CIRCULATING INTERLEUKIN-16 IN SYSTEMIC LUPUS ERYTHEMATOSUS

S. LEE, H. KANEKO, I. SEKIGAWA,* Y. TOKANO, Y. TAKASAKI and H. HASHIMOTO

Department of Internal Medicine and Rheumatology, Juntendo University School of Medicine and *Department of Medicine, Juntendo University Izu-Nagaoka Hospital, Japan

SUMMARY

Objective. To investigate the relationship between interleukin (IL)-16 and systemic lupus erythematosus (SLE).

Methods. Serum levels of IL-16 were examined in SLE patients using an enzyme-linked immunosorbent assay (ELISA).

Results. The serum level of IL-16 in the patients was much higher than that in healthy volunteers \( (P < 0.001) \). An increase in IL-16 was observed in proportion to the activity of SLE assessed by the SLE Disease Activity Index (SLEDAI) score \( (P < 0.0001) \).

Conclusions. Our observations suggest an interaction between disease activity and the production of IL-16 in SLE, and reveal that IL-16 is a useful indicator of disease activity. This is the first report describing the relationship between IL-16 and SLE.

KEY WORDS: Interleukin-16, Systemic lupus erythematosus, CD4\(^{+}\) T-cell activation, CD4\(^{+}\) T-cell anergy, Human immunodeficiency virus.

INTERLEUKIN (IL)-16, originally named lymphocyte chemoattractant factor, is a CD8\(^{+}\) T-cell-derived cytokine that induces chemotaxis of CD4\(^{+}\) T cells, monocytes and eosinophils [1–4]. This cytokine is reported to use CD4 molecules as its receptor and to induce phosphorylation of protein tyrosine kinase p56\(^{\text{ck}}\) in CD4\(^{+}\) T cells through binding or cross-linking of CD4 molecules [3–9]. Besides its chemotactic activity, IL-16 also induces expression of the IL-2 receptor (CD25), human leucocyte antigen (HLA) class II molecules on CD4\(^{+}\) T cells (activated T-cell markers) and CD4\(^{+}\) T-cell anergy [shown as a suppression of the mixed lymphocyte reaction (MLR) and a decline of antigen-induced proliferation of CD4\(^{+}\) T cells] [7–9]. IL-16 is thought to be a competence growth factor for CD4\(^{+}\) T cells, capable of inducing a G\(_0\) to G\(_1\) cell cycle change, but incapable of inducing cell division [9].

T-cell abnormalities induced by IL-16, such as CD4\(^{+}\) T-cell activation and anergy, are also observed in lymphocytes in patients with systemic lupus erythematosus (SLE) [10, 11]. In this study, we examine the circulating level of IL-16 in SLE patients and the relationship between IL-16 and disease activity, and discuss the possible aetiological role of IL-16 in the induction of SLE.

PATIENTS AND METHODS

Patients

Non-heparinized blood was collected from patients with SLE \( (n = 49) \); three males and 46 females, aged 16–62 yr old, and from age- and sex-matched normal controls \( (n = 49) \). There were no pregnant subjects. SLE was diagnosed using the American Rheumatism Association 1982 revised criteria [12]. Disease activity was determined using the SLE Disease Activity Index (SLEDAI) score [13]. Nine patients did not receive any steroid and the remaining patients were treated with 5–10 mg/day of prednisolone for a period of 2–24 months. All patients did not have any immunosuppressive agents before their samples were collected.

Blood samples were centrifuged and the serum was frozen at \(-80^\circ\)C until testing.

ELISA for IL-16

The serum level of IL-16 in SLE patients and normal controls was determined by a sandwich enzyme-linked immunosorbent assay (ELISA) developed by Biosource International (Cytoscreen\(^{\text{®}}\) hIL-16 kit, Camarillo, CA, USA). Briefly, patients’ sera were diluted 1/2 in phosphate-buffered saline (PBS) + 1% bovine serum albumin (BSA) + 0.1% Tween 20, and these samples and standards of known human IL-16 (hIL-16) were added into the wells which were coated with an antibody specific for hIL-16, followed by the addition of a biotinylated second antibody for hIL-16. After removal of excess second antibody, streptavidin-peroxidase (enzyme) was added. After washing to remove all the unbound enzyme, a substrate solution was added, then the optical density (OD) at 450 nm was measured with an automated plate reader (Model 35550-UV Microplate Reader, Bio-Rad, Hercules, CA, USA) and IL-16 levels were determined by comparison with the linear part of this standard curve.

