A characteristic adverse event in the iguratimod group was increased hepatic enzyme. Although this event included transient increase, attention should be paid to hepatic function data during iguratimod therapy based on the frequency of increased hepatic enzymes in our study. Another characteristic adverse event in iguratimod group was dermatological disorder, of which frequency was relatively low. Attention should also be paid to abdominal pain, anemia, and other symptoms and signs related to gastrointestinal disorder during iguratimod therapy because peptic ulcer was reported in the iguratimod group. Hematological disorder does not seem to be an iguratimod-specific adverse event because the disorder reported in the iguratimod group did not differ from that in the salazosulfapyridine group.

In conclusion, the efficacy of iguratimod is not inferior to that of salazosulfapyridine. Iguratimod could be effective even in patients who have a poor response to currently available DMARDs. Adverse reaction profiles of iguratimod are different from those of salazosulfapyridine. If used carefully, iguratimod could become a novel DMARD that is useful to improve physical condition and the quality of life in patients with rheumatoid arthritis.

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Clinical and Immunogenetic Features of Patients With Autoantibodies to Asparaginyl–Transfer RNA Synthetase

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Objective. **We have previously described anti-KS autoantibodies and provided evidence that they are directed against asparaginyl–transfer RNA (tRNA) synthetase (AsnRS). The aim of the present study was to identify patients with anti-AsnRS autoantibodies and elucidate the clinical significance of this sixth antisynthetase antibody. In particular, we studied whether it was associated with the syndrome of myositis (polymyositis or dermatomyositis [DM]), interstitial lung disease (ILD), arthritis, and other features that had been previously associated with the 5 other anti–aminoacyl–tRNA synthetase autoantibodies.**

Methods. **More than 2,500 sera from patients with connective tissue disease (including myositis and ILD) and controls were examined for anti-AsnRS autoantibodies by immunoprecipitation (IP). Positive and control sera were tested for the ability to inhibit AsnRS by preincubation of the enzyme source with the serum. The HLA class II (DRB1, DQA1, DQB1, DPB1) alleles were identified from restriction fragment length polymorphism of polymerase chain reaction–amplified genomic DNA.**

Results. **Anti-AsnRS antibodies were identified in the sera of 8 patients (5 Japanese, 1 American, 1 German, and 1 Korean) by IP of the same distinctive set of tRNA and protein that differed from those precipitated by the other 5 antisynthetases, and these antibodies showed specific inhibition of AsnRS activity. Two of these patients had DM, but 7 of 8 (88%) had ILD. Four patients (50%) had arthritis, and 1 had Raynaud's phenomenon. This antisynthetase was very rare among myositis patients (present in 0% of Japanese myositis patients), but it was found in 3% of Japanese ILD patients. Thus, most patients with anti-AsnRS had chronic ILD with or without features of connective tissue disease. Interestingly, all 4 Japanese patients tested had DR2 (DRB1*1501/1502), compared with 33% of healthy controls.**

Conclusion. **These results indicate that anti-AsnRS autoantibodies, like anti–alanyl–tRNA synthetase autoantibodies, have a stronger association with ILD than with myositis and may be associated with the DR2 phenotype.**

The aminoacyl–transfer RNA (aminoacyl-tRNA) synthetases are a family of cytoplasmic enzymes that catalyze the formation of aminoacyl-tRNA from a specific amino acid and its cognate tRNA and play a crucial role in protein synthesis. Autoantibodies to certain of these synthetases (histidyl–, threonyl–, alanyl–, isoleucyl–, and glycyl–tRNA synthetases) have been identified in patients with inflammatory myopathies (1–6). Among these "antisynthetase autoantibodies," the most common is anti–Jo-1 (anti–histidyl–tRNA synthetase [anti-HisRS]), found in 20% of patients with polymyositis/dermatomyositis (PM/DM) (7–11). Anti–PL-7 (anti–threonyl–tRNA synthetase [anti-ThrRS])

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and anti–PL-12 (anti–alanyl–tRNA synthetase [anti-AlaRS]) autoantibodies are less common, found in 3–4% of all patients with PM/DM (4,5,11–13), while anti-OJ (anti–isoleucyl–tRNA synthetase [anti-IleRS]) and anti-EJ (anti–glycyl–tRNA synthetase [anti-GlyRS]) autoantibodies are the least common, occurring in <2% (6,14,15), although the frequency may vary in different populations (16).

Characteristic clinical features have been found in patients with anti-HisRS and other antisynthetase autoantibodies (1,9,10). These features include myositis, interstitial lung disease (ILD), arthritis, Raynaud's phenomenon, fever with exacerbations, and the skin lesion of the fingers referred to as mechanic's hands, and they appear to form a distinct syndrome referred to as the "antisynthetase syndrome" (8–11). Although the similarity of the clinical features associated with different antisynthetases is impressive (17,18), certain differences have been noted, which must be considered preliminary due to the small reported number of patients with non-HisRS antisynthetases (1,9,19). Patients with anti-AlaRS appear to be more likely than those with anti-HisRS to have ILD and/or arthritis either without myositis or with little evidence of muscle disease. Absence of significant myositis over the full disease course in patients with anti-HisRS is rare (<5%), although it may occur. Clinically significant myositis was seen in 60% of US patients with anti-AlaRS (13), whereas none of 6 Japanese patients with anti-AlaRS autoantibodies fulfilled criteria for myositis (20). Among patients with anti-IleRS, 2 of 10 had ILD without evidence of myositis, and 1 had ILD with subclinical myositis (14). In addition, antisynthetases may occur in either PM or DM, but PM is usually more common with anti-HisRS (10,16,21), and DM is usually more common with other antisynthetases, especially anti-GlyRS (15,22).

We recently described anti-KS autoantibodies and provided evidence that the KS antigen is asparaginyl–tRNA synthetase (AsnRS) (23). This sixth antisynthetase was found in sera from 3 patients with ILD and/or inflammatory arthritis without evidence of myositis. It immunoprecipitated a 65-kd protein and a unique tRNA that was distinct from that precipitated by any previously described antisynthetase or other reported tRNA-related antibody. Each of the 3 sera and their IgG fractions showed significant inhibition of AsnRS activity, but did not inhibit any of the other 19 aminoacyl–tRNA synthetase activities.

In this report, we describe the clinical and immunogenetic features of 5 additional patients with anti-AsnRS autoantibodies, most of whom had the syndrome of ILD with arthritis and/or myositis. Immunoprecipitation (IP) and aminoacylation inhibition studies with sera from these patients provide additional evidence that anti-KS (anti-AsnRS) reacts with asparaginyl–tRNA synthetase.

