

- cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. *Arthritis Rheum* 2009;60:2232–41.
42. Amara K, Steen J, Murray F, Morbach H, Fernandez-Rodriguez BM, Joshua V, et al. Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition. *J Exp Med* 2013;210:445–55.
 43. Harre U, Georgess D, Bang H, Bozec A, Axmann R, Ossipova E, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest* 2012; 122:1791–802.
 44. Genovese MC, Kavanaugh A, Weinblatt ME, Peterfy C, DiCarlo J, White ML, et al. An oral Syk kinase inhibitor in the treatment of rheumatoid arthritis: a three-month randomized, placebo-controlled, phase II study in patients with active rheumatoid arthritis that did not respond to biologic agents. *Arthritis Rheum* 2011;63:337–45.
 45. Karampetsou MP, Andonopoulos AP, Liossis SN. Treatment with TNF α blockers induces phenotypical and functional aberrations in peripheral B cells. *Clin Immunol* 2011;140:8–17.
 46. Platt AM, Gibson VB, Patakas A, Benson RA, Nadler SG, Brewer JM, et al. Abatacept limits breach of self-tolerance in a murine model of arthritis via effects on the generation of T follicular helper cells. *J Immunol* 2010;185:1558–67.
 47. Schwartzberg PL, Mueller KL, Qi H, Cannons JL. SLAM receptors and SAP influence lymphocyte interactions, development and function. *Nat Rev Immunol* 2009;9:39–46.
 48. De Rycke L, Verhelst X, Kruithof E, Van den Bosch F, Hoffman IE, Veys EM, et al. Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. *Ann Rheum Dis* 2005;64: 299–302.
 49. Emery P, Durez P, Dougados M, Legerton CW, Becker JC, Vratsanos G, et al. Impact of T-cell costimulation modulation in patients with undifferentiated inflammatory arthritis or very early rheumatoid arthritis: a clinical and imaging study of abatacept (the ADJUST trial) [published erratum appears in *Ann Rheum Dis* 2011 Aug;70:1519]. *Ann Rheum Dis* 2010;69:510–6.

CASE REPORT

Successful tocilizumab therapy in seven patients with refractory adult-onset Still's disease

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Abstract

To evaluate the effects of tocilizumab (TCZ) on adult-onset Still's disease (AOSD), we reviewed medical records of seven patients with refractory AOSD treated with TCZ at our institution. TCZ therapy might allow rapid corticosteroid tapering and help maintain remission status, that is, resolution of clinical symptoms and normalization of biomarkers such as CRP and ferritin. Patients, however, should be monitored for the development of macrophage activation syndrome when TCZ is administered for active AOSD.

Keywords

Adult-onset Still's disease, Tocilizumab, Macrophage activation syndrome

History

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Introduction

Adult-onset Still's disease (AOSD) is a systemic inflammatory disease of unknown cause that is characterized by remittent fever, an evanescent salmon pink rash, and polyarthralgia, and is frequently accompanied by neutrophilic leukocytosis [1,2]. AOSD treatments include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and immunosuppressive drugs such as methotrexate (MTX) [3]. Corticosteroids are the mainstay of AOSD therapy, despite their various adverse effects.

Recently, several studies have revealed that pro-inflammatory cytokines are involved in the pathogenesis of AOSD, including IL-1, IL-6, IL-18, tumor necrosis factor (TNF), and interferon- γ [4–6]. In fact, AOSD patients have been treated successfully with anti-cytokine therapies such as TNF- α -blocking agents [7], the IL-1 receptor antagonist anakinra [8], and the anti-IL-6 receptor monoclonal antibody tocilizumab (TCZ) [9–14]. Most patients are refractory to conventional therapies, including high-dose corticosteroids and immunosuppressive drugs (e.g., cyclosporine [CsA] and MTX). TCZ seems to be very effective in treating patients refractory to TNF antagonists and anakinra. Recently, reports on the effectiveness of TCZ have been found, but no standard treatment protocol has been developed. This study evaluated the efficacy of a TCZ regimen for refractory AOSD.

Case reports

We assessed retrospectively a series of seven patients with refractory AOSD who were given TCZ in our Division of Rheumatic Disease between 2008 and 2012. All patients met the classification criteria of AOSD proposed by Yamaguchi et al. "Refractory" AOSD is the condition in which AOSD-related

symptoms such as fever, arthritis, and rash persist despite administration of high-dose corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs), such as CyA and MTX, or biologics other than TCZ, thus leaving no option but increasing the dose of corticosteroids. One of the patients (Table 1, Patient 2) has been described previously [13].

All patients who received at least one infusion of TCZ were evaluated. We analyzed the data obtained at visits immediately before, and 1, 3, and 6 months after TCZ therapy. Improvement in systemic features was defined as the resolution of systemic symptoms. Data on routine laboratory indicators of disease activity, including the hematology profile, erythrocyte sedimentation rate, C-reactive protein (CRP) level, and serum ferritin level, were collected and adverse events were recorded.

The profile and outcome of each patient is summarized in Table 1. Four of the patients were male, and the mean age of onset was 33.4 ± 10.4 (range, 25–68) years. The mean follow-up duration (from the first visit to the hospital until the final observation) was 23.4 ± 18.1 months. All seven patients had a polycyclic systemic/intermittent disease course. The clinical manifestations included fever in all seven patients, arthritis in six, skin rash in six, sore throat in three, lymphadenopathy in two, and pericarditis, blepharitis, and splenomegaly in one patient each. Disseminated intravascular coagulation (DIC) was the major complication in four patients. Prior MTX and CsA therapy had been ineffective in three and two patients, respectively, and TNF- α blockers were ineffective in one patient (Patient 1). Of the seven patients, three were seen when the disease first appeared and four during recurrences.

During the active phase, the CRP was 15.9 ± 10.2 (range, 1.4–27.4) mg/dL and ferritin 12934.8 ± 13218.1 (range, 15.5–41860) ng/mL. High ferritin levels are indicative of MAS activity or of AOSD itself. In the active phase, the inflammation was stabilized with high-dose corticosteroids (two pulses of methylprednisolone 1000 mg/day; one pulse of methylprednisolone 500 mg/day; and then oral prednisone [PSL] 1–2 mg/kg/day); TCZ was started at 8 mg/kg weekly or biweekly, except in Patient 2, in whom the dose

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Table 1. Demographics and clinical characteristics of 7 patients with AOSD.

Patient	Age (years)/sex	Disease duration (years/months)	Clinical manifestations	Previous treatment	Dose (starting dose)	Maintenance therapies	Outcome	Corticosteroid dose	Adverse events	Follow-up (months) ^a
1	26/F	3 years	Fever, arthritis, rash, blepharitis	IFX, ETA, MTX, TCR	8 mg/kg/biweekly	TCZ/8 weeks	Good response	Withdrawal		51
2	58/F	< 1 month	Fever, arthritis, rash, lymphadenopathy, sore throat, DIC		8 mg/kg/monthly 8 mg/kg/biweekly	TCZ/6weeks, PSL 3 mg	Good response	Decreased	CMV ^b , MAS, CD sepsis	43
3	31/M	19 years	Fever, pericarditis, DIC	MTX, Buc, TAC, CsA	8 mg/kg/biweekly	TCZ/5 weeks, PSL 3 mg	Good response	Decreased		30
4	31/M	29 years	Fever, rash, DIC		8 mg/kg/biweekly	TCZ/biweekly, PSL 5 mg	Good response	Decreased	CMV, MAS	4 ^c
5	25/F	5 months	Fever, arthritis, rash, lymphadenopathy, sore throat	CsA	8 mg/kg/biweekly	TCZ/8 weeks, PSL 5 mg	Good response	Decreased		27
6	30/M	11 years	Fever, arthritis, rash, sore throat	MTX	8 mg/kg/biweekly	TCZ/biweekly, PSL 12.5 mg	Good response, death ^d	Decreased		2
7	33/F	< 1 month	Fever, arthritis, rash, lymphadenopathy, splenomegaly, DIC		8 mg/kg/biweekly	TCZ/4 weeks, PSL 5 mg	Good response	Decreased		7

IFX, Infliximab; ETA, etanercept; TCZ, tocilizumab; PSL, prednisolone; CsA, Cyclosporin; MTX, methotrexate; Buc, bucillamine; TAC, tacrolimus; DIC, disseminated intravascular coagulation; MAS, macrophage activating syndrome; CMV, cytomegalovirus infection; CD, *Clostridium difficile*

^aDuration from the first visit to the last visit.

^bIt occurred twice to Patient 2, and both was successfully treated with gancyclovir.

^cPatient 4 transferred to other hospitals.

^dPatient 6 took suicide due to schizophrenia despite that AOSD was stabilized after introducing TCZ.

was administered monthly. The mean CRP level before administering TCZ was 0.84 ± 1.3 (range, 0.0–2.8) mg/dL and the mean ferritin level was 912.0 ± 622.5 (range, 12–2083) ng/mL. One month after TCZ was first administered, the mean CRP and ferritin levels were reduced to 0.0 mg/dL and 851.7 ± 1441.1 (range, 2.2–4320) ng/mL, respectively.

All patients responded well to TCZ during the 2- to 51-month follow-up period (median, 27 months), with TCZ inducing remission with no further flares. In one patient, the corticosteroid treatment was withdrawn. Figure 1 shows the changes in the serum CRP and ferritin levels in the patients given TCZ. In these patients, the mean serum CRP and ferritin levels were almost normalized; these inflammatory markers improved steadily without flares thereafter.

Figure 2 shows the corticosteroid-sparing effect of TCZ. The mean reduced corticosteroid dosage was PSL 26.1 ± 37.8 mg after a median treatment duration of 1 month, which shows a tendency toward a larger corticosteroid-sparing effect. In particular, rapid corticosteroid tapering was achieved in Patient 7; 120 mg/day PSL was reduced to 20 mg/day in only 35 days. Except for Patient 4, who dropped out after transfer to another hospital, and Patient 6, who was schizophrenic and committed suicide, the TCZ dose was maintained with no flares. The mean TCZ dosing interval was 5 ± 2.5 (range, 2–8) weeks at the last visit.

Adverse events occurred in two patients (Table 1), and included two episodes of macrophage activation syndrome (MAS), three of cytomegalovirus infection, which occurred twice in Patient 2, and one of *Clostridium difficile* sepsis. The MAS was resolved with lipodexamethasone (0.2 and 0.1 mg/kg/day in Patients 2 and 4, respectively) and a continuous CsA infusion (2 mg/kg/day) in both patients.

Discussion

The overproduction of pro-inflammatory cytokines is involved in the pathophysiology of AOSD [4–6]. Corticosteroids are believed

to help prevent the exacerbation of AOSD symptoms by nonspecifically suppressing inflammatory cytokines. In fact, most (76–95%) AOSD patients can be treated successfully with corticosteroids [15]. Fitzgerald et al. suggested that the cytokine levels increase as a result of an activation cascade. IL-6, the downstream cytokine, plays a pivotal role in the disease. It is associated with systemic symptoms and correlates with increased serum CRP, ferritin, and leukocyte levels [16].

