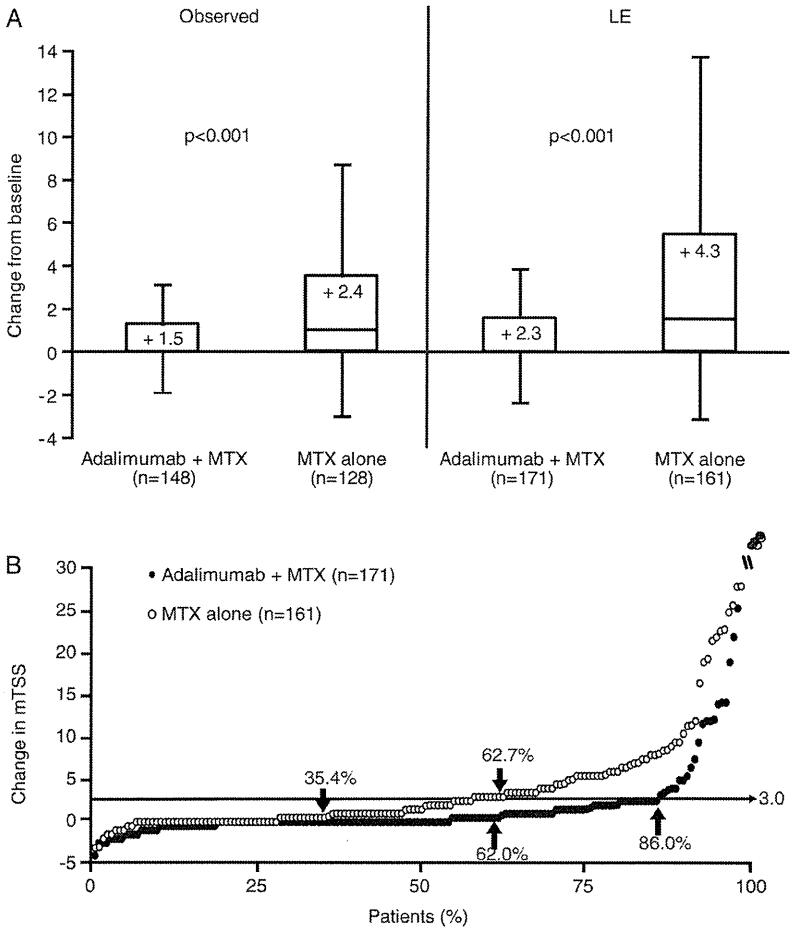

Figure 2 (A) Box plot of change from baseline in mTSS at week 26 with adalimumab+MTX versus MTX alone and (B) cumulative probability plot of mean change from baseline to week 26 in mTSS score (LE). Thickened horizontal lines in (A) indicate median values, the boxes mark the interval between the 25th and 75th percentiles, whiskers indicate the IQR and mean values are reported in the boxes. No radiographic progression (change from baseline in mTSS≤0.5) was reported in 62.0% (106/171) of adalimumab+MTX patients versus 35.4% (57/161) of MTX alone patients (p<0.001). No clinically relevant radiographic progression (change from baseline mTSS≤3) was reported in 86.0% (147/171) of adalimumab+MTX patients versus 62.7% (101/161) of MTX alone patients (p<0.001) (B). LE, linear extrapolation; mTSS, modified total Sharp score; MTX, methotrexate. p Value determined using Wilcoxon rank sum test.

Safety

The mean treatment duration during the double-blind phase was 168.7±36.6 days for adalimumab+MTX patients (mean cumulative adalimumab dose, 477.4±104.5 mg) and 162.8±38.6 days for MTX alone patients. Overall, there were 376 and 302 AEs reported in the adalimumab+MTX group and the MTX alone group, respectively. There were no significant differences in the percentage of patients with AEs in the adalimumab+MTX group (80.7% (138/171)) versus the MTX alone group (71.8% (117/163)), and the incidence of severe AEs was rare (table 2). No significant differences in the incidence of AEs of interest were observed between the two groups, with the exception of injection-site reactions, which were reported in 10.5% of adalimumab+MTX patients and 3.7% of MTX alone patients (p=0.02; table 2). Serious infections were observed in two adalimumab+MTX patients (one case each of pneumonia and infectious enteritis) and one MTX alone patient (*Pneumocystis jiroveci* pneumonia), occurring at rates of 2.5 and 1.4 events per 100 patient-years, respectively. There were no reports of demyelination, tuberculosis or malignancy during the study. One death, due to worsening of interstitial lung disease, occurred in the MTX alone group.

DISCUSSION

The HOPEFUL 1 study was designed to evaluate the efficacy and safety of adalimumab in combination with MTX in Japanese patients with early RA. This is the first description of a clinical trial of anti-TNF therapy+MTX versus MTX alone in MTX-naive Japanese patients with early RA and high disease activity. It is also the first randomised trial evaluating the efficacy of anti-TNF therapy+low-dose MTX versus low-dose MTX alone for the inhibition of radiographic progression in any patient population. This study extends observations from Western studies of adalimumab by demonstrating the superiority of adalimumab+MTX to MTX alone for the inhibition of radiographic progression and improvement in clinical outcomes in Japanese patients with early RA. Moreover, the combination of adalimumab+MTX significantly improved a wide array of clinical and functional disease activity measures and responses versus MTX alone, with improvements observed as early as the first assessment (week 2) and maintained through the 26-week double-blind trial.

Following 26 weeks of treatment, the mean ΔmTSS (primary endpoint) in adalimumab+MTX patients (1.48) in the current study was significantly smaller than observed in MTX alone patients (2.38). In addition, a similar trend in inhibition of radiographic progression in patients with early RA was observed in the OPTIMA study, with a smaller mean ΔmTSS in adalimumab+MTX patients (0.15) versus MTX alone patients (0.96; p<0.001).[12] The difference between the two treatment groups (0.8) at week 26 was similar to the difference observed in the current study (0.9 (observed)).[12] Furthermore, baseline characteristics, including RA duration, in the two studies were generally similar, but the OPTIMA study had a lower percentage of previous DMARD use.

A similar trend in inhibition of radiographic progression in the current study was observed in the PREMIER study, with a