PATIENTS AND METHODS

Sera. Serum samples from a collection of sera from ~800 patients seen at the current or previous collaborating centers of the authors (Keio University, Tokyo, Japan; Kyoto University, Kyoto, Japan; Seoul National University, Seoul, Korea; Clinic and Research Institute for Rheumatic Diseases Aachen, Aachen, Germany; University of Oklahoma Health Sciences Center, Oklahoma City; National Institutes of Health, Bethesda, MD) or sera referred there for testing were stored at −20°C and were tested for the presence of anti-AsnRS autoantibodies. Sera from the following patients were included: 1) patients with PM or DM according to the criteria described by Bohan and Peter (24,25); 2) patients with a condition suggesting the clinical diagnosis of myositis; 3) patients with ILD who had no evidence of myositis and did not meet criteria for other connective tissue diseases; and 4) patients with serum anticytoplasmic antibodies, regardless of diagnosis. Approximately 1,700 other sera have also been tested, including sera from patients with other conditions including systemic lupus erythematosus, systemic sclerosis, and rheumatoid arthritis, as well as sera from normal subjects. Many of the sera were tested in studies of other autoantibodies. All samples were obtained after the patients gave their informed consent, as approved by the corresponding institutional review boards. Stored sera known to contain autoantibodies against synthetases for histidine, threonine, alanine, glycine, and isoleucine were used as controls.

ILD was considered to be present if an interstitial infiltrate was observed on chest radiography. DM was considered to be present if a heliotrope rash and/or Gottron's papules were observed.

IP. IP from HeLa cell extracts was performed as previously described (6,10). Ten microliters of patient sera was mixed with 2 mg of protein A–Sepharose CL-4B (Pharmacia Biotech, Uppsala, Sweden) in 500 μl of IP buffer (10 m*M* Tris HCl at pH 7.5, 500 m*M* NaCl, 0.1% Nonidet P40 [NP40]) and incubated with end-over-end rotation (Labquake shaker; Lab Industries, Berkeley, CA) for 2 hours at 4°C. The IgG-coated Sepharose was washed 4 times in 500 μl of IP buffer using 10-second spins in a microfuge tube, and resuspended in 400 μl of NET-2 buffer (50 m*M* Tris HCl at pH 7.5, 150 m*M* NaCl, 0.05% NP40).

For analysis of RNAs, this suspension was incubated with 100 μl of extracts, derived from 6×10^6 cells, on the rotator for 2 hours at 4°C. The antigen-bound Sepharose was then collected with a 10-second centrifugation in the microfuge, washed 4 times with NET-2 buffer, and resuspended in 300 μl of NET-2 buffer. To extract bound RNAs, 30 μl of 3.0*M* sodium acetate, 30 μl of 10% sodium dodecyl sulfate (SDS), and 300 μl of phenol/chloroform/isoamyl alcohol (50:50:1; containing 0.1% 8-hydroxyquinoline) were added to the Sepharose beads. After agitation in a Vortex mixer and

spinning for 1 minute. RNAs were recovered in the aqueous phase after ethanol precipitation and dissolved in 20 μl of electrophoresis sample buffer, composed of 10*M* urea, 0.025% bromphenol blue, and 0.025% xylene cyanol FF (Bio-Rad, Hercules, CA) in Tris–borate–EDTA buffer (90 m*M* Tris HCl at pH 8.6, 90 m*M* boric acid, and 1 m*M* EDTA). The RNA samples were denatured at 65°C for 5 minutes and then resolved by 7*M* urea–10% polyacrylamide gel electrophoresis (PAGE), with silver staining (Bio-Rad).

For protein studies, antibody-coated Sepharose was mixed with 400 μl of ^{35}S-methionine–labeled HeLa extract derived from 2×10^5 cells and rotated at 4°C for 2 hours. After 4 washes with IP buffer, the Sepharose was resuspended in SDS sample buffer (2% SDS, 10% glycerol, 62.5 m*M* Tris HCl at pH 6.8, 0.005% bromphenol blue). After heating at 90°C for 5 minutes, the proteins were fractionated by 10% SDS-PAGE, enhanced with 0.5*M* sodium salicylate, and dried. Labeled proteins were analyzed by autoradiography.

Aminoacylation. Aminoacylation inhibition reactions were performed as described previously, with minor modification (6,26). Six microliters of HeLa cell extract diluted 1:10 in Tris buffered saline was incubated with 3 μl of a 1:10 dilution of serum for 2 hours at 4°C. This was combined with 17 μl of reaction solution (50 m*M* Tris HCl at pH 7.5, 0.02*M* NaCl, 0.01*M* $MgSO_4$, 1 m*M* dithiothreitol) containing 8 units of yeast tRNA, 3 μl of ^{14}C-asparagine or other ^{3}H-labeled amino acid, and 1 μl of 200 m*M* cold amino acid. Ten-microliter aliquots were tested at 10 minutes and 20 minutes, spotted onto filter paper treated with 5% trichloroacetic acid (TCA), washed 5 times with 5% TCA, then with ethanol, then dried for counting. Results of inhibition testing were expressed as the percent inhibition of the average activity seen with the normal serum included in that experiment, as follows: % inhibition = [(average counts per minute with normal serum) − (cpm with test serum)] × 100/(average cpm with normal serum). Inhibition of >50% compared with the activity with normal serum was considered significant. In previous studies, although nonspecific effects on aminoacylation reactions by serum were common, nonspecific inhibition was usually <25%, and inhibition >50% reliably reflected specific antibody effects (6,7,12,13,26).

DNA typing of the HLA class II (DRB1, DQA1, DQB1, DPB1) alleles by polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP). Genomic DNA was isolated by phenol extraction of SDS-lysed and proteinase K–treated peripheral blood leukocytes, and then amplified by the PCR procedure using an automated PCR thermal cycler (PerkinElmer Cetus, Norwalk, CT). The primers used for specific amplification of the polymorphic exon 2 domains of the DRB1, DQA1, DQB1, and DPB1 genes were previously described (27). Amplified DNA was digested by all-specific restriction endonucleases and subjected to electrophoresis using a 12% polyacrylamide gel. Digested fragments were detected by staining with ethidium bromide, and HLA genotypes were determined on the basis of the RFLP patterns generated as previously described (27).

Other. Ouchterlony double immunodiffusion was performed as described previously, using HeLa cell extract as antigen (10).

Cases. *Patient 1.* The patient, a 61-year-old Japanese woman, noticed chest pain, followed 3 months later by dyspnea on mild exertion. Chest radiography and computed tomography (CT) scanning showed bilateral basilar infiltrates. The patient had hypoxemia, with a restrictive pattern on pulmonary function tests. No muscle weakness was observed, and the creatine kinase (CK) level was normal (67 IU/liter). A lung biopsy specimen obtained by video-assisted thoracic surgery showed mild interstitial chronic inflammation and interstitial fibrosis lacking a temporal heterogeneity pattern, and a diagnosis of fibrotic nonspecific interstitial pneumonia was made.

Patient 2. The patient, a 51-year-old German woman, developed a nonproductive cough and dyspnea on exertion. Chest radiography showed bibasilar interstitial fibrosis, and pulmonary function tests showed a restrictive pattern with decreased diffusing capacity for carbon monoxide (DLco). A diagnosis of ILD was made, and the patient's pulmonary function remained stable throughout her disease course. She had polyarthralgia and developed erythema and keratosis of the palms and fingers consistent with mechanic's hands, but no cutaneous scleroderma, Raynaud's phenomenon, or DM rash (Gottron's papules or heliotrope rash) was observed. No muscle weakness was found, and the CK level was normal (56 IU/liter at the first visit) each time it was measured. When the patient was age 58 years, ovarian carcinoma was found, and surgery with subsequent irradiation was performed. She died of metastatic ovarian carcinoma at age 63 years.