An increasing number of reports have indicated that TCZ is effective in patients with treatment-resistant AOSD [9–18]. “Inflammation” is a multifaceted biological process, and it is difficult to conclude that TCZ-mediated IL-6 blockade alone can stabilize the inflammation. However, a randomized placebo-controlled trial demonstrated the efficacy of TCZ in systemic juvenile idiopathic arthritis (sJIA), which shares some similarities with AOSD [16]. In the cytokine network, which comprises various cytokines, IL-6 could be the key cytokine; although this has not been established yet. Notably, we observed these improvements despite a 61.6% mean reduction in the corticosteroid dose 1 month after introducing TCZ. However, no standard protocols exist for managing such patients, especially regarding the duration of corticosteroid intake or tapering. To prevent the various adverse effects of corticosteroids, a schedule for the introduction of TCZ therapy and a protocol that allows rapid corticosteroid tapering should be developed.

MAS is one of the serious complications of AOSD [19,20]. It occurs in 12% of AOSD patients [20]. Recently, a number of biological agents have been reported to cause MAS-like features [16,21,22]. MAS of noninfectious etiology has been observed as a complication in several cases of sJIA during TCZ therapy, which was administered at various times in the course of sJIA, and the relationship between TCZ therapy and MAS remains unclear [11]. TCZ might induce MAS via an exacerbation of AOSD or MAS because of a transient increase in the serum levels of the target cytokine immediately after the cytokine blockade [11,13]. Direct

Figure 1. Changes in the serum CRP and ferritin levels in seven patients with TCZ administration.

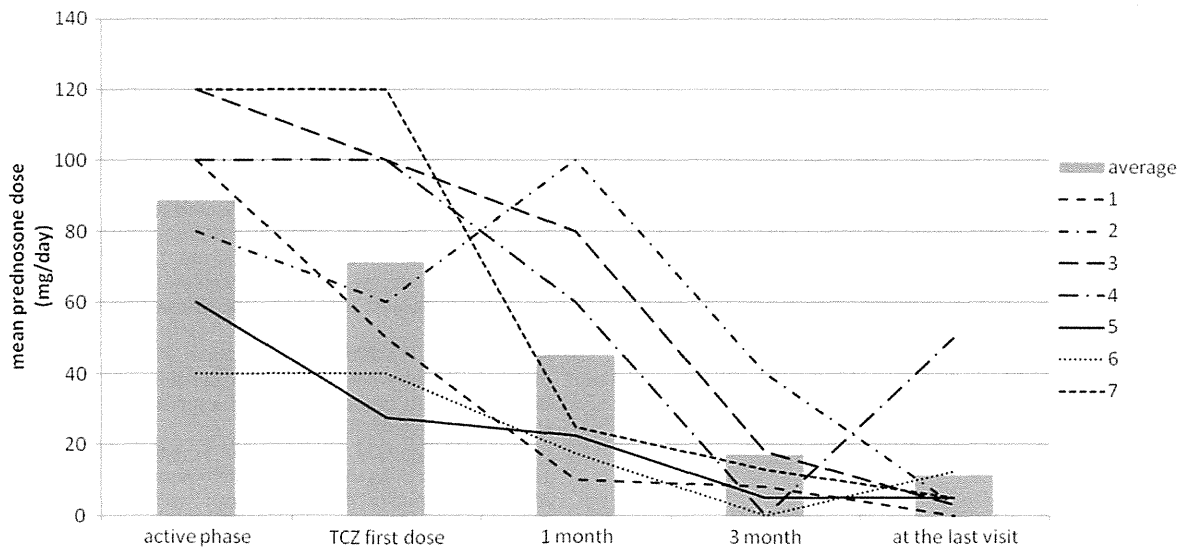
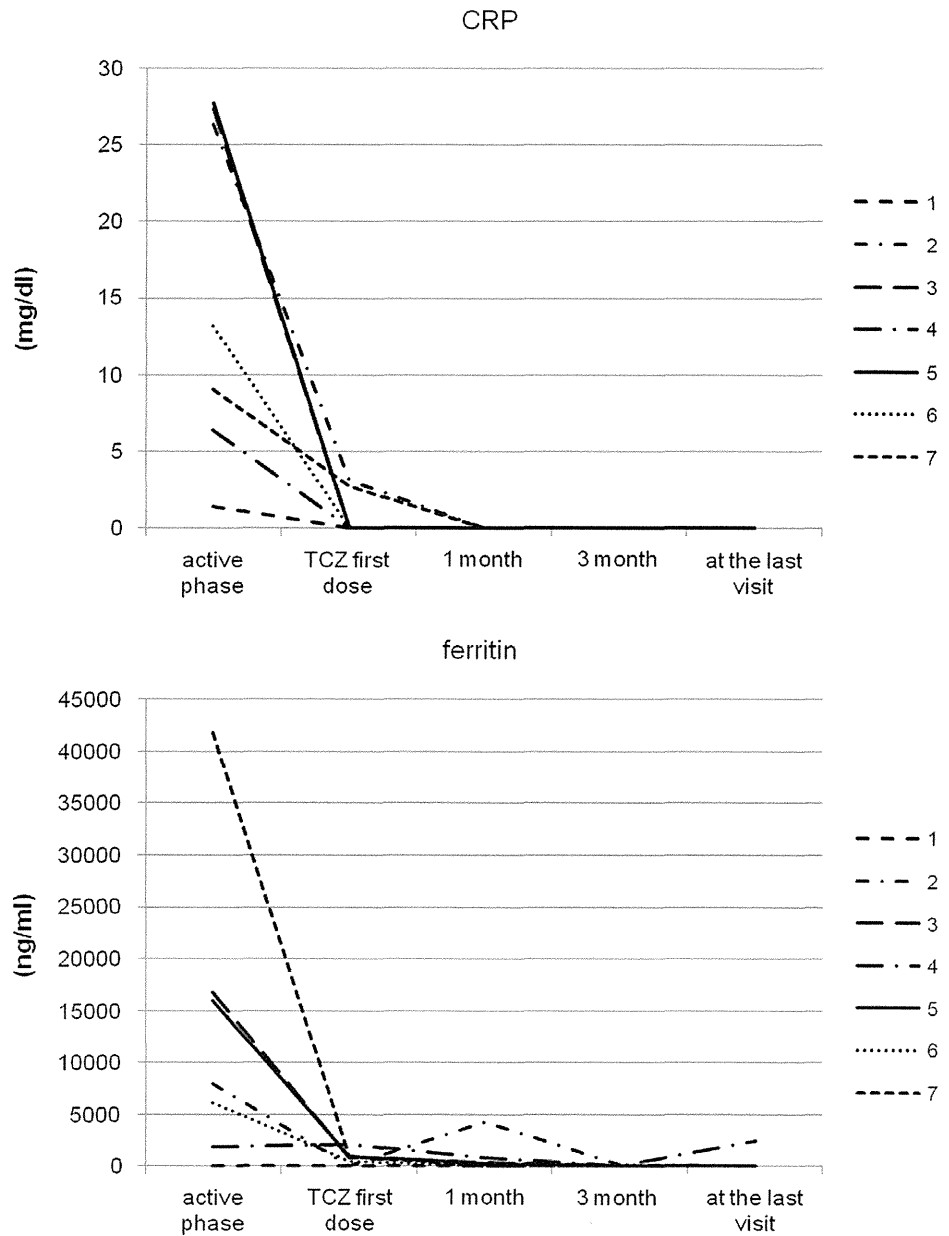


Figure 2. Mean corticosteroid dose and individual dose/day according to TCZ administered.

blockade of the IL-6 receptor also implicates IL-6 in the pathogenesis of AOSD [23]. Another possible reason for the development of MAS during TCZ therapy is aberrant cytokines. During the active phase of AOSD, the number of cytokines being produced is increased. Therefore, inhibiting only IL-6 might lead to an exacerbation of cytokine storms. We speculate that it is risky to treat severe cases with TCZ alone right from the start without suppressing the inflammation with corticosteroids. On the other hand, biweekly infusions of TCZ might be necessary for some AOSD patients during the initial active phase to prevent exacerbation [13]. Moreover, when MAS occurred in our AOSD patients, MAS was suppressed by administering lipodexamethasone and continuous CsA infusion. The mechanism is speculated as follows. CsA inhibits the apoptosis of TNF α by inhibiting the permeability transition of mitochondria, preventing organ- and cell derangement, and vascular endothelial breakdown in MAS [24]. The HLH 2004 protocol recommends that CyA be used concomitantly from an early stage in the cases of AOSD-associated MAS [25]. Liposteroid, an injectable solution of dexamethasone palmitate in adipocyte, is easily and swiftly delivered to the inflammatory site and selectively incorporates in the phagocytes.

Regarding safety, TCZ is well tolerated [26]. Since cytomegalovirus might be reactivated in the highly immunocompromised state induced by the concomitant use of high-dose corticosteroids and TCZ, caution is necessary. To prevent *Pneumocystis jirovecii* infection, all of our patients were administered trimethoprim-sulfamethoxazole.

Patient 7 might serve as a useful reference for the future establishment of a protocol for TCZ therapy for refractory AOSD. One possible protocol is as follows. When the inflammatory response is suppressed by high-dose corticosteroids in the active phase, TCZ therapy is started immediately. Checking for the development of MAS, TCZ is administered every 1–2 weeks while monitoring the inflammatory response, ferritin levels, and clinical symptoms. When the inflammatory response and ferritin levels are maintained stably, the corticosteroids can be tapered off relatively rapidly. When remission is maintained with low-dose corticosteroids, the TCZ dosing interval is extended gradually.

Blockade of the IL-6 receptor has been validated in sJIA and rheumatoid arthritis [27]. However, TCZ is used off-label in AOSD and the optimal duration of TCZ administration remains unclear, but it probably should not be discontinued too soon, as this can lead to relapse. Some authors suggest that the decrease in serum IL-6 observed during TCZ treatment indicates disease remission and might be a guide for discontinuation [23].

Conclusion

We presented seven cases of AOSD that were refractory to corticosteroids, conventional DMARDs, and TNF- α blockers, but were stabilized with TCZ therapy. TCZ therapy for AOSD might enable rapid corticosteroid tapering and maintain remission. Adverse events were improved by prompt, appropriate treatment. Prospective comparative studies are now needed to validate the efficacy and safety of TCZ in this context.

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Conflict of interest

None.