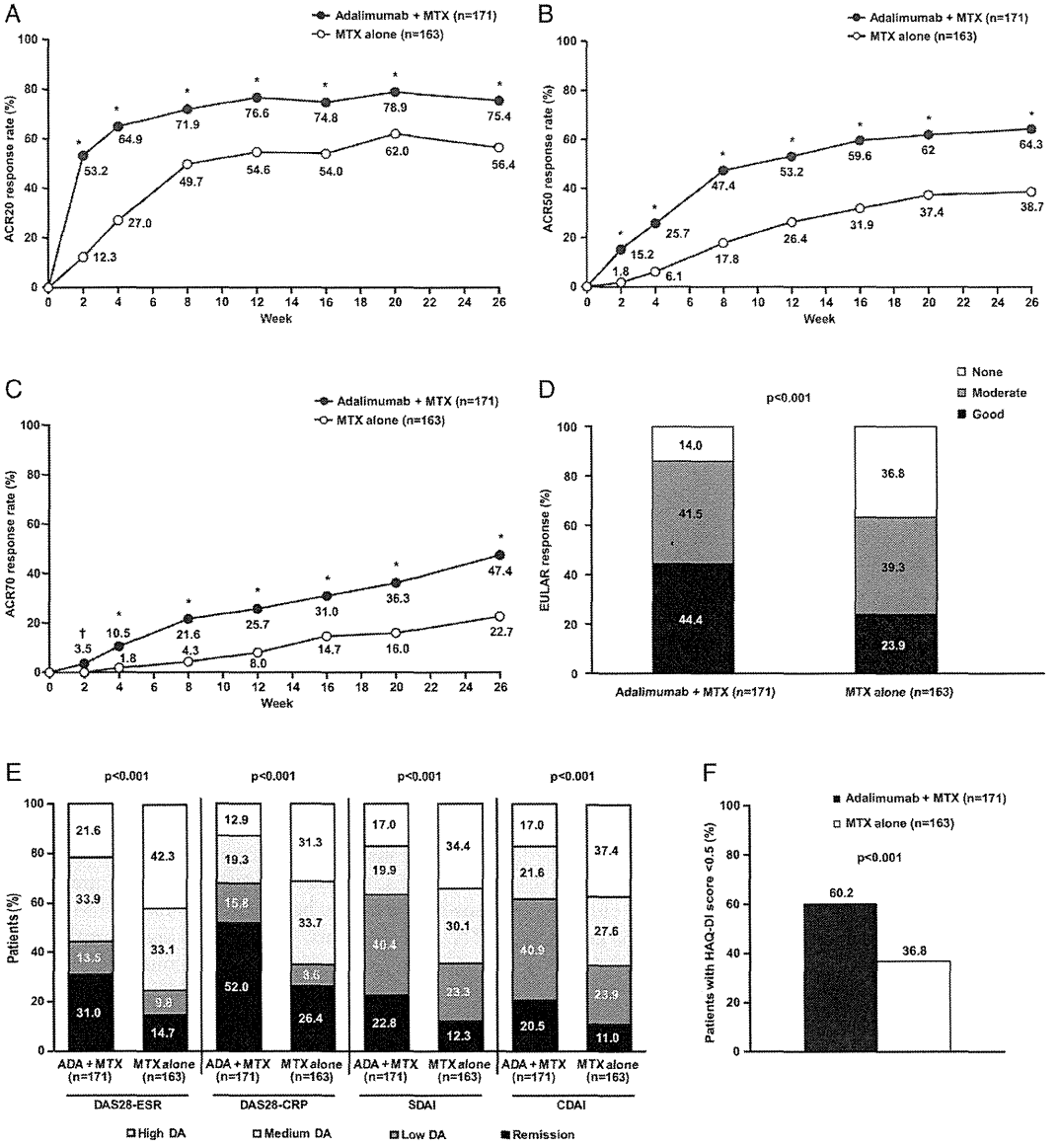

Figure 3 Percentage of patients with an (A) ACR20 response, (B) ACR50 response or (C) ACR70 response over time; (D) the percentage of patients with a EULAR response at week 26; (E) the percentage of patients with low, medium or high disease activity at week 26; and (F) the percentage of patients achieving functional remission (HAQ-DI score<0.5) at week 26. The following values were used to identify remission, low, medium and high disease activity for each clinical assessment in (E): DAS28-ESR or DAS-CRP (<2.6, ≥2.6–<3.2; ≥3.2–≤5.1, >5.1, respectively), SDAI (≤3.3, >3.3–≤11.0, >11.0–≤26.0, >26.0, respectively), and CDAI (≤2.8, >2.8–≤10.0, >10.0–≤22.0, >22.0, respectively). *p<0.001 versus MTX alone. †p=0.03 versus MTX alone. ACR, American College of Rheumatology; ADA, adalimumab; CDAI, clinical disease activity index; DA, disease activity; DAS28-CRP, disease activity score using a 28-joint count and C reactive protein level; DAS28-ESR, disease activity score using a 28-joint count and erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; HAQ-DI, Health Assessment Questionnaire disability index; MTX, methotrexate; SDAI, simplified disease activity index.

smaller mean ΔmTSS in adalimumab+MTX patients (0.8) versus MTX alone patients (3.5; p<0.001). However, the mean difference in radiographic progression between the two treatments groups, although statistically significant, was smaller in the current study (0.9 (observed); 2.0 (LE)) than in the PREMIER study (2.7).

In the current study, the SD for the mean ΔmTSS at week 26 was generally high. When the median ΔmTSS was compared using observed data, results were in good agreement between the PREMIER study (0.0 (adalimumab+MTX) vs 1.3 (MTX alone); data on file) and the current study (0.0 (adalimumab +MTX) vs 1.0 (MTX alone)). Alternatively, the smaller difference in improvement observed in the current study may also be related to the mTSS scoring method used, but this seems unlikely because only two joints assessed in PREMIER were omitted from scoring in the present analysis. The mean duration of RA was also shorter in the current study (0.3 years) versus the PREMIER study (0.7–0.8 years), although the percentage of patients who had previously taken DMARDs was higher (43.3–53.4% vs 31.5–32.5%). There were also slight differences in mean baseline tender and swollen joint counts and CRP levels, which were higher in the PREMIER study and considered

Table 2 Adverse events (AEs)

Parameter	Patients (n (%)) Adalimumab+MTX (n=171)	MTX (n=163)
Any AE	138 (80.7)	117 (71.8)
Severe AE	1 (0.6)	1 (0.6)
Serious AE	7 (4.1)	4 (2.4)
Infectious AE	59 (34.5)	48 (29.4)
Serious infection	2 (1.2)	1 (0.6)
AEs leading to study drug discontinuation	7 (4.1)	6 (3.7)
AEs of interest		
Elevated liver function test level	32 (18.7)†	21 (12.9)†
Injection-site reaction	18 (10.5)*	6 (3.7)
Haematological event	7 (4.1)	8 (4.9)
Allergic reaction	1 (0.6)	2 (1.2)
Interstitial lung disease	1 (0.6)	1 (0.6)
Lupus-like syndrome	0	1 (0.6)
Opportunistic infection	0	1 (0.6)

*p=0.02 versus MTX.
†≥94% of events were mild in severity.
MTX, methotrexate.

related to the longer duration of RA at baseline versus the current study. Furthermore, the MTX dose of 6–8 mg/week, although consistent with the dosage commonly administered in Japan at the time the study was conducted, was substantially lower than that commonly administered in Western countries (eg, 15–20 mg/week). In the PREMIER study, MTX was initiated at 7.5 mg/week, increased to 15 mg/week during weeks 4–8, and increased to 20 mg/week starting at week 9. In addition, the mean MTX dose during the 26 weeks of the current study was significantly lower in the adalimumab+MTX group (6.2±0.8 mg/week) versus the MTX alone group (6.6±0.6 mg/week; $p<0.001$), thereby potentially impacting the ΔmTSS and thus the maximal difference observed between the two treatment groups. Therefore, these multiple differences may have contributed to the small difference in radiographic outcomes between the current study and the PREMIER study. Whether the difference in radiographic outcomes can be explained by differences between Japanese and Western populations remains unclear, although this seems unlikely. Longer-term studies may help elucidate potential differences in outcomes.