Patient 3. The patient, a 72-year-old American woman, developed an itchy red eczematous rash that was thought to be due to a medication for hypertension. The rash was soon accompanied by progressive weakness, myalgias, mild dyspnea, and difficulty swallowing. She was started on prednisone and methotrexate, and 6 months after the rash had first appeared, she was referred to the Arthritis and Rheumatism Branch of the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health. There was a widespread maculopapular rash of the trunk, extremities, and head, and Gottron's papules were observed. Proximal muscle weakness was present, and her CK level was 358 IU/liter. Magnetic resonance imaging of the thighs showed both atrophy and probable inflammation on the STIR images. A biopsy of the deltoid muscle showed changes of an active inflammatory myopathy. No malignancy was identified. She was treated with pulse methylprednisolone. However, her muscle weakness and rash were not significantly improved, and infectious complications limited the therapeutic options. Her disease course was subsequently complicated by herpes zoster and the Ramsay-Hunt syndrome as well as by skin infections and cellulitis, mastoiditis, heart failure, and a cerebrovascular accident.

Patient 4. The patient, a 53-year-old Korean woman with intermittent episodes of productive cough due to bronchiectasis, noticed easy fatigability and myalgia in 1994 and later developed muscle weakness and was admitted to Seoul National University Hospital in February 1995. Proximal muscle weakness in her extremities and a dark pigmentation over the extensor surface of both knees were observed. The CK level was elevated at 3,808 IU/liter. The findings on electromyogram and muscle biopsy were consistent with inflammatory myopathy. A diagnosis of DM associated with ILD was made, and she was treated with prednisolone (60 mg/day). Her muscle enzyme levels gradually normalized, and her muscle weakness improved. Her chest radiograph and high-resolution

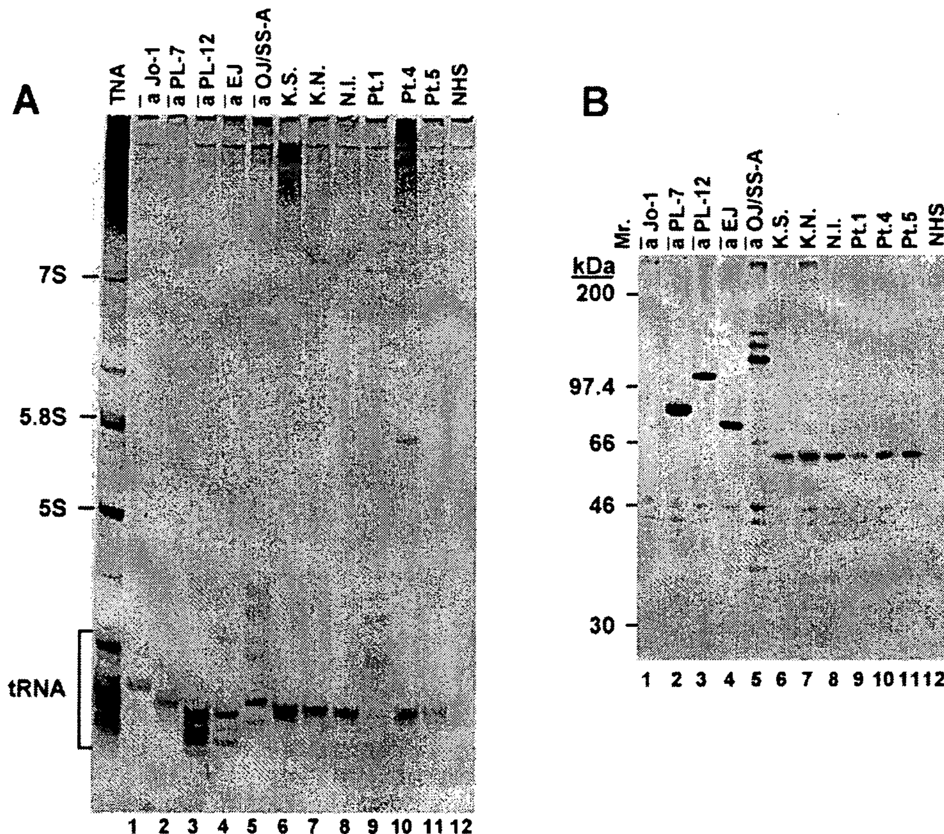

Figure 1. A, Immunoprecipitation (IP) for nucleic acids with anti-KS and control sera. Shown are patterns of transfer RNA (tRNA) resulting from 7*M* urea–10% polyacrylamide gel electrophoresis (PAGE) of phenol-extracted immunoprecipitates from HeLa cell extract, developed with silver stain. TNA = total nucleic acids, with the 5.8S and 5S small ribosomal RNAs and the tRNA region indicated. Antisynthetase sera used for IP are indicated. Lane 1, Anti–histidyl-tRNA synthetase (a Jo-1); lane 2, anti–threonyl-tRNA synthetase (a PL-7); lane 3, anti–alanyl-tRNA synthetase (a PL-12); lane 4, anti–glycyl-tRNA synthetase (a EJ); lane 5, anti–isoleucyl-tRNA synthetase (a OJ/SS-A); lanes 6–11, anti-KS sera from patients KS, KN, and NI in the previous study (23) and from patients 1, 4, and 5 in the present study; lane 12, normal human serum (NHS) control. The tRNA pattern with anti-KS sera is easily distinguishable from that of other antisynthetases. B, IP for proteins with anti-KS and control sera. Autoradiogram of 10% sodium dodecyl sulfate–PAGE of immunoprecipitates from ^{35}S-methionine–labeled HeLa cell extract. Mr. = molecular weight markers. Antisynthetase sera used for IP are indicated as in A. Anti-KS sera immunoprecipitated a very strong protein band from ^{35}S-methionine–labeled HeLa cell extracts (lanes 6–11), migrating at 65 kd, that was clearly different from the bands immunoprecipitated by sera with the described antisynthetases.

CT scan showed bilateral basilar interstitial fibrosis, and pulmonary function tests showed a restrictive pattern with decreased DLco. Her muscle weakness gradually improved, and the CK level normalized in January 1996. Prednisolone was tapered and discontinued in March 1996.

Patient 5. The patient, a 64-year-old Japanese man with a previous history of prostatic carcinoma, was admitted to the hospital due to bilateral infiltrates on chest radiography. He did not notice cough or dyspnea at that time, but a chest CT scan revealed bibasilar interstitial fibrosis. A transbronchial lung biopsy was performed, with histology showing usual interstitial pneumonia. He was started on prednisolone (40 mg/day), resulting in slight improvement seen on his chest radiograph. Prednisolone was tapered and discontinued in April 1998. He then developed polyarthritis and was treated with a nonsteroidal antiinflammatory drug. No muscle weakness was found, and the CK level was normal (50 IU/liter at the first visit) throughout his disease course.

