the culture bottle (the percentage of the monocyte was more than 90%, and residual lymphocytes was <10%). TRIzol was added to the PBMCs for total RNA.

Cell Culture:

Purified monocyte cells (1×10^6 cells per well in a 24-well plate) were cultured for 3 days in complete RPMI-1640 medium (Gibco Invitrogen corporation, UK), supplemented with 20% fetal bovine serum and 1% streptomycin/penicillin.

Real Time PCR Analysis:

Total RNA was isolated from the monocyte (1×10^6 cells) and the knee synovium. The PCR reaction was performed with a 1 µL buffer, 1 µL of 5 mM dNTP, 2.5 µL of Taq polymerase, 1.2 µL of 50 mM MgCl2, and 1 µL of each primer. The PCR condition was as follows: initial denaturation at 94oC for 5 minutes followed by 35 cycles of denaturation at 94oC for 30 seconds, annealing at 56oC for 30 seconds and extension at 72oC for 40 minutes and a final elongation cycle at 72oC for 10 minutes (Corbett 6000 Real Time PCR). PCR products were run on 1% agarose gel (Shanghai Yito Enterprise Company-China) and stained with ethidium bromide. The electrophoresis bands were photographed using the gel doc system and analyzed by quantity gel analysis software.

ELISA (Enzyme -Linked Immunosorbent Assay):

IL-4 levels in serum and monocyte cell culture supernatants of the patients and the control groups were then determined by IL-4 ELISA Kit (R&D system). IL-6 and IL-1β and IFN-γ levels were measured by ELISA kits (R&D system). We also used FLX-800 microplate reader from BioTekinstruments (Bitecs, USA).

Intracellular Staining:

Monocyte cells were obtained from the RA patients and stimulated by PMA/ionomycin for 5 hours. Cells were stained with anti-CD4-FITC. Intracellular staining with antibodies against IL-17 (BD Biosciences) was performed and analyzed by flow cytometry.

Data Analysis:

Data was summarized as mean ± SD. The statistical analysis of the results was performed by the Independent-Sample t test. Values for $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Involvment of IL-4 in RA:

During RA priming, a broad range of cytokines were induced in Monocyte cells and it was found that IFN-γ and IL-17 expressions were higher than that of IL-4 and IL-13. To determine the IL-4 role in inflammation, the expression of IL-4 mRNA was analyzed and the result showed that IL-4 and IL-13 gradually increased during RA (Figures 1A and 1B).

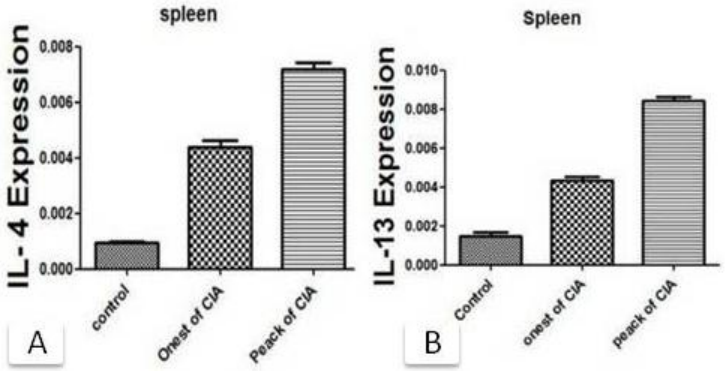

Fig. 1: QPT-PCR analysis of IL-4 and IL-13 mRNA levels in spleen from DBA/1 WT normal the peak of CIA in the spleen ($p < 0.05$).

Expression of IL-4 Can Decrease the Proinflammatory Cytokines:

IL-4 protein levels were determined in serum and monocyte cell culture supernatants by ELISA. The results demonstrated that IL-4 expression increased slightly in the serum ($p<0.05$) and in the monocyte cell culture supernatants ($p<0.03$) at the RA patients. Similarly, IL-4 expression showed no significant change (Figure 2A). IL-6 and IL-1β and TNF-α levels were determined in groups sera by ELISA assay. The results showed that the expression of these cytokines were increased in sera of the RA group ($p<0.05$) (Figure 2B). Also the results from the joint cell studies showed that IL-6 and IFN-γ decrease in the presence of IL-4 (Figure 2C).