Since this study was conducted, the maximum approved MTX dosage in Japan has been increased from 8 to 16 mg/week in patients with RA. Therefore, this study provides important information on the efficacy of low-dose MTX and anti-TNF therapy versus low-dose MTX alone for the inhibition of radiographic progression. Data suggest that patients with early RA who may not tolerate higher doses of MTX will likely benefit from adalimumab+low-dose MTX combination therapy.

Given the lower MTX dose prescribed, one could question whether we might only be seeing natural progression in the MTX only arm. It is ethically difficult to include a true placebo arm in clinical trials of ≥6 months duration for early active RA, particularly when MTX is recommended as first-line therapy to achieve clinical remission/low disease activity. Although an important question to ponder, a placebo arm in long-term clinical trials in early active RA appears to be unrealistic, and further research using highly sensitive and reproducible imaging techniques during a short-term placebo-treatment period in early active RA is warranted.

It is also important to note that the current patient population had severe baseline symptoms, including baseline erosions, despite only several months since RA onset. This scenario is becoming increasingly less common in Western populations due to treat-to-target recommendations and earlier intervention. In Japan, general practitioners are still seeing many early RA patients and referrals to rheumatologists are often delayed. In addition, the diagnosis of RA in this trial was based upon 1987 classification criteria. Thus, these factors may have played a role in the conundrum of more severe baseline clinical symptoms yet shorter mean disease duration.

The clinical results of the current study are supported by the HARMONY study, which retrospectively determined the effectiveness and safety of adalimumab 40 mg every other week with or without MTX (mean dose, 8.5 mg/week) in Japanese patients with RA (mean RA duration, 9.0±9.5 years) with or without prior biologic treatment.[15] Although patients in the HARMONY study had more established disease and the study design was retrospective, adalimumab+MTX patients (n=143) had an improvement from baseline in DAS28-ESR score at week 24 (baseline, 5.3; week 24, 3.3), which was within the range but slightly smaller than the improvement observed in the current study at week 26 (baseline, 6.6; week 26, 3.7; see online supplementary figure 1A). Clinical remission rates for adalimumab+MTX patients were also comparable between the HARMONY study (week 24, 35.0%) and the current study (week 26, 31.0%).

The safety profile of the current study was generally consistent with those in previous clinical studies of adalimumab in patients with RA conducted in Japan.[14–16] There were no reports of demyelination, tuberculosis or malignancy, and there were no statistically significant differences in the incidence of serious AEs, serious infections, opportunistic infections or lupus-like reactions between adalimumab+MTX patients versus MTX alone patients. There was a significantly higher incidence of injection-site reactions for adalimumab+MTX patients versus MTX alone patients, but the incidence (10.5%) was similar to that reported for the 167 adalimumab±MTX patients in the HARMONY study (12.0%). The incidence of injection-site reactions in both of these studies was lower than the 30.8% reported for the 91 adalimumab monotherapy patients (40 mg every other week) in the CHANGE study,[14] possibly related to the immunosuppressive effects of concomitant MTX in the current study and in some of the patients in the HARMONY study.

In the multivariate regression analyses (see online supplementary table 1), lower baseline CRP level was identified as a predictor of radiographic non-progression in adalimumab+MTX patients, whereas normal baseline CRP level (≤0.3 mg/dl) appeared to have an increased likelihood of radiographic non-progression. However, no baseline predictors appeared to predict both the lack of progression and clinical remission. Furthermore, baseline mTSS was not an independent predictor for either treatment group in this study.

Overall, adalimumab+MTX was well tolerated in Japanese patients with early RA with no new safety signals and with a safety and tolerability profile similar to that observed in Western populations. Administration of adalimumab in combination with MTX was efficacious in improving radiographic and clinical responses in MTX-naive patients with early RA, high disease activity and poor prognostic factors (eg, rheumatoid factor positive or with baseline erosive damage) through week 26. Given its radiographic, clinical and functional superiority versus MTX monotherapy, consideration should be given to administration

of anti-TNF-α and MTX combination therapy in patients with early RA and high disease activity.

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Contributors All the authors evaluated the study results, interpreted the data and suggested additional analyses. All authors contributed to the development and critical review of manuscript and approved the final version.

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Patient consent Obtained.

Ethics approval An institutional review board approved the study at each site.

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Adalimumab, a human anti-TNF monoclonal antibody, outcome study for the prevention of joint damage in Japanese patients with early rheumatoid arthritis: the HOPEFUL 1 study

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ORIGINAL ARTICLE

Validation of different sets of criteria for the diagnosis of Sjögren's syndrome in Japanese patients

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Abstract
Objective To validate the revised Japanese Ministry of Health criteria for the diagnosis of Sjögren's syndrome (SS) (JPN) (1999), The American-European Consensus Group classification criteria for SS (AECG) (2002), and American College of Rheumatology classification criteria for SS (ACR) (2012).
Methods The study subjects were 694 patients with SS or suspected SS who were followed-up in June 2012 at ten hospitals that form part of the Research Team for Autoimmune Diseases, The Research Program for Intractable Disease by the Ministry of Health, Labor and Welfare (MHLW). All patients had been checked for all four criteria of the JPN (pathology, oral, ocular, anti-SS-A/SS-B antibodies). We studied the clinical diagnosis made by the physician in charge and the satisfaction of the above criteria.
Results Of the 694 patients, 499 patients did not have other connective tissue diseases (CTDs). SS was diagnosed in 476 patients (primary SS in 302, secondary SS in 174), whereas non-SS was diagnosed in 218 patients (without other CTDs in 197, with other CTDs in 21) by the physician in charge. The sensitivities of JPN, AECG, and ACR in the diagnosis of all forms of SS (both primary and secondary SS) were 79.6, 78.6, and 77.5 %, respectively, with respective specificities of 90.4, 90.4, and 83.5 %. The sensitivities of the same systems in the diagnosis of primary SS were 82.1, 83.1, and 79.1 %, respectively, with specificities of 90.9, 90.9, and 84.8 %, respectively. The sensitivities of the same systems in the diagnosis of secondary SS were 75.3, 70.7, and 74.7 %, respectively, with specificities of 85.7, 85.7, and 71.4 %, respectively.
Conclusion The sensitivity of JPN to all forms of SS and secondary SS, the sensitivity of AECG to primary SS, and the specificities of JPN and AECG for all forms of SS, primary SS, and secondary SS were highest in the diagnosis of SS in Japanese patients. These results indicate that

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the JPN criteria for the diagnosis of SS in Japanese patients are superior to ACR and AECG.

Keywords Sjögren's syndrome · Criteria

Introduction

Sjögren's syndrome (SS) is an autoimmune disease that affects exocrine glands, including the salivary and lacrimal glands. It is characterized by lymphocytic infiltration into the exocrine glands, leading to dry mouth and eyes. A number of autoantibodies, such as anti-SS-A and SS-B antibodies, are detected in patients with SS. SS is subcategorized into primary SS, which is not associated with other well-defined connective tissue diseases (CTDs), and secondary SS, which is associated with other well-defined CTDs [1]. Primary SS is further subcategorized into the glandular form and the extraglandular form.