RESULTS

Identification of anti-KS (anti-AsnRS) antibodies. Sera from all 8 patients (the 3 patients with ILD and/or inflammatory arthritis without evidence of myositis in our previous study [patients KS, KN, and NI; see

Table 1. Clinical features of 8 patients with anti-KS antibodies*

	Patient							
	KS	KN	NI	1	2	3	4	5
Age at onset, years/sex	36/F	44/F	61/F	60/F	51/F	72/F	53/F	65/M
Ethnic background	Japanese	Japanese	Japanese	Japanese	German	US	Korean	Japanese
ILD	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Myositis	No	No	No	No	No	Yes	Yes	No
DM rash	No	No	No	No	No	Yes	Yes	No
Arthritis	Yes	No	No	No	Yes	Yes	No	Yes
Malignancy	No	No	No	No	Ovarian cancer	No	No	Prostate cancer
Raynaud's phenomenon	No	Yes	No	No	No	No	No	No
Other autoantibodies	No	No	No	Anti-SSA/Ro	No	No	No	No
Diagnosis	ILD with arthritis	Idiopathic ILD	Idiopathic ILD	Idiopathic ILD	Idiopathic ILD	DM	DM	ILD with arthritis

* ILD = interstitial lung disease; DM = dermatomyositis.

ref. 23] and the 5 additional patients described above) were shown to immunoprecipitate a characteristic, identical pattern of tRNA, with a strong predominant nucleic acid band of tRNA size, accompanied by a faster faint band (Figure 1A). This gel pattern of tRNA was clearly distinguishable from the pattern of tRNA precipitated by the 5 other described antisynthetases (Figure 1A) and was identical in mobility and appearance to that of serum KS, the originally reported anti-KS serum (23) (Figure 1A).

A very strong band from ^{35}S-methionine–labeled HeLa cell extracts (Figure 1B), migrating at 65 kd, that was also identical in mobility to that of serum KS, was found by IP for all 8 sera, with 5 representative sera shown in Figure 1B. This was clearly different from the characteristic bands immunoprecipitated by sera with the other described antisynthetases (Figure 1B).

Five of the newly recognized anti-KS antibody–positive sera were tested for their ability to inhibit the in vitro enzymatic function of AsnRS (aminoacylation of $tRNA^{Asn}$). Four of the 5 new anti-KS sera significantly inhibited (by >50% at 10 minutes) AsnRS activity compared with normal serum or other controls (serum from patient KS by 87%, serum from patient KN by 99%, serum from patient NI by 91%, serum from patient 1 by 82%, serum from patient 2 by 100%, serum from patient 3 by 18%, serum from patient 4 by 87%, and serum from patient 5 by 91%). This inhibition was strong and comparable with that seen with serum KS, for 4 of the 5 new anti-KS sera. Purified IgG from the third new serum (from patient 3) showed significant, but not strong, inhibition (52%) that increased at 20 minutes (to 84%).

There was no significant inhibition of other synthetases. Normal control serum and anti-KS–negative myositis serum did not show significant inhibition of AsnRS, although sera with other antisynthetases inhibited the expected enzymes. These results indicated that sera with anti-KS by IP showed specific inhibition of AsnRS, further supporting previous data indicating that anti-KS reacted with AsnRS.

Clinical findings. The clinical features of the 5 newly identified patients (patients 1–5) and the 3 patients with anti-AsnRS reported previously (patients KS, KN, and NI) (23) are summarized in Table 1. All patients with anti-AsnRS antibodies were middle-aged or elderly, and 7 of them were women. Five patients were Japanese, 1 was from the US, 1 was German, and 1 was Korean. Seven of these 8 patients (88%) had ILD, documented in each case by both chest radiography and pulmonary function tests. In addition, 2 patients had myositis and a diagnosis of DM. Their clinical courses of ILD were classified as the chronic type. Four patients (50%) had nonerosive arthritis or arthralgia. Raynaud's phenomenon was seen in only 1 patient. None of the patients had sclerodactyly or overlap syndromes with other connective tissue diseases. Malignant diseases (ovarian carcinoma and prostatic carcinoma) were observed in 2 patients. Regarding other autoantibodies, anti-SSA/Ro antibodies were detected in only 1 patient.

Anti-AsnRS was found in 0% of Japanese patients with myositis, but was found in 3% of Japanese patients with "idiopathic" ILD. Thus, most patients with anti-AsnRS antibodies had chronic ILD with or without features of PM/DM or other connective tissue disease.

Immunogenetic features. The HLA class II gene was determined in 4 Japanese patients (Table 2). All 4 patients had DR2 (DRB1*1501 or DRB1*1502) compared with 33% of healthy local controls. It should be noted that all patients with anti-AsnRS antibodies had DR2, but the frequency of DR2 did not reach statistical significance ($P > 0.05$).

Table 2. HLA class II genes in Japanese patients with anti-KS autoantibodies

	Patient			
	KS	KN	NI	1
DR	2/5	2/1	2/2	2/4
DRB1*	1502/1101	1501/0101	1502/1502	1501/0405
DQA1*	0103/0501	0102/0101	0103/0103	0102/0303
DQB1*	0601/0301	0602/0501	0601/0601	0602/0401
DPB1*	0901/1401	0201/0501	0901/0901	0201/0402

DISCUSSION

We have identified anti-KS (anti-AsnRS) autoantibodies in 8 patients with ILD and DM, by IP of the same distinctive set of tRNA and protein that differed from those precipitated by the other 5 antisynthetases. Most of the anti-KS sera showed specific inhibition of the enzyme target, AsnRS, without inhibiting other synthetases.

Several interesting characteristics of the previously studied antisynthetases have been described: 1) they are associated with a distinctive clinical syndrome referred to as the antisynthetase syndrome, 2) they are directed at functionally related enzymes (performing the same function for different amino acids), 3) they do not cross-react with other synthetases, and 4) they tend to be mutually exclusive. Anti-AsnRS antibodies seem to have the same features. No serum with any other antisynthetase has had antibodies to AsnRS, and none of the 8 anti-AsnRS sera reported here showed signs of reaction with other synthetases. The mechanism of this phenomenon remains unknown.

Multiple tRNA bands immunoprecipitated by anti-AsnRS were found on urea-PAGE. The patterns of tRNA for each of the 8 patients were very similar, highly restricted compared with total tRNA, and distinctive compared with the pattern of other anti–aminoacyl tRNA synthetase autoantibodies. These bands are likely to represent different forms of tRNA for asparagine, which can include tRNA with different asparagine anticodons (uracil-uracil-adenine, uracil-uracil-guanine) or tRNA with the same anticodon but differences in other parts of the sequence. Most sera with anti-HisRS, anti-ThrRS, anti-GlyRS, and anti-IleRS had not been described to react directly with tRNA, suggesting indirect precipitation of tRNA. However, approximately one-third of anti-HisRS–positive sera were reported to contain autoantibodies recognizing $tRNA^{His}$ (28). Most anti-AlaRS sera react directly with the sets of $tRNA^{Ala}$ with the inosine-guanine-cytosine anticodon (29). We previously found that the 3 original anti-KS (anti-AsnRS) sera did not immunoprecipitate any RNA from deproteinized HeLa extracts (23). This suggests that anti-AsnRS antibodies can precipitate $tRNA^{Asn}$ indirectly, through its affinity for AsnRS, although the possibility of conformational epitopes on the tRNA has not been excluded (28). Further analysis will be necessary to determine the sequence and specificity of tRNA immunoprecipitated by anti-AsnRS.