Statistical analysis

Statistical analysis was performed with the Mann–Whitney U-test and by determining Spearman rank correlation coefficients. Differences were considered to be significant at \( P < 0.05 \).

RESULTS

The serum levels of IL-16 in SLE patients and normal controls are shown in Fig. 1. Levels in the SLE patients were significantly higher than those in the normal controls. A significant positive correlation was also observed between the serum level of IL-16 and the SLEDAI score. The increase in IL-16 was observed in proportion to the activity of SLE assessed by the SLEDAI score \( (P < 0.0001) \).
Furthermore, CD4⁺ T cells in patients with SLE show T-cell anergy indicated by a low MLR and declines in IL-2 production and cell proliferation induced by mitogens/antigens. In SLE patients, these T-cell abnormalities may be related to a breakdown of self-tolerance and the resultant production of autoantibodies in the presence of polyclonal B-cell activation [10, 14]. In addition, these activated CD4⁺ T cells in SLE patients are reported to promote immunoglobulin production from SLE B cells [15]. CD4⁺ T-cell activation is known to be closely related to anergized CD4⁺ T-cell in SLE patients, because the initial high proportion of HLA-class II⁺ CD4⁺ T cells (in G₁A phase) decreases markedly during culture for a few days, which indicates a return to the G₀ phase of the cell cycle, while pre-culture of patient CD4⁺ T cells restores their ability for IL-2 production and cell proliferation [11, 16]. Thus, it appears that CD4⁺ T cells in SLE patients have an essentially intact capacity to induce IL-2 production and cell proliferation, and that the CD4⁺ T-cell activation occurring continuously in SLE is related to CD4⁺ T-cell anergy. We found that serum levels of IL-16 were greater in SLE patients than in normal volunteers, and that there was a strong correlation of the serum IL-16 level with the activity of SLE (Figs 1 and 2). In addition, there was a significant correlation between serum IL-16 and soluble CD4 (sCD4) levels, which may be released from the CD4⁺ T-cell surface by activation of these cells [17, 18], and no correlation of IL-16 with sCD8 or sCD23 (B-cell surface marker) in SLE patients (data not shown). These results suggest the possibility that the CD4⁺ T cells in SLE patients could receive IL-16 stimulation, with the resultant activation and related anergy occurring in proportion to the disease activity. On the other hand, our preliminary results indicate that serum IL-16 levels in rheumatoid arthritis (RA) patients are significantly lower than those in SLE, although the level of RA patients is slightly higher as compared with normal volunteers (data not shown).

CD4⁺ T-cell abnormalities observed in SLE patients and induced by IL-16 are also observed in human immunodeficiency virus (HIV)-1-infected patients (such as expression of HLA-class II on CD4⁺ T cells, decreases of MLR and IL-2 production) [19, 20]. This may be related to autoimmune phenomena in HIV-1-infected patients [21]. These CD4⁺ T-cell abnormalities can be induced by binding of HIV-1 envelope glycoprotein (gp120) to CD4 and the following phosphorylation of CD4-associated p56 kinase by this binding [22]. Similarities between CD4⁺ T-cell abnormalities induced by IL-16 and gp120 are concerned in the fact that their receptors are located in CD4 molecules, although their precise binding sites in CD4 are different (the binding site of gp120 is situated in the VI domain and that of IL-16 may be located in the V4 domain of CD4) [8, 23]. Regarding SLE, binding of IL-16 to CD4 molecules and subsequent signalling in CD4⁺ T cells may be important for inducing the abnormalities observed in the patient, instead of gp120 in HIV-1-infected patients.

![Fig. 1.—Serum levels of IL-16 in healthy controls (n = 49) and SLE patients (n = 49) (P < 0.001). The line shows the mean value in each population.](image1)

![Fig. 2.—Correlation between serum IL-16 level and SLE disease activity (SLEDAI score) (n = 29, R = 0.753, P < 0.0001).](image2)
The mechanism of the high production of IL-16 in SLE patients is still unknown. It may involve the interaction of other cytokines that influence each other’s production, because several cytokines show changes in SLE patients with disease progression [24–26]. CD8+ T cell and its derived factor are reported to facilitate the low IL-2 production in vitro of SLE, although its precise characterization is still unclear [27–29]. To make clear the production mechanism and pathogenic role of IL-16 in SLE, we are now investigating the mechanism of in vitro IL-16 production in SLE patients.

In conclusion, circulating IL-16 levels are high in SLE patients and are correlated with the disease activity. This may be related to CD4+ T-cell abnormalities observed in patients with SLE.

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References
