interstitial lung disease (ILD), arthritis, and Raynaud's phenomenon [7–11].

The signal recognition particle (SRP) is a cytoplasmic ribonucleoprotein consisting of the 7SL RNA and six associated polypeptides (72, 68, 54, 19, 14 and 9 kDa) that are involved in the recognition of the signal sequences in N-termini of secretory proteins or membrane proteins through binding of its 54 kDa subunit and regulates the translocation of newly synthesized proteins across the endoplasmic reticulum during protein synthesis [12]. Autoantibodies to the SRP have been found mainly in patients with severe myositis that is likely to be often resistant to corticosteroid therapy [13–15]. However, the pathogenic role of these autoantibodies is unknown, and histopathological features of the myopathy associated with anti-SRP antibodies have not been described in detail.

During past years, we have identified 23 patients with anti-SRP antibodies in a large cohort of patients with PM/DM and other connective tissue diseases. In this report, we describe the clinical and histopathological features of these patients.

Materials and methods

Sera

Samples were from a collection of sera from approximately 3,500 patients seen at Keio University Hospital and collaborating medical centers. Sera referred for testing were stored at −20°C and were tested for the presence of MSA and other autoantibodies by immunoprecipitation (IPP). Subjects included: (a) patients with PM or DM, according to the criteria (definite or probable) of Bohan and Peter [16, 17]; (b) patients with a condition suggesting the clinical diagnosis of myositis; and (c) patients with serum anti-cytoplasmic antibodies, regardless of diagnosis. Sera from patients with other conditions including SLE, systemic sclerosis (SSc), rheumatoid arthritis (RA) and normal subjects have also been tested. All samples were obtained after the patients gave their informed consent approved by the corresponding institutional review boards.

RNA immunoprecipitation

IPP using HeLa cell extracts was performed as previously described [9, 18]. Briefly, 10 μl of patient sera was mixed with 2 mg of Protein A-Sepharose CL-4B (Amersham Biosciences AB., Uppsla, Sweden) in 500 μl of IPP buffer (10 mM Tris HCl pH 8.0, 500 mM NaCl,0.1% Nonidet P-40) and incubated with end-over-end rotation (Labquake shaker; Lab Industries, Berkeley, CA, USA) for 2 h at 4°C. The IgG-coated Sepharose was washed four times in 500 μl of IPP buffer using 10-s spins in microfuge tube and resuspended in 400 μl of NET-2 buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.05% Nonidet P-40). For analysis of RNAs, this suspension was incubated with 100 μl of extracts derived from 6×10^6 cells, on the rotator for 2 h at 4°C. The antigen-bound Sepharose beads were then collected with a 10-s centrifugation in the microfuge tubes, washed four times with NET-2 buffer, and were resuspended in 300 μl of NET-2 buffer. To extract bound RNAs, 30 μl of 3 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 with a Vortex mixer and spinning for 1 min, RNAs were recovered in the aqueous phase precipitated with ethanol, and dissolved in 20 μl of electrophoresis sample buffer, composed of 10 M urea, 0.025% bromophenol blue and 0.025% xylene cyanol-FF in TBE buffer (90 mM Tris HCl pH 8.6, 90 mM boric acid, and 1 mM EDTA). The RNA samples were denatured at 65°C for 5 min and then resolved in 7 M urea-10% polyacrylamide gel, which was stained with silver (Bio-Rad Laboratories, Hercules, CA, USA).

Protein immunoprecipitation

For analysis of protein components, antibody-coated Sepharose was mixed with 400 μl of ^{35}S-methionine-labeled HeLa extract derived from 2×10^6 cells, and rotated at 4°C for 2 h. After four washes with IPP buffer, the Sepharose beads were resuspended in SDS-polyacrylamide gel electropheresis (SDS-PAGE) sample buffer (2% SDS, 10% glycerol, 62.5 mM Tris–HCl at pH 6.8, 0.005% bromophenol blue). After heating (90°C for 5 min), the proteins were fractionated by SDS-10% PAGE. Gels were then incubated with 0.5 M sodium salicylate to enhance signals, dried, and exposed to X-ray films for analysis of labeled proteins.

Clinical studies

Standardized data collection forms were used for information, including clinical findings and laboratory data. Muscle weakness of patients with myopathy was evaluated using the ordinal six-point 0–5 of manual muscle strength testing (MMT) (Medical Research Council War Memorandum scale). Severe muscle weakness was defined as muscle weakness grading < 3 by MMT in the weakest muscle and requirement of assistance in daily activities at initial evaluation. DM was diagnosed based on the presence of a Heliotrope rash and/or Gottron's sign. ILD was defined based on interstitial infiltrate on chest radiography or high-resolution computed tomography.

Histopathological studies of muscle

Muscle biopsies were performed on the biceps brachii before treatment with corticosteroid or immunosuppressive agents in all cases. Cryostat sections of the snap frozen muscle were processed in a standard fashion. Muscle biopsy specimens were examined by a neuromuscular pathologist who had no knowledge of the autoantibody status of the patients. Muscle fiber necrosis (degeneration), regeneration, and fibrosis were identified by evaluation of hematoxylin and eosin (H&E)-staining slides. Muscle fiber disruption and atrophy were identified by NADH-tetrazolium reductase (NADH-TR) staining. Muscle fibers were identified as type 1 or 2 fibers using myosin adenosine triphosphatase (ATPase) staining at pH 4.3 and 10.3. Type 1 fiber predominance was defined as greater than 55% of the occupational ratio of type 1 fiber in all muscle fibers [19]. Other special stainings, including modified Gomori trichrome, acid and alkaline phosphatase, nonspecific esterase (NSE), oil red O, periodic acid Schiff, menadione-linked α glycerophosphate dehydrogenase (MAG), succinate dehydrogenase (SDH), cytochrome C oxidase, acetylcholine esterase, phosphorylase, and AMP deaminase, were also performed in histopathological analysis of muscle biopsy specimens [20].

Results

Identification of anti-SRP antibodies

Serum samples from patients with PM/DM and other connective tissue diseases were tested for the presence of antibodies against SRP by immunoprecipitation assays. An anti-SRP prototype serum that immunoprecipitated 7SL RNA and the 54 kDa SRP polypeptide was described previously [10, 14].

Nucleic acid components immunoprecipitated from a whole HeLa cell extract were fractionated on a 7 M urea–10% polyacrylamide gel and detected by silver-staining. Sera from 23 patients were found to immunoprecipitate 7SL RNA (Fig. 1a, lanes 6–16). The pattern of 7SL RNA was clearly distinguishable from that of other RNAs immunoprecipitated by other known described autoantibodies (Fig. 1a, lanes 1–3), and identical in mobility and appearance to 7SL RNA immunoprecipitated by a prototype anti-SRP serum (Fig. 1a, lane 5).

Protein components immunoprecipitated from ^{35}S-methionine-labeled HeLa cell extracts were fractionated on SDS—10% PAGE and detected by autoradiography. The sera that immunoprecipitated 7SL RNA reacted with known protein components of the SRP (Fig. 2b, lanes 2–12). These were clearly different from the characteristic polypeptide bands immunoprecipitated by sera containing other autoantibodies (Fig. 2b, lanes 14–17).

Demographic and clinical features of patients with anti-SRP antibodies

The clinical features of the 23 Japanese patients with anti-SRP antibodies are summarized in Tables 1 and 2. Twenty-one of 23 (91%) patients had myositis; nineteen patients fulfilled the definite criteria of Bohan and Peter and the remaining two patients (patient 10, 14) fulfilled the probable criteria; 14 were type I (pure adult PM), three were type II (pure adult DM), two were type III (myositis associated with malignant disease), and two were type IV (overlap myositis) according to Bohan's classification. Twenty-one patients with myositis ranged in age from 17 to 87 years, (mean ± SD, 51.6 ± 14.9 years) at the onset of muscle weakness, and 15 of them were female. Three patients had typical DM rash including heliotrope rash and/or Gottron's sign. Two patients with PM later developed malignant disease (pharyngeal carcinoma and lung cancer) 10 and 7 years after the onset of PM, respectively. Four (17%), 5 (22%), and 1 (4%) of 23 patients with anti-SRP had arthritis, ILD, and Raynaud's phenomenon, respectively. Two patients had diagnosis of overlap syndrome with RA (Table 1). Three (14%) of 21 patients showed dysphagia that has been reported to be associated with severity and prognosis of myositis or anti-SRP antibodies.

When muscle weakness was clinically present, it was always symmetric and predominant in proximal muscles. Twelve of 20 (60%) patients showed severe muscle weakness (≤3 of MMT) in the weakest muscle at initial evaluation. Muscle weakness developed and progressed rapidly from the time of onset to the maximum severity of weakness, resulting in severe disability. The season of onset of muscle weakness in our 20 patients was seven in the spring, four in the summer, five in the autumn, and four in the winter.