References

- Efthimiou P, Paik PK, Bielory L. Diagnosis and management of adult onset Still's disease. *Ann Rheum Dis*. 2006;65(5):564–72.
- Ohta A, Yamaguchi M, Tsunematsu T, Kasukawa R, Mizushima H, Kashiwagi H, et al. Adult Still's disease: a multicenter survey of Japanese patients. *J Rheumatol*. 1990;17(8):1058–63.
- Aydintug AO, D'Cruz D, Cervera R, Khamashta MA, Hughes GR. Low dose methotrexate treatment in adult Still's disease. *J Rheumatol*. 1992;19(3):431–5.
- Scheinberg MA, Chapira E, Fernandes ML, Hubscher O. Interleukin 6: a possible marker of disease activity in adult onset Still's disease. *Clin Exp Rheumatol*. 1996;14(6):653–5.
- Efthimiou P, Kontzias A, Ward CM, Ogden NS. Adult-onset Still's disease: can recent advances in our understanding of its pathogenesis lead to targeted therapy? *Nat Clin Pract Rheumatol*. 2007;3(6):328–35.
- Chen DY, Lan JL, Lin FJ, Hsieh TY. Proinflammatory cytokine profiles in sera and pathological tissues of patients with active untreated adult onset Still's disease. *J Rheumatol*. 2004;31(11):2189–98.
- Fautrel B, Sibilia J, Mariette X, Combe B; Club Rhumatismes et Inflammation. Tumour necrosis factor alpha blocking agents in refractory adult Still's disease: an observational study of 20 cases. *Ann Rheum Dis*. 2005;64(2):262–6.
- Naumann L, Feist E, Natusch A, Langen S, Krause A, Buttgerit F, Burmester GR. IL-1-receptor antagonist anakinra provides long-lasting efficacy in the treatment of refractory adult-onset Still's disease. *Ann Rheum Dis*. 2010;69(2):466–7.
- de Boysson H, Février J, Nicolle A, Auzary C, Geffray L. Tocilizumab in the treatment of the adult-onset Still's disease: current clinical evidence. *Clin Rheumatol*. 2013;32(1):141–7.
- Tanaka T, Narazaki M, Kishimoto T. Anti-interleukin-6 receptor antibody, tocilizumab, for the treatment of autoimmune diseases. *FEBS Lett*. 2011;585(23):3699–709.
- Sakai R, Nagasawa H, Nishi E, Okuyama A, Takei H, Kurasawa T, et al. Successful treatment of adult-onset Still's disease with tocilizumab monotherapy: two case reports and literature review. *Clin Rheumatol*. 2012;31(3):569–74.
- Suematsu R, Ohta A, Matsuura E, Takahashi H, Fujii T, Horiuchi T, et al. Therapeutic response of patients with adult Still's disease to biologic agents: multicenter results in Japan. *Mod Rheumatol*. 2012;22(5):712–9.
- Kobayashi M, Takahashi Y, Yamashita H, Kaneko H, Mimori A. Benefit and a possible risk of tocilizumab therapy for adult-onset Still's disease accompanied by macrophage-activation syndrome. *Mod Rheumatol*. 2011;21(1):92–6.
- Takahashi Y, Ochi H, Yanai A, Yamashita H, Itoh K, Mimori A. Successful tocilizumab therapy for adult onset Still's disease with intractable conditions over ten years. *Nihon Naika Gakkai Zasshi*. 2010;99(1):130–2.
- Yokota S, Imagawa T, Mori M, Miyamae T, Aihara Y, Takei S, et al. Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. *Lancet*. 2008;371(9617):998–1006.
- Bagnari V, Colina M, Ciancio G, Govoni M, Trotta F. Adult-onset Still's disease. *Rheumatol Int*. 2010;30(7):855–62.
- Ostrowski RA, Tehrani R, Kadanoff R. Refractory adult-onset still disease successfully treated with abatacept. *J Clin Rheumatol*. 2011;17(6):315–7.
- Bartoloni E, Alunno A, Luccioli F, Santoboni G, Gerli R. Successful treatment of refractory adult-onset Still's disease with anti-CD20 monoclonal antibody. *Clin Exp Rheumatol*. 2009;27(5):888–9.
- Maruyama J, Inokuma S. Cytokine profiles of macrophage activation syndrome associated with rheumatic diseases. *J Rheumatol*. 2010;37(5):967–73.
- Arlot JB, Le TH, Marinho A, Amoura Z, Wechsler B, Papo T, Piette JC. Reactive haemophagocytic syndrome in adult-onset Still's disease: a report of six patients and a review of the literature. *Ann Rheum Dis*. 2006;65(12):1596–601.
- Kaneko K, Kaburaki M, Muraoka S, Tanaka N, Yamamoto T, Kusunoki Y, et al. Exacerbation of adult-onset Still's disease, possibly related to elevation of serum tumor necrosis factor-alpha after etanercept administration. *Int J Rheum Dis*. 2010;13(4):e67–9.
- Gianella S, Schaer DJ, Schwarz U, Kurrer M, Heppner FL, Fehr J, Seebach JD. Retinal microangiopathy and rapidly fatal cerebral edema

- in a patient with adult-onset Still's disease and concurrent macrophage activation syndrome. *Am J Hematol.* 2008;83(5):424–7.
23. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Takeuchi T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. *Blood.* 2008;112(10):3959–64.
 24. Mouy R, Stephan JL, Pillet P, Haddad E, Hubert P, Prieur AM. Efficacy of cyclosporine A in the treatment of macrophage activation syndrome in juvenile arthritis: report of five cases. *J Pediatr.* 1996;129(5):750–4.
 25. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2007;48(2):124–31.
 26. Mima T, Nishimoto N. Clinical value of blocking IL-6 receptor. *Curr Opin Rheumatol.* 2009;21(3):224–30.
 27. Nishimoto N, Hashimoto J, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): evidence of clinical and radiographic benefit from an x ray reader-blinded randomised controlled trial of tocilizumab. *Ann Rheum Dis.* 2007;66(9):1162–7.

RAPID COMMUNICATION

Mycophenolate mofetil therapy for rapidly progressive interstitial lung disease in a patient with clinically amyopathic dermatomyositis

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History

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Rapidly progressive interstitial lung disease (ILD) associated with clinically amyopathic dermatomyositis (CADM) is often lethal despite corticosteroid therapy combined with cyclophosphamide and cyclosporine or tacrolimus. Several recent studies have described the treatment of ILD in patients with systemic sclerosis (SSc) or other connective tissue diseases with mycophenolate mofetil (MMF), a non-cytotoxic immunosuppressant [1–9]. To our knowledge, this is the first report of MMF therapy as an alternative to intravenous pulses of cyclophosphamide (IVCY) for rapidly progressive ILD associated with CADM in combination with high-dose corticosteroids and oral tacrolimus.

A 55-year-old female was admitted with a 1-week history of low-grade fever, muscle pain in the thigh, Gottron's papules, and heliotrope rash. The white blood cell count was 3,230/ μ l with 75.9% neutrophils, and C-reactive protein was 0.27 mg/dl (< 0.3 mg/dl). The serum anti-nuclear antibody titre was not elevated and no anti-Jo-1 antibodies were detected. Anti-CADM 140 antibody was positive. She had no muscle weakness and her serum creatine kinase (156 IU/l; normal 45–163 IU/l) and aldolase (6.8 IU/l; normal 2.7–7.5 IU/l) levels were normal. Quadriceps magnetic resonance imaging and electromyography detected muscle inflammation. Slight fine crackles were audible bilaterally. Arterial blood gas analysis showed pH 7.472, P_aO_2 76.4 Torr, and P_aCO_2 34.5 Torr. The serum KL-6 was elevated at 695 U/ml (< 499 U/ml). A chest X-ray revealed faint reticular shadows in both lower lung fields. Chest high-resolution computed tomography (HRCT) showed bilateral subpleural opacity with ground-glass attenuation (GGA) (Fig. 1a).

She was diagnosed with CADM and progressive ILD, and given intravenous pulse methylprednisolone therapy at 1 g/day for 3 days followed by oral prednisolone 1 mg/kg/day (50 mg/day).

After 3 weeks, the muscle pain and skin manifestations had improved, while the chest images had deteriorated gradually with lung shrinkage on chest X-ray and increasing GGA with patchy consolidation on HRCT (Fig. 1b). Biweekly IVCY at 500 mg/body dose and oral tacrolimus were added immediately. The trough serum tacrolimus level was maintained at 8–10 ng/ml. Over the next 13 weeks, she received six sessions of IVCY therapy, the pulmonary lesion extended (Fig. 1c) and hypoxemia requiring oxygen therapy manifested. Intravenous pulse methylprednisolone therapy at 250 mg/day for 3 days was administered followed by prednisolone 30 mg/day. Nevertheless, the chest images and her respiratory condition had not improved 4 weeks after these additional therapies. Furthermore, IVCY could not be continued because of sustained leukocytopenia. We switched from IVCY to oral MMF, and gradual improvement of the hypoxemia and pulmonary lesions was obtained. Thirty-one weeks after starting MMF, when the prednisolone had been tapered to 10 mg/day, the lung GGA and exertional dyspnoea resolved (Fig. 1d) and the serum KL-6 level decreased from 4,837 to 563 U/ml (Fig. 2). During the combination therapy with MMF, she suffered from cytomegalovirus infection, which was successfully treated with ganciclovir. Her ILD and CADM have been well controlled without exacerbation for 2 years.

MMF suppresses lymphocyte proliferation via the inhibition of inosine monophosphate dehydrogenase, a rate-limiting enzyme for the de novo synthesis of guanosine nucleotides, and inhibits fibrosis via direct suppression of fibroblast function.

Recently, MMF has been used to treat ILD in SSc patients. One retrospective study compared 109 MMF-treated patients with 63 patients given other immunosuppressants, and a lower frequency of pulmonary fibrosis and better 5-year survival were seen in the MMF-treated group [7]. Other prospective or retrospective studies documented improved or stabilised pulmonary function tests (PFTs), improved respiratory symptoms, and decreased glucocorticoid doses following MMF treatment [1, 2, 8, 9]. Two small uncontrolled prospective studies of recent-onset SSc-ILD treated

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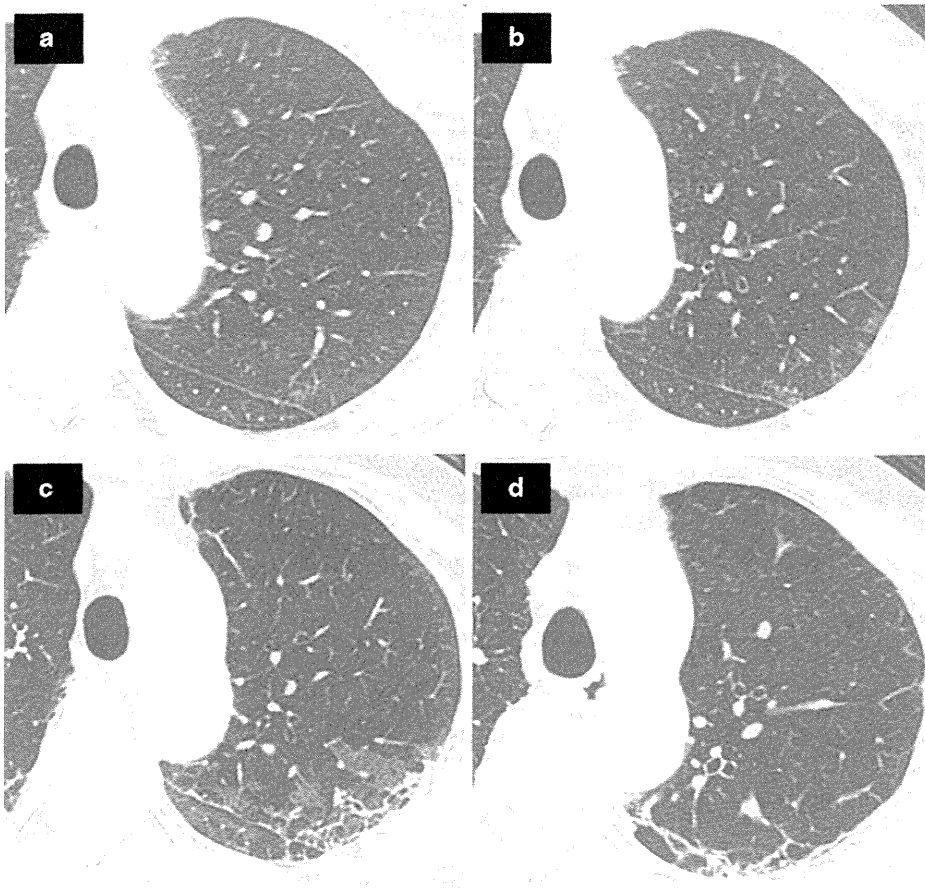


