15th Annual Congress of
Iranian Rheumatology Association

Assessing the Expression of Immunosuppressive Cytokines in the Newly Diagnosed Systemic Lupus Erythematosus Patients: a Focus on B Cells

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Background

The systemic lupus erythematosus (SLE) is a multifactorial chronic autoimmune disease that is more common in women [1]. Evidence showed that genetic and environmental factors, including cigarette smoking, drugs, ultraviolet (UV) light, chemical substances, gut microbiota, and viral infections, could be involved in SLE onset. Regarding the existence of an imbalance between apoptosis and removal of apoptotic substances in SLE patients, nuclear antigens such as histones, centromere proteins, single- and double-stranded deoxyribonucleic acid (ss- and ds-DNA), nucleosome, Smith antigen (Sm Ag), Ro and La proteins, as well as ribonucleoproteins (RNPs) become exposed to the immune system cells and components . These autoantigens are able to stimulate B-cells to produce auto-antibodies such as anti-nuclear antibody (ANA) and anti-doublestranded DNA (ds-DNA) antibody as well as other inflammatory mediators. homeostasis processes .Recent experimental and human studies have further indicated that Bregs can suppress inflammatory responses via production and secretion of anti-inflammatory cytokines like IL-10, IL35, and TGF-β as well as expression of inhibitory molecules. Besides, Bregs come up from common progenitor of transitional 2-marginal zone precursor (T2-MZP) B cells capable of being autoreactive following interaction with pathogens and even activating release anti-inflammatory mediators. Some investigations in this respect have correspondingly suggested that Bregs are impaired in inflammatory autoimmune disorders such as SLE, rheumatoid arthritis (RA), and Graves' disease . However, signals necessary for differentiation of Bregs have remained poorly understood, and previous studies have revealed that, under normal conditions, plasmacytoid dendritic cells (pDCs) would stimulate differentiation of CD19 + $CD24^{hi}CD38^{hi}$ immature B-cells into the $CD24^{+}CD38^{hi}$ mature Bregs, which could produce IL-10 through release of interferon-alpha (IFN-α) and the cluster of differentiation 40 (CD40) engagement. Conversely, the mentioned Bregs can have an inhibitory effect on the generation of IFN-α through pDCs by releasing IL-10 [10]. In SLE, the cross-talk between pDCs and Bregs has defected, and pDCs fail to trigger differentiating $CD19^{+}CD24^{hi}CD38^{hi}$ B-cells into IL-10producing $CD24^{+}CD38^{hi}$ Bregs . In addition, Bregs can suppress $CD4^{+}$ T-cell proliferation, induce $Foxp3^{+}$ regulatory T and Tr1 cells, and suppress T helper (Th) 1 (Th1). Bregs also can stimulate Th17 and $CD8^{+}$ effector T-cells differentiation through cell-cell interactions and release of the anti-inflammatory cytokine, including TGF-β, IL-35, and IL-10. As well, TGF-β and IL-10, which are secreted by Bregs, can have an inhibitory effect on antigen-presenting function, cytokine secretion by DCs, neutrophils, natural killer (NK) cells, M1 macrophages, and conversely, induction of M2 macrophage differentiation.

Moreover, FasL (or CD178) or PD-L1 (or CD274) that are expressed on the Bregs surface are involved in the apoptosis of effector T cells following ligation with PD-1 (CD279) and Fas (CD95) on the surface of mentioned Tcells. Since changes in immune cells phenotype, as well as their number and function, are closely related to the disease activity, measuring the produced mediators and inhibitory molecules by regulatory immune cells before treatment based on diseases activity status could be critical in further understanding the role of the modulatory mechanisms of the immune system in the pathogenesis of SLE. Therefore, this study aimed to measure inhibitory molecules' expression on B cells' surface and anti-inflammatory cytokines produced by B cells in newly diagnosed SLE patients compared to healthy

Results

Twenty-three patients suffering from SLE and thirty normal-age and gender-matched subjects were enrolled in this study. All the patients were classified as individuals having an active disease (SLEDAI ≥4). The mean ± SD of SLEDAI in patients was 9.73 ± 6.01and we divided patients into three groups (SLEDAI I; 4 to 7, SLEDAI II; 7 to10, SLEDAI III more than 10) based on SLEDAI scores (min:5, max: 34, and median: 8). At the time of the blood collection, newly diagnosed SLE patients were not under treatment.

