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Title: Elevated Serum APRIL Levels in Patients with Systemic Lupus Erythematosus

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Abstract

Objective. To determine whether serum levels of a proliferation-inducing ligand (APRIL) are elevated in patients with systemic lupus erythematosus (SLE) and are correlated with autoantibody titers and/or disease activity.

Methods. Sera from 48 patients with SLE, 41 normal healthy individuals and 21 patients with rheumatoid arthritis (RA) were assayed for APRIL by enzyme-linked immunosorbent assay. Medical charts were retrospectively reviewed for autoantibody titers and immunoglobulin levels. Disease activity was assessed using the British Isles Lupus Assessment Group (BILAG) index.

Results. The APRIL levels in the sera from patients with SLE were significantly higher than those from healthy controls and those from patients with RA. Serum APRIL levels were not correlated with serum IgG and IgM levels, but had a tendency to be correlated with anti-double-stranded DNA antibody titers. Moreover, serum APRIL levels were significantly correlated with musculoskeletal manifestations among SLE patients when assessed by the BILAG index.

Conclusion. APRIL may be an important factor in elevated autoantibody titers and musculoskeletal disease in patients with SLE. Patients with elevated serum APRIL levels may be ideal candidates for therapeutic targeting of APRIL.

Key words: APRIL (a proliferation-inducing ligand), systemic lupus erythematosus, ELISA, anti-dsDNA antibody
Introduction

A proliferation-inducing ligand (APRIL, also called TNFSF13), a newly identified member of the TNF ligand family, is a type II membrane binding protein of 250 amino acids (1). Originally, APRIL was reported to have a regulatory role in tumor growth (1). APRIL is a close sequence homologue of the recently reported B cell activation factor (BAFF, also known as BlyS/zTNF4/TALL-1), also a member of the TNF family (2). BAFF promotes B cell differentiation, proliferation and survival of a subset of immature B lymphocytes and BAFF-transgenic mice develop a lupus-like phenotype characterized by high titers of anti-DNA antibodies, hypergammaglobulinemia and glomerulonephritis (3). In APRIL transgenic mice, T cell survival and antigen-specific antibody responses are enhanced (4).

APRIL binds to two of the three BAFF receptors (B cell maturation antigen [BCMA] and transmembrane activator and CAML interactor [TACI]) and is supposed to play a regulatory role in B cell proliferation (5). The treatment of lupus-prone NZBWF1 mice with soluble TACI-Ig fusion protein (soluble decoy receptor for BAFF and APRIL) inhibits the development of proteinuria and prolongs survival of the animals (6). It has also been reported that gene polymorphism of APRIL is associated with SLE (7). These findings indicate that APRIL as well as BAFF may be involved in the development of SLE. The serum levels of BAFF are elevated in patients with SLE, Sjogren’s syndrome and rheumatoid arthritis (RA) (8). Recently, increased levels of BAFF and APRIL were reported in the synovial fluid of inflamed joints (9). However, serum levels of APRIL in patients with SLE have not been reported.

In this article, we report for the first time increased serum APRIL levels in patients with
SLE. Our results indicate that elevated levels of serum APRIL may be an important factor in the pathogenesis of SLE.

Patients and Methods

Subjects
Peripheral blood was obtained from 48 patients (female 46, male 2, mean age 37.9 years) meeting the American College of Rheumatology (ACR) criteria for the classification of SLE. RA patients (n=21; female 18, male 3, mean age=55.7 years) were meeting the ACR criteria and followed by Kyushu University hospital. Control subjects (n=41; female 33, male 8, mean age 35.1) were healthy volunteer donors who were recruited form Kyushu University Graduate School of Medical Sciences personnel. Informed consent was obtained from each patient and control. All were Japanese and the sera were stored at -20 °C until use.

ELISA for measurement of APRIL
The collected sera were assayed for APRIL by an antigen-capture enzyme-linked immunosorbent assay (ELISA). ELISA plates (Becton Dickinson Labware, Franklin lakes, NJ) were coated with 5 μg/ml rabbit anti-human APRIL polyclonal antibody (ED2) (ProSci Inc., Poway, CA) overnight at 4 °C. After non-specific binding had been blocked with 1% BSA-PBS, the collected sera were added, followed by the detection antibody, goat anti-human APRIL antibody (Genzyme Techne, Minneapolis, MN). Rabbit anti-goat IgG-HRP (MBL, Nagoya, Japan) and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Wako Pure
Chemical Industries, Osaka, Japan) were used for detection. Enzyme activities were read at an OD of 405 nm, and a standard curve was generated using recombinant human APRIL (Genzyme Techne). Immunoprecipitation of the APRIL was performed by adding BCMA-Fc chimera (Genzyme Techne), soluble receptor for APRIL, to the serum followed by adding protein A-sepharose (Sigma-Aldrich, St. Louis, MO). Recombinant human BAFF was purchased from Genzyme Techne.

**Measurement of autoantibody and assessment of the disease activity**

The serum levels of anti-dsDNA and anti-Sm antibody were measured by ELISA kit (MBL) according to manufacture’s instructions. We evaluated the disease activity at the time of the serum sampling using the British Isles Lupus Assessment Group (BILAG) index and systemic lupus erythematosus disease activity index (SLEDAI) (10). BILAG has been validated and shown to be reproducible and reliable (11). The index allocates separate scores to each of eight organ-based systems.