Fig. 1 Gradual improvement of the pulmonary lesions was seen after starting MMF: **a** before treatment; **b** after 3 weeks of glucocorticoid therapy; **c** after 17 weeks of combined intravenous pulses of cyclophosphamide, high-dose corticosteroids, and oral tacrolimus; and **d** 31 weeks after switching the immunosuppressant from cyclophosphamide to MMF

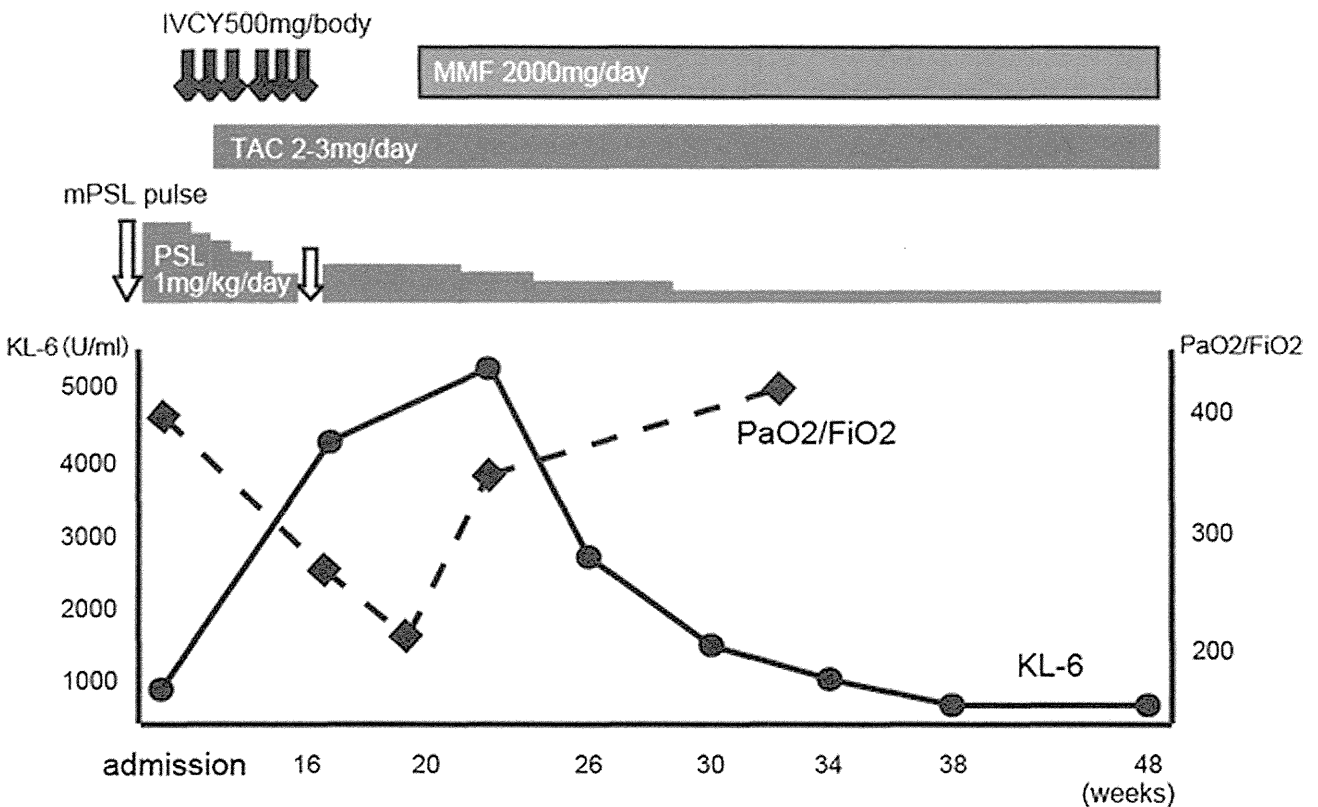


Fig. 2 After starting MMF, gradual improvement of the hypoxemia and decreasing of the serum KL-6 level was obtained. *IVCY* intravenous pulses of cyclophosphamide, *TAC* tacrolimus, *mPSL* methylprednisolone, *PSL* prednisolone

with MMF and glucocorticoids showed improved PFTs, HRCT imaging, and respiratory symptoms in most patients [5, 6]. MMF was well tolerated in those studies.

There are a few reports of MMF therapy for ILD in patients with inflammatory myositis: one case series included two patients with polymyositis [4] and another included one patient with dermatomyositis [3], although the clinical details were unclear. A recent report described successful first-line therapy with MMF and steroid for four patients with dermatomyositis-associated ILD [10]. It was not known whether the ILD in those patients was IVCY resistant.

Our patient had rapidly progressive CADM-associated ILD resistant to combination therapy including IVCY. MMF might be a possible therapeutic option for acute and refractory ILD in CADM patients.

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Conflict of interest

None.

References

1. Gerbino AJ, Goss GH, Molitor JA. Effect of mycophenolate mofetil on pulmonary function in scleroderma-associated interstitial lung disease. *Chest*. 2008;133:455–60.
2. Zamora AC, Wolters PJ, Collard HR, Connolly MK, Elicker BM, Webb WR, et al. Use of mycophenolate mofetil to treat scleroderma-associated interstitial lung disease. *Respir Med*. 2008;102:150–5.
3. Swigris JJ, Olson AL, Fischer A, Lynch DA, Cosgrove GP, Frankel SK, et al. Mycophenolate mofetil is safe, well tolerated, and preserves lung function in patients with connective tissue disease-related interstitial lung disease. *Chest*. 2006;130:30–6.
4. Saketkoo LA, Espinoza LR. Experience of mycophenolate mofetil in 10 patients with autoimmune-related interstitial lung disease demonstrates promising effects. *Am J Med Sci*. 2009;337:329–35.
5. Liossis SN, Bounas A, Andonopoulos AP. Mycophenolate mofetil as first-line treatment improves clinically evident early scleroderma lung disease. *Rheumatology (Oxford)*. 2006;45:1005–8.
6. Vanthuyne M, Blockmans D, Westhovens R, Roufosse F, Cogan E, Coche E, et al. A pilot study of mycophenolate mofetil combined with intravenous methylprednisolone pulses and oral low-dose glucocorticoids in severe early systemic sclerosis. *Clin Exp Rheumatol*. 2007;25:287–92.
7. Nihtyanova SI, Brough GM, Black CM, Denton CP. Mycophenolate mofetil in diffuse cutaneous systemic sclerosis — a retrospective analysis. *Rheumatology*. 2007;46:442–5.
8. Mendoza FA, Nagle SJ, Lee JB, Jimenez SA. A prospective observational study of mycophenolate mofetil treatment in progressive diffuse cutaneous systemic sclerosis of recent onset. *J Rheumatol*. 2012;39:1241–7.
9. Derk CT, Grace E, Shenin M, Naik M, Schulz S, Xiong W. A prospective open-label study of mycophenolate mofetil for the treatment of diffuse systemic sclerosis. *Rheumatology (Oxford)*. 2009;48:1595–9.
10. Morganroth PA, Kreider ME, Werth VP. Mycophenolate mofetil for interstitial lung disease in dermatomyositis. *Arthritis Care Res*. 2010;62:1496–501.

Original article

Identification of novel autoantibodies to GABA_B receptors in patients with neuropsychiatric systemic lupus erythematosusHaruka Tsuchiya^{1,2}, Shiori Haga², Yuko Takahashi¹, Toshikazu Kano¹, Yukihito Ishizaka² and Akio Mimori¹**Abstract**

Objective. The gamma-aminobutyric acid type B receptors (GABA_B) are G-protein coupled receptors for GABA, the main inhibitory neurotransmitter in the brain. We identified GABA_B subunits as candidate antigens in patients with SLE using a random peptide display library. The aim of this study was to investigate the possible link between anti-GABA_B antibodies and disease activity and NPSLE.

Methods. ELISA was performed with recombinant proteins of GABA_{B1b} and GABA_{B2} on serum samples from patients with SLE ($n=88$), scleroderma ($n=20$), myositis ($n=20$) or vasculitis ($n=20$) as well as healthy subjects ($n=20$). Cerebrospinal fluid (CSF) from 23 patients with SLE was also examined.

Results. Autoantibodies to GABA_Bs were exclusive to patients with SLE ($P<0.001$) and positively associated with SLEDAI (anti-GABA_{B1b}, $P=0.001$; anti-GABA_{B2}, $P<0.001$). Of note, autoantibodies were positively linked with NPSLE (anti-GABA_{B1b}, $P=0.02$; anti-GABA_{B2}, $P=0.03$). Moreover, anti-GABA_Bs was detected in 61.5% of CSF samples from patients with active NPSLE, a frequency that was significantly higher than that for patients with non-SLE syndromes.

Conclusion. Anti-GABA_B antibodies could represent novel candidate markers for disease activity and NPSLE.

Key words: systemic lupus erythematosus, neuropsychiatric systemic lupus erythematosus, disease activity, GABA_B receptor, autoantibody.

Introduction

SLE is arguably the most clinically and serologically diverse autoimmune disease, with >100 autoantibodies (anti-double-stranded DNA antibodies and anti-Sm antibodies) found in patients and disease spectra ranging from subtle symptoms to life-threatening multiorgan failure. The hallmark characteristics of SLE, including production of autoantibodies, immune complex depositions and excessive complement activation, are generally

thought to be consequences of immune dysregulation [1–3].

Besides clinical manifestations such as arthritis, nephritis, serositis, blood cytopenias and thrombosis [4], approximately 10–80% of patients with SLE suffer from neuropsychiatric symptoms [5–7]. NPSLE is probably mediated by autoantibodies, microvasculopathy and intracranial production of inflammatory mediators, often in combination. Although several studies have shown a positive correlation between NPSLE and autoantibodies (aPLs, anti-ribosomal P antibodies and autoantibodies that bind to neuronal antigens such as *N*-methyl-D-aspartate glutamate receptor), little is known about how these autoantibodies are involved in disease pathogenesis [8–10].

