FoxP3+ regulatory T cells in inflamed synovia. J Exp Med 2005;201:1793–803.
15. Cao D, Malmstrom V, Baecher-Allan C, Hafler D, Klareskog L, Trollmo C. Isolation and functional characterization of regulatory CD25brightCD4+ T cells from the target organ of patients with rheumatoid arthritis. Eur J Immunol 2003;33:215–23.
16. Van Amelsfort JM, Jacobs KM, Bijlsma JW, Lafeber FP, Taams LS. CD4+CD25+ regulatory T cells in rheumatoid arthritis: differences in the presence, phenotype, and function between peripheral blood and synovial fluid. Arthritis Rheum 2004;50: 2775–85.
17. Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFα therapy. J Exp Med 2004;200:277–85.
18. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155:1151–64.
19. Asano M, Toda M, Sakaguchi N, Sakaguchi S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. J Exp Med 1996;184:387–96.
20. Hori S, Haury M, Coutinho A, Demengeot J. Specificity requirements for selection and effector functions of CD25+4+ regulatory T cells in anti-myelin basic protein T cell receptor transgenic mice. Proc Natl Acad Sci U S A 2002;99:8213–8.
21. Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, et al. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. J Exp Med 2004;199:1455–65.
22. Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. J Exp Med 2004;199:1467–77.
23. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. J Immunol 2003;170:3939–43.
24. Morgan ME, Sutmuller RP, Witteveen HJ, van Duivenvoorde LM, Zanelli E, Melief CJ, et al. CD25+ cell depletion hastens the onset of severe disease in collagen-induced arthritis. Arthritis Rheum 2003;48:1452–60.
25. Morgan ME, Flierman R, van Duivenvoorde LM, Witteveen HJ, van Ewijk W, van Laar JM, et al. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cells. Arthritis Rheum 2005;52:2212–21.
26. Kitamura T, Koshino Y, Shibata F, Oki T, Nakajima H, Nosaka T, et al. Retrovirus-mediated gene transfer and expression cloning: powerful tools in functional genomics. Exp Hematol 2003;31: 1007–14.
27. Morita S, Kojima T, Kitamura T. Plat-E: an efficient and stable system for transient packaging of retroviruses. Gene Ther 2000;7: 1063–6.
28. Walker MR, Kasprowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH, et al. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25− T cells. J Clin Invest 2003;112:1437–43.
29. Abramoff M, Magalhaes P, Ram S. Image Processing with ImageJ. Biophotonics International 2004;11:36–42.
30. Everitt BS, Hothorn T, editors. A handbook of statistical analyses using R. Boca Raton [FL]: Chapman & Hall/CRC Press; 2006.
31. Pinheiro J, Bates D, DebRoy S, Sarkar D (2006). nlme: linear and nonlinear mixed effects models. R package version 3.1-79. 2006.
32. Team RDC. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2006.
33. Puppo F, Murdaca G, Ghio M, Indiveri F. Emerging biologic drugs for the treatment of rheumatoid arthritis. Autoimmun Rev 2005;4:537–41.
34. Thornton AM, Shevach EM. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol 2000;164:183–90.
35. Yamazaki S, Iyoda T, Tarbell K, Olson K, Velinzon K, Inaba K, et al. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. J Exp Med 2003;198: 235–47.
36. Myers LK, Rosloniec EF, Cremer MA, Kang AH. Collagen-induced arthritis, an animal model of autoimmunity. Life Sci 1997;61:1861–78.
37. Luross JA, Williams NA. The genetic and immunopathological processes underlying collagen-induced arthritis. Immunology 2001;103:407–16.
38. Lim HW, Hillsamer P, Banham AH, Kim CH. Cutting edge: direct suppression of B cells by CD4+ CD25+ regulatory T cells. J Immunol 2005;175:4180–3.
39. Baecher-Allan C, Viglietta V, Hafler DA. Inhibition of human CD4+CD25+high regulatory T cell function. J Immunol 2002;169: 6210–7.
40. Nishimura E, Sakihama T, Setoguchi R, Tanaka K, Sakaguchi S. Induction of antigen-specific immunologic tolerance by in vivo and in vitro antigen-specific expansion of naturally arising Foxp3+CD25+CD4+ regulatory T cells. Int Immunol 2004;16: 1189–201.

EXTENDED REPORT

Efficacy of rituximab (anti-CD20) for refractory systemic lupus erythematosus involving the central nervous system

Mikiko Tokunaga, Kazuyoshi Saito, Daisuke Kawabata, Yoshitaka Imura, Takao Fujii, Shingo Nakayamada, Shizuyo Tsujimura, Masao Nawata, Shigeru Iwata, Taeko Azuma, Tsuneyo Mimori, Yoshiya Tanaka

Ann Rheum Dis 2007;66:470–475. doi: 10.1136/ard.2006.057885

See end of article for authors' affiliations

Correspondence to: Dr Y Tanaka, The First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan, 1-1 Iseigaoka Yahata-nishi, Kitakyushu 807-8555 Japan; tanaka@med.uoeh-u.ac.jp

Accepted 30 October 2006
Published Online First 9 November 2006

Aim: Neuropsychiatric systemic lupus erythematosus (NPSLE) is a serious treatment-resistant phenotype of systemic lupus erythematosus. A standard treatment for NPSLE is not available. This report describes the clinical and laboratory tests of 10 patients with NPSLE before and after rituximab treatment, including changes in lymphocyte phenotypes.

Methods: Rituximab was administered at different doses in 10 patients with refractory NPSLE, despite intensive treatment.

Results: Treatment with rituximab resulted in rapid improvement of central nervous system-related manifestations, particularly acute confusional state. Rituximab also improved cognitive dysfunction, psychosis and seizure, and reduced the SLE Disease Activity Index Score at day 28 in all 10 patients. These effects lasted for >1 year in five patients. Flow cytometric analysis showed that rituximab down regulated CD40 and CD80 on B cells and CD40L, CD69 and inducible costimulator on CD4+ T cells.

Conclusions: Rituximab rapidly improved refractory NPSLE, as evident by resolution of various clinical signs and symptoms and improvement of radiographic findings. The down regulation of functional molecules on B and T cells suggests that rituximab modulates the interaction of activated B and T cells through costimulatory molecules. These results warrant further analysis of rituximab as treatment for NPSLE.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by multiple lesions induced by activation of autoreactive T cells and overproduction of autoantibodies by B cells. The involvement of the central nervous system (CNS) in SLE is often intractable, complicating the course of the disease in about 12–75% of patients with SLE. The involvement of the CNS has a negative clinical impact with a 5-year survival of 55–85% and is associated with poor prognosis.[1 2] Neuropsychiatric systemic lupus erythaematosus (NPSLE) exhibits a wide range of symptoms unrelated to SLE activation, which include organic and mental disorders, often associated with impairment of consciousness and/or convulsions. These organic disorders may become permanent, eventually leading to long-term or irreversible decline in higher mental functions.

CNS immune abnormalities have an important role in such disease states. Therefore, a trial of intensive treatment, including the combination of potent immunosuppressive treatment and plasma exchange (PE), depending on the disease type and its severity, may be advisable in an effort to control autoreactive lymphocytes.[3–10] Although the severity of NPSLE correlates with prognosis, there is no established treatment protocol and many cases are resistant to treatment making this condition difficult to control.

This study describes the results of treatment of patients with NPSLE who had previously failed to respond to various immunosuppressants. Our approach was based mainly on the use of anti-CD20 antibody (rituximab), a chimeric antibody that directly targets B cells.[11 12] Rituximab is a biological preparation that eliminates B cells through a variety of mechanisms such as antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity and apoptosis. Rituximab has recently been used for the treatment of a variety of SLE disease conditions and good therapeutic response has been reported.[13–16] We investigated the short-term and long-term responses to rituximab treatment in 10 patients with NPSLE, and report that some showed marked improvement following rituximab treatment. Moreover, the results showed that rituximab modulated the functional molecules of activated lymphocytes, implying the efficacy of anti-CD20 antibody treatment for CNS lesions in patients with SLE, otherwise resistant to other treatments.

MATERIALS AND METHODS

Patients

The study subjects were 10 patients who had been previously diagnosed with SLE based on the American College of Rheumatology criteria.[17] The inclusion criteria were (1) the presence of a highly active disease and (2) CNS lesions resistant to conventional treatment. None of the patients showed improvement in CNS-related symptoms in response to conventional immunosuppressive treatment such as intravenous cyclophosphamide pulse treatment (IV-CY), cyclosporine A (CsA), PE and immunoadsorption therapy. All patients completed the course of anti-CD20 antibody treatment described in this study. Patients 1–8, and patients 9 and 10 were treated at the University of Occupational and Environmental Health Hospital and Kyoto University Hospital, respectively, from 2000 to 2005. Informed consent was obtained from all patients in accordance with the

Abbreviations: CNS, central nervous system; FACS, fluorescence-activated cell sorter; NPSLE, neuropsychiatric systemic lupus erythematosus; PBS, phosphate-buffered saline; PE, plasma exchange; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index; SPECT, single-photon-emission computed tomography

regulations of the aforementioned two hospitals, and rituximab was administered in accordance with the study protocol approved by the ethics committee of each hospital.

Treatment protocol

Patients 1–5 and 10 were treated with 375 mg/m^2 rituximab once a week for 2 weeks, and patient 9 received a single administration of the same dose. Patients 6 and 7 received 500 mg rituximab once a week for 4 weeks, while patient 8 was treated with 1000 mg once biweekly for 4 weeks. Blood pressure and ECG were monitored within the first 3.5 h of the administration to check for any reaction to the drug infusion.