All patients with anti-SRP antibodies except for one patient with PM and one patient with RA were treated with corticosteroids therapy. Most of them partially responded to treatment, but showed persistent muscle weakness and relative resistance to corticosteroid therapy. Prolonged corticosteroid therapy resulted in severe adverse effects such as diabetes mellitus, cataracts, osteoporosis, vertebral compression fractures, and psychosis in some cases. Twelve of 19 (63%) patients showed disease flares of myositis when corticosteroids were tapered. In addition to corticosteroids, eight of 21 (38%) patients with myositis required treatment with immunosuppressive agents such as methotrexate, azathioprine, cyclosporine or intravenous immunoglobulin. No specific immunosuppressive agents was found to be effective for intractable myopathy of all

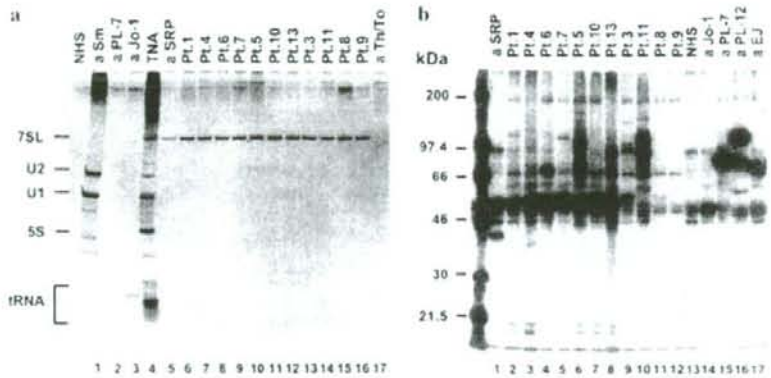


Fig. 1 **a** Nucleic acid components immunoprecipitated by anti-SRP antibody positive sera. Immunoprecipitated RNAs from HeLa cell extract were fractionated on a 7 M urea-10% polyacrylamide gel and detected by silver staining. The normal human serum (*NHS*) precipitates no RNAs. Anti-Sm (*lane 1*), anti-PL-7 (*lane 2*), anti-Jo-1 (*lane 3*) and anti-Th/To (*lane 17*) are reference sera containing the indicated antibodies. Anti-SRP (*lane 5*) is a previously described prototype serum. *TNA* is the total nucleic acids phenol-extracted from HeLa cells. Eleven representative anti-SRP sera (*lanes 6–16*) showed an intense band that is compatible in size with 7SL RNA of the SRP. **b** Protein components immunoprecipitated by anti-SRP antibody positive sera. Proteins immunoprecipitated from ^{35}S-methionine-labeled HeLa cell extracts were fractionated on SDS—10% polyacrylamide gels and detected by autoradiography. Anti-SRP (*lane 1*), anti-Jo-1 (*lane 14*), anti-PL-7 (*lane 15*), anti-PL-12 (*lane 16*), and anti-EJ (*lane 17*) are reference sera. *Lanes 2–12* are 11 anti-SRP positive representative sera that immunoprecipitated 7SL RNA

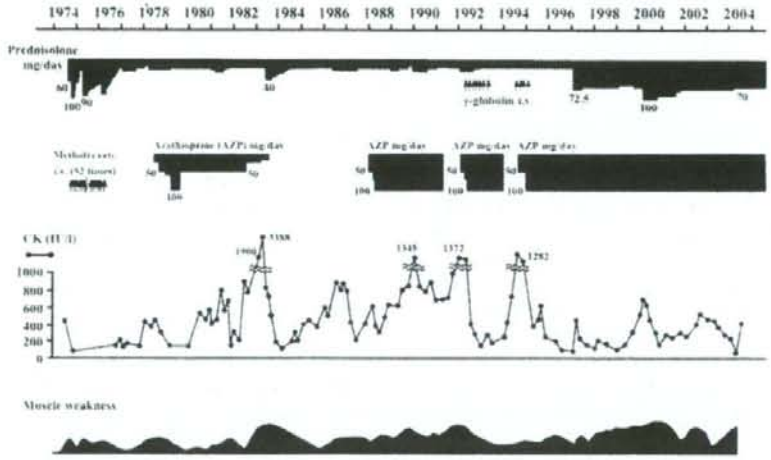


Fig. 2 Clinical course of a typical anti-SRP positive myositis patient. A 41-year-old woman developed dysphasia and gait disturbance in June 1974. A diagnosis of PM was made based on proximal muscle weakness, elevated levels of CK and other muscle enzymes, and muscle biopsy finding consistent with myositis. Her muscle weakness was severe and required continuous corticosteroid therapy which resulted in various serious side effects including cataract and generalized osteoporosis associated with compression fracture of spinal bones. The therapeutic efficacy of steroid to myositis was limited and cytotoxic agent (azathioprine) and γ globulin therapy were also applied

patients with anti-SRP. Three patients died of heart failure at age 73 (patient 1), myocardial infarction at age 48 (patient 3), and pneumonia at age 83 (patient 11), respectively. Clinical course of the representative case with anti-SRP antibodies is shown in Fig. 2.

Laboratory findings

Serum creatine kinase (CK) was remarkably elevated in all myositis patients with anti-SRP antibodies, ranging from 1,387 to 9,900 IU/l (mean ± SD, 5016 ± 2452 IU/l). The

Table 1 Clinical features of 23 patients with anti-SRP antibodies (1)

Patient	Diagnosis	Age at symptom onset/sex	DM rash	Malignancy	Arthritis	ILD	Raynaud's phenomenon	Overlap syndrome	Other autoantibodies
1	DM	40 F	+	−	−	−	−	−	−
2	PM	55 F	−	−	−	−	−	−	−
3	PM	31 M	−	−	−	−	−	−	SS-A/Ro
4	PM	52 F	−	−	−	+	+	−	−
5	PM	60 M	−	−	−	+	−	−	SS-A/Ro
6	PM	34 F	−	−	−	−	−	−	−
7	PM/RA	37 F	−	−	+	−	−	+	SS-A/Ro
8	PM	56 M	−	−	−	−	−	−	−
9	PM	38 M	−	−	−	−	−	−	−
10	PM	56 F	−	−	−	−	−	−	SS-A/Ro
11	PM/RA	68 F	−	−	+	−	−	+	−
12	PM	48 F	−	−	−	−	−	−	−
13	PM	75 F	−	−	−	−	−	−	Th/To
14	PM	87 F	−	−	−	+	−	−	−
15	PM	53 F	−	+	−	−	−	−	−
16	DM	56 F	+	−	−	+	−	−	−
17	DM	17 F	+	−	−	−	−	−	−
18	PM	56 M	−	−	−	−	−	−	−
19	PM	55 M	−	+	−	+	−	−	−
20	PM	44 F	−	−	−	−	−	−	−
21	PM	63 F	−	−	−	−	−	−	−
22	RA	53 F	−	−	+	−	−	−	−
23	RA	53 F	−	−	+	−	−	−	SS-A/Ro

ILD interstitial lung disease, *RA* rheumatoid arthritis

EMG finding showed myogenic changes, including myopathic motor unit potentials and prominent spontaneous activity in proximal muscles in all the patients examined. Both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were highly elevated, but AST/ALT ratio < 1.0 was observed in 13 of 18 (72%) patients when the highest value of CK was recorded (Table 2). Most of patients showed a diffuse cytoplasmic staining in indirect immunofluorescence using HEp-2 cells. Other autoantibodies detected in the 23 patients with anti-SRP included 5 patients with anti-SS-A/Ro (Table 1). One patient had anti-Th/To, a specificity usually found in SSc; however, this patient did not have features of SSc.

Histopathological findings of muscle biopsies

Myopathic change of muscle biopsies from all 21 myositis patients with anti-SRP were verified in their medical records. Out of them, a total of 11 muscle biopsy specimens from patients with anti-SRP antibodies were evaluated in detail and their pathological findings were summarized in Table 3. All muscle biopsies were performed during the initial diagnostic evaluation and showed histological changes consistent with myositis. Muscle biopsy specimens showed myopathic changes including prominent variation of myofiber size, and none of them exhibited perifascicular atrophy. Muscle fiber necrosis (degeneration) was present in 9 of 11 (82%) patients. Muscle fiber regeneration was observed in all biopsy specimens. It is noteworthy that all but one showed absence of inflammatory infiltration, and none had muscle fibers with vacuoles. Four patients (36%) had ragged red fiber, which indicated mitochondrial damages. Neurogenic change was seen only in one patient. It should be noted that six of 11 (55%) patients with anti-SRP antibodies showed the predominance of type 1 fiber, while eight myositis patients without these antibodies did not (55% vs. 0%, $P = 0.04$ by Fisher's exact test) (Table 3).

Discussion

The present study is the first comprehensive analysis of clinical and histological features of Japanese patients with anti-SRP antibodies. We have identified anti-SRP antibodies in 23 Japanese patients with PM/DM, RA, and

Table 2 Clinical features of 23 patients with anti-SRP antibodies (2)

Patient	Month at onset	Severe muscle weakness[a]	Creatine kinase (maximum) (IU/l)	AST/ALT < 1.0	Initial dose of prednisolone (mg/day)	Recurrence	Cytotoxic agent	Outcome
1	June	+	3,388	+	100	+	MTX, AZP	Dead
2	June	+	9,900	+	60	+	–	NA
3	June	+	3,354	+	60	+	AZP	Dead
4	February	+	3,179	–	30	NA	–	NA
5	February	–	3,139	+	60	+	–	Alive
6	September	+	2,977	–	1,250	–	–	Alive
7	ND	ND	3,280	ND	50	+	AZP	NA
8	February	+	3,443	+	50	+	–	Alive
9	November	–	6,400	+	50	+	AZP	Alive
10	May	–	1,387	–	0	–	–	NA
11	April	+	3,035	–	50	–	–	Dead
12	April	–	5,140	+	60	+	–	NA
13	January	+	4,307	+	45	–	6MP	Alive
14	May	–	2,960	ND	40	NA	–	NA
15	October	–	7,169	ND	60	+	–	Alive
16	September	+	9,089	+	60	+	CyA	Alive
17	March	–	6,453	+	50	+	–	Alive
18	August	+	9,325	+	75	–	AZP	Alive
19	November	+	7,000	+	60	–	–	Alive
20	April	+	6,758	–	60	+	AZP	Alive
21	May	–	3,646	+	60	–	–	Alive
22	ND	–	68	–	7	ND	MTX	Alive
23	ND	–	89	–	0	ND	MTX	Alive

ND not determined, *NA* not available, *MTX* methotrexate, *AZP* azathioprine, *6MP* mercaptopurine, *CyA* cyclosporin