The revised criteria for the diagnosis of SS issued by the Japanese Ministry of Health (JPN) (1999) (Table 1) [2], as well as the American-European Consensus Group classification criteria for SS (AECG) (2002) (Tables 2, 3) [1], are usually used in both daily clinical practice and clinical studies in Japan. Thus, two sets of diagnostic systems are being applied for the same disease. This could result in a heterogeneous pool of SS patients. This heterogeneity of SS patients makes it difficult to analyze the diagnosis, efficacy of treatment, and prognosis of SS patients. A better alternative would be to use a unified set of criteria for the diagnosis of SS in Japan. Recently, The American College of Rheumatology (ACR) published the ACR classification criteria for SS (2012) (Table 4), which were proposed by the Sjögren's International Collaborative Clinical Alliance (SICCA) [3]. The new set of criteria is designed to be used worldwide, not only in advanced countries but also in developing countries. The SICCA established a uniform classification for SS based on a combination of objective tests that have known specificity to SS [3].

Table 1 The revised Japanese Ministry of Health criteria for the diagnosis of SS (1999)

1. Histopathology
Definition: Positive for at least one of (A) or (B)
(A) Focus score ≥1 (periductal lymphoid cell infiltration ≥50) in a 4 mm^2 minor salivary gland biopsy
(B) Focus score ≥1 (periductal lymphoid cell infiltration ≥50) in a 4 mm^2 lacrimal gland biopsy
2. Oral examination
Definition: Positive for at least one of (A) or (B)
(A) Abnormal findings in sialography ≥stage 1 (diffuse punctate shadows of <1 mm)
(B) Decreased salivary secretion (flow rate ≤10 ml/10 min according to the chewing gum test or ≤2 g/2 min according to the Saxon test) and decreased salivary function according to salivary gland scintigraphy
3. Ocular examination
Definition: Positive for at least one of (A) or (B)
(A) Schirmer's test ≤5 mm/5 min and rose bengal test ≥3 according to the van Bijsterveld score
(B) Schirmer's test ≤5 mm/5 min and positive fluorescein staining test
4. Serological examination
Definition: Positive for at least one of (A) or (B)
(A) Anti-Ro/SS-A antibody
(B) Anti-La/SS-B antibody
Diagnostic criteria: diagnosis of SS can be made when the patient meets at least two of the above four criteria

Upon comparing these three classification sets, there are some differences among them in their purpose and the items adopted in the set (Table 5). The JPN criteria (1999) are intended as an aid for diagnosis, whereas the AECG criteria (2002) and the ACR criteria (2012) are intended for classification purposes in clinical studies and trials. Although the ACR criteria include only three objective items (Tables 4, 5) and are the simplest among the three sets, the ACR criteria may not identify SS patients with negative findings in labial salivary gland biopsy, because the ACR criteria do not include salivary secretion analysis and imaging studies. On the other hand, the JPN criteria combined oral examinations such as salivary secretion, sialography, and salivary gland scintigraphy with three objective items adopted in the ACR criteria (Table 5). Only the AECG criteria include ocular and oral symptoms, which may cause false positives in patients with non-SS conditions such as aging or visual display terminals (VDT) syndrome (Table 5).

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Table 2 The American-European Consensus Group classification criteria for SS (2002)

I. Ocular symptoms: a positive response to at least one of the following questions
1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than 3 times a day?
II. Oral symptoms: a positive response to at least one of the following questions
1. Have you had a daily feeling of dry mouth for than 3 months?
2. Have you had recurrently or persistently swollen salivary glands as an adult?
3. Do you frequently drink liquids to aid in swallowing dry food?
III. Ocular signs—that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests
1. Schirmer's test, performed without anaesthesia (≤5 mm in 5 min)
2. Rose bengal score or other ocular dry eye score (≥4 according to van Bijsterveld's scoring system)
IV. Histopathology: in minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score ≥1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acuni and contain more than 50 lymphocytes) per 4 mm^2 of glandular tissue
V. Salivary gland involvement: objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests
1. Unstimulated whole salivary flow (≤1.5 ml in 15 min)
2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts
3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer
VI. Autoantibodies: presence in the serum of the following autoantibodies
1. Antibodies to Ro (SS-A) or La (SS-B) antigens, or both

Table 3 The American-European Consensus Group classification criteria for SS (2002) rules for classification

For primary SS
In patients without any potentially associated disease, primary SS may be defined as follows:
(A) The presence of any 4 of the 6 items is indicative of primary SS, as long as either item IV (histopathology) or VI (serology) is positive
(B) The presence of any 3 of the 4 objective criteria items (that is, items III, IV, V, VI)
For secondary SS
In patients with a potentially associated disease (for instance, another well-defined connective tissue disease), the presence of item I or item II plus any 2 from among items III, IV, and V may be considered as indicative of secondary SS
Exclusion criteria:
Past head and neck radiation treatment
Hepatitis C infection
Acquired immunodeficiency disease (AIDS)
Pre-existing lymphoma
Sarcoidosis
Graft vs. host disease
Use of anticholinergic drugs (for a time shorter than 4-fold the half life of the drug)

The purpose of the present study was to validate the JPN criteria, AECG criteria, and ACR criteria for the diagnosis of SS in Japanese patients. The study identified the differences among these three classification sets.

Patients and methods

Study population

The study subjects were 694 patients (51 males and 643 females) with a diagnosis of SS or suspected SS who had been checked for all four criteria of the JPN (pathology, oral, ocular, anti-SS-A/SS-B antibody), and were followed up in June 2012 at ten hospitals across Japan (Kanazawa Medical University Hospital, Nagasaki University Hospital, Hyogo Medical University Hospital, Keio University Hospital, Tokyo Women's Medical University Hospital, Tsurumi University Hospital, Kyushu University Hospital, University of Occupational and Environmental Health Hospital, Kyoto University Hospital, and University of Tsukuba Hospital) that form part of the Research Team for Autoimmune Diseases, The Research Program for Intractable Disease of the Ministry of Health, Labor and Welfare (MHLW).

Data collection and analysis

We collected clinical data from the above ten hospitals using a questionnaire. We retrospectively examined the clinical diagnosis made by the physician in charge, as well as the satisfaction of the JPN, AECG, and ACR criteria. Because lissamine green ocular staining had not been adopted in Japan at the time of clinical examination, we regarded patients who had a positive rose bengal test or fluorescein staining test as having satisfied the ocular staining score in the ACR classification system.

We regarded the clinical diagnosis made by the physician in charge as the gold standard for the diagnosis of SS in this study. We compared the sensitivities and specificities of the JPN, AECG, and ACR diagnostic systems in the diagnosis of SS (both primary and secondary SS), primary

Table 4 The American College of Rheumatology classification criteria for SS (2012)

The classification of SS, which applies to individuals with signs/symptoms that may be suggestive of SS, will be met in patients who have at least 2 of the following 3 objective features:
1. Positive serum anti-SS-A/Ro and/or anti-SS-B/La or (positive rheumatoid factor and ANA titer ≥1:320)
2. Labial salivary gland biopsy exhibiting focal lymphocytic sialadenitis with a focus score ≥1 focus/4 mm^2
3. Keratoconjunctivitis sicca with ocular staining score ≥3 (assuming that individual is not currently using daily eye drops for glaucoma and has not had corneal surgery or cosmetic eyelid surgery in the last 5 years)
Prior diagnosis of any of the following conditions would exclude participation in SS studies or therapeutic trials because of overlapping clinical features or interference with criteria tests:
History of head and neck radiation treatment
Hepatitis C infection
Acquired immunodeficiency syndrome
Sarcoidosis
Amyloidosis
Graft vs. host disease
IgG4-related disease

SS, and secondary SS. Agreement between the three was assessed via the kappa coefficient.