The gamma-aminobutyric acid type B receptors (GABA_Bs) are G-protein coupled receptors for GABA, the main inhibitory neurotransmitter in the brain. GABA_B is composed of GABA_{B1} and GABA_{B2}

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subunits, sharing a high degree of identity. The GABAR_{B1} subunit, existing as splice variants GABAR_{B1a} and GABAR_{B1b}, differs in two Sushi domains at the N-terminus. GABAR_B is widely expressed in the brain and spinal cord, most highly in the hippocampus, thalamus and cerebellum [11, 12], and GABAergic signalling is related to anxiety-related behaviour, cognitive processing, discrimination of information and sensorimotor gating. Depending on their subcellular localization, GABAR_B exerts distinct regulatory effects on synaptic transmission. Pre-synaptically, GABAR_B inhibits calcium influx by closing voltage-gated calcium channels, thereby regulating neurotransmitter release [13, 14]. Post-synaptically, GABAR_B activates inwardly rectifying potassium channels, leading to hyperpolarization of the postsynaptic membrane [15] and inducing a slow inhibitory postsynaptic current [16]. GABAR_B limits the duration of network high-activity states, thereby preventing excessive neuronal synchronization and allowing new stimuli to break synchronous activity [17, 18].

In this study, using a random peptide display library (RPDL), we identified GABAR_B subunits as candidate antigens in patients with SLE. Based on reactivity with serum and cerebrospinal fluid (CSF) samples from patients with SLE, we looked at the possible link between anti-GABAR_B antibodies and disease activity and NPSLE.

Methods

Patients

All 88 enrolled patients were adults admitted to the National Center for Global Health and Medicine (NCGM) hospital from January 2002 to December 2012. Diagnosis of SLE was based on ACR classification criteria [19]. Clinical data were obtained from medical records. Consent was obtained from the patients for storage of the residual CSF and serum samples that had been originally collected for clinical purposes. Written informed consent was newly obtained from the patients prior to use of the samples for research purposes. The study was approved by the Institutional Review Board of the NCGM, Tokyo (NCGM-G-001292-00).

Data collection

Disease activity was assessed using the SLEDAI [20]. Nephritis was defined as class III, IV or V (proliferative, membranous or membranoproliferative) according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification [21, 22]. NPSLE was divided into 19 neuropsychiatric syndromes classified according to the ACR [23] and diagnosed based on neurological examination, brain/spinal cord MRI, electroencephalogram, CSF, nerve conduction examination, psychiatric interviews and a short battery of neuropsychological tests recommended by the ACR committee [23]. Cognitive function was evaluated in all patients except those with a disturbance of consciousness or poor general condition. Other possible aetiologies of NPSLE, such as infection and drugs, were excluded. APS was

diagnosed based on the Sydney criteria [24]. Laboratory tests included evaluation of complete blood count and immunological markers [complement component 3 (C3)]. Anti-dsDNA antibody titres (normal values <20 IU/ml) and autoantibodies to SSA, Sm and U1-RNP were measured by enzyme immunoassay.

RPDL screening

The FliTrx Random Peptide Library was amplified according to the manufacturer's instructions (Life Technologies, Carlsbad, CA, USA). Briefly, *Escherichia coli* that possesses an RPDL inserted into its flagellar protein was grown at 25°C in IMC medium [1 × M9 salts (6 g Na₂HPO₄, 3 g KH₂PO₄, 0.5 g NaCl and 1 g NH₄Cl for 1 l), 0.2% casamino acids, 0.5% glucose, 1 mM MgCl₂] in the presence of 100 µg/ml ampicillin and then fusion peptides were expressed in the same medium supplemented with 100 µg/ml tryptophan. After washing with a blocking buffer composed of 1% skimmed milk, 150 mM NaCl and 1% α-methyl mannoside, bacteria were reacted for 1 h at room temperature with 150 µl of SLE patients' serum, which was pre-cleaned three times with a bacterial clone that expressed a truncated version of FliTrx protein. Then, protein G sepharose was incubated with pre-cleaned serum for 1 h at room temperature. After centrifugation at 120 g, the pellet was rinsed five times with a washing buffer composed of the IMC medium with 1% α-methyl mannoside and recovered bacteria were vigorously agitated then inoculated into the IMC medium with ampicillin (100 µg/ml). After overnight culture, the same procedures were carried out using the same volume of the patients' serum. After five rounds of biopanning procedures, the enriched library was grown. Then, ~1000 single colonies were picked up, and plasmid DNA from each clone was purified and sequenced (QIAprep Spin Miniprep Kit, Qiagen, Leusden, The Netherlands). After sequencing, the deduced amino acid sequence of each clone was applied to a Basic Local Alignment Search Tool (BLAST) program for searching the homology of proteins. As candidate antigens of GABAR autoantibodies in patients with SLE, we identified peptides composed of amino acids (aa) GGWPGG (aa 63–68), RKLRLH (aa 336–340) and GGRSGVR (aa 371–377) for GABAR_{B1b} and VCPSVT (aa 134–139), LSFAAT (aa 154–159), YQWKRVG (aa 193–199), ASSRHQR (aa 378–384) and VGEYNAV (aa 439–445) for GABAR_{B2}.

Preparation of recombinant proteins of GABAR_{Bs}

We prepared recombinant proteins of the amino-terminal (N) region of GABAR_{Bs}, where epitopes of candidate autoantibodies were identified by RPDL.

Full-length human GABAR_{B1b} cDNA was purchased from the National Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Chiba, Japan. An amino-terminal (N) region of the extracellular domain (aa 1–475) was amplified by PCR and cloned into pcDNA3.1 (–) vector (Life Technologies). The FLAG sequence, consisting of eight aa (DYKDDDDK), was inserted just after the C-terminus. For the GABAR_{B2}

N-terminal extracellular domain (aa 1–480), cDNA with the FLAG sequence inserted just after its N-terminal signal sequence was synthesized using Eurofins MWG Operon (Huntsville, AL, USA) and cloned into pcDNA3.1 (–) vector. FreeStyle 293 cells (Life Technologies) were transfected with plasmid DNA encoding GABAR_{B1b}-FLAG or FLAG-GABAR_{B2} according to the manufacturer's protocol. On day 2 after transfection, supernatants were collected and purified by column chromatography using diethylaminoethyl cellulose.

Detection of anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies in patients' serum and CSF samples

ELISA was performed as previously described [25] and the researchers performing these assays were blinded to the underlying clinical information. Briefly, 96-well plates (Immuno MaxiSorp, Nunc, Roskilde, Denmark) were coated with 250 ng of GABAR_{B1b}-FLAG or FLAG-GABAR_{B2} in bicarbonate buffer (pH 9.6) overnight at 4°C. Wells were washed and blocked with 5% skim milk in Tris-buffered saline Tween 20 [TBS-T; 20 mM Tris-HCl (pH 7.5), 150 mM NaCl and 0.1% Tween 20]. Sera were diluted 1000-fold with 0.25% skim milk in TBS-T and undiluted CSF was added. After incubation for 1 h at room temperature, the wells were rinsed five times with TBS-T and developed with horseradish peroxidase (HRP)-conjugated anti-human IgG antibody. SureBlue TMB was used as an HRP substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) and optical density (OD) at 450 nm was measured. All samples were independently assayed twice. OD values were normalized to samples from one patient. The intra-assay coefficients of variation for anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies were 9.0% and 9.2%, respectively. The cut-off values for positive and negative assignments were determined as mean values + 3 s.d. compared with controls. We defined serum samples as positive when OD values were >0.39 (anti-GABAR_{B1b}) or >0.31 (anti-GABAR_{B2}). We defined CSF samples as positive when OD values were >0.47 (anti-GABAR_{B1b}) or >0.46 (anti-GABAR_{B2}).

Statistical analysis

Statistical analyses were performed using Fisher's exact test to compare frequencies and the Mann-Whitney *U*-test to compare median values. Correlation coefficients (r_s) were calculated as Spearman's rank correlation coefficients. Logistic regression analysis was performed to evaluate the association between anti-GABAR_{B1b} or anti-GABAR_{B2} antibodies and various organ involvements using a backwards stepwise model. Data were analysed using JMP software (SAS Institute, Cary, NC, USA). Statistical significance was defined at $P < 0.05$.

Results

Patient demographics

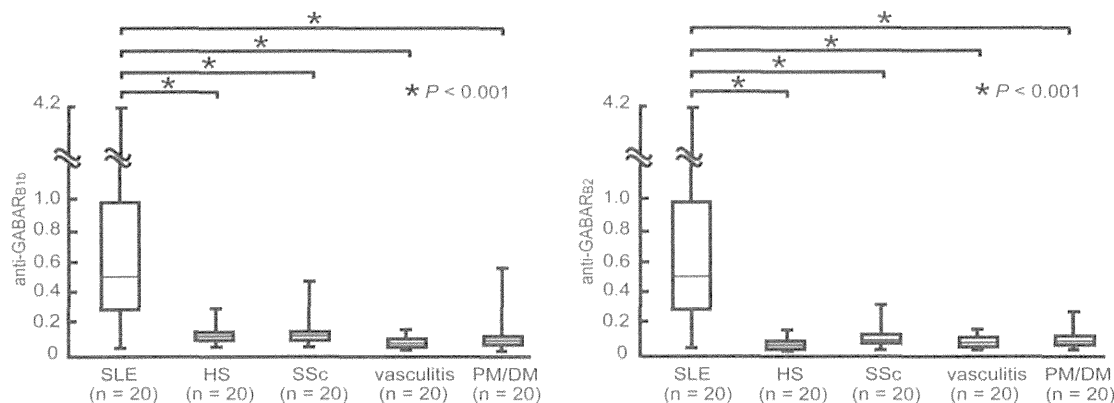
The clinical characteristics of 88 patients with SLE are summarized in supplementary Table S1, available at

Rheumatology Online. The mean age of all patients with SLE was 47 years (s.d. 18) and 90% were female. Fifty patients suffered from symptoms associated with SLE on admission (26 patients were admitted for the first treatment, whereas 24 patients were recurrent and received low- or maintenance-dose steroids and/or immunosuppressants; active SLE). The other 38 patients had no symptoms or abnormal clinical results related to SLE and were being treated with or without low- or maintenance-dose steroids and/or immunosuppressants (chronic SLE). The median SLEDAI score was 10 [interquartile range (IQR) 2–17]. The frequency of each clinical feature was as follows: malar erythema, 22%; arthritis, 16%; serositis, 11%; nephritis, 28%; and NPSLE, 17% [including aseptic meningitis ($n = 4$), cerebrovascular disease ($n = 1$), headache ($n = 2$), movement disorder ($n = 1$), myelopathy ($n = 3$), seizure ($n = 1$), acute confusional state ($n = 2$), other psychiatric syndromes ($n = 2$) and peripheral nervous system disorders ($n = 2$)]. Combined neuropsychiatric forms were observed in two patients with SLE. APS coexisted in 7% of patients. The median dosage of prednisolone was 9 mg/day (IQR 1.8–15) and concurrent immunosuppressant use was reported in 32% of patients. The positive frequency of each autoantibody was as follows: anti-dsDNA, 53%; anti-SSA, 48%; anti-Sm, 48%; and anti-U1 RNP, 55%. Patients with non-SLE syndromes included those with SSC, vasculitis and PM/DM diagnosed using established criteria [26–28]. All patients with non-SLE syndromes were newly diagnosed and had never been treated with steroids and/or immunosuppressants. The mean age and frequency of female patients with non-SLE syndromes were matched with those of patients with SLE.