Assessment

Clinical symptoms and treatment-induced adverse reactions were assessed before treatment, every week during treatment, every week within 1 month after treatment and once monthly thereafter. Laboratory tests included blood count, erythrocyte sedimentation rate, liver and renal function tests, urinary protein, serum complement titre and autoantibody level (such as anti-ds-DNA antibody). To evaluate the impact of rituximab on CNS lesions, we measured the immunoglobulin (Ig)G index and interleukin (IL)6 level in the cerebrospinal fluid, MRI, cerebral flood flow scintillator (single-photon-emission computed tomography (SPECT), and ^{18}FTG-positron emission tomography. To assess SLE activity, the SLE Disease Activity Index (SLEDAI) was determined before and after treatment. The level of expression of functional molecules on the lymphocyte cell surface was assessed by flow cytometry.

Flow cytometry

Mononuclear cells were isolated from peripheral blood using lymphocyte separation medium (ICN/Cappel Pharmaceuticals, Aurora, Ohio, USA). After washing twice with phosphate-buffered saline (PBS), the cells were incubated in blocking buffer (0.25% human globulin, 0.5% human albumin (Yoshitomi, Osaka, Japan), and 0.1% NaN_3 (Sigma Aldrich, St Louis, Missouri, USA) in PBS) and left to stand in a 96-well plate at 4℃ for 15 min. In the next step, the cells were incubated in 100 µl of fluorescence-activated cell sorter (FACS) solution (0.5% human albumin and 0.1% NaN_3 in PBS) and then treated with fluorescein isothiocyanate-labelled mouse IgG_1 and antihuman CD40, CD69, inducible costimulator (ICOS), CD19, CD4 (Pharmingen, San Diego, California, USA), CD80 (Chemicon Europe, Chandlers Ford, UK), or CD40L (Ancell, Bayport, USA) antibody, and left to react for 30 min at 4℃. The cells were washed three times with FACS solution and analysed using FACScalibar (Becton–Dickinson, San Jose, California, USA).

Statistical analysis

All data were expressed as mean (SD). Differences between data collected before and after treatment were examined for statistical significance using the Student's t test. $p<0.05$ denoted the presence of a significant difference.

RESULTS

Characteristics of patients

Table 1 summarises the NPSLE classification and laboratory data of the 10 patients. All patients were females with a mean (range) age of 31 (20–55) years. The mean (range) duration of illness from the onset of SLE to administration of rituximab was 9.6 years (3 months to 25 years). Immunosuppressants used for treatment before enrollment in the rituximab protocol included CsA, cydophosphamide, mizoribine, and azathioprine. In addition, five patients with intractable disease did not respond to the combination treatment, and thus received PE as well.

With regard to CNS-related symptoms, acute confusional state was noted in 5, psychosis in 4, seizures in 2, mood disorders in 2, and one patient each had headache, demyelinating syndrome, myelopathy, anxiety disorder and cognitive dysfunction, based on the NPSLE classification of the American College of Rheumatology.[18 19] MRI findings included abnormal signals in the cerebral white matter in six patients. SPECT showed reduced cerebral blood flow in eight patients. Although a high IgG index[20] was noted in five patients (>0.66), an increase in IL6 was confirmed in only one patient.

Serious haemolytic anaemia, cardiomyopathy-associated decreased cardiac function, muscle pain, mucocutaneous disorders, peripheral neural deficits such as abnormal sensation and neurogenic bladder were also seen in these patients, in addition to the CNS-related changes (tables 1 and 2). In all participants, conventional immunosuppressive therapy produced either no improvement of symptoms or only a poor response. The SLEDAI values (range, 2–49) reflected the presence or absence of organ system-specific activity, with large scores representing involvement of CNS and low scores reflecting haematological activity. In the present study, involvement of organs was limited to those that could be confirmed objectively, while subjective signs such as fatigue and paresthesia were not recorded. Thus, using this approach, the SLEDAI scores of patients with objective signs reflecting multiple involvement of CNS were high whereas those of patients with subjective symptoms only were low. In our study, patients 1 and 3 had multiple CNS signs, patients 1 (49 points) and 3 (37 points) had seizures, psychosis and organic brain syndrome. On the other hand, patient 2 had MRI abnormality in the medulla oblongata but had only paresthesia as a subjective symptom (2 points), and patient 7 had MRI abnormality in the dorsal medulla spinalis and paralysis of the lower extremities, mood and anxiety disorders. However, the SLEDAI scores of both patients were based on subjective symptoms, and thus the scores were low (2 and 3, respectively).

Clinical outcome

At the start of rituximab treatment, patients were treated with low to moderate doses of corticosteroids (15–40 mg of prednisolone, 1–3 mg betamethasone), and continued to use this treatment during the rituximab arm of the study. However, immunosuppressants were stopped at entry to the study in all patients except for patient 8 who continued her treatment of 50 mg azathioprine. The postrituximab follow-up period was 7–45 months. Table 2 provides details of the clinical symptoms and laboratory tests before and 28 days after rituximab treatment (unless otherwise indicated in the table). Improvement in the skin and mucocutaneous lesions was fast, and the ejection fraction recovered from 44% to 72.1% in patient 4. All patients showed improvement in haematopenia and complement titre and marked falls in PE-resistant autoantibodies after treatment. Analysis of SLE activity before and after the treatment showed a significant decrease in SLEDAI from 19.9 (range, 49–2) before treatment to 6.2 (range, 15–0) after treatment ($p = 0.013$, fig 1). Moreover, SLEDAI decreased to 0 in 9 of the 10 patients at 1–6 months after rituximab treatment.

Rituximab treatment was also effective against CNS lesions in all patients. In particular, the consciousness state of all the five patients who were in acute confusional state before treatment, improved rapidly after the treatment. For example, the GCS score of patient 1 improved from 7–11 to 15 after 5 days of treatment, and that of patient 2 from 3 to 14 after 2 days of treatment. This rapid recovery was clinically significant. In addition, even in three patients who were in a dazed state and needed to be woken up before rituximab

Table 1 Characteristics of 10 female patients with neuropsychiatric systemic lupus erythaematosus at study entry

Patient	Age (years)	Duration of disease	Previous treatment	NP classification	MRI/SPECT	IgG index /IL6 (pg/ml)	Clinical manifestations	SLEDAI
1	35	19 years	CS (40 mg, pulse 14), IV-CY (22), VCR (10 mg), CsA (300 mg, 3 years), AZ (100 mg, 2 months), MTX (8 mg/w, 4 months), PE (11), IA (15)	Acute confusional state, seizure, psychosis	Normal/abnormal	Not done/not done	Fever, fatigue, nephritic syndrome, leukopenia, low Hb, high ESR, CH50, anti-ds DNA ↑	49
2	55	25 years	CS (40 mg, pulse 3), IV-CY (7), PE (2)	Acute confusional state	II, III/abnormal	0.73 ↑ / 1.8	Paresthesia of fingers, severe AIHA, anti-ds DNA ↑	2
3	46	3 months	CS (50 mg), IV-CY (1), PE (2), IA (3)	Acute confusional state, seizure	II, III/abnormal	0.46/33.8 ↑	Leukopenia, low Hb, thrombocytopenia, proteinuria, AIH, anti-ds DNA ↑	37
4	20	1 year	CS (50 mg), CsA (175 mg, 1 m)	Headache	Normal/not done	1.05 ↑ /3.1	Fever, fatigue, skin rash, alopecia, cardiomyopathy, polyneuropathy, leukopenia, C4 ↓, anti-ds DNA ↑	16
5	34	3 years	CS (60 mg), IV-CY (8), MZ (150 mg, 25 months)	Demyelinating syndrome	II, III/normal	0.85 ↑ /0.9	Sensory deficit, photosensitivity, mouth ulcer, lymphocytopenia, C4 ↓	16
6	30	22 years	CS (40 mg), MZ (150 mg, 22 years)	Mood disorder	Normal/abnormal	0.54/1.5	Polyneuropathy, muscular pain, skin rash, leukopenia, anti-ds DNA ↑	17
7	21	7 years	CS (60 mg, pulse 3), IV-CY (14), MTX (intrathecal 30 mg), MZ (300 mg, 2 years)	Myelopathy, mood disorder, anxiety disorder	II, III/abnormal	0.80 ↑ /4.7	Periungual erythaema, leukopenia	3
8	20	9 months	CS (45 mg), IV-CY (6), AZ (50 mg, 1 month)	Psychosis, cognitive dysfunction	III/abnormal	0.56/1.0	Lymphadenopathy, alopecia, malar rash, lymphocytopenia	18
9	20	8 months	CS (60 mg, pulse 3), IV-CY, DFPP (4)	Acute confusional state, psychosis	III/abnormal	0.98 ↑ /4.2	Fever, lymphadenopathy, low Hb, lymphocytopenia, high ESR, anti-Sm ↑	28
10	29	17 years	CS (40 mg, pulse 2), AZ (100 mg, iy), CsA (300 mg, 1 month), IV-CY (2), PE (4)	Acute confusional state, psychosis	Normal/abnormal	0.60/2.4	Severe AIHA, CH50 ↓	18

The disease activity was high in all patients and none had responded to conventional immunosuppressants.
AIHA, autoimmune haemolytic anaemia; AZ, azathioprine; CS, corticosteroid; CsA, cyclosporine; CY, cyclophosphamide; DFPP, double filtration plasmapheresis; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; IA, immunoadsorption; MTX, methotrexate; MZ, mizoribine; PE, plasma exchange; SLE-DAI, Systemic Lupus Erythaematosus Disease Activity Index; VCR, vincristine.
For IV-CY, PE and IA, numbers in parentheses represent the number of treatments. For CS, CsA, AZ and MZ, the doses in parentheses represent maximum dosage. For VCR in patient 1 and MTX in patient 7, the dose in parentheses expresses total dosage. MRI finding: II, small areas of increased signal intensity secondary to microinfarctions; III, focal areas of signal intensity in grey matter (*Am J Roentgenol* 1985;144:1027–31).