**Statistical analysis**

All statistical analyses were performed using StatView software (Abacus concept Inc., Cary, NC). Nonparametric testing was performed by the Mann-Whitney’s U Test between two groups. Correlation was determined by Pearson’s product-moment correlation for interval data or by Spearman’s rank order correlation for ordinal data or for interval data that did not follow normal distribution. When we investigate the correlation of BILAG with serum APRIL levels, we used 4, 3, 2, 1 instead of A,B,C,D of BILAG score, respectively.
Results

Elevated serum levels of APRIL in the patients with SLE

We measured the serum levels of APRIL in 48 patients with SLE, 21 patients with RA and in 41 healthy controls using a sandwich ELISA. The serum levels of APRIL in SLE patients (84.1 +/-106.1, mean +/-S.D.) were significantly higher than those in the normal cohorts (16.0+-25.6, p=0.0004) and RA patients (25.1+-34.5, p=0.046), while those in RA patients were not higher than those in normal controls (Figure 1). The serum APRIL levels in most of the normal controls were below 50 ng/ml, and only three of 41 controls (7.32%) had levels higher than 100 ng/ml. In contrast, the APRIL levels in the majority of SLE patients were higher than 50 ng/ml, and 20 of 48 patients (42%) had levels above 100 ng/ml. Immunoprecipitation of the sera with BCMA-Fc chimera, resulted in a significant decrease in the APRIL level by this ELISA system. In addition, recombinant human BAFF was not detected by this ELISA (data not shown). This result suggested the specificity of our ELISA system to APRIL.

Correlation between serum APRIL levels and elevated autoantibody titers

Retrospective chart review revealed that 45 SLE patients were screened for serum anti-dsDNA antibodies within the same collection of blood for serum APRIL determination. The anti-dsDNA antibody titers had a tendency to be correlated (r=0.277, p=0.065) with serum APRIL levels. Anti-Sm antibody was not correlated with the serum APRIL levels in SLE patients. The serum levels of APRIL were not correlated with IgG,
IgM or anti-Sm antibody titers in SLE patients. There was no correlation between serum levels of APRIL and the dose of corticosteroids or immunosuppressants.

**Correlation between serum APRIL levels and disease activity**

We also analyzed the clinical data of the SLE patients using BILAG (Figure 2). The serum APRIL levels were significantly correlated with the BILAG score of musculoskeletal disease \( p=0.0151 \), but there was no correlation with other seven organs including nervous system and renal involvement. There was also no correlation between the serum APRIL level and SLEDAI.

**DISCUSSION**

In this study, we showed for the first time that APRIL levels were elevated in the sera from patients with SLE and were associated with a phenotype of SLE.

SLE is a prototypic systemic autoimmune disease characterized by the production of autoantibodies against a spectrum of nuclear antigens (12). These autoantibodies are produced by autoreactive B lymphocytes in the presence or absence of autoreactive T lymphocytes (12). APRIL has a regulatory role in B cell proliferation (5), and affects the T cell response (4), and dendritic cells induced CD40-independent immunoglobulin class switching through APRIL (13). Furthermore, the soluble decoy receptors for APRIL prolong the survival of lupus-prone NZBWF1 mice (6, 14). These lines of evidence suggest that increased serum APRIL may be involved in the pathogenesis of SLE. The mechanism of the increased serum APRIL levels might be explained as follows. APRIL is
synthesized by monocytes and dendritic cells in response to interferons. Since type I interferon-regulated genes are up-regulated in peripheral blood mononuclear cells from patients with SLE, elevated APRIL could be explained by the interferon-induced expression in these cell types (13). Another possible mechanism for elevated serum APRIL levels in SLE is that there may be polymorphisms in the promoter region of APRIL gene, and these might affect gene expression.

The serum APRIL levels tended to be correlated with anti-dsDNA antibody titers (p=0.065). In fact recombinant APRIL costimulates B cells in vitro and in vivo, although its potency is less than that of BAFF (5). It is thus suggested that APRIL may be an important factor in elevated autoantibody titers.

It is noteworthy that serum APRIL levels were significantly correlated with the musculoskeletal manifestations of SLE, mostly arthritis in the present study. It has been reported that joint inflammation and joint destruction of collagen-induced arthritis, a model of noninfectious inflammatory arthritis, are inhibited by the treatment with TACI-Ig, an antagonist of APRIL and BAFF (15). Recently, it was reported that the levels of APRIL and BAFF are increased in the synovial fluid of inflamed joints, but not in the sera, indicating increased local production of BAFF and APRIL (9). Because B cells have a role in activation and cytokine production of T cells in joints, prolonged survival or activation of B cells by APRIL may lead to joint inflammation. Although we could not analyze the APRIL levels in synovial fluid from SLE patients, elevation of APRIL may lead to the worsening of arthritis in patients with SLE.

Our findings suggest that APRIL antagonist may be a suitable therapy for patients with SLE. Further studies on the regulation of APRIL gene in patients with SLE, and the association of serum APRIL with other autoimmune diseases, are warranted.
REFERENCES


Figure legends

Figure 1. The serum APRIL levels in patients with SLE.
The serum levels of APRIL in the patients with SLE (n=48) were significantly higher than those in healthy controls (n=41), p=0.0004.

Figure 2. The correlation between the serum APRIL levels and musculoskeletal manifestations among SLE patients. The correlation coefficient was determined by the Spearman’s rank order correlation.
BILAG (joint and tendon)

serum APRIL (ng/ml)

(p=0.0151)
Elevated serum APRIL levels in patients with systemic lupus erythematosus

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