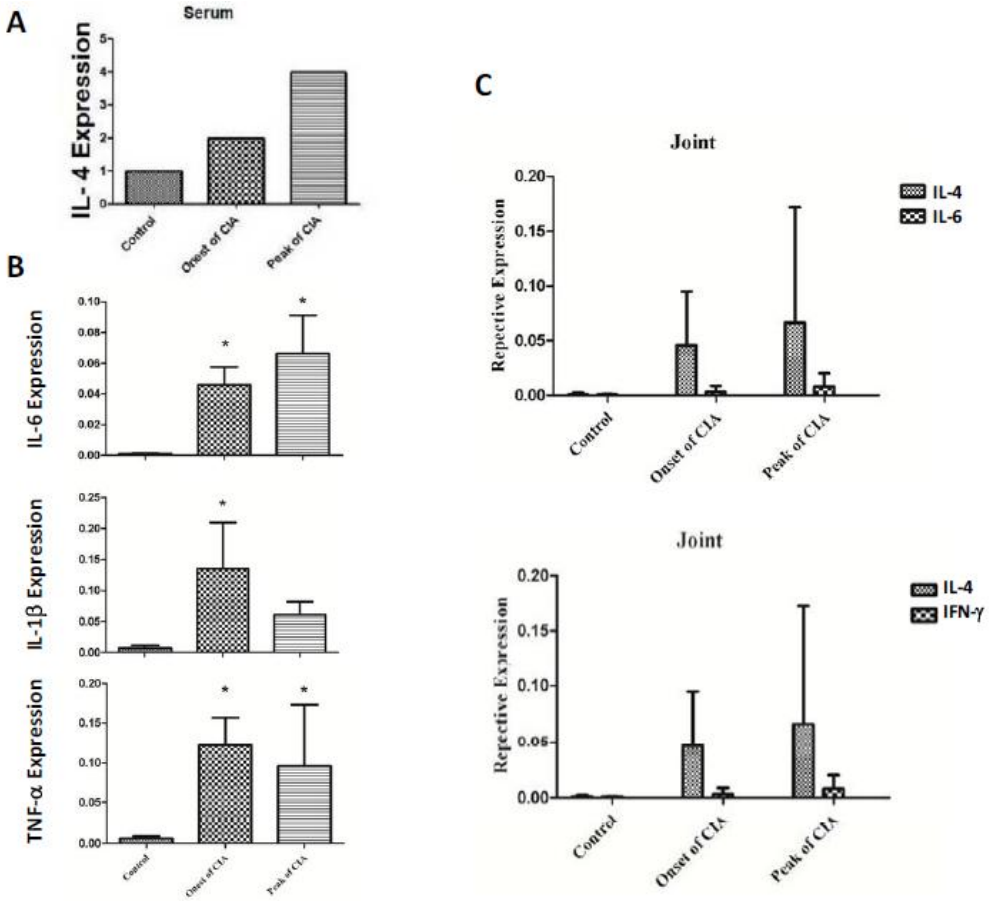

Fig. 2: A: IL-4 expression in serum and spleen cell culture in DBA mice increases during the CIA ($p<0.05$) **B:** ELISA results showing that IL-1β, TNF-α and IL-6 expression in serum is higher in CIA mice ($p<0.05$) **C:** ELISA results from joint cells showing the relationship between IL-4 and IL-6 or IFN-γ. IL-4 can inhibit both IL-6 and IFN-γ expression ($p<0.05$).

Discussion:

Rheumatoid arthritis is a chronic inflammatory disease that ultimately leads to the progressive destruction of cartilage and bone in numerous joints [9-11]. There is mounting evidence for an important function of innate

immunity in the pathogenesis of RA. Th17 cells and their specific transcription factor or related cytokines are being recognized as important mediators in inflammatory and autoimmune diseases including RA [12]. All of these Th17-associated cytokines are found in RA synovial tissue. In addition, IL-17 can synergize with IL-1β, a cytokine known to play an important role in the pathogenesis of RA [13, 14]. Although there have been significant advances in understanding the development and maintenance of Th17 cells in vitro, the endogenous regulation of Th17 responses during the development of arthritis is still under investigation [16]. In this study, we demonstrated the effects of IL-4 on Th1/Th2 balance following antigenic stimulation. Studies have found that IL-4 and IL-17 play opposing roles in certain diseases. CD4+CD25+Treg cells serve an important function in the regulation of autoimmune diseases [17, 18]. IL-4 production is important to NK T-cells, which secrete IL-4, IL-5 and IL-13 during the immune response. During inflammation, IL-4 expression was increased. Several studies have shown that a balance of Th1/Th2-type cytokines may have a substantial role in the regulation of autoimmune diseases. Our data also showed that IFN-γ was always at high levels in the course of the disease. Results of our study suggest that disease outcome is not determined solely by the absolute level of the pathogenic cytokine, but rather by the balance between pathogenic and protective signals. One possibility is that, these signals modulate trafficking of Th17 cells to the joint, either by altering the ex-pression of chemokines by cells of the synovium or expression of chemokine receptors by T cells [19, 20]. Once in the joint, Th17 cells can then induce inflammation and recruitment other inflammatory cells .In summary, these observations support the role of Th-17 cells in the pathogenesis of RA and also support the role of IL-4 in the inhibition of IL-17 which may imply that the inhibition of IL-17 can decrease the expression of IL-1β and IL-6 production which will result in the aggravation of arthritis.

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