Table 2 Clinical outcomes of neuropsychiatric systemic lupus erythaematosus after anti-CD20 antibody treatment

Patient	Dose of rituximab	Other treatments at study entry (mg)	CNS manifestations before	CNS manifestations after	Objective NPSLE findings after treatment	Duration of remission (m)
1	375 mg/m² day 1, 8	Bet 1.0	Consciousness disorder, seizure, psychosis	Complete recovery (GCS 7–11→15/5 days)	Improvement of SPECT	22
2	375 mg/m² day 1, 15	Bet 1.5	Consciousness disorder	Improved consciousness	No follow-up data	18
3	375 mg/m² day 1, 8	Bet 1.0	Consciousness disorder, seizure	Complete recovery (GCS 3→14/2 days)	No improvement in MRI and SPECT	23
4	375 mg/m² day 1, 8	m-PSL 20	Headache	Resolution of headache	Improved IgG index (1.05 →0.84/4 w)	29
5	375 mg/m² day 1, 8	Bet 1.25	Paresthesia of fingers, toes and left precordial-back	Resolution of paresthesia	Improvement of neck MRI	7
6	500 mg day 1, 8, 15, 22	Bet 2.5	Depressive state, insomnia	Improvement of depressive state	Improvement of SPECT	7
7	500 mg day 1, 8, 15, 22	Bet 1.25	Paresis of both lower limbs, muscle weakness, depressive state	Reduction of paresis, improvement of depressive state (SDS 58→50/2 w)	Improvement of SPECT, improvement of IgG index (0.80 →0.72/3 m)	14
8	1000 mg day 1, 15	Bet 1.25, AZ 50	Psychosis, cognitive dysfunction	Improvement of psychosis (BPRS 26→7/8 w)	Improvement of SPECT	11
9	375 mg/m² day 1	PSL 45	Consciousness disorder, psychosis, paresis of both lower limbs, neurological bladder	Complete recovery	Improvement of PET and MRI, improved IgG index (0.98 →0.61/2 w)	10
10	375 mg/m² day 1, 8	Bet 3	Consciousness disorder, hallucination, cataplexy	Complete recovery	No significant improvement in objective findings	4

Bet, betamethasone; BPRS, brief psychiatric rating scale; CNS, central nervous system; GCS, Glasgow Coma Scale; m-PSL, methylprednisolone; MRI, magnetic resonance imaging; NPSLE, neuropsychiatric systemic lupus erythaematosus; PET, 18FTG-positron emission tomography; PSL, prednisolone; SDS, self-rating depression scale; SPECT, single photon emission computed tomography. For other abbreviations, see table 1.

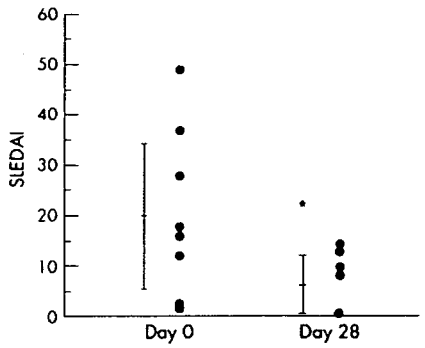

Figure 1 Systemic lupus erythaematosus disease activity index (SLEDAI) score before and 28 days after rituximab treatment. A decrease in SLEDAI score was detected in 9 of the 10 patients. Data are mean (SD). *p<0.05.

treatment, became alert the next day (patient 2) or after a few days of treatment (patients 9 and 10). Furthermore, rituximab also improved neuropsychiatric symptoms such as psychosis and mood disorder within a few weeks to a few months after treatment. For example, the Brief Psychiatric Rating Scale, which is used for the assessment of schizophrenia, markedly decreased in patient 8 from 26 to 7 points within 2 months, together with recovery of communication skills. In addition, patients 1 and 9 showed rehabilitation into society after rituximab treatment although they had serious neuropsychiatric symptoms before treatment. In addition to the improvement in SLE activity and clinical symptoms, rituximab also improved the quality of life of the patients.

We also assessed the effects of rituximab treatment by comparing the findings of MRI and SPECT before and after treatment. In four patients (patients 1, 6, 7 and 8), rituximab treatment improved cerebral blood flow as determined by SPECT; in patient 1, such improvement was noted at the early stage of treatment and paralleled the improvement in clinical symptoms. For patient 5, rituximab treatment resulted in improvement in the abnormal findings in T2-weighted images of the cervical cord on MRI, along with the improvement in sensory deficits due to inflammation at the same site. For patient 9, rituximab treatment resulted in reduction of the high-intensity lesion in the head MRI T2-weighted image.

Four of our patients had peripheral neuropathies in addition to CNS lesions. Treatment with rituximab resulted in remission or marked improvement of paresthesia in patient 2, radiculopathy in patient 4, ulnar neuropathy in patient 6, and neurological bladder in patient 9. Rituximab also improved quality of life based on improvement of peripheral neuropathy-related symptoms although such symptoms tended to persist after treatment.

While the overall therapeutic effect of rituximab was excellent, some patients developed relapse after long-term remission. Six of the 10 patients showed reactivation of SLE including reappearance of CNS-related symptoms. For patient 1, remission was maintained with low-dose steroid for 22 months after rituximab treatment. However, the patient showed recurrence associated with an increase in autoantibodies and proteinuria. Recurrence was also noted 18 months after treatment in patient 2, associated with haemolysis. Both patients 1 and 2 required retreatment with rituximab. At 23 months after completion of rituximab treatment, patient 3 showed worsening of the head MRI findings and cerebrospinal fluid abnormalities and developed witnessed seizure attacks. In patient 5, a reduction in the steroid dose was followed by recurrence of CNS-related symptoms after 7 months. Generalised skin rashes appeared in patient 9 after 10 months and patient 10 reported worsening of lupus headache after 4 months. Patients 3 and 5 received IV-CY treatment, and patient 9 and 10 required an increase in the steroid dose. However, four patients (patients 4, 6, 7 and 8) maintain a remission state at the time of writing this report (at 35 months in patient 4, at 7 months in patient 6, at 19 months in patient 7 and 16 months in patient 8) after the completion of rituximab treatment.

Adverse effects

Of the 10 patients, two developed pneumonia, one had herpes zoster, one developed chickenpox and one had intractable infection of decubitus ulceration. These infections were successfully controlled with antibiotics.

Phenotypic analysis of SLE lymphocytes

T cells and B cells are activated by antigen stimulation via T cell receptors and signals from costimulatory molecules. The responsible costimulatory molecules, such as CD40/40L, CD80, CD86/CD28 and ICOS/B7h, are known to be expressed in patients with active SLE.[21-26]

We performed serial analysis of the expression of functional molecules in eight patients with SLE before and after rituximab treatment by flow cytometry. Rituximab treatment resulted in rapid disappearance of CD20, a specific antigen to B cells, marked decrease in CD19-positive cells, within several days to 2 weeks after treatment. Rituximab also resulted in rapid falls in the percentages of CD40-expressing and CD80-expressing CD19 cells within 1 day and both were hardly detected after the second day (fig 2). The expression levels of these molecules were still low at 3 months after completion of rituximab treatment.

We also assessed the effects of treatment on the expression levels of CD40L (a costimulatory molecule on CD4-positive cells), ICOS and CD69 (an early activation antigen). While only three patients showed high expression of these molecules before treatment, rituximab treatment reduced the expression levels of these molecules in all three patients (fig 3), suggesting that rituximab does not only affect B cells but also T cells in patients with SLE.

DISCUSSION

To date, reports on rituximab treatment for autoimmune diseases have covered various conditions, including RA, SLE, dermatomyositis, Sjögren's syndrome and vasculitis.[27-30] Rituximab treatment resulted in improvement, manifested by a decrease in the British Disease Activity score and SLE DAI score, of arthropathy, nephropathy, thrombocytopenia and haemolytic anaemia.[11-16]

Although few reports described the efficacy of rituximab treatment in patients with SLE with CNS lesions,[11 14 31] to our knowledge, there are no published reports that provide detailed analysis of the effects of such treatment in a large group of patients. Rituximab has a large molecular weight of 146 kDa, and hence cannot readily cross the blood–brain barrier; therefore, it is unlikely to reach the cerebrospinal fluid following systemic administration. We measured rituximab concentration in the cerebrospinal fluid of patient 8 at 24 h after treatment. The value (0.3 μg/ml) was slightly higher than the lower detection limit of the assay, whereas the serum concentration was 279 μg/ml. Based on this finding, we assume that the central effects of rituximab are mediated through another mechanism, not through antibody-dependent cellular cytotoxicity and/or complement-dependent cytotoxicity.[32]

To assess autoreactive lymphocyte activity, we determined the expression of various functional molecules on the surface of peripheral blood lymphocytes before and after rituximab treatment by using flow cytometry. We previously proposed that