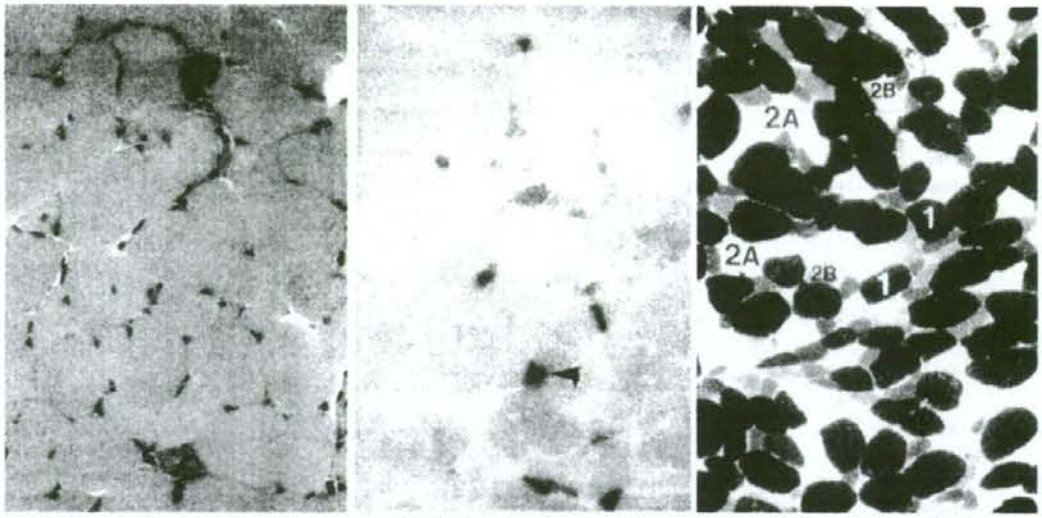
[a] Severe muscle weakness: ≤3 on manual muscle strength testing

Table 3 Histopathological features of myopathy in patients with anti-SRP antibodies

Anti-SRP antibodies	Anti-SRP (+) 11 patients											Anti-SRP (–) 8 patients
Patient No.	3	5	8	11	12	13	15	18	19	20	21	
Diagnosis	PM	PM	PM	PM	PM	PM	PM	PM	PM	PM	PM	PM
Necrosis (degeneration)	+	+	+	+	+	+	–	+	+	–	+	8/8 (100%)
Regeneration	+	+	+	+	+	+	+	+	+	+	+	8/8 (100%)
Inflammation	–	–	–	–	–	–	–	–	–	–	+	4/8 (50%)
Ragged-red fiber	–	–	–	+	–	+	–	–	–	+	+	2/8 (25%)
Neurogenic change	–	–	–	–	–	–	–	–	+	–	–	0/8 (0%)
Type 1 fiber predominance	+	–	–	+	+	+	–	–	–	+	+	0/8 (0%)

overlap syndrome by immunoprecipitation of 7SL RNA and protein components that are consistent with SRP (Fig. 3).

Most of our anti-SRP-positive myositis patients had severe muscle weakness and showed rapid clinical course, consistent with previous studies [15, 21]. Serum CK levels were markedly elevated. It should be noted that 72% of patients with anti-SRP antibodies exhibited elevation of aminotransferase with the serum AST/ALT raio < 1, an unusual pattern for muscle injury. AST have been thought to be more specific for inflammatory myopathies than ALT, which is found primarily in the liver. The serum AST/ALT ratio < 1 usually indicate liver injury such as viral hepatitis. Thus, the serum AST/ALT ratios < 1 in many anti-SRP-positive myositis patients suggest that the mechanisms of muscle injury in this subset of patients may



(a) Hematoxylin and eosin staining (b) Alkaline phosphatase staining (c) ATPase staining (pH 4.5)

Fig. 3 Muscle pathology in patients with anti-SRP associated myopathy. **a** Atrophic and hypertrophic muscle fibers in H&E staining. Muscle fiber regeneration shown in basophilic color on H&E staining was distributed through most biopsies. Muscle fiber necrosis was also present (pale staining with H&E staining). Note the absence of inflammatory cell infiltration. **b** Muscle fiber regeneration shown in *small dark color* on alkaline phosphatase staining. Small regenerating muscle fibers were distributed randomly through most biopsy specimens. **c** It showed the predominance of type 1 fiber (*dark color* in ATPase staining, pH 4.3)

be different from other myositis patients. Further analysis to identify the type of injured muscle fiber will be required to elucidate the unique myopathy associated with anti-SRP antibodies.

A distinct seasonal pattern in the onset of muscle weakness associated with MSA was described [22]. The predominant month in the onset of myositis was November in patients with anti-SRP antibodies, whereas it was April in patients with anti-Jo-1 antibodies, suggesting the role of an antecedent environmental trigger, such as viral infection in development of myositis [22]. However, our patients with anti-SRP did not have recognizable seasonal pattern and apparent prodromal illness. This may suggest that no single mechanism can explain the production of these antibodies. An intensive investigation for genetic factors as well as the triggering environmental agents that determine susceptibility to the production of anti-SRP antibodies is required.

In this study, most of anti-SRP-positive patients had type 1 disease (pure adult PM) and the frequencies of DM rash, malignant disease and overlap syndrome were low, consistent with our previous study that these antibodies were detected in two of 27 patients with PM, one of 22 patients with DM, none of 15 patients with other connective tissue diseases [10]. The clinical features associated with anti-ARS antibodies, including arthritis, ILD, Raynaud's phenomenon, and mechanic's hands, have been reported to be uncommon in patients with anti-SRP antibodies [8, 15]. In our patients, the frequencies of ILD and arthritis were significantly lower than those of patients with anti-ARS antibodies (22% vs. 96%, $P < 0.001$; 17% vs. 96%, $P < 0.001$ by Fisher's exact test, respectively), consistent with observations by others [10]. It should be noted that we have found two patients with RA and two with PM overlap syndrome with RA. Okada et al. found one patient with anti-SRP who had polyarthritis [14]. Kao et al. [23] described two patients with SSc and one patient with anti-synthetase syndrome, and two of these with arthritis. Taken together, it should be noted that anti-SRP antibodies appear to be nearly specific for PM; however, some patients may still present without evidence for myositis. In addition, the patients without myositis may present with arthritis as a predominant clinical manifestation, especially as RA in Japanese population.

In the present study, there were three deaths among the 19 myositis patients with anti-SRP antibodies, but all three patients died in more than 7 years, after onset of myositis. Regarding the prognosis, Kao et al. [23] described that the 5-year cumulative survival rate in the SRP-positive PM patients (86%) was not significantly different from SRP-negative PM patients (the ARS-negative patients (83%) and the ARS-positive patients (75%). These results

may indicate that the prognosis of the SRP-positive PM patients is not so poor, compared with the SRP-negative PM patients, although myositis associated with anti-SRP is severe and resistant to corticosteroid therapy. The prospective comparative study of survival rate among patients with each myositis-specific antibody should be fully established.

Anti-SS-A/Ro antibodies were detected in five of the 23 (22%) anti-SRP-positive patients and anti-Th/To in 1, consistent with the report by Kao et al. [23]. This is likely to reflect the high frequency of anti-SS-A/Ro antibodies in myositis patients in general and is not particularly higher than the frequency of anti-SS-A/Ro in other subsets of PM/DM patients.

The typical pathologic feature of inflammatory muscle disease is mononuclear cell infiltration of the endomysium and focal cellular invasion of muscle fibers with various degree of muscle fiber necrosis and regeneration. Several different pathological changes may occur in immune-mediated myopathy, and the presence of inflammatory cell infiltration in the affected muscles is one of the most common findings. In the muscle pathology of patients with anti-Jo-1 antibodies, macrophage predominant inflammation and fragmentation in perimysial connective tissue was described as the most prominent feature [24]. In contrast, our histopathological examination in biopsy specimens from anti-SRP-positive patients showed significant muscle fiber necrosis (degeneration) and regeneration with no or little inflammatory cell infiltrations, consistent with "necrotizing myopathy" reported by Miller et al. and Hengstman et al. [21, 25]. Furthermore, we have found a unique feature of "type 1 fiber predominance" in anti-SRP-positive PM patients, compared with the SRP-negative PM patients. The "type I fiber predominance" has been reported to occur in non-inflammatory muscle diseases, including congenital muscular dystrophy, myotubular myopathy, nemaline myopathy, infantile acid maltase deficiency, infantile myotonic dystrophy, cerebellar hypoplasia, and Krabbe's disease [26, 27]. These a distinctive histological findings of anti-SRP associated myopathy, necrotic myofibers without inflammatory cell infiltration and type 1 fiber predominance might explain the corticosteroid resistance of myositis in anti-SRP-positive patients. Further histopathological studies associated with MSA are needed to confirm these observations.

In summary, anti-SRP antibodies appear nearly specific for a small group of Japanese patients with PM who had severe proximal muscle weakness at initial presentation and resistance to corticosteroid therapy, consistent with the clinical manifestations observed in Caucasian patients. This specificity is also occasionally found in patients with other autoimmune disorders, such as RA especially in Japanese patients in the absence of myositis. Unlike the patients with anti-ARS antibodies, the extramuscular symptoms including arthritis, ILD, and Raynaud's phenomenon are uncommon, and resistance to standard corticosteroid therapy are frequently observed in our patients with anti-SRP antibodies. Furthermore, it was found that anti-SRP associated myopathy is pathologically characterized by active muscle fiber necrosis with little or no mononuclear cell inflammation and type 1 fiber predominance. All these results indicate that anti-SRP antibodies are closely associated with a clinically as well as pathologically distinct subset of myositis. However, the pathogenesis of this disorder that might be included in the spectrum of autoimmune-mediated myopathies, remains unknown. Further studies will be required to elucidate the pathogenic role of anti-SRP antibodies and develop appropriate treatment strategies.

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CASE REPORT

Protein-losing gastroenteropathy associated with primary Sjögren's syndrome: a characteristic oriental variant

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Abstract Protein-losing gastroenteropathy (PLGE) is a rare manifestation of primary Sjögren's syndrome (SS). We report a case of a 41-year-old Japanese man, who is the first male patient, with PLGE associated with primary SS. Although serum anti-SSA and SSB antibodies were detected, he had no subjective sicca symptoms. He had multiple annular erythema: a characteristic skin manifestation of Asian SS patients. A diagnosis of PLGE was made from results of ^{99m}Tc-labelled albumin scintigraphy and a faecal alpha-1-antitrypsin clearance test. Intravenous administration of high-dose glucocorticoid was not effective, but pulse methylprednisolone therapy alleviated disease manifestations. As all cases of PLGE associated with primary SS have been reported from East Asia, this complication could be essentially limited to Asian patients.