Results

Diagnosis of SS (primary and secondary SS) and non-SS

Of the 694 patients, 499 patients did not have other well-defined CTDs, whereas 195 patients did. SS was diagnosed in 476 patients (302 primary SS, 174 secondary SS), whereas non-SS was diagnosed in 218 patients (197 without other CTDs, 21 with other CTDs) by the physician in charge (Table 6).

Sensitivities and specificities of the three diagnostic systems for SS

The sensitivities of JPN, AECG, and ACR in the diagnosis of all SS (302 primary SS and 174 secondary SS) were 79.6, 78.6, and 77.5 %, respectively, whereas the respective specificities in the diagnosis of all SS were 90.4, 90.4, and 83.5 %. The sensitivities of JPN, AECG, and ACR in the diagnosis of 302 primary SS were 82.1, 83.1, and 79.1 %, respectively, with specificities of 90.9, 90.9, and 84.8 %, respectively. The sensitivities of JPN, AECG, and ACR in the diagnosis of 174 secondary SS were 75.3, 70.7, and 74.7 %, respectively, with specificities of 85.7, 85.7, and 71.4 % (Table 7).

Table 5 Comparison of the items adopted in the JPN and AECG and ACR criteria

	JPN	AECG	ACR
Ocular symptoms	×	○	×
Oral symptoms	×	○	×
Ocular signs			
Schirmer's test	○	○	×
Ocular staining	○	○	○
Labial salivary gland biopsy	○	○	○
Salivary gland involvements			
Salivary secretion	○	○	×
Sialography	○	○	×
Scintigraphy	○	○	×
Autoantibodies			
SS-A	○	○	○
SS-B	○	○	○
ANA	×	×	○
RF	×	×	○

SS-A anti-SS-A antibody, *SS-B* anti-SS-B antibody, *ANA* anti-nuclear antibody, *RF* rheumatoid factor, ○ adopted, × not adopted, *JPN* the revised Japanese Ministry of Health criteria for the diagnosis of Sjögren's syndrome (1999), *AECG* The American-European Consensus Group classification criteria for Sjögren's syndrome (2002), *ACR* American College of Rheumatology classification criteria for Sjögren's syndrome (2012)

Table 6 Diagnosis of SS and non-SS

	Associated with other CTDs		Total
	No	Yes	
Clinical diagnosis			
SS	302 (primary SS)	174 (secondary SS)	476
Non-SS	197	21	218
Total	499	195	694

Clinical diagnosis diagnosis of SS by the physician in charge
CTDs connective tissue diseases

Comparisons of the satisfaction of the three diagnostic systems

Figure 1 displays Venn diagrams showing comparisons of the satisfaction of the three diagnostic systems. Among all SS patients ($n = 476$), more patients satisfied only the AECG criteria ($n = 42$) rather than only the JPN criteria ($n = 8$) or the ACR criteria ($n = 6$). The same tendency was also observed in patients with primary SS only and in those with secondary SS only. The diagrams indicate that the JPN and ACR diagnostic systems are similar, whereas the AECG diagnostic system is different from the other two. Table 8 shows the agreement among the three

Table 7 Sensitivities and specificities of the three tested systems for diagnosing SS

	Entire group		Without other CTDs		With other CTDs	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
JPN	79.6	90.4	82.1	90.9	75.3	85.7
AECG	78.6	90.4	83.1	90.9	70.7	85.7
ACR	77.5	83.5	79.1	84.8	74.7	71.4

The "entire group" comprised 694 patients, including 476 with SS (302 patients with primary SS and 174 with secondary SS) and 218 patients with non-SS. The "without other CTDs" group of 499 patients included 302 patients with primary SS and 197 with non-SS. The "with other CTDs" group of 195 patients included 174 patients with secondary SS and 21 with non-SS

JPN Japanese Ministry of Health criteria for the diagnosis of Sjögren's syndrome (1999), *AECG* The American-European Consensus Group classification criteria for Sjögren's syndrome (2002), *ACR* The American College of Rheumatology classification criteria for Sjögren's syndrome (2012)

diagnostic systems, as assessed using the kappa coefficient. The data indicate a high level of agreement between the JPN and ACR diagnostic systems (kappa coefficient 0.74), but a low level of agreement between AECG and the other two (kappa coefficient 0.10–0.46) in the diagnosis of all SS, primary SS, and secondary SS.

Discussion

While it is difficult to select the best gold standard system for the diagnosis of CTDs such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and SS, this issue is clinically relevant and important. In SLE, the ACR revised criteria for the classification of SLE (1997) [4] has been adopted for diagnosis in daily clinical practice and for classification purposes in clinical studies. Recently, the Systemic Lupus International Collaborating Clinics (SLICC) has proposed new classification criteria for SLE [5], which has generated interesting discussion about these two criteria among expert rheumatologists. On the other hand, for RA, the 2010 RA classification criteria: an ACR/European League Against Rheumatism (EULAR) collaborative initiative [6] was published recently and is currently used not only in clinical studies for the classification of RA but also in daily clinical practice for the diagnosis of RA. Therefore, these available diagnostic systems for SLE and RA could be regarded as the gold standard for both clinical studies and daily clinical practice. The AECG criteria have been adopted in Western countries for the diagnosis of SS. In Japan, however, both the AECG and JPN criteria are currently being used simultaneously for the classification and diagnosis of SS. On the other hand, the new ACR criteria have been proposed as a uniform classification for SS. At present, there is no gold standard system for the diagnosis of SS in both clinical studies and daily clinical practice, except for expert judgment. This state could create a heterogeneous pool of SS patients, which makes it difficult to analyze the diagnosis, efficacy of treatment, and prognosis of SS patients. Establishing a single set of criteria for SS and selecting a gold standard system for the diagnosis of SS is an important task in Japan.

The present study demonstrated that the sensitivity of the JPN system for all SS and secondary SS, the sensitivity of the AECG system for primary SS, and the specificities of the JPN and AECG systems for all SS, primary SS, and secondary SS were highest among the three systems for diagnosing SS in Japanese patients (relative to clinical judgment as the gold standard). The results also showed high agreement between the JPN and ACR systems, but low agreement between AECG and the other two diagnostic systems for all SS, primary SS, and secondary SS. These results indicate that the JPN and ACR criteria covered similar patient populations, although the sensitivity and specificity were higher for the JPN system than the ACR system. Among the 302 patients with primary SS, 14 did not satisfy the ACR criteria for the diagnosis of SS, although they did meet the criteria of both JPN and AECG. Further analysis of these 14 SS patients also showed that 50 % of these patients had negative pathological findings, 70 % had negative ocular staining, and 50 % were negative for autoantibodies (data not shown). These SS patients could be misdiagnosed by the ACR criteria, resulting in the lower sensitivity of the ACR diagnostic system. On the other hand, among 197 non-SS patients without other CTDs, ten patients satisfied the ACR criteria but not the JPN nor the AECG criteria (data not shown). Further analysis of these ten patients indicated that 80 % were positive for lissamine green ocular staining (Schirmer's test, rose bengal staining, and fluorescein staining were not performed), and 60 % were positive for anti-SS-A antibody (data not shown). Although these patients might be misdiagnosed as primary SS by the ACR criteria, this could not be confirmed because these patients could be positive for other ocular tests adopted by the JPN and AECG diagnostic systems.