The specific inhibition of AsnRS function by most of the sera found to have anti-KS is consistent with findings observed for other antisynthetases. It should be noted that our anti-KS sera also demonstrated inhibition of enzymatically active recombinant AsnRS (30). Most sera with any of the 5 reported antisynthetases specifically inhibit the aminoacylation of the respective tRNA, indicating inhibition of the enzymatic function of the synthetase (3,5–7,12). This functional inhibition may indicate that the autoantibodies are recognizing the active sites of the synthetases. In contrast, it has been reported that animal antisera raised against synthetases do not consistently show such inhibition, suggesting that active sites tend not to be immunogenic for animals (31). Hypothetically, this could relate to relative conservation of the active site. However, there might be an alternative mechanism for inhibition. For example, binding of antibodies outside the active site may alter the structure of the enzyme or interfere with enzyme activity sterically. Further studies of the precise epitope on the aminoacyl–tRNA synthetase might help to explain the development of these autoantibodies.

Each of the 5 previous antisynthetases was first identified in patients with myositis and then found to be associated with ILD. In previous studies, these autoantibodies were associated with myositis with a high frequency of ILD (50–80%) and arthritis (50–90%) (1,2,17,18), as well as an increase in Raynaud's phenomenon (60%), fever with exacerbations (80%), and the skin lesion of the fingers referred to as mechanic's hands (70%) when compared with the overall population of patients with myositis (9–11). The similarities between patients with different antisynthetases have been noted, whereas certain differences have been found, which must be considered preliminary due to the small reported number of patients with non-HisRS antisynthetases. Absence of significant myositis over the full disease course in patients with anti-HisRS is rare (<5%) (32), whereas patients with anti-AlaRS are more likely than patients with anti-HisRS to have ILD and/or arthritis without clinical evidence of myositis (19). Anti-ThrRS

resembles anti-HisRS more than anti-AlaRS in Japanese patients (33).

In the present study, 7 of 8 patients (88%) with anti-AsnRS autoantibodies had ILD, some with other associated features of connective tissue disease including arthritis and Raynaud's phenomenon. In this respect, anti-AsnRS appears to resemble anti-AlaRS more than anti-HisRS. It is noteworthy that the 2 patients with both anti-AsnRS and myositis were among the 3 patients from outside Japan, while none of 5 patients from Japan had myositis. Thus, as with patients with anti-AlaRS, for patients with anti-AsnRS, the frequency of ILD without myositis may be higher in Japanese patients. However, most of the group of patients with ILD without myositis who were tested in this study were from Japan.

The features of these 8 patients with anti-KS appeared to reside within the spectrum of the antisynthetase syndrome that has been associated with other antisynthetases. ILD is one of the most important features of the antisynthetase syndrome, and Raynaud's phenomenon and arthritis, as seen in some patients with anti-AsnRS, are also likely to be part of the syndrome. The syndrome associated with anti-AsnRS may be one end of the spectrum of patients with antisynthetase. This highlights the clinical importance of looking for such antibodies in patients with ILD even if there are no signs of myositis or connective tissue diseases.

The typical cutaneous features of DM were observed in 2 patients with anti-AsnRS antibodies. PM has been reported to be much more common (60–80% or more) than DM in patients with anti-HisRS in most studies, whereas DM was most frequent with anti-GlyRS (15) and was also found to be common among patients with anti-AlaRS (13). Like anti-GlyRS and anti-AlaRS antibodies, anti-AsnRS antibodies were more associated with DM in the small number of patients available.

Malignancy has been reported to be unusual in patients with antisynthetases. In our studies, 2 patients were found to have malignancy during their disease course. However, malignancy in these patients may not be related to the DM or ILD, since these malignancies occurred separated in time from each other.

Immunogenetic studies of connective tissue disease have been performed, but HLA associations produced conflicting results. However, a strong correlation of HLA class II antigens with some autoantibodies has been reported (34). With regard to antisynthetase antibodies, HLA-DR3 (DRB1*0301), DQA1*0501, or DQA1*0401 was found to be significantly increased in myositis patients with antisynthetases (9,21). In Japanese patients, we have reported that 7 of 9 patients (78%) with anti-HisRS tested had the HLA class II DRB1*0405;DQA1*0302;DQB1*0401 haplotype, compared with 22% of healthy controls (odds ratio [OR] 13, $P = 0.002$), while 4 of 7 patients (57%) with anti-AlaRS had the DRB1*1501;DQA1*0102;DQB1*0602 haplotype, compared with 9% of healthy controls (OR 14, $P = 0.006$) (35). Interestingly, all 4 Japanese patients tested had DR2 (DRB1*1501/1502), compared with 33% of healthy controls, although a definite statistical association could not be established. These results suggest that the stronger association of anti-AlaRS and anti-KS with ILD may be related to the DR2 phenotype. However, it has been noted that different ethnic groups exhibit different immunogenetic profiles that link with specific autoantibodies (36). Therefore, further studies including analysis of more patients with anti-KS antibodies in different ethnic groups and major histocompatibility complex–restricted T cell responses could provide important clues for understanding the possible mechanisms for the development of antisynthetase antibodies.

The mechanism for the association of antisynthetases with ILD is unknown, but it seems to be related to etiologic factors (37). Recently, a new association of anti-HisRS–positive PM and ILD was reported in a patient with hepatitis C virus infection (38). It was hypothesized that viruses might interact with the synthetases and induce autoantibodies by molecular mimicry or antiidiotype mechanisms in the anti-HisRS–positive patient with myositis associated with ILD (3,39). Another mechanism for generating autoantigenic epitopes of synthetase by granzyme B cleavage in apoptosis was also described recently (40,41). However, these proposed mechanisms remain speculative, and further studies could provide important clues for understanding the possible mechanisms for the development of these antibodies. Studies of these antibodies may provide insight into the etiologic and pathogenetic mechanisms of ILD and myositis.

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AUTHOR CONTRIBUTIONS

Dr. Hirakata had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Hirakata.
Acquisition of data. Hirakata, Nagai, Genth, Song, Targoff.
Analysis and interpretation of data. Hirakata, Suwa, Takada, Sato, Mimori.
Manuscript preparation. Hirakata, Takada, Targoff.
Statistical analysis. Hirakata, Suwa, Targoff.

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Concise Report

Expression of B-cell activating factor of the tumour necrosis factor family (BAFF) in T cells in active systemic lupus erythematosus: the role of BAFF in T cell-dependent B cell pathogenic autoantibody production

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Objectives. To determine whether B cell activating factor of the tumour necrosis factor family (BAFF) is involved in T cell-dependent B cell pathogenic autoantibody production in systemic lupus erythematosus (SLE).
Methods. Peripheral blood mononuclear cells (PBMCs) from 23 SLE patients were analysed by flow cytometry to examine the intracellular expression of BAFF in $CD4^+$ and $CD8^+$ T cells and the surface expression of BAFF-receptor (R) and TACI on $CD20^+$ B cells. Moreover, peripheral blood was used to determine the level of BAFF messenger RNA (mRNA) in $CD4^+$ and $CD8^+$ T cells and the level of BAFF-R mRNA in $CD20^+$ B cells. Blocking of BAFF function with TACI-Ig measured anti-double-stranded DNA (dsDNA) antibodies by enzyme-linked immunosorbent assay (ELISA).
Results. $CD4^+$ and $CD8^+$ T cells from patients with active SLE expressed intracellular BAFF whereas those from normal subjects did not. BAFF-R and TACI were expressed on B cells from both normal controls and patients with active SLE and there was no significant difference. $CD4^+$ and $CD8^+$ T cells from SLE patients expressed BAFF mRNA whereas those from normal controls did not. Expression of BAFF-R mRNA in $CD20^+$ B cells showed no significant difference between SLE patients and normal controls. TACI-Ig suppressed spontaneous *in vitro* T cell-dependent B cell anti-dsDNA antibodies production on active SLE with kidney involvement.
Conclusions. BAFF may play a pathogenic role in SLE by stimulating T cell-dependent B cell autoantibodies production. Blockade of BAFF is a promising therapeutic approach for SLE especially in patients with kidney involvement.

