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CASE REPORT

Atsushi Ogata · Masayasu Kitano · Maki Fukamizu
Teruaki Hamano · Hajime Sano

Increased serum interleukin-18 in a patient with systemic lupus erythematosus and T-cell large granular lymphocytic leukemia

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Abstract Interleukin-18 (IL-18) is a potent cofactor for T-helper (Th-1) cell development and inducer of cytotoxic T lymphocytes (CTL), and is reported to contribute to autoimmune diseases. T-cell large granular lymphocyte (T-LGL) leukemia involves the proliferation of autoreactive CTL that is often associated with autoimmune disorders. We found increased serum IL-18 concentrations in a 55-year-old woman with systemic lupus erythematosus (SLE) and T-LGL-leukemia. Her serum IL-18 concentrations correlated with the intensity of her SLE symptoms and the number of T-LGL cells in peripheral blood. This evidence suggests that IL-18 is involved in T-LGL-related autoimmune disorders.

Key words Interleukin 18 (IL-18) · Systemic lupus erythematosus (SLE) · T-cell large granular lymphocyte (T-LGL) leukemia

Introduction

T-cell large granular lymphocyte (T-LGL) leukemia is a chronic disease characterized by a proliferation of CD3+, CD16+, and CD57+ cells.[1] The lymphocytes are CD8+ cytotoxic T lymphocytes (CTL) in almost all patients, although rare cases of CD4+ cells have also been described.[2] Autoreactive CTL often accumulates in patients with T-LGL leukemia, and is frequently associated with autoimmune phenomena.[1] Rheumatoid arthritis (RA) is frequently associated with T-LGL leukemia; in such cases, RA resembles Felty's syndrome.[3]

Interleukin-18 (IL-18) is a potent cofactor for T-helper (Th-1) cell development and inducer of CTL. CD8+ cytotoxic T cells are elevated in IL-18 transgenic mice.[4] Recently, IL-18 has been found to play a pathogenetic role in certain autoimmune diseases. Elevated serum IL-18 concentrations have been reported in patients with such autoimmune disorders as adult-onset Still's disease, RA, and systemic lupus erythematosus (SLE).[5–7] In autoimmune MRL lpr/lpr mice, lymphocytes overexpress the IL-18 receptor accessory chain and hyperrespond to IL-18.[8] These observations suggest that IL-18 contributes to the pathogenesis of autoimmune disorders through the induction and activation of autoreactive CTL. This case report describes a patient with T-LGL leukemia who also exhibited symptoms of SLE. Her serum IL-18 concentrations correlated with her SLE symptoms and peripheral T-LGL cell counts.

A. Ogata (✉) · M. Kitano · M. Fukamizu · T. Hamano · H. Sano
Division of Rheumatology, Department of Internal Medicine, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya 663-8131, Japan
Tel. +81-798-45-6591; Fax +81-798-45-6593
e-mail: a-ogata@hyo-med.ac.jp

Case report

A 55-year-old woman was admitted to the Hyogo College of Medicine for treatment for SLE in June 2000. In June 1982, she had been diagnosed with idiopathic thrombocytopenic purpura (ITP) based on the presence of purpura and thrombocytopenia. Following splenectomy and azathioprine and low-dose steroid therapy, she was able to maintain adequate peripheral platelet counts of 5–10 $\times$ 10^4/μl. Laboratory tests in 1987 were negative for antinuclear antibody (ANA), and her serum IgG concentration was 1800 mg/dl. CD4 and CD8 surface markers were present on 40.4% and 33.1% of peripheral lymphocytes, respectively. In 1997, her ANA titer was found to be elevated (1:320), as was her serum IgG (4210 mg/dl). However, she exhibited no symptoms of SLE, and her thrombocytopenia was stable in 1997.

Early in 1998, the patient began to experience cyclic neutropenia with transient lymphocytosis 2–3 times a year. She manifested livedo reticularis in December 1999, and polyarticular arthritis in April 2000. Laboratory test results in June 2000 were as follows: ANA titer, 1:320; serum IgG, 3430 mg/dl; direct and indirect Coombs tests, negative; rheumatoid factor (RF), negative. Anti-double-stranded (ds) DNA antibody and antiphospholipid antibodies were