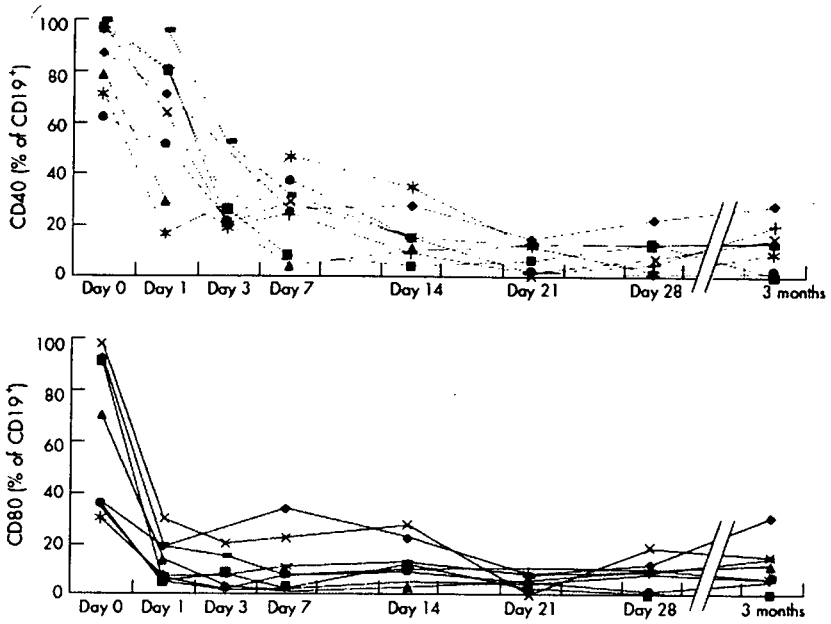

Figure 2 Serial changes in CD40 and CD80 expression on CD19-positive cells after rituximab treatment in eight patients with systemic lupus erythaematosus. CD40 and CD80 expression was measured before and 28 days after rituximab treatment.

rituximab could regulate SLE disease activity and correct autoimmune abnormalities.[12] The present results showed a rapid decrease in the expression of functional surface molecules and maintenance of long-term control following rituximab treatment (fig 2). Specifically, a marked decrease in the proportion of CD40-expressing and CD80-expressing cells was detected on the day after initiation of rituximab treatment. In this regard, Leng *et al*[13] found CD40 overexpression in CD19 cells in patients with rheumatoid arthritis compared with healthy controls. Others also reported that the percentage of CD80-positive cells among activated B cell subset was higher in SLE than the controls.[14] These results suggest that the target of rituximab treatment is activated B cells. Anolik *et al*[15] examined B cell phenotypes after rituximab treatment and reported that the proportion of autoreactive memory B cells was decreased after rituximab treatment. Considered together, the above results and those of the present study suggest that T cell activation is negatively influenced by a rapid decrease in B cell to T cell stimulation in

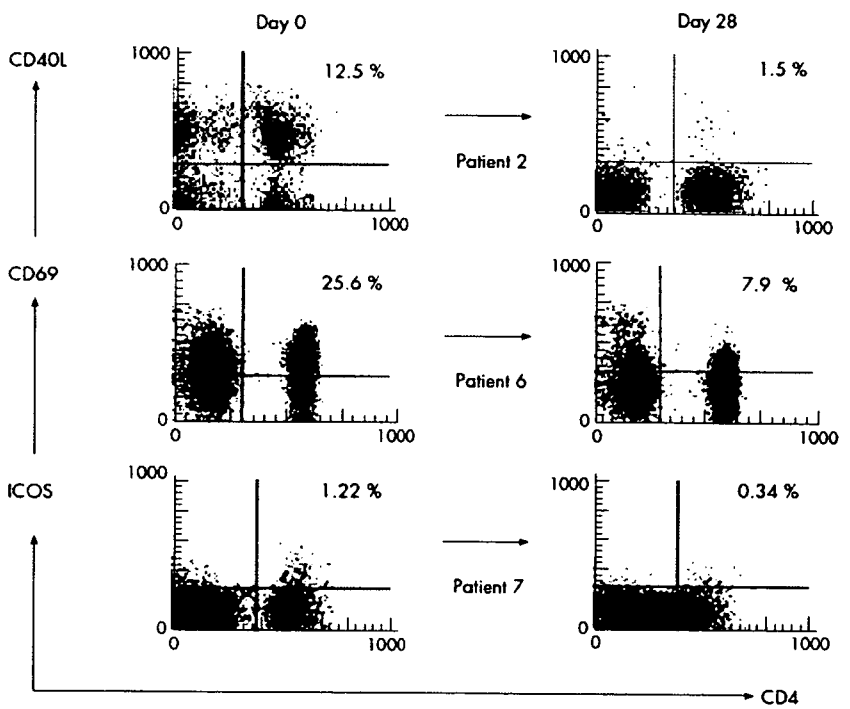

Figure 3 Changes in expression of functional molecules on CD4-positive cells induced by rituximab treatment. The expression of CD40L (patient 2), CD69 (patient 6) and ICOS (patient 7) on CD4-positive cells was measured before (day 0) and 28 days after rituximab treatment. Percentages represent the percentage of CD4-positive cells expressing the functional molecules.

parallel with the loss of B cells. Our results also showed that rituximab down regulated CD40L, ICOS and CD69 on CD4-positive cells in patients with active SLE (fig 3). Sfikakis *et al*[36] also reported that rituximab treatment decreased CD40L and CD69 expression in patients with SLE. These results imply that rituximab could eliminate B cells bearing functional molecules and inhibit the interaction between these B cells and activated T cells by down regulating costimulatory molecules, and also possibly by reducing the production of certain cytokines and complement activation, which could lead to rapid improvement of CNS manifestations of the disease.

At present, there is no treatment strategy for patients with NPSLE who fail to respond to conventional therapies. In such patients, large doses of steroids are provided on long-term basis, and IV-CY is administered continuously. Our study showed that rituximab is useful as a new treatment for such cases. However, recurrence after rituximab treatment was noted in our patients, as has been reported previously in patients with rheumatoid diseases.[28] Two of our patients who experienced recurrence received rituximab re-treatment. However, these patients experienced recurrence at 18 and 22 months after rituximab treatment, suggesting that remission could be maintained for a comparatively long period of time with rituximab treatment. Further studies are needed to develop strategies for the prevention of recurrence and counter measures for inhibiting the production of antichimeric antibodies.[37 38] There is also a need to investigate the long-term effects of rituximab treatment and its organ specificity.

ACKNOWLEDGEMENTS

This work was supported in part by a Research Grant-In-Aid for Scientific Research by the Ministry of Health, Labor and Welfare of Japan, the Ministry of Education, Culture, Sports, Science and Technology of Japan and University of Occupational and Environmental Health, Japan.

Authors' affiliations

Mikiko Tokunaga, Kazuyoshi Saito, Shingo Nakayamada, Shizuyo Tsujimura, Masao Nawata, Shigeru Iwata, Taeko Azuma, Yoshiya Tanaka, The First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Kitakyusyu, Japan
Daisuke Kawabata, Yoshitaka Imura, Takao Fujii, Tsuneyo Mimori, Department of Rheumatology and Clinical Immunology, Kyoto University, Graduate School of Medicine, Kyoto, Japan

Competing interests: None declared.