Key words: Systemic lupus erythematosus, BAFF, T cell, Autoantibody production.

Introduction

B cell activating factor of the tumour necrosis factor (TNF) family (BAFF; also known as BLyS, TALL-1, THANK, TNFSF13B and zTNF4) is a 285-amino-acid member of the TNF ligand superfamily [1–6]. It is expressed as a type II transmembrane protein which is cleaved at the cell surface by a furin protease, resulting in release of a soluble, biologically active 17-kDa molecule [7]. Expression of BAFF is highly restricted to myeloid lineage cells (e.g. monocytes, macrophages, dendritic cells, neutrophils), and levels of BAFF mRNA and protein are up-regulated by interferon (IFN) γ, interleukin (IL)-10 and CD40L. Expression of the three known BAFF receptors (BCMA, TACI and BAFF-R) is also highly restricted. TACI and BCMA bind both BAFF and APRIL, another TNF superfamily member, and their roles are more controversial. The agonist effects of BAFF on B cells are mediated mainly via BAFF-R [8–10].

Systemic lupus erythematosus (SLE) is characterized by loss of B cell tolerance and the presence of polyclonal B cell activation [11–13]. Recent studies have shown that the serum levels of BAFF are elevated in patients with SLE and Sjögren's syndrome and in the synovial fluid of patients with rheumatoid arthritis [14–18]. The association of each these diseases with autoantibody production suggests a potential role of increased BAFF in the disease process. Moreover, cross-sectional studies have demonstrated elevated levels of circulating BAFF in SLE [15, 16]. However, the role of T cell-dependent B cell autoantibody production by the BAFF system in SLE is still unclear.

In the present study, we examined whether BAFF is involved in T cell-dependent B cell pathogenic autoantibody production in SLE.

Materials and methods

Subjects

Twenty-three patients with SLE who had been admitted to Juntendo University Hospital were recruited for this study. The clinical diagnosis in all patients was made in accordance with the American College of Rheumatology 1982 revised criteria for the SLE [17]. In order to be enrolled, each patient had to be suffering from active SLE as assessed subjectively by the patient's physician, and was required to provide informed consent. Disease activity was assessed by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [18]. Eighteen patients had nephritis [CH50: 19.3 ± 10.2 U/ml, DNA/RIA: 58.3 ± 69.5 IU/ml, SLEDAI: 23.0 ± 6.8, prednisone (median): 21.25 mg/day (range; 0–55)] and five had neuropsychiatric involvement [CH50: 39.2 ± 6.9 U/ml, DNA/RIA: 7.35 ± 4.2 IU/ml, SLEDAI: 19.5 ± 4.9, prednisone (median): 8 mg/day (range: 5–10)]. Twenty-three healthy controls were recruited from personnel at Juntendo University School of Medicine. Ethical approval was not required under the present rules of our university when using and investigating the peripheral blood of patients or healthy donors. All the patients and healthy donors were fully informed and gave their consent to participate in our study. All information and data about patients or healthy donors is kept confidential and the data are fully available to patients or donors upon request.

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Antibodies and reagents

Fluorescein isothiocyanate-conjugated anti-CD45RA, anti-CD45RO, anti-CD20, anti-CD27, anti-BAFF, phycoerythrin/Cy5-conjugated anti-CD14, anti-CD20, anti-CD38, anti-BAFF-R, anti-TACI and allophycocyaninn-conjugatted anti-CD4, anti-CD8 and anti-CD20 monoclonal Ab (mAb) were purchased from BD Biosciences (San Jose, CA). Unconjugated antibodies against BAFF (1D6; mouse IgG1) and BAFF-R (8A7; mouse IgG2a) and the matched isotype control were conjugated to biotin, and the specificites of mAbs 1D6 for BAFF and 8A7 for BAFF-R have been documented previously [19, 20]. Recombinant human TACI/Fc chimera (R&D systems, MN) and recombinant human Fas/Fc chimera (Sigma, St Louis, MO) for *in vitro* blocking of the BAFF systems was used.

Flow cytometric analysis

In order to prepare peripheral lymphocytes, venous blood samples were collected from SLE patients and healthy controls after obtaining informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by Ficoll density-gradient centrifugation and were triple-stained with fluorescein isothiocyanate-conjugated anti-CD45RA, anti-CD45RO, anti-CD20, anti-CD27, anti-BAFF and phycoerythrin/Cy5-conjugated anti-CD14, anti-CD20, anti-CD38, anti-BAFF-R, anti-TACI and allophycocyanin-conjugated anti-CD4, anti-CD8 and anti-CD20 mAb. For intracellular staining of BAFF, we used Intraprep (Beckman Coulter, Miami, FL) for fixation and membrization according to their manufacturer instruction. Flow cytometric analysis was performed using FACSAria (Becton Dickinson, San Jose, CA), and data were processed using the Cell Quest program (Becton Dickinson).

Determination of BAFF and BAFF-R mRNA levels in peripheral blood

For isolation of peripheral blood $CD4^+$, $CD8^+$ T cells or $CD20^+$ B cells, 5 ml of peripheral blood was labelled with 40 μl of anti-human CD4, CD8 or CD20 antibody coupled to colloidal paramagnetic microbeads (Miltenyi Biotech, Bergisch-Gladbach, Germany) and isolated using AutoMACS (Miltenyi Biotech), respectively. $CD4^+$, $CD8^+$ or $CD20^+$ cells were isolated at a purity of more than 93% and the resulting cell population was <2% $CD14^+$ and <2% $CD57^+$ as assessed by flow cytometric analysis. Total RNA was isolated from 1×10^6 $CD4^+$, $CD8^+$ T cells or $CD20^+$ B cells using an RNeasy Mini kit (QIAGEN, Valencia, CA). Real-time semi-quantitative RT–PCR was performed in a single 50 μl reaction volume containing 25 μl of One-step RT–PCR SYBR Green Master Mix (Applied BioSystems, Foster City, CA) with 1.0 μl of AmpliTaq Gold DNA polymerase (Applied BioSystems), 0.25 μl of 40 × MultiScribe reverse transcriptase (Applied BioSystems), and the following sense and antisense primers at 10 nM: BAFF: 5′-GGAGAAGGCAACTCCAGTC AGAAC-3′ and 5′-CAATTCATCCCCAAAGACATGGAC-3′, BAFF-R: 5′-CAAGGTCATCATTCTGTCCG-3′ and 5′-CGGC TCCCTGCTATTGTTGCTCA-3′, APRIL: 5′-ATGCCAGC CTCATCTCCTTTC-3′ and 5′-TCACAGTTTCACAAACCCCA GG-3′, β-actin: 5′-GGACTTCGAGCAAGAGATG and 3′-AGC ACTGTGTTGGCGTACA. The terminal cycling conditions were 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of complication at 95°C for 15 s and 60°C for 1 min for denaturing and annealing-extension, respectively. Expression of the message level was measured with an ABI PRISM 7500 Sequence Detection System (Applied BioSystems) and normalized to β-actin mRNA.