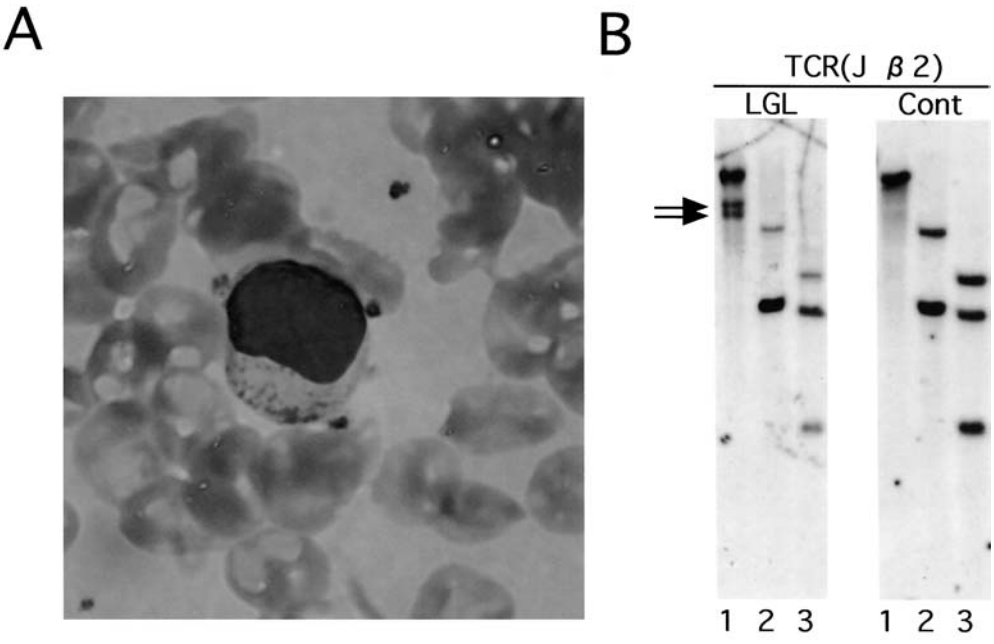
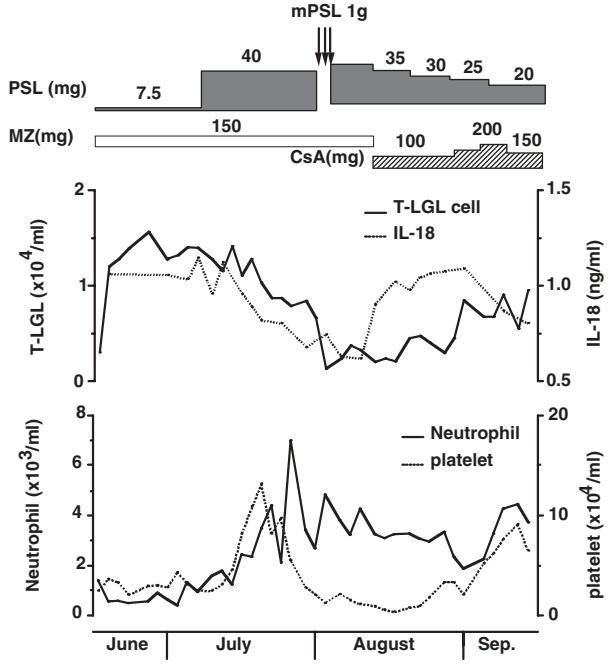


Fig. 1. **A** Peripheral blood smear showing characteristic large, mature-seeming granular lymphocytes (Giemsa, ×400). **B** Southern blot analysis of the T-cell receptor *TCR*(*Jβ2*) gene in large granular lymphocytes (*LGL*) showing clonal rearrangement. The restriction enzymes used were Bam HI (1), Eco RV (2), and Hind III (3). Human placenta was used as a negative control (*Cont*). *Arrows* identify rearranged bands

present. The patient was diagnosed with SLE based on the presence of arthritis, thrombocytopenia, anemia, ANA, anti-dsDNA antibody, antiphospholipid antibodies, and LE cells. The patient exhibited pancytopenia (neutrophils, 0.5 $\times$ 10^3/μl; platelets, 1.8 $\times$ 10^4/μl; red blood cells, 291 $\times$ 10^4/μl). Conversely, the number of peripheral blood mononuclear cells that were morphologically similar to LGL cells (Fig. 1A) was increased (12.1 $\times$ 10^3/μl). These cells expressed CD2, CD3, CD8, and CD57, but not CD20, CD4, CD16, or CD56. In addition, CD8+ lymphocytes had massively invaded her bone marrow (32.1% of all cells). A diagnosis of T-LGL leukemia was confirmed by the detection of a clonal rearrangement of the T-cell receptor (TCR) β gene (Fig. 1B). In July 2000, the patient was also diagnosed with T-LGL leukemia.

The patient's arthritis and laboratory abnormalities improved after administration of 40 mg prednisolone (PSL), pulse steroid therapy, and cyclosporine A (Fig. 2). In September 2000, anti-dsDNA antibody and antiphospholipid antibodies had become undetectable. Furthermore, her ANA titer (1:80) and plasma IgG concentration decreased (1310 mg/dl). Her hematological status also improved (neutrophils, 5.6 $\times$ 10^3/μl; platelets, 9.2 $\times$ 10^4/μl; red blood cells, 314 $\times$ 10^4/μl) in parallel with a decrease in the number of T-LGL cells (5.6 $\times$ 10^3/μl). Her arthritis and livedo reticularis also improved. Her serum IL-18 was measured using an enzyme-linked immunossorbent assay (ELISA) kit (Medical and Biological Laboratories, Nagoya, Japan). Her serum IL-18 concentration showed increases, and these correlated with the number of peripheral T-LGL cells (Fig. 2). Interleukin-2, IFNα, interleukin-12, and soluble Fas ligand (FasL) were not detectable in the serum.