REFERENCES

1 Blanco FJ, Gomez-Reino JJ, de la Mata J, Corrales A, Rodriguez-Valverde V, Rosas JC, *et al.* Survival analysis of 306 European Spanish patients with systemic lupus erythematosus. *Lupus* 1998;7:159–63.
2 Kasitanon N, Louthrenoo W, Sukitawut W, Vichainun R. Causes of death and prognostic factors in Thai patients with systemic lupus erythematosus. *Asian Pac J Allergy Immunol* 2002;20:85–91.
3 Hahn BH. Systemic lupus erythematosus. In: Kasper DL, eds. *Origins of Harrison's principles of internal medicine*.16th edn. Columbus: McGraw-Hill, 2005:1960–7.
4 Ad Hoc Working Group on Steroid-Sparing Criteria in Lupus. Criteria for steroid-sparing ability of interventions in systemic lupus erythematosus: report of a consensus meeting. *Arthritis Rheum* 2004;50:3427–31.
5 Goldblatt F, Isenberg DA. New therapies for systemic lupus erythematosus. *Clin Exp Immunol* 2005;140:205–12.
6 Petri M. Cyclophosphamide: new approaches for systemic lupus erythematosus. *Lupus* 2004;13:366–71.
7 Boumpas DT, Yamada H, Patronas NJ, Scott D, Klippel JH, Balow JE. Pulse cyclophosphamide for severe neuropsychiatric lupus. *Q J Med* 1991;81:975–84.
8 Barile-Fabris L, Ariza-Andraca R, Olguin-Ortega L, Jara LJ, Fraga-Mouret A, Miranda-Limon JM, *et al.* Controlled clinical trial of IV cyclophosphamide versus IV methylprednisolone in severe neurological manifestations in systemic lupus erythematosus. *Ann Rheum Dis* 2005;64:620–5.
9 Sherer Y, Levy Y, Langevitz P, Lorber M, Fabrizzi F, Shoenfeld Y. Successful treatment of systemic lupus erythematosus cerebritis with intravenous immunoglobulin. *Clin Rheumatol* 1999;18:170–3.
10 Valesini G, Priori R, Francia A, Balestrieri G, Tincani A, Airo P, *et al.* Central nervous system involvement in systemic lupus erythematosus: a new therapeutic approach with intrathecal dexamethasone and methotrexate. *Springer Semin Immunopathol* 1994;16:313–21.
11 Saito K, Nawata M, Nakayamada S, Tokunaga M, Tsukada J, Tanaka Y. Successful treatment with anti-CD20 monoclonal antibody (rituximab) of life-threatening refractory systemic lupus erythematosus with renal and central nervous system involvement. *Lupus* 2003;12:798–800.
12 Tokunaga M, Fujii K, Saito K, Nakayamada S, Tsujimura S, Nawata M, *et al.* Down-regulation of CD40 and CD80 on B cells in patients with life-threatening systemic lupus erythematosus after successful treatment with rituximab. *Rheumatology* 2005;44:176–82.
13 Perrotta S, Locatelli F, La Manna A, Cennamo L, De Stefano P, Nobili B. Anti-CD20 monoclonal antibody (Rituximab) for life-threatening autoimmune haemolytic anaemia in a patient with systemic lupus erythematosus. *Br J Haematol* 2002;116:465–7.
14 Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR, Isenberg DA. An open study of B lymphocyte depletion in systemic lupus erythematosus. *Arthritis Rheum* 2002;46:2673–7.
15 Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, *et al.* B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. *Arthritis Rheum* 2004;50:2580–9.
16 Leandro MJ, Cambridge G, Edwards JC, Ehrenstein MR, Isenberg DA. B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. *Rheumatology* 2005;44:1542–5.
17 Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
18 ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology nomenclature and classification and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 1999;42:599–608.
19 Borchers AT, Aoki CA, Naguwa SM, Keen CL, Shoenfeld Y, Gershwin ME. Neuropsychiatric features of systemic lupus erythematosus. *Autoimmun Rev* 2005;4:329–44.
20 French CA, Tracy RP, Rudick RA, Kraemer AM, Arvan DA. Simultaneous determination of cerebrospinal fluid oligoclonal bands and the "gamma-protein index" by agarose electrophoresis and densitometry. *Clin Chem* 1986;32:84–7.
21 Datta SK, Kalled SL. CD40-CD40 ligand interaction in autoimmune disease. *Arthritis Rheum* 1997;40:1735–45.
22 Koshy M, Berger D, Crow MK. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J Clin Invest* 1996;98:826–37.
23 Yellin MJ, Thienel U. T cells in the pathogenesis of systemic lupus erythematosus: potential roles of CD154-CD40 interactions and costimulatory molecules. *Curr Rheumatol Rep* 2000;2:24–31.
24 Kovacs B, Thomas DE, Tsokos GC. Elevated *in vivo* expression of the costimulatory molecule B7-BB1 (CD80) on antigen presenting cells from a patient with SLE. *Clin Exp Rheumatol* 1996;14:695–7.
25 Bijl M, Horst G, Limburg PC, Kallenberg CG. Expression of costimulatory molecules on peripheral blood lymphocytes of patients with systemic lupus erythematosus. *Ann Rheum Dis* 2001;60:523–6.
26 Andreas H, Kerstin B, Karin R, Hans JB, Marcus O, Annett J, *et al.* Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus. *Arthritis Rheum* 2004;50:3211–20.
27 Leandro MJ, Edwards JC W, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. *Ann Rheum Dis* 2002;61:883–8.
28 Keogh KA, Ytterberg SR, Fervenza FC, Carlson KA, Schroeder DR, Specks U. Rituximab for refractory Wegener's granulomatosis: report of a prospective, open-label pilot trial. *Am J Respir Crit Care Med* 2006;173:180–7.
29 Levine TD. Rituximab in the treatment of dermatomyositis: an open-label pilot study. *Arthritis Rheum* 2005;52:601–7.
30 Pijpe J, van Imhoff GW, Spijkervet FK, Roodenburg JL, Wolbink GJ, Mansour K, *et al.* Rituximab treatment in patients with primary Sjogren's syndrome: an open-label phase II study. *Arthritis Rheum* 2005;52:2740–50.
31 Armstrong DJ, McCarron MT, Wright GD. SLE-associated transverse myelitis successfully treated with Rituximab (anti-CD20 monoclonal antibody). *Rheumatol Int* 2005;18:1–2.
32 Reff ME, Carner K, Chambers KS, Leonard JE, Raab R, Newman RA, *et al.* Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994;83:435–45.
33 Leng JH, Hu ZY, Zhuo GC, Wang KY, Wang ZW, Peng L, *et al.* The expression and significance of costimulatory molecule in peripheral blood B lymphocytes in rheumatoid arthritis. *Zhonghua Nei Ke Za Zhi* 2004;43:519–21.
34 Folzenlogen D, Hofer MF, Leung DY, Freed JH, Newell MK. Analysis of CD80 and CD86 expression on peripheral blood B lymphocytes reveals increased expression of CD86 in lupus patients. *Clin Immunol Immunopathol* 1997;83:199–204.
35 Anolik JH, Barnard J, Cappione A, Pugh-Bernard AE, Pugh-Bernard AE, Felgar RE, *et al.* Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. *Arthritis Rheum* 2004;50:3580–90.
36 Sfikakis PP, Boletis JN, Lionaki S, Vigklis V, Fragiadaki KG, Iniotaki A, *et al.* Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. *Arthritis Rheum* 2005;52:501–13.
37 Weide R, Heymanns J, Pandorf A, Koppler H. Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy. *Lupus* 2003;12:779–82.
38 Edelbauer M, Jungraithmayr T, Zimmerhackl LB. Rituximab in childhood systemic lupus erythematosus refractory to conventional immunosuppression: case report. *Pediatr Nephrol* 2005;20:811–13.

ARTHRITIS & RHEUMATISM
Vol. 56, No. 4, April 2007, pp 1295–1303
DOI 10.1002/art.22506

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

Michito Hirakata,[1] Akira Suwa,[2] Tetsuya Takada,[1] Shinji Sato,[1] Sonoko Nagai,[3] Ekkehard Genth,[4] Yeong W. Song,[5] Tsuneyo Mimori,[3] and Ira N. Targoff[6]

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])

Supported in part by grants from the Japanese Ministry of Education, Science, Culture, Sports, and Technology, the Japanese Ministry of Health, Labor, and Welfare, and the Keio University School of Medicine. Dr. Targoff's work was supported in part by the US Department of Veterans Affairs.

[1]Michito Hirakata, MD, Tetsuya Takada, MD, Shinji Sato, MD: Keio University School of Medicine, Tokyo, Japan; [2]Akira Suwa, MD: Tokai University School of Medicine, Ischara, Japan; [3]Sonoko Nagai, MD, Tsuneyo Mimori, MD: Kyoto University Graduate School of Medicine, Kyoto, Japan; [4]Ekkehard Genth, MD: Clinic and Research Institute for Rheumatic Diseases Aachen, Aachen, Germany; [5]Yeong W. Song, MD: Seoul National University Hospital, Seoul, Korea; [6]Ira N. Targoff, MD: Veterans Affairs Medical Center, University of Oklahoma Health Sciences Center, and Oklahoma Medical Research Foundation, Oklahoma City.

Dr. Targoff serves as a technical consultant to the Oklahoma Medical Research Foundation Clinical Immunology Laboratory.

Address correspondence and reprint requests to Michito Hirakata, MD, Section of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: mhirakat@sc.itc.keio.ac.jp.

Submitted for publication September 25, 2005; accepted in revised form January 4, 2007.

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

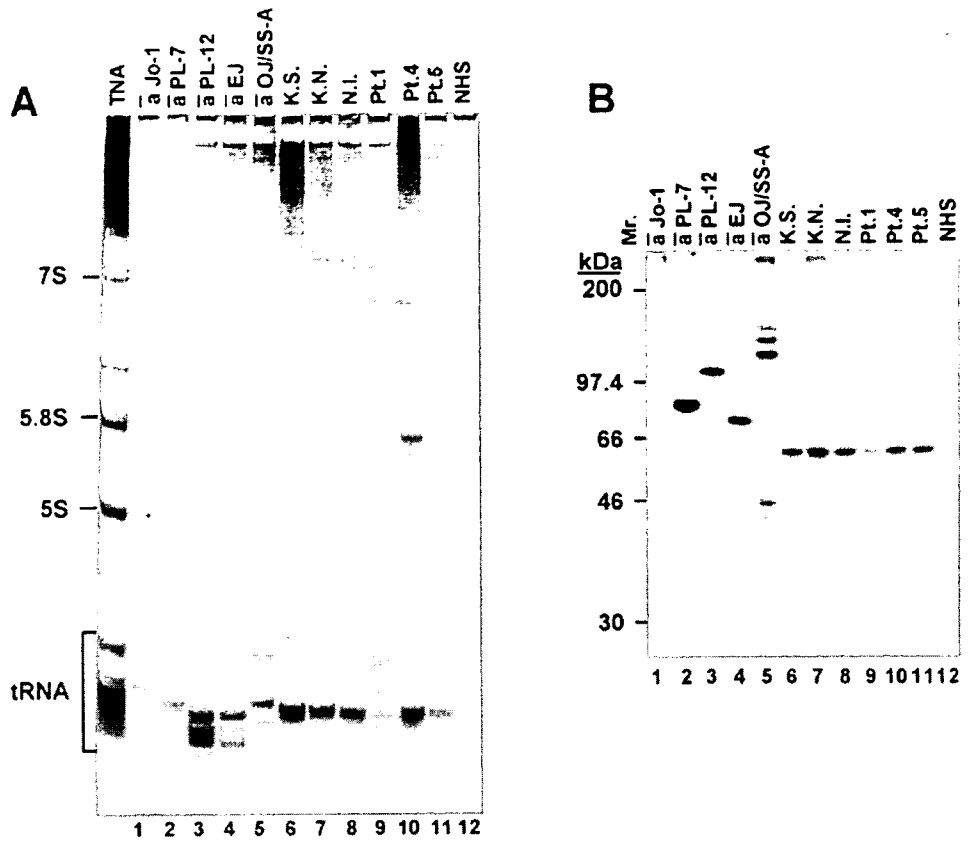

Figure 1. A. Immunoprecipitation (IP) for nucleic acids with anti-KS and control sera. Shown are patterns of transfer RNA (tRNA) resulting from 7*M* urea–10% polyacrylamide gel electrophoresis (PAGE) of phenol-extracted immunoprecipitates from HeLa cell extract, developed with silver stain. TNA = total 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	I
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.