BAFF secretion by T cells

PBMCs were isolated from active SLE and healthy controls by Ficoll–Hypaque (Pharmacia, Piscataway, NJ) density-gradient centrifugation. PBMCs were separated by the E rosette-positive and E rosette-negative populations with 5% sheep erythrocytes. The E rosette-positive cells were depleted of monocytes by adherence to the plastic surface of culture dishes and further purified T cells by complement (Cedarlane, Ontario, Canada) lysis with anti-CD57 (HNK-1; mouse IgM), anti-CD14 (63D3; mouse IgG1) plus rat anti-mouse IgG1 mAb (Becton Dickinson). The resultant T cell population was <2% CD19 and CD14, <2% CD57 and >93% CD3. T cells (2×10^5/well) were cultured in 96-well round-bottom plates in 0.2 ml of culture medium for 10 days at 37°C in a humidified atmosphere with 5% CO_2. The culture supernatant was harvested and soluble (s) BAFF titres were determined using human BAFF ELISA kit (Bender Medsystems Gmbh, Vienna, Austria). Assays were performed according to the manufacturer's instructions.

Suppression of anti-dsDNA antibodies by TACI-Ig

PBMCs were depleted of monocytes by adhesion to the plastic culture dishes and further purified into lymphocytes by complement (Cedarlane, Ontario, Canada) lysis with HNK-1 and 63D3 plus rat anti-mouse IgG1 mAb (PharMingen). The resulting cell population was <2% $CD14^+$ and <2% $CD57^+$. All cultures were conducted in RPMI 1640 medium supplemented with 10% FCS, 2 mM l-glutamine, penicillin G (200 U/ml) and gentamicine (10 μg/ml). Lymphocytes (2×10^5/well) were cultured in 96-well round-bottom plates in 0.2 ml of culture medium for 10 days at 37°C in a humidified atmosphere with 5% CO_2. Nil, TACI-Ig or control-Ig was added at the beginning of the experiment. The culture supernatants were harvested and anti-dsDNA titres were determined using an ELISA kit (Bio-Rad, CA, USA). Assays were performed according to the manufacturer's instructions.

Statistics

Statistical analysis was performed using non-parametric test for comparison of population samples. A value of $P < 0.05$ was used to reject the null hypothesis.

Results

Expression of BAFF or BAFF-R on circulating lymphocytes

To determine whether the increased BAFF antigen is produced by circulating T cells in patients with active SLE, we first examined its surface expression by flow cytometric analysis. Monocyte from active SLE was highly expressed BAFF antigen ($CD14^+$: 28.6 ± 3.4) [mean fluorescence intensity (MFI)]. However, we did not detect any cell surface expression of BAFF on T cells from either control subjects or patients with active SLE (data not shown). We then looked for intracellular expression of BAFF in circulating T cells. A striking finding was that $CD4^+$ T cells from patients with active SLE showed the intracellular BAFF expression, whereas those from normal controls did not (Fig. 1A). This $CD4^+$ T cell population comprised almost entirely memory ($CD45RO^+$) T cells (data not shown). Another unexpected finding was that $CD8^+$ T cells from patients with active SLE also expressed the BAFF antigen (Fig. 1B), whereas circulating $CD8^+$ T cells from normal controls did not.

In particular, patients with kidney involvement had significantly higher MFI of intracellular BAFF expression on $CD4^+$ T cells (23.4 ± 8.74) and $CD8^+$ T cells (20.1 ± 4.80) in comparison with non-kidney involvement ($CD4^+$: 6.40 ± 0.57, $CD8^+$: 19.7 ± 11.46) and normal controls ($CD4^+$: 4.19 ± 0.74, $CD8^+$: 6.01 ± 1.56), respectively ($P < 0.01$).

We next examined the expression of BAFF receptors on B cells. BAFF-R and TACI were expressed on B cells from both normal controls and patients with active SLE and the expression levels in the two groups did not differ significantly (Fig. 1C and D). Within the sensitivity limits of flow cytometric analysis, the expression

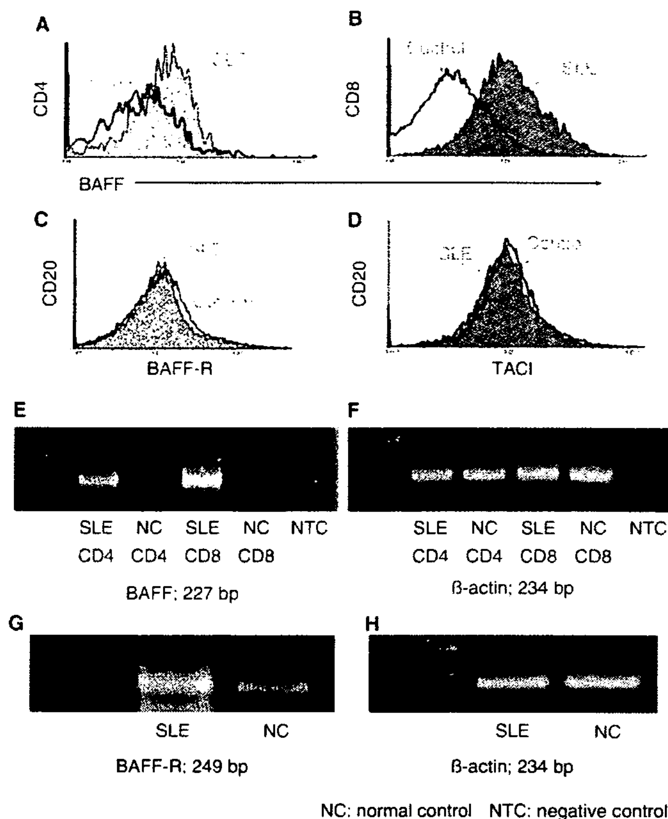

FIG. 1. Expression of BAFF and BAFF receptor in the peripheral blood of representative patients with active SLE and normal controls. (**A**) CD4$^+$ T cells appear above the vertical line and BAFF appear above the horizontal line. (**B**) CD8$^+$ T cells are shown above the vertical line and BAFF is shown above the horizontal line. For intracellular staining of BAFF, we used Intraprep for fixation and membrization according to the instructions supplied by the manufacturer. (**C**) CD20$^+$ cell expression is shown above the vertical line and BAFF-R is shown above the horizontal line. (**D**) CD20$^+$ cells are shown above the vertical line and TACI is shown above the horizontal line. PBMCs obtained from representative normal controls and representative patients with active SLE were double stained for surface CD20 and surface BAFF-R or TACI. (**E**) Real-time semi-quantitative RT–PCR for the expression of BAFF mRNA levels on CD4$^+$ and CD8$^+$ T cells in the blood of representative patients with active SLE and normal controls. Peripheral blood CD4$^+$ and CD8$^+$ T cells were isolated by AutoMACS using anti-human CD4 and CD8 antibody coupled to colloidal paramagnetic microbeads, respectively. The sizes of the products for BAFF and β-actin were 227 and 234 bp, respectively. (**F**) Real-time semi-quantitative RT–PCR for the expression of BAFF-R mRNA on CD20$^+$ B cells in the blood of representative patients with active SLE and normal controls. Peripheral blood CD20$^+$ B cells were isolated by AutoMACS using anti-human CD20 antibody coupled to colloidal paramagnetic microbeads, respectively. The sizes of the products for BAFF-R and β-actin were 249 and 234 bp, respectively.

of BAFF receptors on B cells from SLE was similar to that on the same B cell subsets in healthy controls [21].