Keywords Sjögren's syndrome · Protein-losing gastroenteropathy · Annular erythema · Anti-SSA antibody

Introduction

Protein-losing gastroenteropathy (PLGE), an uncommon disorder, often presents with marked oedema and pleural effusion in the absence of heart failure, malnutrition, or nephrotic syndrome. PLGE sometimes occurs in association with systemic autoimmune diseases, particularly systemic lupus erythematosus (SLE) [1]. Patients with PLGE associated with SLE characteristically lack typical presenting symptoms of lupus such as malar rash or lupus nephritis [2]. Serologic features include antinuclear antibodies (ANA) with a speckled pattern, negative anti-double stranded (ds) DNA antibody, and low serum complement. More than 30 cases have been reported in English nearly all in women [1]. PLGE complicating primary Sjögren's syndrome (SS) also has been described; so far, only four cases had been reported in English [3–5], all from East Asia. Here, we report an additional Asian case of PLGE complicating SS, in the first male patient. He had annular erythema, a characteristic skin manifestation of Asian SS.

Case report

A 41-year-old Japanese man was admitted to our hospital because of bilateral pleural effusion and ascites. He had noted facial oedema 2 months before; distal leg oedema and abdominal fullness developed 1 month later. He consulted a local physician because of shortness of breath, general fatigue, and oedema. He had gained more than 15 kg over a few months. He did not complain of diarrhoea. Under follow-up by a dermatologist beginning 3 years previously, he had recurrent erythema affecting his cheek, ear, neck, and trunk. Serum anti-single stranded DNA, anti-SSA, and anti-SSB antibodies were positive, but anti-ds DNA antibody was negative. He therefore was diagnosed with SS, although he had noted no oral or conjunctival dryness.

On physical examination, height was 166 cm; body weight, 74.3 kg; blood pressure, 122/78 mmHg; pulse, 78/min; and body temperature, 36.5°C. No cardiac murmurs or pulmonary rales were heard, but respiratory sounds were diminished. The abdomen was distended, and bowel sounds were hypoactive. Eyelids were oedematous; oedema also

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was prominent in the lower extremities. The skin showed multiple horseshoe-shaped and annular lesions with erythema over the left cheek, chest and back (Fig. 1).

Laboratory results indicated white blood cell count, 9100/μl with 19% lymphocytes; red blood cell count, 463 × 10^4/μl; haemoglobin, 14.8 g/dl; platelets, 41 × 10^4/μl; and erythrocyte sedimentation rate, 107 mm/h (normal < 17). Total protein was 4.8 g/dl; albumin, 1.3 g/dl; urea nitrogen, 12 mg/dl; and creatinine, 0.86 mg/dl. Liver function and electrolyte values were normal. Total cholesterol was 328 mg/dl (normal < 220); triglyceride, 286 mg/dl (normal < 150); complement C3, 54 mg/dl (normal 86–160); and C4, 18 mg/dl (normal 18–25). CA125 antigen was 8,641 U/ml (normal < 35); and fibrinogen, 1,154 mg/dl (normal 129–271). C-reactive protein was 0.75 mg/dl. Serum IgG, IgA, and IgM respectively were 1,059 mg/dl (normal 870–1,700); 116 mg/dl (normal 110–410); and 137 mg/dl (normal 46–260). Cryoglobulin was not detected. ANA was positive at a dilution of 1:1280, with nucleolar and speckled patterns. The anti-SSA antibody titre was 1:64; anti-SSB antibody was 1:32. Anti-ds DNA, anti-Sm, anti-RNP, and anti-β2 glycoprotein I antibodies were negative. Anti-*Helicobacter pylori* antibody was positive. Urinalysis disclosed urinary protein of 0.16 g/day with no casts. Occult blood testing of stool was negative, but stool fat was detected by Sudan III staining. The pleural effusion showed characteristics of a transdate, and culture of the effusion grew no bacteria. Thoracic and abdominal computed tomography (CT) depicted massive pleural effusions and ascites. No lymphadenopathy was observed. Cardiac ultrasonography indicated a pericardial effusion of moderate size, with no evidence of heart failure. Ocular examinations including Schirmer's test, Rose-Bengal test, and fluorescein staining detected no abnormalities. Only 2 ml of salivary secretion occurred during 10 min of gum chewing (normal > 10 ml), ^{99m}Tc sialoscintigraphy demonstrated decreased radioisotope uptake by both parotid and submandibular glands, and decreased salivary secretion after stimulation. The patient declined diagnostic minor salivary gland biopsy. A diagnosis of SS was made according to the 1999 Revised Japanese Criteria for SS, which were adopted by the Japanese Ministry of Health and Welfare.

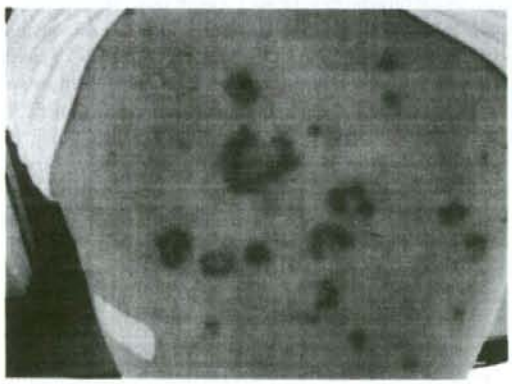

Fig. 1 A view of the back of the patient. Multiple annular or horseshoe-shaped erythema of various sizes were seen

The patient was suspected to have protein-losing gastroenteropathy based upon hypoalbuminaema, massive pleural effusion, and ascites in the absence of proteinuria or anorexia. Gastrointestinal endoscopy disclosed atrophic gastritis and a duodenal ulcer scar. By double-balloon endoscopy, villi in the jejunum were not oedematous and had normal shape, being fingerlike and varying in size. Colonoscopy demonstrated mild nonspecific erosions in the left colon. Biopsy specimens of gastric and colonic mucosa showed normal findings with only minimal oedema, and a specimen of jejunal mucosa disclosed mild infiltration of mononuclear cells into villi and mild lymphatic vessel dilation near the muscularis mucosae. Biopsy specimens of gastric and jejunum mucosa demonstrated *H. pylori* infection. Immunologic studies to assess complement deposition were not performed. Average alpha-1-antitrypsin clearance in the stool for three consecutive days was greatly elevated, at 280.5 ml/day (normal < 19 ml/day). ^{99m}Tc-labelled human serum albumin abdominal scintigraphy showed increased secretion of radioisotope from the stomach and small intestine (Fig. 2). From these findings, a diagnosis of PLGE was made. Treatment with intravenous prednisolone (PSL) at 70 mg daily (1 mg/kg) was started, together with diuretics. After failing to respond after 2 weeks, the patient was given intravenous pulse methylprednisolone (1 g/day for 3 days) followed by oral PSL. After this therapy, body weight gradually decreased, and serum albumin slowly increased. After 1 year of therapy, serum total protein and albumin were within the normal range, and PSL was tapered successfully to 7 mg per day.

Discussion

This report describes the first known man with primary SS in association with protein-losing gastroenteropathy. Recently, reports of protein-losing gastroenteropathy associated with autoimmune diseases have been increasing. SLE is the most common associated disease; more than 30 occurrences have been reported so far [1]. The cause of PLGE generally is unknown, but when PLGE is associated with collagen diseases, an immune-mediated mechanism is suspected since corticosteroids or other immunosuppressive drugs are reported to be effective; further, patients often have low serum complement. Disturbance of vascular permeability by complement activation in the intestinal

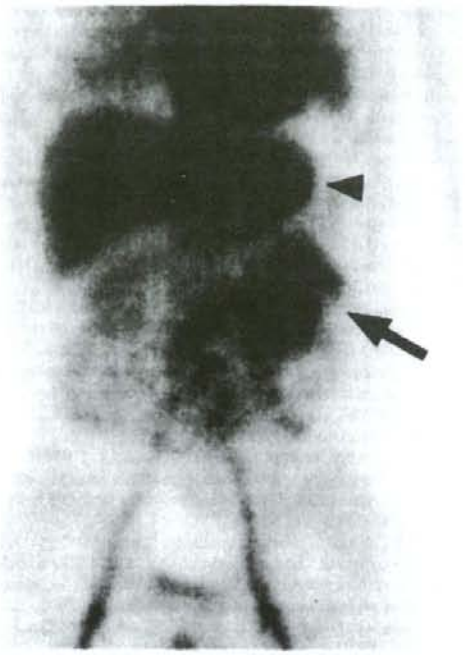

Fig. 2 ^{99m}Tc-labelled human serum albumin abdominal scintigram. Twenty minutes after intravenous injection of ^{99m}Tc-labelled albumin, radioactivity could be detected in the stomach (*arrowhead*) and left upper small intestine (*arrow*)

wall is speculated to be a mechanism of PLGE in autoimmune diseases, but complement deposition in the intestinal wall rarely has been confirmed [3, 6, 7].

Although we could not obtain histopathologic evidence of salivary gland involvement, a diagnosis could be made based on Revised Japanese Criteria for SS: positive anti-SSA and SSB antibodies, and decreased salivary flow demonstrated by a chewing gum test and sialoscintigraphy. This testing is considered more sensitive and accurate than that required by the 2002 American–European Revised Classification Criteria for SS [8, 9]. Because our patient reported no xerostomia or xerophthalmos—features of the classification criteria proposed by the American–European Consensus Group—the patient satisfies fewer than four of six criteria for SS. Since two of the six criteria involve subjective complaints, some patients with true primary SS easily could be misclassified. The Japanese criteria do not include subjective items. In one study, 15% of patients with primary SS did not complain of xerostomia [10], while in an other only 67.5% of patients complained of dry eye [11].