ACKNOWLEDGMENTS

We would like to thank Dr. Paul H. Plotz for providing the clinical information and serum, and Ms Mutsuko Ishida and Mr. Edward Trieu for expert technical assistance. We wish to thank Dr. John A. Hardin for helpful discussion and critical review of the manuscript.

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.

REFERENCES

1. Targoff IN. Laboratory testing in the diagnosis and management of idiopathic inflammatory myopathies. Rheum Dis Clin North Am 2002;28:859–90.
2. Targoff IN. Myositis specific autoantibodies. Curr Rheumatol Rep 2006;8:196–203.
3. Mathews MB, Bernstein RM. Myositis autoantibody inhibits histidyl-tRNA synthetase: a model for autoimmunity. Nature 1983; 304:177–9.
4. Mathews MB, Reichlin M, Hughes GR, Bernstein RM. Anti-threonyl-tRNA synthetase, a second myositis-related autoantibody. J Exp Med 1984;160:420–34.
5. Bunn CC, Bernstein RM, Mathews MB. Autoantibodies against alanyl tRNA synthetase and $tRNA^{Ala}$ coexist and are associated with myositis. J Exp Med 1986;163:1281–91.
6. Targoff IN. Autoantibodies to aminoacyl-transfer RNA synthetases for isoleucine and glycine: two additional synthetases are antigenic in myositis. J Immunol 1990;144:1737–43.
7. Targoff IN, Reichlin M. Measurement of antibody to Jo-1 by ELISA and comparison to enzyme inhibitory activity. J Immunol 1987;138:2874–82.
8. Oddis CV, Medsger TA Jr, Cooperstein LA. A subluxing arthropathy associated with the anti-Jo-1 antibody in polymyositis/dermatomyositis. Arthritis Rheum 1990;33:1640–5.
9. Love LA, Leff RL, Fraser DD, Targoff IN, Dalakas MC, Plotz PH, et al. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine (Baltimore) 1991;70:360–74.
10. Hirakata M, Mimori T, Akizuki A, Craft J, Hardin JA, Homma M. Autoantibodies to small nuclear and cytoplasmic ribonucleoproteins in Japanese patients with inflammatory muscle disease. Arthritis Rheum 1992;35:449–56.
11. Marguerie C, Bunn CC, Beynon HL, Bernstein RM, Hughes JM, So AK, et al. Polymyositis, pulmonary fibrosis and autoantibodies to aminoacyl-tRNA synthetase enzymes. Q J Med 1990;77:1019–38.
12. Targoff IN, Arnett FC, Reichlin M. Antibody to threonyl-transfer RNA synthetase in myositis sera. Arthritis Rheum 1988;31:515–24.
13. Targoff IN, Arnett FC. Clinical manifestations in patients with antibody to PL-12 antigen (alanyl-tRNA synthetase). Am J Med 1990;88:241–51.
14. Targoff IN, Trieu EP, Miller FW. Reaction of anti-OJ autoantibodies with components of the multi-enzyme complex of aminoacyl-tRNA synthetases in addition to isoleucyl-tRNA synthetase. J Clin Invest 1993;91:2556–64.
15. Targoff IN, Trieu EP, Plotz PH, Miller FW. Antibodies to glycyl–transfer RNA synthetase in patients with myositis and interstitial lung disease. Arthritis Rheum 1992;35:821–30.
16. Furuya T, Hakoda M, Tsuchiya N, Kotake S, Ichikawa N, Nanke Y, et al. Immunogenetic features in 120 Japanese patients with idiopathic inflammatory myopathy. J Rheumatol 2004;31: 1768–74.
17. Yoshida S, Akizuki M, Mimori T, Yamagata H, Inada S, Homma M. The precipitating antibody to an acidic nuclear protein antigen, the Jo-1, in connective tissue diseases: a marker for a subset of polymyositis with interstitial pulmonary fibrosis. Arthritis Rheum 1983;26:604–11.
18. Bernstein RM, Morgan SH, Chapman J, Bunn CC, Mathews MB, Turner-Warwick M, et al. Anti-Jo-1 antibody: a marker for myositis with interstitial lung disease. Br Med J 1984;289:151–2.
19. Friedman AW, Targoff IN, Arnett FC. Interstitial lung disease with autoantibodies against aminoacyl-tRNA synthetase in the absence of clinically apparent myositis. Semin Arthritis Rheum 1996;26:459–67.
20. Hirakata M, Nakamura K, Okano Y, Suwa A, Inada S, Akizuki M, et al. Anti-alanyl tRNA synthetase (PL-12) antibodies are associated with interstitial lung disease in Japanese patients [abstract]. Arthritis Rheum 1995;38:S321.
21. Arnett FC, Targoff IN, Mimori T, Goldstein R, Warner NB, Reveille JD. Interrelationship of major histocompatibility complex class II alleles and autoantibodies in four ethnic groups with various forms of myositis. Arthritis Rheum 1996;39: 1507–18.
22. Hirakata M, Suwa A, Takeda Y, Matsuoka Y, Irimajiri S, Targoff IN, et al. Autoantibodies to glycyl–transfer RNA synthetase in myositis: association with dermatomyositis and immunologic heterogeneity. Arthritis Rheum 1996;39:146–51.
23. Hirakata M, Suwa A, Nagai S, Kron MA, Trieu EP, Mimori T, et al. Anti-KS: identification of autoantibodies to asparaginyl-transfer RNA synthetase associated with interstitial lung disease. J Immunol 1999;162:2315–20.
24. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344–7.
25. Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975;292:403–7.
26. Targoff IN, Arnett FC, Berman L, O'Brien CA, Reichlin M. Anti-KJ: a new antibody associated with the myositis/lung syndrome that reacts with translation-related protein. J Clin Invest 1989;84:162–72.
27. Inoko H, Ota M. PCR-RFLP. In: Hui KM, Bidwell J, editors. Handbook of HLA typing techniques. Boca Raton (FL): CRC Press; 1993. p. 9–70.
28. Brouwer R, Vree Egberts W, Jongen PH, van Engelen BG, van Venrooij WJ. Frequent occurrence of anti-$tRNA^{His}$ autoantibodies that recognize a conformational epitope in sera of patients with myositis. Arthritis Rheum 1998;41:1428–37.
29. Bunn CC, Mathews MB. Two human tRNA(Ala) families are recognized by autoantibodies in polymyositis sera. Mol Biol Med 1987;4:21–36.
30. Beaulande M, Tarbouriech N, Hartlein M. Human cytosolic asparaginyl-tRNA synthetase: cDNA sequence, functional expression in Escherichia coli and characterization as human autoantigen. Nucleic Acids Res 1998;26:521–4.
31. Miller FW, Waite KA, Biswas T, Plotz PH. The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. Proc Natl Acad Sci U S A 1990;87: 9933–7.
32. Schmidt WA, Wetzel W, Friedlander R, Lange R, Sorensen HF, Lichey HJ, et al. Clinical and serological aspects of patients with anti-Jo-1 antibodies: an evolving spectrum of disease manifestations. Clin Rheumatol 2000;19:371–7.
33. Sato S, Hirakata M, Kuwana M, Nakamura K, Suwa A, Inada S, et al. Clinical characteristics of Japanese patients with anti-PL-7 (anti-threonyl-tRNA synthetase) autoantibodies. Clin Exp Rheumatol 2005;23:609–15.
34. Arnett FC. The genetics of human lupus. In: Wallace DL, Hahn BH, editors. Dubois' lupus erythematosus. 5th ed. Baltimore: Williams & Wilkins; 1997. p.77–117.
35. Hirakata M, Satoh S, Suwa A, Nakamura K, Hama N, Ohsone Y, et al. Clinical and immunogenetic features of anti-aminoacyl

tRNA synthetase autoantibodies in Japanese patients [abstract]. Arthritis Rheum 1997;40 Suppl 9:S83.
36. Hirakata M, Suwa A, Kuwana M, Sato S, Mimori T, Hardin JA. Association between autoantibodies to the Ku protein and DPBI. Arthritis Rheum 2005;52:668–9.
37. Plotz PH, Dalakas M, Leff RL, Love LA, Miller FW, Cronin ME. Current concepts in the idiopathic inflammatory myopathies: polymyositis, dermatomyositis, and related disorders. Ann Intern Med 1989;111:143–57.
38. Weidensaul D, Imam T, Holyst MM, King PD, McMurray RW. Polymyositis, pulmonary fibrosis, and hepatitis C. Arthritis Rheum 1995;38:437–9.
39. Plotz PH. Autoantibodies are anti-idiotype antibodies to antiviral antibodies. Lancet 1983;2:824–6.
40. Casciola-Rosen L, Andrade F, Ulanet D, Wong WB, Rosen A. Cleavage by granzyme B is strongly predictive of autoantigen status: implications for initiation of autoimmunity. J Exp Med 1999;190:815–26.
41. Plotz PH. The autoantibody repertoire: searching for order. Nat Rev Immunol 2003;3:73–8.

Autoantibodies in idiopathic inflammatory myopathy: an update on clinical and pathophysiological significance

Tsuneyo Mimori, Yoshitaka Imura, Ran Nakashima and Hajime Yoshifuji

Purpose of review

Idiopathic inflammatory myopathy is characterized by the production of autoantibodies to various cellular constituents. These autoantibodies closely correlate with certain clinical conditions and prognosis of disease. This review examines recent progress in myositis-specific autoantibodies, particularly in their clinical significance and pathophysiological roles.