ANA	X320	X320	X160	X160	X160
IgG(mg/dl)	3430	2840	1510	1250	1310
LE	(+)	(+)	(-)	(-)	(-)
anti-ssDNA(U/ml)	97.1	75.7	24.6	21.6	13.5
anti-dsDNA(U/ml)	30.7	20.3	<5.0	<5.0	<5.0
αCLβ2GPI(U/ml)	30.6	15.3	9.1	8.0	6.4

Fig. 2. Clinical course of the patient in the year 2000. Although neutropenia improved after daily oral administration of 40 mg PSL, lymphocytosis and serum IL-18 concentrations did not improved. Lymphocytosis and serum IL-18 concentrations improved after pulse mPSL therapy. Thrombocytopenia improved after CsA treatment. Anti-ssDNA, normal range <20 U/ml; anti-dsDNA, normal range <20 U/ml; αCL β2GPI (anti-cardiolipin β2-glycoprotein I), normal range <3.5 U/ml. *PSL*, prednisolone; *mPSL*, methylprednisolone; *MZ*, mizoribine; *CsA*, cyclosporine A; *T-LGL*, T-cell large granular lymphocyte; *IL-18*, interleukin-18

Discussion

This patient's T-LGL cells increased rapidly as her serum IL-18 concentrations increased. Both IL-18 and T-LGL cells decreased after immunosuppressive therapy, which included pulse steroid therapy and cyclosporine A. Interestingly, IL-18 increased before the T-LGL cells increased in August (shown in Fig. 2). This evidence suggests that serum IL-18 activates T-LGL cells and contributes to the development of autoimmune disorders. The precise pathogenic role of IL-18 in this patient remains unclear. Although no studies have reported on the direct relationship between IL-18 and T-LGL leukemia, CD8+ cytotoxic T cells are elevated in IL-18 transgenic mice,[4] which suggests that IL-18 may be a possible activator of CD8+ T-LGL leukemic cells.

The relationship between T-LGL leukemia and autoimmune phenomena has been reported.[3,13–15] However, the mechanism by which autoimmune phenomena develop in patients with T-LGL leukemia is not well understood. CD8+ T-LGL cells that mediate cytotoxicity are thought to represent in vivo-activated cytotoxic T lymphocytes. The pattern of gene expression in leukemic-LGL resembles that seen in activated CTL.[9] However, the bone marrow cells of T-LGL leukemia patients with cytopenia are not directly lysed by T-LGL cells.[10] Baker et al.[11] showed both humoral and cellular suppression of granulopoiesis in a patient with neutropenia associated with a CD3+ CD8+ CD57+ population. In Baker's study, marrow colony forming unit-GM (CFU-GM) growth was markedly reduced, but normalized after T-cell depletion in the absence of autologous plasma. The addition of either autologous T cells or autologous plasma to cultures caused marked growth inhibition. Humoral suppression of CFU-GM was shown to be mediated by the IgG fraction, and seemed to involve a complement-independent mechanism. Our patient exhibited increased IgG and autoreactive antibodies, including ANA, antiphospholipid antibody, and ssDNA and dsDNA antibodies. Antibody-dependent cell-mediated cytotoxicity[12] may therefore have played an important role in our patient.

Recent studies reported an accumulation of autoreactive lymphocytes in T-LGL-leukemia patients due to a dysregulation of the Fas/FasL–apoptotic pathway.[16,17] Also, excessive accumulations of autoreactive T cells by dysregulation of the Fas/FasL–apoptotic pathway have been observed in autoimmune *gld* or *lpr* mice and in patients with autoimmune lymphoproliferative syndrome (ALPS) or Canale–Smith syndrome.[18–20] Taken together, not only the Fas/FasL-apoptotic pathway but also IL-18 may be important to the mechanism of the accumulation of autoreactive T lymphocytes.

Similarly, the pathogenic roles of IL-18 have been focused in cases of SLE. Sera from patients with SLE were found to contain significantly higher concentrations of IL-18 than normal individuals.[7] MRL/lpr mice, which develop spontaneous lupus-like autoimmune disease, have also been found to have higher serum levels of IL-18 and to be hyperresponsive to IL-18.[8,21] Daily injections of IL-18 have been shown to enhance disease progression and severity even in lupus-prone lpr mice.[21] Furthermore, in vivo IL-18 inhibition by IL-18 cDNA vaccination has been shown to reduce spontaneous lupus-like autoimmune disease.[22] These results suggest that IL-18 is able to accelerate spontaneous autoimmune lupus disease.

SLE is generally considered a prototype of dominant Th-2 cytokine disease. However, Th-1 as well as Th-2 cytokines can be elevated in SLE patients.[23,24] Furthermore, recent reports have stressed the role of Th-1 cytokines in the pathogenesis of SLE, especially with regard to nephritis.[25,26] The role of IL-18 and Th-1 cells in the pathogenesis of SLE may be important.

This is the first report of the possible contribution of IL-18 to T-LGL leukemia associated with autoimmune disorders, and identifies directions for the future study of the pathogenetic role of autoreactive T-LGL cells in autoimmune disease. Further analysis of CTL activation by IL-18 may clarify the mechanism underlying both autoimmune disease and T-LGL leukemia.

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