Stool fat was demonstrable in our case. Generally, steatorrhoea implies obstructed lymphatic flow. Thoracoabdominal CT showed no lymphadenopathy, and a biopsy specimen from the jejunum demonstrated only slight lymphatic vessel dilation. In typical intestinal lymphangiectasia, serum albumin, gamma globulin, cholesterol, fibrinogen, and absolute lymphocyte count all are decreased [12], while our patient had only hypoalbuminaemia. Gamma globulin and lymphocyte count were normal, while serum total cholesterol and fibrinogen were increased. From these laboratory findings, this patient's PLGE would not be idiopathic intestinal lymphangiectasia, but more likely autoimmune-associated PLGE. PLGE resembles lymphangiectasia in terms of protein-rich exudation reflecting hyperpermeability of intestinal microvascular vessels with intestinal interstitial oedema, which leads to obstruction of lymphatic flow and dilation of lymphatic vessels [1, 13]. Accordingly, stool fat can be positive even in PLGE.

Helicobacter pylori also is reported to be associated with PLGE. Eradication of *H. pylori* decreased protein loss in patients with hypertrophic gastritis and Ménétrier's disease [14–16]. Our patient was diagnosed with *H. pylori* infection by anti- *H. pylori* antibody positivity and by examining biopsy specimens from stomach and jejunum. However, gastrointestinal endoscopy indicated no hypertrophy of the gastric mucosa. Moreover, pulse corticosteroid therapy improved protein loss without *H. pylori* eradication. Eradication of *H. pylori* in a case of PLGE associated with autoimmune disease did not abolish PLGE [17]. *H. pylori* infection is associated with atrophic gastritis; the prevalence of atrophic gastritis and *H. pylori* infection is more than 50% in our patient's age group in Japan [18]. Atrophic gastritis is considered a possible extraglandular manifestation of SS [19, 20], but our patient's atrophic gastritis could have resulted from either SS or *H. pylori* infection. The exact role of *H. pylori* in PLGE remains to be determined.

Our patient had recurrent erythema 3 years before a diagnosis of PLGE was made. Annular erythema has been reported to be relatively frequent in Asian SS patients, especially Japanese [21, 22]. This skin manifestation also is seen in subacute cutaneous lupus erythematosus (SCLE), described as a distinct subset of lupus erythematosus [23]. The difference between SS and SCLE has been a matter of discussion, since SCLE is significantly associated with anti-SSA antibody in patients of Occidental descent [24]. Racial differences could underlie the difference in cutaneous expression between Oriental and Occidental patients with demonstrable anti-SSA antibody [25]. Annular erythema in SS can be distinguished clinically and histopathologically from that associated with SCLE [26]. Fifty percent of SS patients with annular erythema did not complain of either ocular or oral dryness [26]. Therefore, we consider our patient's diagnosis to be correct.

We used an intravenous route to administer high-dose steroids in view of possible poor gut absorption, but this treatment was ineffective. After substituting 1 g of intravenous

methylprednisolone daily for three doses, hypoalbuminaemia, bilateral pleural effusion, and lower leg oedema began to gradually improve. All four patients with both SS and PLGE were treated with corticosteroids. Three of these patients also underwent intravenous methylprednisolone pulse therapy; two concurrently received hydroxychloroquine; and one required addition of cyclophosphamide. In PLGE associated with SLE, 7 of 29 patients needed addition of pulse steroid therapy after failure of oral steroids [1]. Intravenous pulse steroid therapy may be effective in most autoimmune disease-associated PLGE. One reported patient was diagnosed with hypertrophic gastropathy [5], but presence of *H. pylori* infection was not described.

All cases of primary SS associated with PLGE have been reported from East Asia, particularly Japan, Taiwan, and China. More than a dozen cases of PLGE with SS have been reported in the Japanese-language literature [27]. This suggests that SS associated with PLGE is not rare in Japan. Mok et al. [28] suggest that PLGE might be fairly common among Oriental SLE patients. We similarly propose that PLGE is a distinctive complication in Asian SS patients. Accordingly, patients with PLGE associated with positive ANA in a speckled pattern require careful serologic, ocular, and oral examination for diagnosis of subclinical SS.

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High prevalence of autoantibodies to muscarinic-3 acetylcholine receptor in patients with juvenile-onset Sjögren syndrome

Sjögren syndrome (SS) is an autoimmune disease characterised pathologically by lymphocytic infiltration into the lacrimal and salivary glands, and clinically by dry eyes and mouth. Lymphocytic infiltration is also found in the kidneys, lungs, thyroid, and liver. Immunohistochemical studies have shown that most infiltrating lymphocytes around the labial salivary and lacrimal glands and the kidneys are CD4-positive αβT cells.[1] Candidate autoantigens recognised by T cells that infiltrate the labial salivary glands of SS have been analysed and Ro/SS-A 52 kDa,[2] α-amylase, heat shock protein, and TCR BV6[3] have been identified, although Ro/SS-A 52 kDa reactive T cells were not increased in peripheral blood.[4]

In contrast, various autoantibodies (autoAbs) have been identified in the sera of patients with SS, and some of these autoAbs, such as anti-SS-A antibody (Ab) and anti-SS-B Ab, are used as diagnostic markers. Muscarinic-3 acetylcholine receptor (M3R) is involved in activation of salivary and lacrimal glands. This receptor is G-protein-linked and its activation triggers a second-messenger cascade that culminates in a rise in intracellular calcium and activation of K^+ and Cl^- channels that drive fluid secretion.[5] Although autoAbs to M3R have been demonstrated in patients with SS,[6] the location of B cell epitopes on M3R remain controversial.[7 8] We previously reported the presence of autoAbs against the second loop domain of M3R in 11.2% of patients with adult SS.[9] Anti-M3R Ab is specific for SS because it is not present in patients with other autoimmune diseases such as rheumatoid arthritis and systemic lupus erythaematosus. Based on these early findings, we hypothesised that the presence of anti-M3R Ab may be directly related to defective salivary and lacrimal secretion in SS patients. The prevalence of M3R Ab in juvenile SS is still unknown. To examine this issue, we screened sera of patients with juvenile SS for anti-M3R Ab.

Serum samples were collected from 38 Japanese paediatric patients with juvenile-onset SS (JSS) followed-up at the Departments of Pediatrics of Graduate School of Medicine, Chiba University and Yokohama City University School of Medicine, Yokohama. We recruited 76 healthy control subjects from the Division of Clinical Immunology, Major of Advanced Biological Applications, Graduate School Comprehensive Human Science, University of Tsukuba. The mean (SD) age of the patients was 15 (5) and 22 (2) years for the JSS and control groups, respectively. The 25mer synthetic amino acid encoding the second extracellular domain of M3R was used as the antigen, because this portion plays an important role in intracellular signalling. Figure 1 shows that the mean titre of anti-M3R Ab in patients with JSS (0.329 (0.189)) was significantly higher than that of controls (0.105 (0.089), $p<0.001$). Moreover, the prevalence of anti-M3R Ab in patients with JSS (52.6%) was significantly higher than that

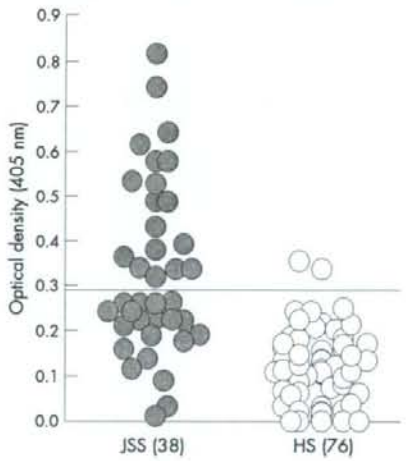

Figure 1 Comparison of anti-M3R Abs in patients with juvenile Sjögren syndrome (JSS) and control. A 25mer peptide (KRTVPPGECFIQFLSEPTITFGTAI) corresponding to the sequence of the second extracellular loop domain of the human M3R was synthesised (Sigma-Aldrich Japan, Ishikari, Japan). A 25mer peptide (SGSGSGSGSGSGSGSGSGSGSGS) was also synthesised as a negative control (Sigma-Aldrich Japan). Peptide solution (100 μl/well at 10 μg/ml) in 0.1M Na_2CO_3 buffer, pH 9.6, was adsorbed onto a Nunc-Immuno plate (Nalge Nunc International, Rochester, New York, USA) overnight at 4°C, and blocked with 5% bovine serum albumin (Wako Pure Chemical Industries, Osaka, Japan) in phosphate buffered saline (PBS) for 1 h at 37°C. Serum at 1:50 dilution in blocking buffer was incubated for 2 h at 37°C. The plates were then washed twice with 0.05% Tween 20 in PBS, and 100 μl of alkaline phosphatase-conjugated goat antihuman IgG (Fc; American Qualex, San Clemente, California, USA) diluted 1:000 in PBS was added for 1 h at room temperature. After three washes, 100 μl of *p*-nitrophenyl phosphate (Sigma) solution (final concentration 1 mg/ml) was added as alkaline phosphate substrate. Plates were incubated for 30 min at room temperature and the optical density at 405 nm was measured by plate spectrophotometry (Bio-Rad Laboratories, Hercules, California, USA). Optimal density was used to express the titre of anti-M3R Abs. Measurements were performed in triplicate and standardised between experiments. Numbers in parentheses represent the number of patients in each group.

in controls (2.9%, $p<0.001$). These results indicate the high prevalence of anti-M3R in JSS patients, compared to adult-onset SS patients. The presence of anti-SS-A Ab or anti-SS-B Ab were not associated with the presence of anti-M3R Ab in patients with JSS.

In conclusion, the high titre and prevalence of anti-M3R Abs in patients with JSS suggest that anti-M3R Ab could be potentially useful as a diagnostic marker for JSS.

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Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial

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Summary

Background Systemic-onset juvenile idiopathic arthritis does not always respond to available treatments, including antitumour necrosis factor agents. We investigated the efficacy and safety of tocilizumab, an anti-interleukin-6-receptor monoclonal antibody, in children with this disorder.