Recent findings

During the 1-year review period, novel myositis-specific autoantibodies were identified in clinically amyopathic dermatomyositis (anti-CADM-140 antibody) and malignancy-associated myositis (anti-p155 and anti-p155/p140 antibodies). These new autoantibodies are extremely important because it is thought that myositis-specific autoantibodies are negative in these subgroups, and may enable a new classification of idiopathic inflammatory myopathy. New clinical aspects of other myositis-specific autoantibodies (anti-aminoacyl-tRNA synthetases, anti-signal recognition particles and anti-Mi-2) are also described. The possibility was raised that the high expression of myositis-specific autoantigens in regenerating muscle cells and certain cancers may be involved in initiating and perpetuating the autoimmune response in myositis.

Summary

Myositis-specific autoantibodies are useful markers for clinical diagnosis, classification and predicting prognosis of idiopathic inflammatory myopathy. To understand the etiopathogenic mechanisms of the disease it is particularly important to elucidate the nature of target autoantigens recognized by these myositis-specific autoantibodies.

Keywords

aminoacyl-tRNA synthetase, amyopathic dermatomyositis, malignancy-associated myositis, myositis-specific autoantibody

Curr Opin Rheumatol 19:523–529.

Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Kyoto, Japan

Correspondence to Tsuneyo Mimori, Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, 35 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan
Tel: +81 75 751 4379; fax: +81 75 751 4338;
e-mail: mimorit@kuhp.kyoto-u.ac.jp

Current Opinion in Rheumatology 2007, 19:523–529

Abbreviations

ADM	amyopathic dermatomyositis
ARS	aminoacyl-tRNA synthetase
C-ADM	clinically amyopathic dermatomyositis
CTD	connective tissue disease
IIM	idiopathic inflammatory myopathy
ILD	interstitial lung disease
MAA	myositis-associated autoantibody
MSA	myositis-specific autoantibody
SRP	signal recognition particle

1040-8711

Introduction

Polymyositis and dermatomyositis are forms of idiopathic inflammatory myopathy (IIM) that involve skeletal muscle as well as many other organs, such as lung, heart, joint and skin. As in other connective tissue diseases (CTDs), polymyositis/dermatomyositis are characterized by the production of a series of autoantibodies to various cellular constituents. Some of these autoantibodies are found specifically in patients with polymyositis/dermatomyositis (known as myositis-specific autoantibodies – MSAs) or myositis overlap syndrome (known as myositis-associated autoantibodies – MAAs), and correlate with certain clinical and pathophysiological conditions of myositis [1–3]. Autoantibodies detected in myositis are listed and summarized in Table 1. In this article, we review recent advances and update the clinical and possible pathophysiological significance of autoantibodies and their target autoantigens in IIM.

Newly identified myositis-specific autoantibodies

Novel MSAs have been identified in amyopathic dermatomyositis and malignancy-associated myositis. These new autoantibodies are important because previously it had been thought that MSAs are negative in these subgroups of myositis.

Anti-CADM-140 antibody: a new marker for clinically amyopathic dermatomyositis

The concept of amyopathic dermatomyositis (ADM) has been discussed among dermatologists since the 1970s [4], and is also termed dermatomyositis sine myositis [5]. Euwer and Sontheimer [6] suggested diagnostic criteria for ADM in which patients with typical dermatomyositis rashes but without muscle symptoms

Table 1 Autoantibodies found in myositis, their target antigens and clinical significance

Antibodies	Nature of target antigens	Frequency in IIM (%)	Clinical significance
Myositis-specific autoantibodies (MSAs)			
anti-ARS			
anti-Jo-1	Histidyl-tRNA synthetase	15–20	Antisynthetase syndrome (myositis, interstitial pneumonia, polyarthritis, mechanic's hand, Raynaud's phenomenon, fever)
anti-PL-7	Threonyl-tRNA synthetase	5–10	
anti-PL-12	Alanyl-tRNA synthetase	<5	
anti-EJ	Glycyl-tRNA synthetase	5–10	
anti-OJ	Isoleucyl-tRNA synthetase	<5	
anti-KS	Asparaginyl-tRNA synthetase	<5	
anti-Zo	Phenylalanyl-tRNA synthetase	<1	
anti-YRS	Tyrosyl-tRNA synthetase	<1	
anti-SRP	Signal recognition particle	5–10	Necrotizing myopathy
anti-Mi-2	218/240 kDa helicase family proteins	5–10	Dermatomyositis
anti-CADM-140	Unknown 140 kDa protein	50 (C-ADM)	Specific in C-ADM
anti-p155(/p140)	Transcriptional intermediary factor 1-γ	20 (dermatomyositis)	Dermatomyositis, especially in cancer-associated dermatomyositis
anti-MJ	Unknown 140 kDa protein	<5	Juvenile dermatomyositis
anti-PMS1	DNA repair mismatch enzyme	<5	
Myositis-associated autoantibodies (MAAs)			
anti-U1RNP	U1 small nuclear RNP	10	Overlap myositis, MCTD
anti-Ku	70/80 kDa DNA-PK regulatory subunit	20–30	Polymyositis–SSc overlap in Japanese
anti-PM-Scl	Nucleolar protein complex of 11–16 proteins	8–10	Polymyositis–SSc overlap in Caucasians

ARS, aminoacyl-tRNA synthetases; C-ADM, clinically amyopathic dermatomyositis; DNA-PK, DNA-dependent protein kinase; MCTD, mixed connective tissue disease; SSc-scleroderma.

for longer than 2 years were diagnosed as confirmed ADM [6]. Recently, Sontheimer [7] corrected the definition of ADM so that patients with no clinical muscle symptoms for more than 2 years but with at the very least an abnormality in laboratory tests should be defined as hypomyopathic dermatomyositis. He suggested that patients with ADM or hypomyopathic dermatomyositis should be defined as clinically ADM (C-ADM), since they have no clinically apparent muscle symptoms. Such patients with ADM or C-ADM have been reported to develop sometimes life-threatening acute progressive interstitial pneumonia [8–12]. So far, however, MSAs have not been detected in patients with C-ADM and this appears to be the characteristic feature of C-ADM.

Recently, a specific autoantibody was identified in C-ADM patients by Sato *et al.* [13]. They screened 306 patient sera, including 15 of C-ADM by ^{35}S-protein immunoprecipitation and immunoblotting techniques using leukemia-derived K562 cells, and eight of 15 patients (53%) with C-ADM immunoprecipitated a 140 kDa protein. None of the sera from other connective tissue diseases or healthy controls recognized the same protein. This newly identified autoantibody was termed anti-CADM-140. All eight patients with anti-CADM-140 had typical dermatomyositis rashes such as heliotrope erythema and Gottron's sign, but no muscle weakness or muscular pain and normal serum creatinine kinase level. In 15 patients with C-ADM, 13 developed interstitial lung disease (ILD), and five were acute progressive type. In five patients with acute ILD, four had anti-CADM-140. Thus, anti-CADM-140 antibody may be useful as a new specific serologic marker for a subset of myositis and acute ILD.

The nature of the target antigen of anti-CADM-140 is still unknown, but it shows a granular or reticular cytoplasmic staining resembling cytoskeletal components in indirect immunofluorescence. Anti-CADM-140 is not detected from HeLa cells but shows stronger reaction with alveolar epithelia-derived A549 cells, suggesting a possibility that this autoantibody may be involved in an alveolar injury by antigen–antibody interaction.

Anti-p155 antibody: a new marker for dermatomyositis including cancer-associated myositis

Targoff *et al.* [14••] reported a new autoantibody that recognized a 155 kDa protein (p155) in dermatomyositis. They tested 244 patient sera from IIM, 138 sera from nonmyositis CTD and healthy volunteers for autoantibodies by ^{35}S-protein immunoprecipitation using HeLa cells and immunoblotting using K562 cells. They detected a previously unrecognized autoantibody that immunoprecipitated a 155 kDa protein along with a weaker 140 kDa protein. Fifty-one of 244 myositis patients (21%) were positive for anti-p155, including 30 with juvenile dermatomyositis (29%), five with juvenile CTD-associated myositis (33%), eight with adult dermatomyositis (21%), two with adult CTD-associated myositis (15%), and, interestingly, six with cancer-associated dermatomyositis (75%). Since only one patient with systemic lupus erythematosus had this antibody in nonmyositis CTD, it was supposed to be a dermatomyositis-specific antibody. Clinically, patients with anti-p155 frequently revealed a V-sign rash and shawl sign but none of the patients developed ILD. The authors examined the immunogenetic association of anti-p155 and showed that DQA1*0301 was found to be a unique HLA risk factor in Caucasian patients.

In a preliminary report, Targoff *et al.* [15] identified the target antigen of anti-p155 antibody as the transcriptional intermediary factor 1-γ (TIF1-γ) [15]. It should be noted that the main immunological targets of polymyositis-specific autoantibodies are intracytoplasmic systems of protein translation, whereas dermatomyositis-specific autoantibodies target nuclear transcription factors, suggesting different pathogenic mechanisms or different production systems of MSAs between polymyositis and dermatomyositis.