Measurement of serum anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies in patients with SLE and non-SLE syndrome

First, we analysed the presence of anti-GABAR_B antibodies in 20 randomly selected serum samples from patients who were either active SLE or non-SLE (Fig. 1). The median OD values of serum anti-GABAR_{B1b} antibodies in samples of patients with SLE, SSC, vasculitis and PM/DM were 0.512 (IQR 0.324–0.985), 0.078 (0.063–0.107), 0.051 (0.036–0.061) and 0.07 (0.054–0.09), respectively; the mean OD value of healthy subjects was 0.07 (0.063–0.13). The values for serum anti-GABAR_{B2} antibodies in patients with SLE, SSC, vasculitis and PM/DM were 0.48 (IQR 0.307–0.995), 0.086 (0.066–0.109), 0.055 (0.043–0.071) and 0.079 (0.061–0.118), respectively; the value in healthy subjects was 0.064 (0.046–0.102). The OD values of autoantibodies were significantly different between patients with SLE and non-SLE syndromes ($P < 0.001$), and specificity was confirmed by excluding the FLAG-tag reactivity (supplementary Fig. S1, available at *Rheumatology* Online). These results indicated that autoantibodies against GABAR_Bs were uniquely detectable in patients with SLE.

Fig. 1 Serum antibodies in SLE and non-SLE patients were measured for anti-GABAR_{B1b} (left) and anti-GABAR_{B2} (right) antibodies



* $P < 0.001$.

Comparison of clinical characteristics between serum anti-GABAR_{B1b} and anti-GABAR_{B2} antibody-positive and -negative SLE patients

Next we compared clinical characteristics of patients who were positive or negative for autoantibodies (Table 1). The positive association of the SLEDAI with anti-GABAR_B antibodies was marked (anti-GABAR_{B1b}, $P < 0.001$; anti-GABAR_{B2}, $P < 0.001$). Of note, the frequency of patients with NPSLE was also greater in the autoantibody-positive groups (anti-GABAR_{B1b}, $P < 0.001$; anti-GABAR_{B2}, $P < 0.001$). In the autoantibody-positive groups, the value of anti-dsDNA antibodies increased (anti-GABAR_{B1b}, $P = 0.03$; anti-GABAR_{B2}, $P = 0.002$) and the haemoglobin content decreased (anti-GABAR_{B1b}, $P = 0.007$; anti-GABAR_{B2}, $P < 0.001$). In the anti-GABAR_{B2}-positive group, C3 abundance decreased ($P = 0.01$). Mean age; gender; frequencies of malar erythema, arthritis, serositis, nephritis and APS; leucocyte and platelet counts; median prednisolone dosage; frequency of concurrent immunosuppressant use and antibody positivity rates of anti-SSA, anti-Sm and anti-U1 RNP showed no significant differences between the autoantibody-positive and -negative groups. There were four patients in whom the anti-GABAR_{B2} antibody was the only antibody that could be demonstrated. There were no patients with peripheral nervous system disorders or diffuse psychiatric syndromes except for acute confusional states in the autoantibody-positive groups. No significant differences in other clinical characteristics (age, gender, SLEDAI, treatment at baseline, complete blood cell count, C3, anti-dsDNA antibody, anti-SSA antibody, anti-Sm antibody or anti-U1 RNP antibody) were found between autoantibody-positive and -negative patients with NPSLE (data not shown).

The presence of autoantibodies was assayed in active and chronic patients and titres were significantly higher in patients with active SLE than in chronic patients ($P < 0.001$; supplementary Fig. S2, available at

Rheumatology Online). The titres markedly decreased after immunosuppressive treatment in all eight pairs examined (anti-GABAR_{B1b}, $P = 0.008$; anti-GABAR_{B2}, $P = 0.006$; supplementary Fig. S3, available at *Rheumatology* Online).

Correlations between clinical parameters and serum anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies

Next we determined correlations among autoantibody titres and clinical parameters in 88 patients with SLE (Table 2). A strong correlation was detected between anti-GABAR_{B1b} and anti-GABAR_{B2} antibody titres ($r_s = 0.87$, $P < 0.001$). A moderate correlation was identified between anti-GABAR_B and anti-dsDNA antibodies (anti-GABAR_{B1b}, $r_s = 0.46$; anti-GABAR_{B2}, $r_s = 0.58$; $P < 0.001$). Of note, a significant correlation was detected between anti-GABAR_B antibodies and the SLEDAI (anti-GABAR_{B1b}, $r_s = 0.51$; anti-GABAR_{B2}, $r_s = 0.72$; $P < 0.001$) and between anti-dsDNA antibodies and the SLEDAI ($r_s = 0.65$, $P < 0.001$). A moderate correlation was detected between C3 and anti-GABAR_{B2} ($r_s = -0.45$, $P < 0.001$) or anti-dsDNA ($r_s = -0.58$, $P < 0.001$) and a significant but weak correlation between C3 and anti-GABAR_{B1b} antibodies was detected ($r_s = -0.33$, $P = 0.002$). No strong correlation was found between either of the antibodies and other laboratory markers tested.

Association between serum anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies and various organ involvements and clinical parameters

Logistic regression analysis was performed to evaluate associations between autoantibodies, organ involvements and clinical parameters. Titres of anti-GABAR_B antibodies were the dependent variables. Independent variables included the SLEDAI, NPSLE, leucocytes, haemoglobin, C3 and anti-dsDNA antibodies. The SLEDAI showed the best association with anti-GABAR_B antibodies, with an odds ratio of 1.14 (95% CI 1.05, 1.26, $P = 0.001$) for anti-GABAR_{B1b} antibodies and 1.13 (1.05, 1.24, $P < 0.001$) for

TABLE 1 Comparison of clinical characteristics between serum anti-GABAR_B antibody-positive and -negative SLE patients

Variables	Anti-GABAR _{B1b} antibody			Anti-GABAR _{B2} antibody		
	Positive (n = 14)	Negative (n = 74)	P-value ^a	Positive (n = 18)	Negative (n = 70)	P-value ^a
Age, mean (s.d.), years	41 (12)	48 (19)	0.21	41 (13)	48 (19)	0.12
Gender, n (%), female	14 (100)	65 (88)	0.17	18 (100)	61 (87)	0.11
SLEDAI, median (IQR)	20 (13–30)	7 (1–15)	<0.001**	19 (13–27)	6 (1–15)	<0.001**
Malar erythema, n (%)	4 (29)	15 (20)	0.49	5 (28)	14 (20)	0.47
Arthritis, n (%)	4 (29)	10 (14)	0.16	5 (28)	9 (13)	0.12
Serositis, n (%)	3 (21)	8 (11)	0.27	3 (17)	8 (11)	0.55
Nephritis, n (%)	7 (50)	18 (24)	0.05	8 (44)	17 (24)	0.09
NPSLE, n (%)	8 (57)	7 (9)	<0.001**	9 (50)	6 (9)	<0.001**
Neurological syndromes, n						
Aseptic meningitis	2	2		2	2	
Cerebrovascular disease	1	0		1	0	
Demyelinating syndrome	0	0		0	0	
Headache	2	0		2	0	
Movement disorder	0	1		0	1	
Myelopathy	2	1		3	0	
Seizure	1	0		1	0	
Diffuse psychiatric syndromes, n						
Acute confusional state	2	0		2	0	
Other psychiatric syndromes	0	2		0	2	
Peripheral nervous system disorders	0	2		0	2	
APS, n (%)	1 (7)	5 (7)	>0.99	1 (6)	5 (7)	>0.99
Prednisolone, median (IQR), mg/day	8 (1.3–17.5)	9 (2.5–15)	0.89	6.5 (0–10)	9.5 (5–15)	0.28
Concurrent immunosuppressant, n (%)	2 (14)	26 (35)	0.21	3 (17)	25 (36)	0.16
Leucocytes, median (IQR), × 10 ³ /μl	4.4 (3.3–7.7)	6.0 (4.0–7.5)	0.47	4.0 (3.3–7.3)	6.2 (4.2–7.6)	0.14
Haemoglobin, median (IQR), g/dl	10.3 (9.2–11.1)	12.2 (10.7–12.9)	0.007**	10.8 (9.2–11.1)	12.3 (10.7–13)	<0.001**
Platelets, median (IQR), × 10 ⁴ /μl	16.2 (12.9–18.1)	18.5 (13.6–23.9)	0.25	16.5 (14.1–20.3)	18.5 (13.1–23.6)	0.52
C3, median (IQR), mg/dl	49.1 (35.3–86.5)	76.3 (58.3–101)	0.07	49.1 (34–83.2)	77.3 (60.2–102.5)	0.01*
Anti-dsDNA antibody, median (IQR), IU/ml	63.1 (16.9–328.8)	21.6 (5.1–56.9)	0.03*	111 (22.3–320.5)	17.2 (4.9–48.9)	0.002**
Antibody positivity, n (%)						
Anti-SSA	8 (57)	34 (46)	0.56	10 (56)	32 (46)	0.60
Anti-Sm	10 (71)	32 (43)	0.08	12 (67)	30 (43)	0.11
Anti-U1 RNP	9 (64)	39 (53)	0.56	11 (61)	37 (53)	0.60

^aMann-Whitney *U*-test *P*-value. **P* < 0.05 and ***P* < 0.01 indicate significant differences in the positive and negative groups, respectively.

TABLE 2 Correlation coefficients between the OD values of anti-GABAR_B and anti-dsDNA antibodies and clinical parameters

Variables	Anti-GABAR _{B1b} antibody		Anti-GABAR _{B2} antibody		Anti-dsDNA antibody	
	r_s^a	P-value	r_s^a	P-value	r_s^a	P-value
vs SLEDAI	0.51	<0.001**	0.72	<0.001**	0.65	<0.001**
vs anti-dsDNA	0.46	<0.001**	0.58	<0.001**	—	—
vs anti-GABAR _{B1b}	—	—	0.87	<0.001**	0.46	<0.001**
vs anti-GABAR _{B2}	0.87	<0.001**	—	—	0.58	<0.001**
vs C3 value	-0.33	0.002**	-0.45	<0.001**	-0.58	<0.001**
vs leucocyte count	-0.21	0.046*	-0.36	0.001**	-0.4	<0.001**
vs haemoglobin count	-0.25	0.02*	-0.34	0.001**	-0.29	0.007**
vs platelet count	-0.14	0.2	-0.15	0.16	-0.09	0.41

^aCorrelation coefficient estimated by Spearman's rank correlation coefficient. * $P < 0.05$ and ** $P < 0.01$ indicate significant differences between the autoantibody titres with clinical parameters.

anti-GABAR_{B2} antibodies. For the association of NPSLE with antibodies the odds ratio was 5.51 (95% CI 1.26, 24.74, $P = 0.02$) for anti-GABAR_{B1b} antibodies and 4.64 (1.16, 19.13, $P = 0.03$) for anti-GABAR_{B2} antibodies.