mRNA and protein expression of cytokines and inhibitory molecules

The data obtained from RT-PCR demonstrated the considerable rising trend in expressions of IL-10, IL-35 (EBI3 or IL-12P35), TGF-β, PDL-1, and FasL genes ($P<0.0001$) in the B-cell population of the SLE patients compared with ones isolated from the healthy subjects (Fig. 1). Additionally, serum levels of IL-10 (control:115 ± 22.1; patient: 158 ± 31.7;$P< 0.001$), IL-35 (control:377 ± 63.84; patient: 499 ± 42.95; $P < 0.001$), and TGF-β(control: 28 ± 6.87; patient: 112 ± 19.47; $P< 0.005$) were significantly increased in SLE patients compared with the healthy subjects. The multiple comparison analysis in three groups of patients based on the SLEDAI score showed that by increasing disease activity, serum levels of IL-10 and TGF-β decreased significantly ($P< 0.05$), while in the case of IL-35, a significant difference was not observed. Cytokines, autoantibodies, inhibitory molecules and disease activity The correlation analysis between disease activity scores and titres of autoantibodies showed that there was a positive association between ANA, anti-dsDNA, and anti-SSA/Ro with disease activity score but the correlations were not statistically significant ($r = 0.2$, $p= 0.359$; $r = 0.108$, $p= 0.623$; and $r = 0.353$,$p= 0.1$; respectively). Correlation matrix analysis showed that there was an association between serum level of TGF-β and SLEDAI ($r =−0.54$,$p= 0.007$); PDL-1 gene and IL-35 serum level($r = 0.53$, $p= 0.008$); IL-10 and TGF-βserum levels ($r =0.64$, $p= 0.001$); IL-10 and EBI3 genes ($r =−0.56$, $p=0.05$.

Moreover, information of nine variables related to studied cytokines (EBI3 gene, TGF-β, IL-10, IL-12p35, PDL-1, FasL genes and IL-10, TGF-β, and IL-35 serum levels) were extracted and converted into two components 1, and 2 using PCA. The cumulative percent of the variance of the two components was 88.47%. HCA was also used to cluster patients based on cytokine obtained data. Data showed that healthy subjects (n= 30) were placed in clusters 1 and 2 (blue and yellow) and to the left of the dendrogram while SLE patients (n= 23) were placed in clusters 3 and 4 (red and gray) and to the right of the dendrogram Altogether, the results showed that considering components 1 and 2 data, SLE patients could be differentiated from healthy subjects.

Additionally, the results of regression (PCR) showed that there is only a significant inverse relationship between component 2 and SLEDAI scores because the estimated confidence interval for component 2 did not include zero; therefore, on average, with the increase of one unit of component 2 in which IL-10 plays an important role, the SLEDAI score has decreased 1.39 units . These results suggest that there is an inverse association between SLEDAI scores and immunomodulatory cytokines.

Material and methods

A total number of 23 new cases of SLE patients and 30 normal-age and sex-matched subjects were enrolled in this study. Peripheral blood samples were collected. The serum levels of IL-10, TGF-β, and IL-35 were subsequently measured via ELISA as well as expressions of IL-10, TGF-β, and IL-35 (EBI3 or IL-12p35,(programmed death-ligand 1 (PD-L1 or CD274), and Fas ligand (FasL or CD178) examined in isolated B-cells using the real-time polymerase chain reaction (RT-PCR) technique.

Discussion

In this study, the expression of some inhibitory molecules and immunosuppressive cytokine in isolated B cells and sera from newly diagnosed SLE patients whose treatment had not been started was investigated. The
study findings revealed that mRNA levels of IL-35 (EBI3or IL-12p35), IL-10, and TGF-βin isolated B-cells from SLE patients were elevated compared to healthy subjects. Moreover, the level of IL-10, TGF-β, and IL-35 serum
increased in the patients' peripheral blood affected by the SLE compared with healthy subjects. The findings in three groups of patients based on SLEDAI score also demonstrated that by increasing disease activity, serum levels of IL-10 and TGF-βdecreased significantly ($P<0.05$), although, in the case of IL-35, there was no remarkable difference between groups. Additionally, the mRNA levels of PDL-1 and FasL were significantly upregulated in B-cells of the SLE patients compared to healthy subjects.

Taken together, the results of this study indicated that a group of SLE patients B cells might modulate immune responses in mild to moderate disease activity by producing anti-inflammatory cytokines and expressing inhibitory molecules. The findings can also clarify SLE disease activity's effect on the fluctuation and expression of IL-10, TGF-β serum levels. However, the source of the studied cytokines might be other regulatory immune cells. Future studies are needed to fully elucidate the source of released antiinflammatory cytokines and evaluate the producer regulatory cells' number and activity to a well understanding of the immunomodulatory mechanisms in SLE.