Japanese investigators independently reported almost identical results. Kaji *et al.* [16••] screened 52 patients with myositis by immunoprecipitation using leukemic K562 cells, and detected an autoantibody that immunoprecipitated 155 and 140 kDa proteins in seven of 52 (13%) myositis patients. These anti-p155/p140-positive patients had typical dermatomyositis rash more frequently, but in contrast, none of these patients had ILD. Notably, internal malignancies were found at significantly higher frequency in positive patients than in negative patients (71% versus 11%). This autoantibody seems to be identical to anti-p155 reported by Targoff *et al.*, although Kaji *et al.* claim that they are different because the 155 kDa band is always associated with a 140 kDa band in their study. The final confirmation should be necessary by exchanging sera each other.

The problem is whether anti-CADM-140 and anti-p155 are different autoantibodies. In a preliminary report, a novel anti-MJ antibody, which was also found to recognize a 140 kDa protein, was identified mainly in juvenile dermatomyositis [17]. In the reports by Sato *et al.* [13] and Targoff *et al.* [14••], anti-MJ was confirmed to be different from anti-CADM-140 and anti-p155, respectively. Anti-CADM-140 shows cytoplasmic staining in immunofluorescence [13], in contrast to the nuclear staining by anti-p155 [14••]. Moreover, the clinical features of anti-p155 are quite different from those of anti-CADM-140. Therefore, anti-CADM-140 and anti-p155 appear to be different autoantibodies. In order to elucidate the racial difference in the frequency and distribution of these autoantibodies, a larger number of patients from different countries and populations need to be examined.

Recent development of other myositis-specific autoantibodies

New findings of classical myositis-specific autoantibodies are reviewed in this section.

Anti-aminoacyl-tRNA synthetases autoantibodies

Aminoacyl-tRNA synthetases (ARSs) are the enzymes that catalyze the binding of amino acids to their corresponding tRNAs in an energy-dependent manner. ARSs are the major targets of autoimmune response in polymyositis/dermatomyositis, and six different autoantibodies reacting with different ARSs have been recognized so far: anti-Jo-1 (histidyl) [18,19], anti-PL-7 (threonyl) [20,21], anti-PL-12 (alanyl) [22,23], anti-EJ (glycyl) [24], anti-OJ (isoleucyl) [25] and anti-KS (asparaginyl) [26]. With a few exceptions, each patient has only one of these autoantibodies, yet patients have disease with similar clinical manifestation, including myositis, ILD, polyarthritis, fever, Raynaud's phenomenon and mechanic's hand, called 'antisynthetase syndrome' [27].

In addition to the six classical anti-ARS autoantibodies, two classes of novel anti-ARS antibodies were reported. One is an autoantibody that recognizes phenylalanyl-tRNA synthetase [28•]. This autoantibody, termed anti-Zo, was found in a patient with ILD, proximal myopathy, Raynaud's phenomenon and arthralgia. The patient serum immunoprecipitated 60 and 70 kDa proteins as well as tRNAs corresponding to a tRNA synthetase. Using proteomic analysis of immunoprecipitation, immunoblotting and matrix assisted laser desorption/ionization-time of flight (MALDI-TOF), the target proteins were identified as phenylalanyl-tRNA synthetase α and β chains. Another class of anti-ARS antibodies was reported to recognize tyrosyl-tRNA synthetase [29]. This preliminary report showed that the autoantibody was detected in a patient with skin rash, muscle weakness, ILD and arthritis. The patient serum coimmunoprecipitated distinct tRNA bands and a 61–63 kDa protein. The identification of tyrosyl-tRNA synthetase for this protein was done by inhibition study of aminoacylation reaction and mass spectrometry. Although only one patient for each novel anti-ARS antibody has been identified so far, it is noteworthy that these two patients reveal typical clinical manifestations of antisynthetase syndrome.

More detailed clinical features of patients with anti-ARS antibodies have been described in several recent reports. Yoshifuji *et al.* [30•] reported the usefulness of anti-ARS antibodies in clinical course prediction of ILD with IIM patients. This retrospective study analyzed 74 patients with myositis in whom 41 had ILD. Any of the anti-ARS antibodies were detected in 28% of whole IIM patients and in 49% of IIM patients with ILD. Anti-ARS-positive patients had significantly higher frequency of ILD (95%) than negative patients (40%), and ILD of the most positive patients was diagnosed at the same time or before developing myositis. ILD of patients with anti-ARS showed a better response to initial corticosteroid therapy but revealed significant higher recurrence than those without anti-ARS. As a result, the 2-year prognosis of pulmonary function was not different between the two groups of each anti-ARS status. The detection of anti-ARS antibodies may be useful to predict late-onset myopathy in ILD-preceding patients and to predict the clinical course of ILD in myositis patients.

Although patients with anti-ARS antibodies develop a similar clinical manifestation known as antisynthetase syndrome, the detailed clinical analyses demonstrate that each anti-ARS antibody appears to correlate with distinct clinical characteristics of antisynthetase symptoms and signs. Sato *et al.* [31] described the clinical characteristics of seven Japanese patients with anti-PL-7 (threonyl-tRNA synthetase). All seven patients with anti-PL-7 had polymyositis/dermatomyositis or ILD. Five patients had polymyositis–scleroderma overlap syndrome, one was dermatomyositis and one was idiopathic interstitial pneumonitis. All patients had chronic ILD, six had arthritis and five sclerodactyly (diagnosed as polymyositis–scleroderma overlap). Another report [32] showed that anti-PL-7 was found in relatively higher frequency (17%) of Japanese polymyositis/dermatomyositis patients in contrast to previous studies, and was associated with lower muscle enzyme levels and milder muscle symptoms compared with anti-Jo-1 antibody. Hirakata *et al.* [33] reported clinical and immunogenetic features of anti-KS (asparaginyl-tRNA synthetase) antibody. In eight patients (five Japanese, one American, one German and one Korean) with anti-KS, only two had dermatomyositis, but seven of eight had ILD. Japanese anti-KS patients correlated with DR2 (DRB1*1501/1502). Thus, anti-KS appears to have a stronger association with ILD than with myositis. Clinical characteristics of patients with anti-OJ (isoleucyl-tRNA synthetase) antibody were also described [34]. In this report, seven patients with anti-OJ were examined and three were diagnosed as having polymyositis, three with ILD and one with polymyositis–rheumatoid arthritis overlap. All patients had ILD but myopathy was seen in only four, suggesting that anti-OJ may be more closely associated with ILD than myositis. All these results indicate that each anti-ARS antibody is associated with a unique clinical syndrome, while being characterized as an antisynthetase syndrome.

Anti-signal recognition particle antibody

Signal recognition particle (SRP) is a cytoplasmic small RNA–protein complex that consists of 7SL-RNA and six polypeptides of 72, 68, 54, 19, 14, and 9 kDa. The biological function of SRP is to recognize signal sequences in N-termini of secretary proteins or membrane proteins via binding to the 54 kDa subunit and to regulate the translocation of newly synthesized proteins across the endoplasmic reticulum membrane. Five to ten percent of polymyositis/dermatomyositis patients produce an autoantibody to SRP [35,36].

Patients with anti-SRP reveal severe myositis with relatively acute onset. These patients are usually resistant to standard treatment with corticosteroids and show frequent exacerbation [35]. In a study of 23 patients with anti-SRP antibody by Hengstman *et al.* [37••], the histopathological examination of muscle specimens showed that none of the patients had the typical histological features of myositis but most biopsy specimens showed necrotic muscle fibers and no inflammatory infiltrates. Miller *et al.* [38] reported a similar observation that muscle biopsy from anti-SRP-positive patients was associated with necrotizing myopathy characterized by muscle fiber necrosis, endomysial capillary change and no inflammation. These data indicate that the anti-SRP antibody may be a marker for a syndrome of necrotizing myopathy that is different from typical polymyositis. Although such patients are often resistant to corticosteroid therapy, rituximab, monoclonal anti-CD20, may be effective in anti-SRP-positive myositis patients refractory to conventional therapy [39•]. Anti-SRP antibody may define a new spectrum of IIM both clinically and histopathologically. Anti-SRP, however, sometimes appears to be detected in patients without myositis, such as scleroderma and ILD [40], although the frequency in nonmyositis patients is low.

A new autoantibody that recognizes 7SL-RNA, an RNA component of SRP, has been reported [41]. In screening of polymyositis/dermatomyositis patient sera containing anti-SRP, five sera from 10 Japanese patients and two sera from 22 North American patients with anti-SRP recognized a deproteinized 7SL-RNA. The presence of this antibody appears to be associated with ethnic background. Moreover, the seasonal onset of the disease was different for patients with anti-7SL-RNA (who developed the disease between October and January) from that of antibody-negative patients (between June and August). The production of this antibody may be associated with both genetic and environmental factors.

Anti-Mi-2 antibody

Anti-Mi-2 antibody is more common in dermatomyositis than in polymyositis and therefore appears to be a marker for dermatomyositis. Anti-Mi-2 antibody was detected in 8% of whole myositis patients and 15–20% of dermatomyositis patients, including juvenile dermatomyositis in an earlier report [42,43]. There are two proteins of the target Mi-2 antigen, known as Mi-2α (240 kDa) and Mi-2β (218 kDa) [44–46]. Both forms of Mi-2 are different molecules but are found to have a series of helicase motifs, suggesting that they may have a similar function as helicases in nucleus. Mi-2β forms a protein complex with histone deacetylases, termed nucleosome remodeling deacetylase (NuRD) complex [47], and may play a role in gene transcription by histone acetylation, resulting in nucleosome structure remodeling.

Many studies of anti-Mi-2 antibody describe that anti-Mi-2 is associated mainly with dermatomyositis (both adult and juvenile) as opposed to polymyositis, fewer complication of ILD and a relatively good prognosis. In contrast, Hengstman *et al.* [48•] recently reported a rather