Expression of BAFF or BAFF receptors mRNA in active SLE

A previous study has demonstrated overexpression of BAFF mRNA in peripheral blood leucocytes from SLE [22], although the subpopulation of lymphocytes expressing the BAFF mRNA was unclear. Therefore, we investigated the expression of mRNA for BAFF in CD4$^+$or CD8$^+$lymphocytes. CD4$^+$ T lymphocytes in SLE expressed the BAFF mRNA, but those from normal controls did not (Fig. 1E and F). Moreover, CD8$^+$ T lymphocytes from SLE was also expressed the BAFF mRNA (Fig. 1E and F). We think that these results neglect the monocyte contamination by flow-cytometric analysis (CD14$^+$ <2%). However, CD4$^+$ and CD8$^+$ T cells did not express APRIL mRNA (data not shown).

We then investigated the expression of mRNA for BAFF-R in CD20$^+$ B cells from SLE and normal controls, and found that both groups expressed the BAFF-R mRNA, with no significant expression level between them (Fig. 1G and H).

BAFF secretion by T cells from active SLE

Then, we examined the BAFF secretion by T cells from active SLE. T cells from active SLE and normal controls produced 7.2 ± 1.1 and 1.3 ± 0.7 (ng/ml), respectively, of sBAFF *in vitro* without any stimulation ($P < 0.05$). We found T cells from active SLE produced higher amount of sBAFF than T cells from normal control under basal conditions.

In vitro *suppression of anti-dsDNA antibodies production by TACI-Ig*

To investigate the direct involvement of BAFF in T cell-dependent B cell autoantibody production, we then examined whether TACI-Ig inhibited spontaneous production of anti-dsDNA antibodies by cultured T and B cells from six patients with active SLE showing kidney involvement. Table 1 shows the characteristics of the individual SLE and the extent of suppression of anti-dsDNA antibody titres by TACI-Ig. The addition of TACI-Ig, but not control-Ig, significantly suppressed *in vitro* T cell-dependent anti-dsDNA antibodies production by B cells. These results strongly suggest that BAFF plays an important role in T-cell-dependent anti-dsDNA antibodies production in SLE patients through BAFF-R and/or TACI.

Discussion

In the present study, we have demonstrated abnormal production of BAFF in T cells from SLE (Fig. 1), especially in patients with kidney involvement. Furthermore, we showed that blocking of BAFF in T cell–B cell interaction reduced the production of autoantibody by TACI-Ig. These results suggest that another mechanism operates in the pathogenesis in SLE, i.e. autoantibody production driven by BAFF produced in part by T cells, supporting a previous study indicating expression of BAFF by T cells [4]. A recent study has also shown that BAFF is expressed in T cells infiltrating salivary glands in patients with Sjögrne's syndrome [14, 23]. Moreover, a very recent report has indicated that SLE T cells produce soluble BAFF upon stimulation and that the BAFF mRNA robustly induced by a human TE cell line, Loucy [24]. Therefore, we tried to stimulate T cells with anti-CD3 to study a possible increase of BAFF expression by intracellular cytometric assay (data not shown). The result was controversial. We speculate this reason why CD3/TCR-mediated response of purified T cells in SLE ranges normal to enhanced [25] and T cells from SLE display a number of signalling abnormalities (e.g. decreased expression of TCR ζ chain) [26]. However, previous reports and our present data suggest that the role of T cell-derived BAFF in the production of autoantibodies may provide insight into the pathogenesis and development of SLE, especially that with kidney involvement.

Another unexpected finding in this study was expression of BAFF and increased levels of mRNA in CD8$^+$ T cells from SLE, but not in those from normal controls (Fig. 1B and E). This result leads us to hypothesize that CD8$^+$ T cells synergize with CD4$^+$ T cells to support pathogenic autoantibody production in SLE. A previous report indicating that CD8$^+$ T cells can have positive rather than negative effects on antibody production in SLE has important implications in relation to the mechanism of autoantibody formation in this disease [27]. This report indicated that removal of either CD8$^+$ or CD4$^+$ lymphocytes markedly decreased the spontaneous *in vitro* production of polyclonal IgG and/or anti-dsDNA antibodies production by PBMC in SLE. Thus, it seems that, in human SLE, there is a requirement for both

TABLE 1. Characteristics of individual members of patients in SLE with kidney involvement and suppression of anti-double-stranded DNA (dsDNA) antibodies blockade of BAFF system by TACI-Ig

						Suppression of anti-dsDNA (mU/ml)	
	Age	CH50 (U/ml)	DNA/RIA (IU/ml)	SLEDAI	Predonisone (mg/day)	(nil)-(add control-Ig)	(nil)-(add TACI-Ig)
Median	32.3	19.1	40.7	23.5	21.7	0.1	1.85*
Range	18–57	7–34.3	10.1–129	18–38	7–50	0.01–0.12	0.22–3.80

Sex: female ($n = 4$), male ($n = 2$).
RIA, radioimmunoassay; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; *$P < 0.01$: compare with (nil)-(add control-Ig).

$CD8^+$ and $CD4^+$ T cells for generation of pathogenic autoantibodies and that regulation of homoeostatic T cells is defective.

Moreover, present study showed that blocking of BAFF in T cell–B cell interaction reduced the production of autoantibody by TACI-Ig which is a soluble decoy receptor for BAFF and APRIL. Patients with SLE have elevated serum levels of BAFF correlated with elevated levels of autoreactive Abs [15]. Therefore, BAFF may be an appropriate target for intervention in autoimmune diseases in which elevated levels of autoantibodies contribute to disease pathology. In NZB/W F1 mice, administration of TACI-Ig and/or BAFF-R-Ig prolongs the life span [28] and prevents the emergence of IgG anti-DNA antibodies [9]. That study has shown that treatment of NZB/W F1 mice with BAFF-R-Ig reduced the circulating levels of anti-dsDNA antibody titres in parallel with clinical improvement [9]. These results and our present data suggest that BAFF derived from T cells may also play a pathogenic role of SLE and blockade of BAFF as a promising therapeutic approach for SLE, especially in patients with kidney involvement.

Rheumatology key messages

- BAFF is present in intracellular T cells in active SLE patients.
- BAFF derived from T cells may play a pathogenic role of SLE, especially in patients with kidney involvement.
- BAFF is a therapeutic approach for SLE, especially in patients with kidney involvement.

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