The specificities of the criteria for all SS, primary SS, and secondary SS patients used in the JPN and AECG

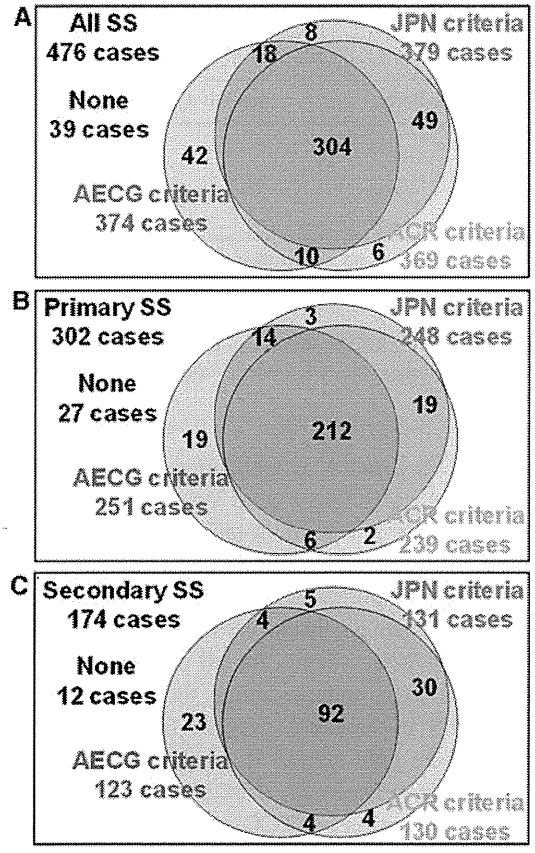

Fig. 1 Venn diagrams showing a comparison of the satisfaction of the three tested systems. **a** Comparison of the satisfaction of the three tested systems, performed using data from all 476 SS patients (302 primary SS and 174 secondary SS). **b** Comparison of the satisfaction of the three tested systems using data on 302 patients with primary SS. **c** Comparison of the satisfaction of the three tested systems using data on 174 patients with secondary SS. *Numbers* show the numbers of patients who satisfied each set of criteria, *None* indicates the number of patients who did not satisfy the criteria of any of the three systems. *JPN criteria* the revised Japanese Ministry of Health criteria for the diagnosis of SS (1999), *AECG criteria* The American-European Consensus Group classification criteria for SS (2002), *ACR criteria* American College of Rheumatology classification criteria for SS (2012)

systems were the same in this study. The reason for the same specificities of the JPN and AECG criteria may be the identical number of non-SS patients (21 patients, including 18 patients without CTDs and 3 patients with CTDs) who satisfied JPN and AECG. However, the JPN and AECG profiles for 20 out of these 21 non-SS patients were completely different, highlighting the low agreement between JPN and AECG, as shown in Table 8.

The sensitivity of AECG for primary SS was highest among the three systems, whereas that of JPN for all SS and secondary SS was highest. Among the 302 primary SS patients, 19 patients only satisfied the AECG criteria. These 19 primary SS patients had high frequencies of dry eye (84.2 %) and dry mouth (100.0 %) but low frequencies of anti-SS-A antibody (10.5 %) and anti-SS-B antibody (0 %). These seronegative primary SS patients with symptoms of dryness could only be diagnosed by the AECG criteria, because only the AECG criteria include symptoms of dryness. This may be the sensitivity of AECG for primary SS was highest among the three systems.

The above findings suggest that JPN provided the best set of criteria necessary for the diagnosis of Japanese patients with SS. Admittedly, however, the results of the present study do not allow us to confirm the superiority of JPN due to the inherent limitations of the study. First, we used the clinical judgment of the physician in charge as the gold standard. In Japan, because the JPN criteria are the criteria used most commonly in daily clinical practice, the clinical judgment could depend on the satisfaction of the JPN criteria. It is better to rely on expert committee consensus based on clinical case scenarios as the gold standard for diagnosis in order to avoid this bias. Second, patients who had been checked for all four criteria of the JPN diagnostic system (pathology, oral, ocular, anti-SS-A/SS-B antibodies) were included in this study, but the methods used for ocular staining varied among the participating institutions. Third, the results of the study could include selection bias. For these reasons, we need a more

Table 8 Agreement among the three tested systems, as assessed using the kappa coefficient

All SS (n = 476)	All SS (n = 476) (primary SS, n = 302, secondary SS, n = 174)	Primary SS (n = 302)	Secondary SS (n = 174)
JPN vs. AECG	0.31	0.46	0.10
JPN vs. ACR	0.74	0.74	0.74
AECG vs. ACR	0.30	0.42	0.12

The "entire group" comprised 694 patients, including 476 with SS (302 patients with primary SS and 174 with secondary SS) and 218 patients with non-SS. The "without other CTDs" group of 499 patients included 302 patients with primary SS and 197 with non-SS. The "with other CTDs" group of 195 patients included 174 patients with secondary SS and 21 with non-SS.

JPN Japanese Ministry of Health criteria for the diagnosis of Sjögren's syndrome (1999), *AECG* The American-European Consensus Group classification criteria for Sjögren's syndrome (2002), *ACR* The American College of Rheumatology classification criteria for Sjögren's syndrome (2012)

sophisticated validation study using randomly selected clinical case scenarios from various institutions and expert committee consensus diagnosis as the golden standard to test the three diagnostic systems for SS, to unify the criteria used for the diagnosis of SS, and ultimately to select the gold standard set of criteria for the diagnosis of SS in Japan.

Currently, the JPN diagnostic system is only used in Japan, because ACR and EULAR have never validated the JPN system. Therefore, we strongly hope that an ACR/EULAR collaborative initiative will validate JPN as well as the AECG and ACR systems.

In conclusion, although this study has a few limitations, the results obtained from it indicate the superiority of the JPN criteria, as it has higher sensitivity and specificity values for the diagnosis of SS in Japanese patients with SS than those of ACR and AECG.

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Conflict of interest None.

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Review

CD247 variants and single-nucleotide polymorphisms observed in systemic lupus erythematosus patients

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Abstract

SLE is associated with a deficiency in cluster of differentiation 247 (CD247, also known as CD3 zeta chain), a component of the T-cell receptor (TCR)–CD3 complex. A comprehensive analysis showed that in more than half of SLE patients tested CD247 expression was either attenuated or absent. Recent evidence suggests that these variations in expression profiles may be due, at least in part, to polymorphisms in the *CD247* gene. Aberrant *CD247* transcript variants displaying either spliced exon 7 or short 3′-untranslated region have been detected in SLE T cells, and a recent genome-wide association study reported the existence of new *CD247* single-nucleotide polymorphisms in SLE patients. Here, we review these unique and significant features of defective CD247 observed in SLE.

Key words: systemic lupus erythematosus, T-cell receptor, signal transduction, CD247, splice variants.