Detection of anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies in the CSF of patients with NPSLE

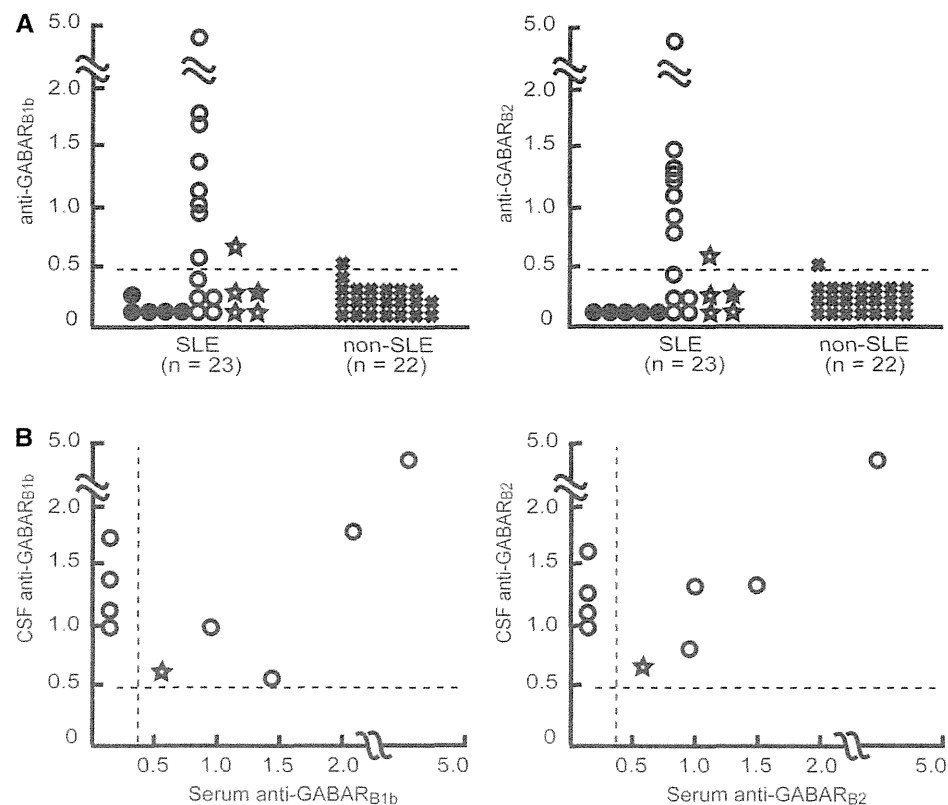
Thirteen of the 23 SLE samples examined were from patients with active NPSLE (Fig. 2A), while 22 CSF control samples from patients with non-SLE syndromes, including SSc ($n = 5$), vasculitis ($n = 12$) and PM/DM ($n = 5$), were examined. The mean age and frequency of females in the non-SLE group were matched with those of patients in the SLE group. Nine samples in the SLE group, including eight samples from patients with active NPSLE, were positive for anti-GABAR_{B1b} and anti-GABAR_{B2} antibodies (39.1%), whereas only one sample in the non-SLE group was positive (4.5%). CSF anti-GABAR_B antibodies and the clinical characteristics of 13 patients with active NPSLE are summarized in Table 3. Neuropsychiatric symptoms in patients with anti-GABAR_B antibodies included acute confusional state (patient 1), visual field defect (patient 2), meningitis (patients 3, 4, 5 and 8), myelitis (patients 4 and 5), brainstem findings (patient 6), chorea (patient 7) and aphasia (patient 7). Patients without anti-GABAR_B antibodies presented with cranial neuropathy (patient 9), psychosis (patients 10 and 12) and mononeuropathy (patients 11 and 13). The IgG index, which is one of the markers of IgG production in the central nervous system, increased (>0.8) in seven of eight patients with CSF anti-GABAR_B antibodies. Interestingly, we observed that four of nine patients with SLE (patients 5–8 in Table 3) were positive for anti-GABAR_B antibodies in CSF but not in serum (Fig. 2B).

Discussion

In 15–20% of SLE patients we detected serum autoantibodies against GABAR_Bs that were associated with disease activity. First, the SLEDAI in the patients who were

positive for serum anti-GABAR_B antibodies significantly increased (Table 1). Second, the SLEDAI was the most significantly associated clinical parameter with serum anti-GABAR_B antibodies, and anti-dsDNA antibodies, which have been reported as both diagnostic and disease activity markers for SLE [29], significantly correlated with serum anti-GABAR_B antibodies (Table 2). Finally, therapeutic intervention significantly decreased serum anti-GABAR_B antibody titres in all eight patient pairs examined (supplementary Fig. S3, available at *Rheumatology* Online). Taken together with observations of a significant difference in serum anti-GABAR_B antibody titres in patients with active and chronic SLE (supplementary Fig. S2, available at *Rheumatology* Online), our data suggest that serum anti-GABAR_B antibodies could be a novel biomarker for the disease activity of SLE. Although anti-GABAR_B antibodies have been detected in patients with limbic encephalitis and have been reported to block receptor function [30, 31], our study is the first to show that serum anti-GABAR_B antibodies are present in patients with SLE and have significant correlations with disease activity.

Although GABA is known purely as an inhibitory neurotransmitter, receptors and enzymes involved in GABA metabolism and catabolism have been shown to be widely distributed outside the brain, notably in the immune system. GABAR_B is expressed on haematopoietic progenitor and stem cells (HSPCs), neutrophil granulocytes and peripheral blood mononuclear cells (PBMCs) and has recently been reported to influence neutrophil chemotaxis, HSPC locomotion and cytokine production by PBMCs [32]. GABA primarily has an inhibitory effect on the immune system, although the effect of activating GABAR_B seems to be more complex and includes immune stimulation [33]. One plausible explanation of our findings that serum anti-GABAR_B antibodies significantly correlated with disease activity of SLE is that autoantibodies have a disruptive effect on GABA-mediated immune response.

Fig. 2 CSF from SLE and non-SLE patients were measured for anti-GABAR_{B1b} (left) and anti-GABAR_{B2} (right) antibodies

Bars indicate cut-off values. (A) The non-SLE subjects included 22 patients with SSc, vasculitis and PM/DM. Open circles (○) represent patients with active NPSLE; filled circles (●) represent patients with chronic NPSLE; open stars (☆) represent patients with active SLE (except for NPSLE); filled stars (★) represent patients with chronic SLE (except for NPSLE), and cross marks (×) represent patients with non-SLE. Active SLE (NPSLE) patients were defined as patients admitted for a first treatment or recurrence. Chronic SLE (NPSLE) patients were defined as patients who had no symptoms or abnormal clinical results related to SLE, treated with or without low-dose steroids and/or immunosuppressants. (B) Comparison between serum and CSF anti-GABAR_B antibodies in nine patients who were positive for the CSF autoantibodies.

Intriguingly, the current study demonstrated that serum anti-GABAR_B antibodies are positively linked with NPSLE and that autoantibodies were present in 61.5% of CSF samples from patients with active NPSLE. Although our sample size was small and further study will be required, our results suggest that anti-GABAR_B antibodies have specificity for some neuropsychiatric manifestations. For instance, in serum or CSF anti-GABAR_B antibody-positive groups there were no patients with peripheral nervous system disorders or diffuse psychiatric syndromes except for acute confusional states.

Blood-brain barrier (BBB) permeability increases in patients with SLE, particularly in those with neuropsychiatric manifestations [34, 35]. Abbott *et al.* [36] described the following two main mechanisms for BBB damage in SLE: microthrombi in cerebral vessels and immune-mediated attack of the endothelium. Thus the presence of anti-GABAR_B antibodies in CSF may be the result of a

transfer of antibodies from the blood into intrathecal regions. However, four patients with NPSLE were positive for anti-GABAR_B antibodies in the CSF but negative in serum, and the IgG index increased in those patients. These results imply that anti-GABAR_B antibodies could be produced within the central nervous system, consistent with previous studies showing that intrathecal IgG production increased in patients with NPSLE [34, 37].

Given the wide variety of clinical manifestations of NPSLE, it is unlikely that a single pathogenic mechanism is responsible for all of them. In addition to autoantibodies, there is evidence to support the notion that proinflammatory cytokines and chemokines, which have been identified in the CSF of patients in the SLE group with neuropsychiatric disease, have a pathogenic role [38–40]. With respect to autoantibodies, the present data suggest that aPL antibodies cause focal neuropsychiatric diseases (stroke or seizures) by promoting

TABLE 3 CSF anti-GABAR_B antibodies and clinical characteristics of 13 patients with active NPSLE

Patient	Age/gender	Neuropsychiatric symptoms	CSF		Serum		
			Anti-GABAR _{B1b} / GABAR _{B2}	Anti-GABAR _{B2}	Anti-GABAR _{B1b} / GABAR _{B2}	IgG index	
CSF anti-GABAR _B positive							
1	31/F	Acute confusional state	4.99/4.70		3.57 ^a /2.72 ^a	1.09 ^b	
2	31/F	Visual field defect	0.50/0.67		1.46 ^a /0.96 ^a	0.2	
3	36/F	Meningitis	0.93/1.29		1.00 ^a /1.00 ^a	1.06 ^b	
4	40/F	Meningitis, myelitis	1.67/1.28		2.35 ^a /1.54 ^a	2.08 ^b	
5	49/M	Meningitis, myelitis	1.15/0.93		0.07/0.10	1.26 ^b	
6	18/F	Brainstem findings— impaired consciousness, diplopia, nystagmus, ataxia	1.38/1.28		0.15/0.22	1.22 ^b	
7	33/F	Chorea, aphasia	1.75/1.47		0.10/0.08	0.97 ^b	
8	35/F	Meningitis	1.00/1.08		0.09/0.10	1.11 ^b	
CSF anti-GABAR _B negative							
9	66/F	Cranial neuropathy	0.10/0.12		0.06/0.08		
10	45/F	Psychosis	0.12/0.09		0.04/0.05		
11	32/F	Mononeuropathy	0.37/0.19		0.12/0.10		
12	24/F	Psychosis	0.26/0.23		0.08/0.12		
13	67/F	Mononeuropathy	0.08/0.06		0.28/0.30		

IgG index = (CSF IgG/CSF albumin)/(serum IgG/serum albumin). ^aSerum anti-GABAR_B antibodies positive. ^bIgG index >0.8.

intravascular thrombosis [6]. In contrast, anti-ribosomal P antibodies [41, 42], and possibly anti-NR2 antibodies [43, 44], cause diffuse neuropsychiatric events (psychosis, depression and cognitive impairment) via a direct effect on neuronal cells. It is possible that a combination of biomarkers reflecting different components of the pathogenic model of NPSLE will best explain clinical events. Anti-GABAR_B antibodies could play a pathogenic role in NPSLE.

Despite the novel aspects of our study, several limitations should be considered. First, a limited number of patients with NPSLE were included, which could raise concerns about the generalization of our results. In addition, access to CSF samples in our study was infrequent and restricted to situations when a lumbar puncture was clinically indicated. Second, clinical data were retrospectively obtained, meaning that some underestimation in the clinical manifestations may be present. Third, we did not perform an in-depth screening for neuropsychiatric manifestations among patients lacking histories and could not exclude that they had mild or subclinical neuropsychiatric manifestations. Finally, the procedure detected autoantibodies towards only the ectodomain of GABAR_Bs and may accordingly have excluded antibodies against epitopes on the GABAR_Bs.