Methods 56 children (aged 2–19 years) with disease refractory to conventional treatment were given three doses of tocilizumab 8 mg/kg every 2 weeks during a 6-week open-label lead-in phase. Patients achieving an American College of Rheumatology Pediatric (ACR Pedi) 30 response and a C-reactive protein concentration (CRP) of less than 5 mg/L were randomly assigned to receive placebo or to continue tocilizumab treatment for 12 weeks or until withdrawal for rescue medication in a double-blind phase. The primary endpoint of the double-blind phase was an ACR Pedi 30 response and CRP concentration of less than 15 mg/L. Patients responding to tocilizumab and needing further treatment were enrolled in an open-label extension phase for at least 48 weeks. The analysis was done by intention to treat. This study is registered with ClinicalTrials.gov, numbers NCT00144599 (for the open-label lead-in and double-blind phases) and NCT00144612 (for the open-label extension phase).

Findings At the end of the open-label lead-in phase, ACR Pedi 30, 50, and 70 responses were achieved by 51 (91%), 48 (86%), and 38 (68%) patients, respectively. 43 patients continued to the double-blind phase and were included in the efficacy analysis. Four (17%) of 23 patients in the placebo group maintained an ACR Pedi 30 response and a CRP concentration of less than 15 mg/L compared with 16 (80%) of 20 in the tocilizumab group ($p<0·0001$). By week 48 of the open-label extension phase, ACR Pedi 30, 50, and 70 responses were achieved by 47 (98%), 45 (94%), and 43 (90%) of 48 patients, respectively. Serious adverse events were anaphylactoid reaction, gastrointestinal haemorrhage, bronchitis, and gastroenteritis.

Interpretation Tocilizumab is effective in children with systemic-onset juvenile idiopathic arthritis. It might therefore be a suitable treatment in the control of this disorder, which has so far been difficult to manage.

Funding Chugai Pharmaceuticals.

Introduction

Systemic-onset juvenile idiopathic arthritis is a subtype of chronic childhood arthritis of unknown cause, manifested by spiking fever, erythematous skin rash, pericarditis, and hepatosplenomegaly.[1] Half of patients given non-steroidal anti-inflammatory drugs or corticosteroids continue to show progressive involvement of increasing number of joints and severe functional disability with striking growth impairment. Moreover, long-term use of systemic corticosteroids leads to various disorders, including iatrogenic Cushing's disease, growth suppression, bone fracture, or cataracts. Sometimes systemic-onset juvenile idiopathic arthritis progresses to macrophage-activation syndrome, in which the inflammation might be caused by cytokine storm;[2–4] therefore, effective and tolerable treatments are much needed.

One major development in rheumatology was the introduction of biological-response modifiers. Tumour necrosis factor α (TNFα) concentrations are increased in serum and synovial fluid of children with juvenile idiopathic arthritis, and concentrations are correlated with disease activity.[5] Etanercept has proven effective in the treatment of children with this type of arthritis, which is resistant to methotrexate.[6] To protect joints from inflammatory destruction, use of biological-response modifiers as early as possible is appropriate in patients with rheumatoid arthritis.[7] These findings also encourage the use of these drugs for treatment of juvenile idiopathic arthritis. However, patients with this type of arthritis have a higher rate of etanercept failure than those with other chronic arthritis subtypes,[8] indicating that TNFα is not the only cytokine implicated in the pathogenesis of the disease. Macrophage-activation syndrome has been reported during treatment with etanercept;[9] therefore inhibition of TNFα does not always prevent this potentially fatal complication.

Although serum concentrations of interleukin 1 are not increased in systemic-onset juvenile idiopathic arthritis, dysregulation of this cytokine might play a part in the pathogenesis.[10] Case reports and an early uncontrolled

study have suggested that treatment with anakinra, an interleukin-1-receptor antagonist, might be effective in patients with this illness,[10–13] but macrophage-activation syndrome still occurred despite treatment with anakinra.[14,15]

The pathogenesis of systemic-onset juvenile idiopathic arthritis remains obscure. However, interleukin 6 and soluble interleukin-6 receptor play a part as inflammatory mediators.[16] The serum interleukin-6 concentrations are related to the extent and severity of joint involvement, fever patterns, platelet counts,[17] growth retardation, and osteoporosis.[18] Transgenic mice with increased expression of human interleukin 6 were growth retarded, as are children with systemic-onset juvenile idiopathic arthritis.[19] The clinical use of tocilizumab, an anti-interleukin-6-receptor monoclonal antibody, in early trials had striking and long-lasting effects on both the systemic and articular manifestations of systemic-onset juvenile idiopathic arthritis, even in patients with severe disease that was refractory to other therapies.[20,21]

In the search to improve treatment of this difficult and debilitating childhood disease, we undertook a placebo-controlled trial of the efficacy and safety of tocilizumab (Chugai Pharmaceuticals, Tokyo, Japan) in systemic-onset juvenile idiopathic arthritis.

Methods

Patients

Patients were eligible if they were 2–19 years of age with disease onset before their 16th birthday and if they met the International League of Associations for Rheumatology classification criteria for systemic-onset juvenile idiopathic arthritis.[22] Treatment with intra-articular corticosteroids, methylprednisolone pulse treatment, immunosuppressive drugs, and disease-modifying antirheumatic drugs (DMARDs)—such as methotrexate, ciclosporin, sulfasalazine, azathioprine, and cyclophosphamide—for 2 weeks before the first administration of tocilizumab was not allowed; and treatment with TNF agents was not allowed for 12 weeks before patients started tocilizumab. Doses of oral corticosteroids had to be stable for 2 weeks before the trial.

Active disease was defined by an increase in C-reactive protein (CRP; ≥15 mg/L) concentrations and an inadequate response to corticosteroids (at ≥0·2 mg/kg prednisolone equivalent) for longer than 3 months. Clinical manifestations were carefully monitored, especially when synovitis was present with other systemic features.

Patients were excluded if they had important concurrent medical or surgical disorders, with leucopenia ($<3·5\times10^9$/L) or thrombocytopenia ($<100\times10^9$/L); cardiac disease (assessed by a paediatric cardiologist before enrolment); or developed macrophage-activation syndrome during the prestudy hospital admission. All patients were examined during screening for active infections, especially pneumonia and tuberculosis, and suspected cases were further examined by chest radiography or computed tomography.

Patients were admitted 2 weeks before the start of the trial and until the completion of the double-blind phase of the study for safety monitoring. Children were cared for by child-life specialists or they went to an in-hospital school or nursery when not accompanied by their family.

The protocols and amendments were approved by the Japanese ministry of welfare, health, and labour and the institutional review boards at every centre. The parent or legal guardian of every child gave written informed consent and the child gave assent when appropriate.

Procedures

This study consisted of three phases—an open-label lead-in phase of 6 weeks, a double-blind, randomised, placebo-controlled phase of 12 weeks, and an open-label extension phase of at least 48 weeks, and was undertaken in eight university hospitals and children's hospitals in Japan. The primary endpoints in the open-label lead-in phase of the study were the proportion of children achieving an American College of Rheumatology Pediatric (ACR Pedi) 30 response and the proportion of those showing improvements in CRP concentrations (<5 mg/L) at the end of the 6-week treatment. All patients were to receive three doses as intravenous infusions of tocilizumab at 8 mg/kg every 2 weeks. The children were assessed for improvement, defined as achievement of the ACR Pedi 30 response—ie, at least three of six ACR Pedi variables improved by at least 30% with no more than one variable worsening by more than 30%.[23] The ACR Pedi variables were physician's and patients'/parents' general assessment on a 10 cm visual-analogue scale, functional ability (childhood health assessment questionnaire, Japanese version, which has been validated and will be published), number of active joints defined by the presence of swelling or, if no swelling is present, restriction of motion accompanied by pain, or tenderness, or both, and number of joints with restriction of movement, and erythrocyte sedimentation rate (ESR). Children were also assessed for ACR Pedi 50 and 70 responses—ie, at least three of six response variables improved by at least 50% and at least 70%, respectively, with no more than one variable worsening by more than 30%.

Patients who completed the open-label lead-in phase and achieved both an ACR Pedi 30 response and CRP concentrations of less than 5 mg/L were randomly assigned to receive an infusion of tocilizumab 8 mg/kg or placebo every 2 weeks (give or take 3 days) for 12 weeks in a double-blind manner. The primary endpoint was the proportion of patients in each treatment group who completed the 12-week period and maintained an ACR Pedi 30 response and CRP concentrations of less than 15 mg/L. Patients who did not maintain an ACR Pedi 30 response or those whose CRP concentrations increased to at least 15 mg/L were withdrawn for rescue medication. Methotrexate, ciclosporin, and other DMARDs and