Introduction

SLE is a prototype autoimmune disease characterized by an abundant production of autoantibodies and the subsequent formation of immune complexes that lead to tissue damage and clinical phenotypes such as butterfly rash and GN [1–3]. The factors of this pathogenic process are thought to be multiple and complex. For example, plasmacytoid dendritic cells activated by the innate immune system produce high levels of type I IFNs (IFN-α and IFN-β) in SLE patients. Type I IFNs affect myeloid dendritic cells and produce a number of other pro-inflammatory cytokines, resulting in the activation of immune cells such as T cells [2, 4].

T cells play a central role in both acquired immune system and immune tolerance and have been shown to be involved in various abnormalities and dysfunctions in SLE patients [4]. Functional activation of T cells is dependent on their surface expression of unique T-cell antigen receptor–cluster of differentiation 3 (TCR–CD3) complexes. TCR–CD3 complexes consist of the alpha and beta chains of TCR, associated with two epsilon, one gamma and one delta chains of CD3 and with a zeta chain [also known as CD3-zeta, TCR zeta chain or cluster of differentiation 247 (CD247)]. Here, we focus on CD247 abnormalities in SLE patients, with particular attention to gene variants and single-nucleotide polymorphisms (SNPs), and discuss how these abnormalities develop into SLE from an immunopathological perspective.

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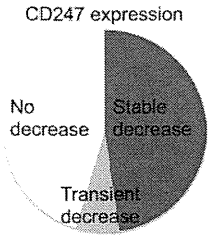
Defective CD247 expression in SLE T cells

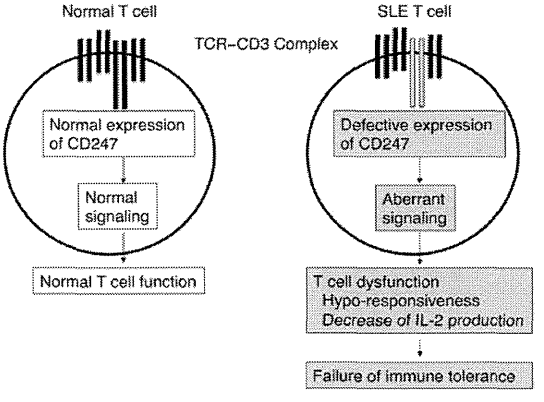
CD247 plays an important role in coupling antigen recognition to several intracellular signal transduction pathways. Our early immunoblotting analysis showed that 54.5% of SLE patients (24 out of 44) had lower (>2 S.D.) levels of CD247 protein than did healthy controls. CD247 expression, which seems to be disease-specific in the disease controls (including RA, SSc and primary SS), was not decreased. Among 44 SLE patients, CD247 expression decreased stably in 21 cases and transiently in the remaining three, suggesting the existence of several mechanisms leading to CD247 defect (Fig. 1). The relationship of CD247 expression and SLEDAI with the amount of corticosteroid administered was not significant. Furthermore, direct comparison between active and inactive phases in SLE patients showed no change in CD247 expression [5]. A decrease in TCR-initiated tyrosine phosphorylation was observed in peripheral blood T cells of SLE patients. CD247 protein expression in T-cell subpopulations, including $CD4^+$, $CD8^+$, $CD45RA^+$ (naïve phenotype) and $CD45RO^+$ (memory phenotype), was decreased. The mean CD247 fluorescence intensity in all subpopulations demonstrated a remarkably similar decrease. These results confirm the defective expression and altered tyrosine phosphorylation of CD247 in a large

Fig. 1 Defects of CD247 expression in SLE patients.



Percentage of decrease in CD247 protein less than mean ± 2 S.D. of healthy controls is shown. In 21 patients, it was stably decreased and in three patients it was transiently decreased. In total, defective CD247 expression in 54.5% of SLE patients were observed [4]. Adapted from Autoimmunity 2005;38:339–46.

Fig. 2 Defective expression of CD247 in SLE T cells.



In normal T cells, signals through the TCR–CD3 complex are transduced into internal cascades, resulting in normal T-cell function while defective expression of CD247 is observed in SLE T cells. Consequently, aberrant signalling causes T-cell dysfunction such as hyporesponsiveness and a decrease in IL-2 production, which leads to immune tolerance failure.

proportion of SLE patients, suggesting that defective expression may play an important role in SLE T-cell dysfunction [5].

In normal T cells, the TCR–CD3 complex induces intracellular signalling cascades that lead to normal T-cell function (Fig. 2), while in SLE patients diminished CD247 protein expression [6, 7] undermines the TCR–CD3 complex signalling, leading to T-cell dysfunction such as hyporesponsiveness and decreased IL-2 production, resulting in an overall immune tolerance failure.

The mechanisms responsible for this decrease in CD247 expression include low transcription activity [7], splice variant generation [6, 8, 9], increased ubiquitination [10], increased caspase-3-dependent proteolysis [10], heat stress [11], chronic pro-inflammatory cytokines exposure [12] and direct contact with activated macrophages [13]. An early CD247 northern blot analysis in T cells showed that *CD247* mRNA was undetected in three, decreased in three and normal in two out of eight SLE patients tested [6].

CD247 splice variants in SLE T cells

RNA splicing is the process by which pre-mRNA is converted into mature mRNA by removal of introns and joining of exons. Variations in splicing of the same pre-mRNA can result in the generation of splice variants that display different exon combinations.

Human *CD247* is located in chromosome 1 (1q22–q23) and consists of eight exons (Fig. 3). The existence of abnormal *CD247* transcripts was previously reported, including splice variants lacking exon 7 and variants with a short 3′-untranslated region (UTR) [5, 7, 14, 15], both of which were exclusively observed in SLE patients [16]. Other variants such as eta (exons 1–7 plus exon 9, see Fig. 3) and iota (exons 1–7 plus exon 10, not shown) are generated by alternative splicing of *CD247*.

The role of unique splice variants in defective CD247 expression

In vitro analysis of *CD247* in SLE T cells showed that mRNA instability was responsible for the lower protein expression of both the short 3′-UTR and the exon 7(–) variants. Furthermore, a T-cell transfectant model with these variants showed similar functional defects to those seen in SLE T cells [8, 15, 17–19].

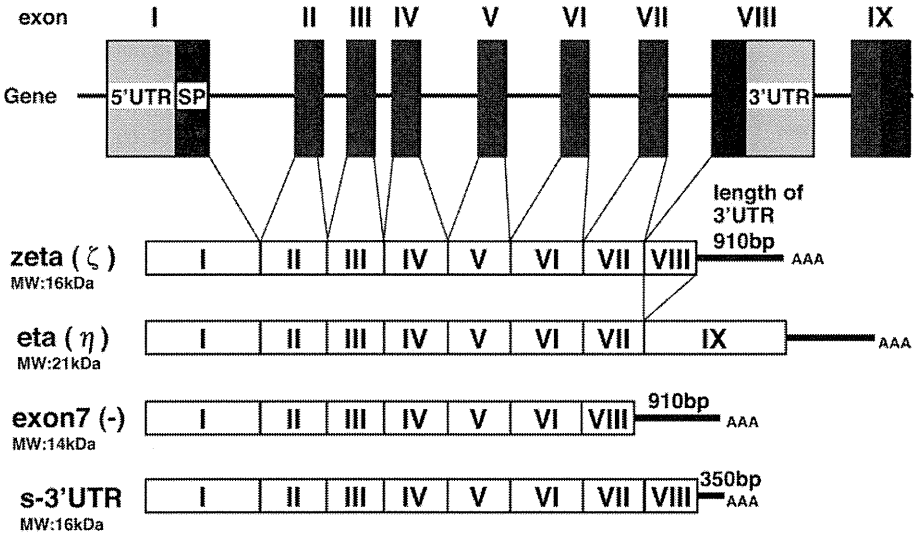
Mice bearing reduced immunoreceptor tyrosine-based activation motif (ITAM) domains in CD247 similar to those of mutated CD247 produced a substantial amount of cytokines including IFN-γ [20], which suggests that CD247 defects are linked to IFN-γ signature expression. IL-2 production from splenic T cells with all these six ITAMs of CD247 mutated was reduced in the same murine model. This is similar to human SLE T cells stimulated *in vitro*.