Nevertheless, as far as we know, this is the first study that demonstrates the presence of anti-GABAR_B antibodies in serum and CSF from SLE patients and suggests the positive link between anti-GABAR_B antibodies and disease activity and NPSLE.

Conclusions

In conclusion, anti-GABAR_B antibodies could represent novel candidate markers for disease activity and NPSLE.

Further studies will shed light on the biological activity of anti-GABAR_B antibodies on GABA-mediated signalling neuronal cells.

Rheumatology key messages

- Anti-gamma-aminobutyric acid type B receptor (anti-GABAR_B) antibodies are present in serum and cerebrospinal fluid from SLE patients.
- Anti-GABAR_B antibodies could represent novel candidate markers for disease activity and NPSLE.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

- 1 Hahn BH. An overview of the pathogenesis of systemic lupus erythematosus. In: Wallace DJ, Hahn BH, eds.

- Dubois' Lupus Erythematosus. Philadelphia: Williams and Wilkins, 1993:69–76.
- 2 Winchester RJ. Systemic lupus erythematosus pathogenesis. In: Koopman W, ed. *Arthritis and Allied Conditions*. Philadelphia: Williams and Wilkins, 1996:1361–91.
 - 3 Arbuckle MR, McClain MT, Rubertone MV *et al*. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003;349:1526–33.
 - 4 Edworthy S. Clinical manifestations of systemic lupus erythematosus. In: Harris ED, Budd RC, Firestein GS, eds. *Kelly's Textbook of Rheumatology*. Philadelphia: Elsevier Saunders, 2004:1204–15.
 - 5 Ainiala H, Loukkola J, Peltola J *et al*. The prevalence of neuropsychiatric syndromes in systemic lupus erythematosus. *Neurology* 2001;57:496–500.
 - 6 Sanna G, Bertolaccini ML, Cuadrado MJ *et al*. Neuropsychiatric manifestations in systemic lupus erythematosus: prevalence and association with antiphospholipid antibodies. *J Rheumatol* 2003;30:985–92.
 - 7 Loukkola J, Laine M, Ainiala H *et al*. Cognitive impairment in systemic lupus erythematosus and neuropsychiatric systemic lupus erythematosus: a population-based neuropsychological study. *J Clin Exp Neuropsychol* 2003;25:145–51.
 - 8 Reichlin M. Ribosomal P antibodies and CNS lupus. *Lupus* 2003;12:916–8.
 - 9 Kurki P, Helve T, Dahl D *et al*. Neurofilament antibodies in systemic lupus erythematosus. *J Rheumatol* 1986;13:69–73.
 - 10 Williams RC Jr, Sugiura K, Tan EM. Antibodies to microtubule-associated protein 2 in patients with neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum* 2004;50:1239–47.
 - 11 Enna SJ, Bowery NG. GABA(B) receptor alterations as indicators of physiological and pharmacological function. *Biochem Pharmacol* 2004;68:1541–8.
 - 12 Emson PC. GABA(B) receptors: structure and function. *Prog Brain Res* 2007;160:43–57.
 - 13 Ladera C, del Carmen Godino M, Jose Cabanero M *et al*. Pre-synaptic GABA receptors inhibit glutamate release through GIRK channels in rat cerebral cortex. *J Neurochem* 2008;107:1506–17.
 - 14 Kaneda K, Tachibana Y, Imanishi M *et al*. Down-regulation of metabotropic glutamate receptor 1 α in globus pallidus and substantia nigra of parkinsonian monkeys. *Eur J Neurosci* 2005;22:3241–54.
 - 15 Nicoll RA. My close encounter with GABA(B) receptors. *Biochem Pharmacol* 2004;68:1667–74.
 - 16 Kaneda K, Kita H. Synaptically released GABA activates both pre- and postsynaptic GABA(B) receptors in the rat globus pallidus. *J Neurophysiol* 2005;94:1104–14.
 - 17 Mann EO, Kohl MM, Paulsen O. Distinct roles of GABA(A) and GABA(B) receptors in balancing and terminating persistent cortical activity. *J Neurosci* 2009;29:7513–8.
 - 18 Brown JT, Davies CH, Randall AD. Synaptic activation of GABA(B) receptors regulates neuronal network activity and entrainment. *Eur J Neurosci* 2007;25:2982–90.
 - 19 Tan EM, Cohen AS, Fries JF *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
 - 20 Bombardier C, Gladman DD, Urowitz MB *et al*. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992;35:630–40.
 - 21 Weening JJ, D'Agati VD, Schwartz MM *et al*. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 2004;15:241–50.
 - 22 Weening JJ, D'Agati VD, Schwartz MM *et al*. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004;65:521–30.
 - 23 The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 1999;42:599–608.
 - 24 Miyakis S, Lockshin MD, Atsumi T *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006;4:295–306.
 - 25 Takahashi Y, Haga S, Ishizaka Y *et al*. Autoantibodies to angiotensin-converting enzyme 2 in patients with connective tissue diseases. *Arthritis Res Ther* 2010;12:R85.
 - 26 Hunder GG, Arend WP, Bloch DA *et al*. The American College of Rheumatology 1990 criteria for the classification of vasculitis. *Arthritis Rheum* 1990;33:1065–144.
 - 27 Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* 1975;292:344–7.
 - 28 Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980;23:581–90.
 - 29 Lefkowitz JB, Kiehl M, Rubenstein J *et al*. Heterogeneity and clinical significance of glomerular-binding antibodies in systemic lupus erythematosus. *J Clin Invest* 1996;15:1373–80.
 - 30 Graus F, Saiz A, Dalmau J. Antibodies and neuronal autoimmune disorders of the CNS. *J Neurol* 2010;257:509–17.
 - 31 Lancaster E, Lai M, Peng X *et al*. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol* 2010;9:67–76.
 - 32 Duthey B, Hübner A, Diehl S *et al*. Anti-inflammatory effects of the GABA(B) receptor agonist baclofen in allergic contact dermatitis. *Exp Dermatol* 2010;19:661–6.
 - 33 Jin Z, Mendu SK, Birnir B. GABA is an effective immunomodulatory molecule. *Amino Acids* 2013;45:87–94.
 - 34 Winfield JB, Shaw M, Silverman LM *et al*. Intrathecal IgG synthesis and blood-brain barrier impairment in patients with systemic lupus erythematosus and central nervous system dysfunction. *Am J Med* 1983;74:837–44.
 - 35 McLean BN, Miller D, Thompson EJ. Oligoclonal banding of IgG in CSF, blood-brain barrier function, and MRI findings in patients with sarcoidosis, systemic lupus erythematosus, and Behçet's disease involving the

- nervous system. *J Neurol Neurosurg Psychiatry* 1995;58: 548-54.
- 36 Abbott NJ, Mendonca LL, Dolman DE. The blood-brain barrier in systemic lupus erythematosus. *Lupus* 2003;12: 908-1.
- 37 Hirohata S, Hirose S, Miyamoto T. Cerebrospinal fluid IgM, IgA, and IgG indexes in systemic lupus erythematosus: their use as estimates of central nervous system disease activity. *Arch Intern Med* 1985;145: 1843-6.
- 38 Dellalibera-Joviliano R, Dos Reis ML, Cunha Fde Q *et al.* Kinins and cytokines in plasma and cerebrospinal fluid of patients with neuropsychiatric lupus. *J Rheumatol* 2003; 30:485-92.
- 39 Trysberg E, Carlsten H, Tarkowski A. Intrathecal cytokines in systemic lupus erythematosus with central nervous system involvement. *Lupus* 2000;9:498-503.
- 40 Fragoso-Loyo H, Richaud-Patin Y, Orozco-Narváez A *et al.* Interleukin-6 and chemokines in the neuropsychiatric manifestations of systemic lupus erythematosus. *Arthritis Rheum* 2007;56:1242-50.
- 41 Arnett FC, Reveille JD, Moutsopoulos HM *et al.* Ribosomal P autoantibodies in systemic lupus erythematosus. Frequencies in different ethnic groups and clinical and immunogenetic associations. *Arthritis Rheum* 1996;39: 1833-9.
- 42 Karassa FB, Afeltra A, Ambrozic A *et al.* Accuracy of anti-ribosomal P protein antibody testing for the diagnosis of neuropsychiatric systemic lupus erythematosus: an international meta-analysis. *Arthritis Rheum* 2006;54: 312-24.
- 43 DeGiorgio LA, Konstantinov KN, Lee SC *et al.* A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nat Med* 2001;7:1189-93.
- 44 Kowal C, DeGiorgio LA, Nakaoka T *et al.* Cognition and immunity; antibody impairs memory. *Immunity* 2004;21: 179-88.

LUPUS AROUND THE WORLD

A nationwide study of SLE in Japanese identified subgroups of patients with clear signs patterns and associations between signs and age or sex

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We performed a nationwide study to determine the distributions of the signs and clinical markers of systemic lupus erythematosus (SLE) and identify any patterns in their distributions to allow patient subclassification. We obtained 256,999 patient-year records describing the disease status of SLE patients from 2003 to 2010. Of these, 14,779 involved patients diagnosed within the last year, and 242,220 involved patients being followed up. Along with basic descriptive statistics, we analyzed the effects of sex, age and disease duration on the frequencies of signs in the first year and follow-up years. The patients and major signs were clustered using the Ward method. The female patients were younger at onset. Renal involvement and discoid eczema were more frequent in males, whereas arthritis, photosensitivity and cytopenia were less. Autoantibody production and malar rash were positively associated with young age, and serositis and arthritis were negatively associated. Photosensitivity was positively associated with a long disease duration, and autoantibody production, serositis and cytopenia were negatively associated. The SLE patients were clustered into subgroups, as were the major signs. We identified differences in SLE clinical features according to sex, age and disease duration. Subgroups of SLE patients and the major signs of SLE exist. *Lupus* (2014) 23, 1435–1442.

Key words: Systemic lupus erythematosus; anti-dsDNA antibodies; anticardiolipin; antibodies; hematologic changes; renal lupus; musculoskeletal

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder that involves multiple organs and can lead to severe complications including cerebral infarction, myocardial infarction, infection, renal failure and a poor prognosis.^{1–4} SLE is characterized by the heterogeneity of its clinical features, and we have yet to fully understand this heterogeneity, which is one of the reasons why classification criteria for SLE⁵ were developed, and new criteria were recently proposed.⁶

Epidemiological studies of SLE

Epidemiological studies of SLE can be classified into two types. The first type involves studies on relatively detailed issues, including the clinical features of SLE, that included only a limited number of patients. The second type involves studies on limited epidemiological indices, such as the incidence and prevalence of the condition, that included many registrants. Most of the first type of studies were hospital-, clinic- or limited region-based studies with fewer than 1000 SLE patients,^{7–11} although some of these studies recruited participants from multiple regions within a nation.¹² There have been only two national registry-based studies of SLE, which were performed in Taiwan and Japan.^{13,14} The sample sizes of these two studies were 22,182 and 21,405, respectively. SLE is three to 10 times more common in females than in males.¹⁵ The age at onset of SLE peaks from 15 to 30, and the female:male ratio has been reported to be highest

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