	Open-label lead-in phase (n=56)			Double-blind phase						Open-label extension phase (n=50)	
	Baseline	6 weeks*	Improvement†	Placebo (n=23)			Tocilizumab (n=20)			48 weeks	Improvement†
				Baseline	6 weeks*	Last observation‡	Baseline	6 weeks*	Last observation‡		
Juvenile idiopathic arthritis core set criteria											
Number of active joints	4·0 (0–39·0)	0 (0–34·0)	73%	4·0 (0–21·0)	0 (0–13·0)	0 (0–34·0)	3·5 (0–18·0)	0 (0–4·0)	0 (0–4·0)	0 (0–4·0)	88%
Number of joints with restricted motion	0·5 (0–47·0)	0 (0–45·0)	54%	0 (0–37·0)	0 (0–41·0)	0 (0–42·0)	0·50 (0–47·0)	0 (0–45·0)	0 (0–46·0)	0 (0–62·0)	72%
Physician's global assessment of disease severity§	52·0 (18·0–100)	8·5 (0–97·0)	75%	51·0 (18·0–95·0)	5·0 (1·0–60)	14·0 (0–84·0)	51·0 (21·0–96·0)	7·50 (0–42·0)	5·50 (0–47·0)	3·5 (0–22·0)	89%
Patient's or parent's global assessment of wellbeing§	53·0 (0–90)	13·5 (0–69·0)	63%	55·0 (18·0–85·0)	10 (1·0–49·0)	39·0 (2·0–94·0)	51·5 (0–76·0)	12·0 (0–63·0)	4·5 (0–34·0)	8·5 (0–70)	75%
Score on childhood health assessment questionnaire¶	0·88 (0–3·00)	0·38 (0–3·00)	43%	0·63 (0–3·00)	0·25 (0–2·75)	0·38 (0–3·00)	0·88 (0–2·38)	0·38 (0–2·63)	0·38 (0–1·63)	0·13 (0–2·13)	67%
Erythrocyte sedimentation rate (mm/h)	44·5 (8·0–125·0)	4·0 (0–64·0)	82%	35·0 (8·0–68·0)	3·0 (1·0–13·0)	11·0 (1·0–41·0)	39·5 (8·0–103·0)	4·0 (0–9·0)	4·0 (0–7·0)	3·0 (0–12·0)	91%
Additional assessments											
Number of patients with total systemic feature score‖	1·0 (0–3·0)	1·0 (0–2·0)	34%	1·0 (0–2·0)	1·0 (0–2·0)	1·0 (0–2·0)	1·0 (0–3·0)	1·0 (0–2·0)	0·5 (0–2·0)	0 (0–1·0)	98%
0	7	23		6	11	8	1	8	10	47	
1	40	27		14	11	12	16	10	9	1	
2	8	6		3	1	3	2	2	1	0	
3	1	0		0	0	0	1	0	0	0	
White-blood-cell count ($\times10^3$ per μL)	12·4 (4·9–30·8)	8·4 (2·5–21·9)	29%	12·8 (6·4–21·0)	8·1 (3·5–14·1)	12·2 (4·4–24·6)	11·2 (4·9–16·1)	6·6 (2·5–10·4)	7·4 (4·1–11·6)	6·4 (3·4–37·4)	36%
C-reactive protein (mg/L)	43·5 (16·0–190·0)	0·5 (0–99·0)	90%	38·0 (17·0–131·0)	0·2 (0–1·0)	15·0 (0–101·0)	35·0 (16·0–190·0)	0·1 (0–1·0)	0·1 (0–21·8)	0·1 (0–2·0)	99%

Data are median (range), unless otherwise indicated. *Measurements at the end of open-label lead-in phase or at withdrawal. †Improvement between baseline and the end of open-label lead-in phase. ‡Measurements at the end of double-blind phase or at withdrawal. §Score on a visual-analogue scale could range from 0 mm (best) to 100 mm (worst). ¶Score could range from 0 (best) to 3 (worst). ‖Systemic feature score includes febrile episode, rheumatoid rash, lymphadenopathy, hepatosplenomegaly, and serositis; score could range from 0 (best) to 8 (worst).

Table 2: **Measurement of disease activity and improvement from baseline**

Role of the funding source

The sponsor of the study supplied the study medication and was responsible for data processing and management, statistical analysis, and reporting of serious adverse events. All authors had full access to the study data on request. The corresponding author had final responsibility to submit the report for publication.

Results

Table 1 summarises the baseline demographic and clinical characteristics of the 56 patients who took part in the open-label and double-blind phases of the study. All patients had onset of systemic-onset juvenile idiopathic arthritis before their 16th birthday (range 6 months to 12 years). All patients had previously received oral corticosteroids. Most patients had previously received at least two DMARDs or immunosuppressive drugs, or both, such as methotrexate and ciclosporin.

Patients had moderate disease activity at entry in the open-label lead-in phase of the study, despite background corticosteroid treatment (table 2) as shown by the ESR, CRP concentrations, systemic feature score (median 1, range 0–3), and fever (>38°C, present in 49 [88%] of 56 patients). The baseline demographic characteristics of patients in the placebo and active treatment groups during the double-blind phase showed minor but not significant differences in baseline disease severity (table 2). The distribution of ACR Pedi 30, 50, and 70 responses at completion of the open-label lead-in phase was similar in the placebo and tocilizumab groups, and median ESR values and CRP concentrations were low and much the same in both groups in the double-blind phase.

Figure 1 shows the trial profile. Six patients were withdrawn during the open-label lead-in phase: three developed anti-tocilizumab IgE antibodies, two had serious adverse events (one anaphylactoid reaction, one gastrointestinal haemorrhage), and one because of absence of efficacy. Six patients did not meet the response criteria for randomisation—CRP concentrations (<5 mg/L) and ACR Pedi 30 response—for the double-blind phase of the study. In the double-blind phase, one patient in the tocilizumab group had to be excluded from the efficacy analysis because the study mask for this patient was broken by

immunosuppressive drugs were not allowed throughout the study, with the exception of stable doses of oral corticosteroids.

ACR Pedi responses, systemic feature score,[24] and CRP concentrations were assessed every 2 weeks. Systemic features assessed were fever, rash, lymphadenopathy in cervical, axillary, and inguinal regions, and hepatomegaly, splenomegaly, and serositis. These features were scored as either absent (0 point) or present (1 point for each one of the eight features) and the score therefore could range from 0 to 8 points. CRP concentrations were measured to assess the disease response as a surrogate marker of interleukin-6 function.

Patients were monitored for safety by routine physical examinations every day during their hospital stay. Urinalysis and blood examinations—white-blood-cell and platelet counts and measurements of haemoglobin, aminotransferases, creatinine, total cholesterol, and ESR—were done every 2 weeks. CRP and ferritin concentrations were measured every week to monitor disease activity and the development of macrophage-activation syndrome. Anti-tocilizumab IgG and IgE antibodies were measured before each administration of tocilizumab. Serum tocilizumab, interleukin 6, and soluble interleukin-6-receptor concentrations were measured every 2 weeks, but results were masked to the investigators and other study personnel during the double-blind phase. Maximum body temperature was recorded daily. Patients who developed anti-tocilizumab antibodies, had grade-3 laboratory test abnormalities according to the National Cancer Institute common terminology criteria for adverse events,[25] or had important safety or compliance difficulties were withdrawn from the study.

In addition to patients who were randomised in the double-blind phase, those who did not meet the criteria for randomisation but completed the open-label lead-in phase with reductions in CRP concentrations were eligible for the open-label extension phase. All eligible patients were to receive tocilizumab 8 mg/kg every 2 weeks for at least 48 weeks. The dosing interval was adjusted according to the disease activity measured by ACR Pedi responses and CRP concentrations and it could be shortened, but not to less than 1 week. In the open-label extension phase, efficacy variables were assessed every 6 weeks and ACR Pedi variables and CRP concentrations were assessed every 2 weeks. The primary endpoint was the proportion of patients achieving an ACR Pedi 30 at the final visit. Corticosteroid-sparing effect was assessed by the reduction in corticosteroid doses, which were allowed to be adjusted during the extension phase. Safety laboratory tests were done every 2 weeks during the first 6 weeks and every 6 weeks thereafter.

All patients who left the study were required to return for a follow-up assessment 2 weeks after discontinuation of treatment. Any adverse events and laboratory abnormalities reported during the study, especially those thought to be drug-related, were followed up until resolution or stabilisation.

Statistical analysis

The method of analysis was by intention to treat. In the double-blind phase, patients were randomly (1 to 1 ratio) assigned to the two treatment groups. A dynamic allocation was done after the balance between the strata (CRP concentrations <30 mg/L or ≥30 mg/L) within the site was checked and for all patients randomly assigned up to that point. Treatment groups were assessed by the exact χ^2 test with a two-tailed significance level of 5%. Each treatment group had to include at least 20 patients to provide 90% power to detect a difference in the proportions of patients achieving both an ACR Pedi 30 response and CRP concentrations of less than 15 mg/L, so that the response was 2 (60%) of 20 patients in the tocilizumab group and 2 (10%) of 20 patients in the placebo group. Time to withdrawal was estimated by the Kaplan-Meier method and assessed by the log-rank test. Secondary endpoints included the time courses of efficacy variables. Comparisons of these variables at each time point were done with a *t* test.

Safety analysis was done for all patients who received at least one dose of the study drug. A last-observation-carried-forward approach was used for missing data for the patients who withdrew early.

This study is registered with ClinicalTrials.gov, numbers NCT00144599 (for the open-label lead-in and double-blind phases) and NCT00144612 (for the open-label extension phase).

	Open-label lead-in phase (n=56)	Double-blind phase	
		Placebo (n=23)	Tocilizumab (n=20)
Patients*			
Male	21 (38%)	8 (35%)	7 (35%)
Female	35 (63%)	15 (65%)	13 (65%)
Age (years)	8·3 (4·4)	9·3 (4·5)	8·0 (4·3)
Age group (years)			
2–5	20 (36%)	5 (22%)	9 (45%)
6–10	19 (34%)	11 (48%)	5 (25%)
11–15	13 (23%)	4 (17%)	5 (25%)
16–19	4 (7%)	3 (13%)	1 (5%)
Age at disease onset (years)†	4·3 (2·6)	5·1 (3·0)	3·9 (2·2)
Disease duration (years)	4·5 (3·6)	4·7 (4·0)	4·6 (3·5)
Number of past treatments‡	2·1 (1·0)	2·0 (1·0)	2·1 (1·0)
Study entry prednisolone-equivalent steroid dose (mg/kg per day)	0·51 (0·36)	0·46 (0·33)	0·42 (0·27)

Data are number (%) or mean (SD). *Total of percentages might not equal 100% because numbers were rounded up or down. †All patients developed the disease before their 16th birthday. ‡Disease-modifying antirheumatic drugs or immunosuppressive agents.

Table 1: Baseline demographic and disease characteristics

mistake and pharmacokinetic data were unexpectedly unmasked; therefore, 43 patients were included in the efficacy analysis. One patient was withdrawn from each treatment group in the double-blind phase because of adverse events (figure 1).