Although CD247 expression levels in SLE patients were found to be inversely correlated with levels of IFN-γ, both in serum and *in vitro* [21], microarray analysis of mouse transfectants carrying the human spliced variant did not detect any IFN-γ signature [22]. Further investigation on the clinical and experimental aspects of SLE will therefore be needed.

CD247 single SNPs and genome-wide association studies

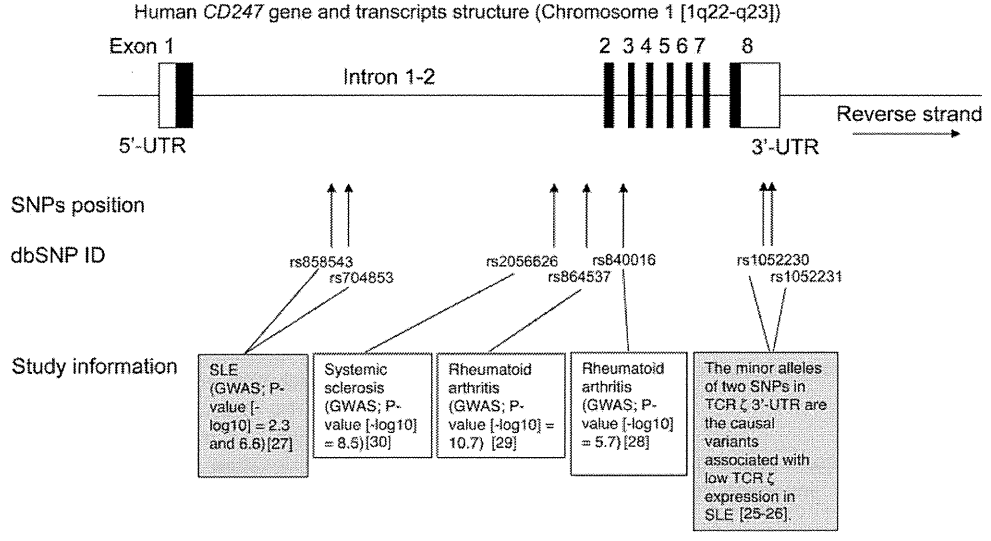
The mechanism responsible for the generation of spliced *CD247* variants in SLE patients is not yet fully understood, and conflicting observations have been reported regarding the presence or absence of mutations or deletions in the 5′-flanking region of the *CD247* gene [15, 23]. Splicing donor and acceptor sites have been reported to carry no such polymorphisms [24]. The National Center for Biotechnology Information database currently harbours

FIG. 3 Structure of known normal and spliced variants of human *CD247*.



Exon–intron organization of human *CD247* genes and their transcripts for zeta, eta and spliced variants (exon 7 deletion and short 3′-UTR) found in SLE [4]. Reproduced with permission from Autoimmunity 2005;38:339–46.

FIG. 4 SNPs of human *CD247* observed in systemic rheumatic diseases.



Schemata of human *CD247* genome and transcripts are shown with summary information of systemic rheumatic diseases related to reported SNPs (position, dbSNP ID and study information).

seven *CD247* gene SNPs that are known to be associated with systemic autoimmune diseases (http://www.ncbi.nlm.nih.gov/gene/919).

Two groups reported the existence of SNPs in the *CD247* 3′-UTR region [25, 26] (Fig. 4). They showed that the minor alleles of two of these SNPs were causal variants associated with low CD247 expression and that one-third of their mRNA was identical to that of the major alleles. The haplotype carrying the low-expression variants predisposes carriers to develop SLE [25].

CD247 was recently shown to be associated with SLE in Asian populations. A genome-wide association study in people of Chinese ethnicity identified two SNPs (rs858543 and rs704853) in the 78-kb intron 1–2 region, one of which

(rs704853) was linked to oral ulcers, haematological disorders and anti-dsDNA antibody production [27].

Two meta-analyses on RA [28, 29] and a study on systemic sclerosis [30] have reported two *CD247* SNPs located in the intron 1-2 region, one associated with RA and the other with SSc. Future analyses should focus on the functional influences of these SNPs on CD247 expression. The strength of effect of known polymorphism may not be substantial, and therefore, variation in CD247 expression must act in concert with other defects.

Conclusions

CD247 splice variants are associated in SLE with aberrant expression through either ITAM deficiency such as exon 7(−) or mRNA instability. Although the molecular mechanisms of RNA splicing are not yet fully understood, various RNA processing dysfunctions, including splicing abnormalities, were recently identified in neurological diseases [31]. We discussed here that abnormal RNA splicing processes were also found to be important in SLE pathogenesis, which suggests that more attention should be focused on new RNA-dependent diseases. Genome-wide analysis of splice variants using high-throughput sequencing and RNA processing functional assessments may improve current understanding of the topic.

Rheumatology key messages

- In SLE, defective expression of CD247 leads to T-cell dysfunction.
- *CD247* splice variants and SNPs may play a key role in SLE pathogenesis.

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ORIGINAL ARTICLE

Phase II dose–response study of abatacept in Japanese patients with active rheumatoid arthritis with an inadequate response to methotrexate

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Abstract
Objective The objective of this study was to assess the response to abatacept at doses of 2 mg/kg and 10 mg/kg compared to placebo in patients with active rheumatoid arthritis (RA) with an inadequate clinical response to methotrexate (MTX).
Methods In this multicenter, placebo-controlled, double-blind, parallel-group, dose–response study, 195 Japanese patients with active RA with an inadequate response to MTX were randomized 1:1:1 to receive 10 mg/kg or 2 mg/kg abatacept plus MTX, or placebo plus MTX, for 24 weeks.
Results Abatacept demonstrated a dose–response relationship when given at 2 and 10 mg/kg. Based on the American College of Rheumatology criteria (20, 50, and 70 %), the responses to 10 mg/kg abatacept were significantly greater than those to placebo at week 24 ($p < 0.001$). Smaller yet statistically significant responses were also seen in the 2 mg/kg abatacept group. Overall rates of adverse events, serious adverse events, and treatment discontinuations because of adverse events were comparable in all three groups.
Conclusions Abatacept (2 mg/kg and 10 mg/kg) showed a dose–response relationship in Japanese patients with active RA with an inadequate clinical response to MTX. Administration of abatacept in combination with MTX for 24 weeks was well tolerated.

Keywords Abatacept · Active rheumatoid arthritis · Clinical response · Japan · Methotrexate

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