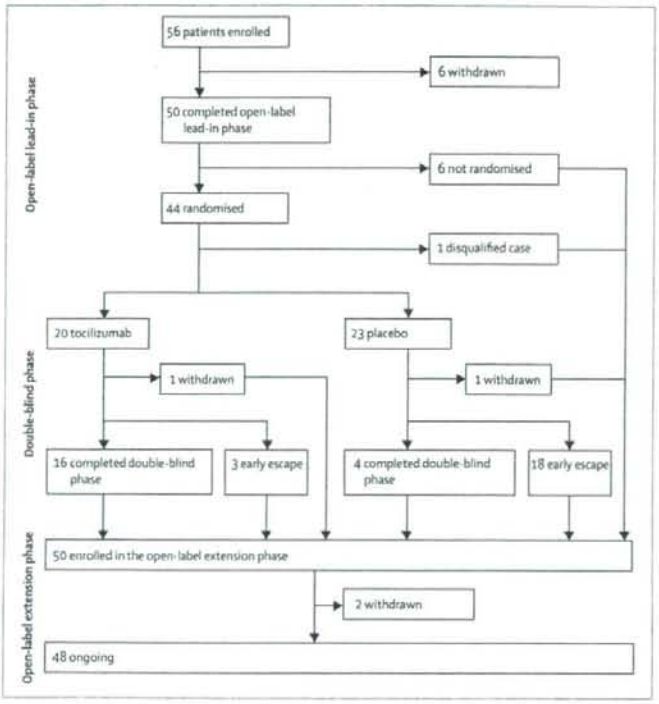

Figure 1: Trial profile

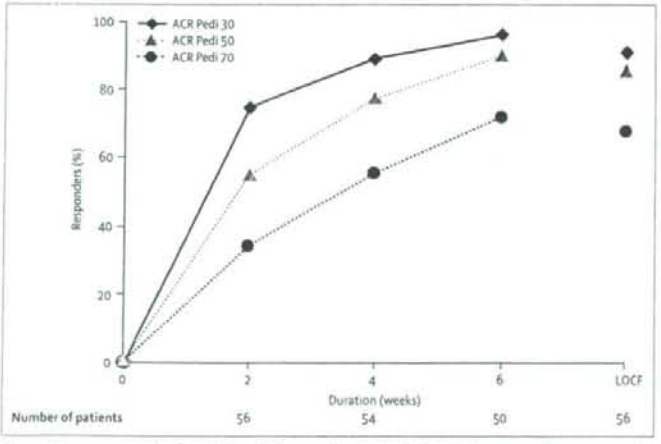

Figure 2: Time courses of American College of Rheumatology Pediatric (ACR Pedi) 30, 50, and 70 responses in initial open-label lead-in phase
LOCF=last observation carried forward.

In the open-label extension phase, patients randomly assigned in the double-blind phase and six patients who were not randomly assigned but completed the open-label lead-in phase were given tocilizumab. These six patients had limited treatment options and showed benefit to some extent in the open-label extension phase. All patients started tocilizumab treatment in the open-label extension phase immediately after they left or completed the initial phases. Two of 50 patients continuing tocilizumab treatment were withdrawn during the open-label extension phase because of adverse events—anaphylactoid reaction in one and development of anti-tocilizumab antibodies in the other.

All 56 patients enrolled in the open-label lead-in phase were included in the efficacy analysis. Figure 2 shows the proportion who had ACR Pedi 30, 50, and 70 responses. At the last observation, the ACR Pedi 30, 50, and 70 response rates were seen in 51 (91%), 48 (86%), and 38 (68%) of 56 patients, respectively. 48 (86%) of 56 patients had an improvement in their actual CRP concentrations to less than 5 mg/L and this reduction took place within 2 weeks of starting tocilizumab. Overall 44 (79%) of 56 patients achieved both an ACR Pedi 30 and CRP concentrations of less than 5 mg/L at week 6. Moreover, every ACR Pedi variable showed a sustained response to tocilizumab treatment. Similar to CRP concentrations, median ESR rapidly fell within 2 weeks, joint counts and childhood health assessment questionnaire decreased by week 4, and general assessments continued to improve until week 6 (table 2). The proportion of patients with the systemic feature score of at least 1 decreased from 49 (88%) of 56 patients to 33 (59%) of 56 patients during the lead-in open-label phase (table 2).

Four (17%) of 23 patients in the placebo group and 16 (80%) of 20 patients in the tocilizumab group ($p<0·0001$) completed the 12-week double-blind phase and maintained an ACR Pedi 30 response and CRP concentrations of less than 15 mg/L. Similarly, four (17%) of 23 patients had an ACR Pedi 50 response and CRP concentrations of less than 15 mg/L in the placebo group compared with 16 (80%) of 20 patients in the tocilizumab group; and three (13%) of 23 patients had an ACR Pedi 70 response and CRP concentrations of less than 15 mg/L in the placebo group versus 15 (75%) of 20 patients in the tocilizumab group. Figure 3 shows the time to early escape. Duration of sustained efficacy was increased in the tocilizumab group compared with the placebo group ($p<0·0001$). The median time to early escape was 4·9 weeks in the placebo group, but longer than 12 weeks in the tocilizumab group.

Both CRP concentrations and ESR remained low in the tocilizumab group, but increased in the placebo group after patients entered the double-blind phase. Median values for both indices on the last observation day were lower in the tocilizumab group than in the placebo (table 2).

Figure 4 shows the changes in ACR Pedi 30, 50, and 70 responses from the end of the open-label lead-in phase to the last study visit of the double-blind phase. More patients in the placebo group than in the tocilizumab group lost their response or had a reduction in their response; patients in the tocilizumab group showed further improvement in ACR Pedi 70 with continued treatment.

Four patients on placebo completed the double-blind phase of the study despite having undetectable serum tocilizumab concentrations 3–5 weeks after randomisation; these patients remained responders at the end of the double-blind phase. Three of these patients had mild disease at study entry, which might have been the reason for their lasting response to tocilizumab treatment during the open-label lead-in phase.

At the 48-week analysis in the open-label extension phase, 48 (96%) of 50 patients were still receiving tocilizumab. Median duration of treatment for the 50 patients from the initial open-label lead-in phase was 61·1 (range 8·7–98·9) weeks; 48 of these patients completed 48-week assessments. The numbers of patients who achieved ACR Pedi 30, 50, and 70 responses at 48 weeks were 47 (98%), 45 (94%), and 43 (90%), respectively. The median absolute change from baseline in ESR at week 48 was −34 (−121 to −7) mm/h and median percentage change was −93·2% (−100·0% to −78·6%). The median absolute change from baseline in CRP concentrations at the same time point was −43·1 (−190·0 to −16·0) mg/L and median percentage change was −99·7% (−100·0% to −95·1%).

Haemoglobin concentrations and platelet counts showed improvement after patients started tocilizumab. Median haemoglobin concentration increased from 111 (range 74–151) g/L at baseline to 124 (73–179) g/L at week 48. Median platelet count decreased from $41·8\times10^{10}$ ($16·8\times10^{10}$ to $86·2\times10^{10}$) per L at baseline to $30·2\times10^{10}$ ($13·1\times10^{10}$ to $55·6\times10^{10}$) per L at week 48. All 48 patients with 48-week efficacy data were given stable doses of oral corticosteroids throughout the initial open-label lead-in and double-blind phases and during the open-label extension phase, 33 (69%) and 22 (46%) were able to reduce their doses by at least 30% and at least 50%, respectively. Figure 5 shows the efficacy responses in each treatment group during the double-blind phase and open-label extension phase. The efficacy response of 21 patients who met the rescue criteria in the double-blind phase improved immediately after they resumed tocilizumab infusion in the extension phase. Patients lost their response to tocilizumab during placebo treatment in the double-blind phase but regained it once tocilizumab treatment was restarted in the open-label extension phase.

No deaths or cases of macrophage-activation syndrome occurred during the lead-in and double-blind phases of the study. Two serious adverse events were reported during the open-label lead-in phase: one anaphylactoid

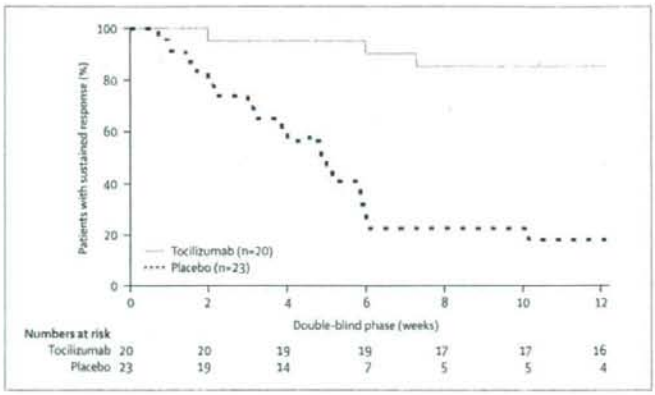

Figure 3: Time course of early escape for rescue medication

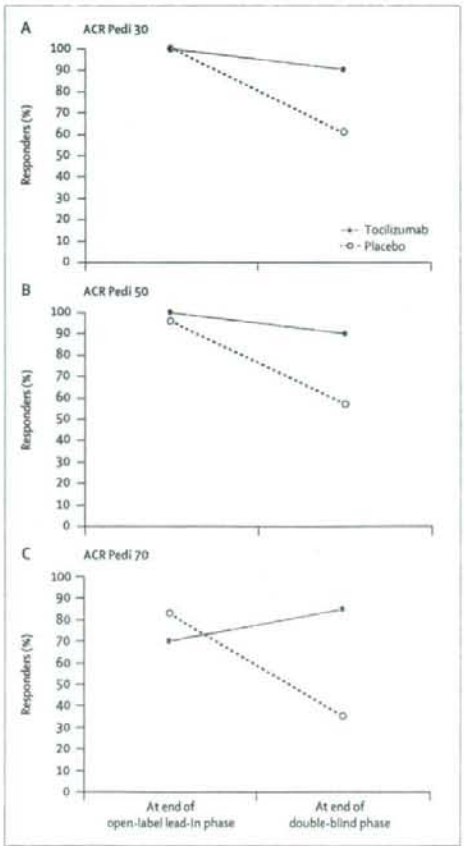

Figure 4: Changes in American College of Rheumatology Pediatric (ACR Pedi) responses from the open-label phase to the double-blind phase