Figure 6. Cytokine production in culture supernatants of splenocytes stimulated with CAWS in vivo from CAWS-administered mice. CAWS (0 or 4mg/mouse) was administered i.p. to DBA/2, C57BL/6, C3H/HeN and CBA/J mice for five consecutive days in the 1st and 5th week. In the 9th week, splenocytes were collected from each mouse. The splenocytes were cultured with CAWS (0, 2.5, 5 or 10 μg/ml) for 48 hour at a density of 1×10^7 cells/ml. The culture supernatants were collected and the level of each cytokine was measured by ELISA. The data shows one of four (C3H/HeN and CBA/J), three (DBA/2) or two (C57BL/6) experiments performed with similar results. The results show the mean ± standard deviation (S.D.). *; P < 0.05 compared with the control using Student's *t-test*.
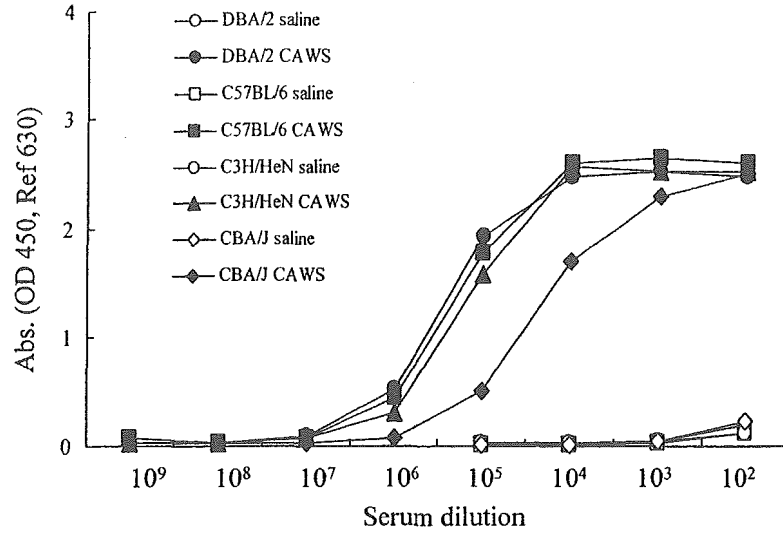
Figure 7. Anti-CAWS antibody in sera from CAWS-administered mice. CAWS (0 or 4mg/ mouse) was administered i.p. to DBA/2, C57BL/6, C3H/HeN and CBA/J mice for five consecutive days in the 1st and 5th week. In the 9th week, sera were collected from each mouse. Anti-CAWS antibody was measured by ELISA. Color development was stopped after 10 min.
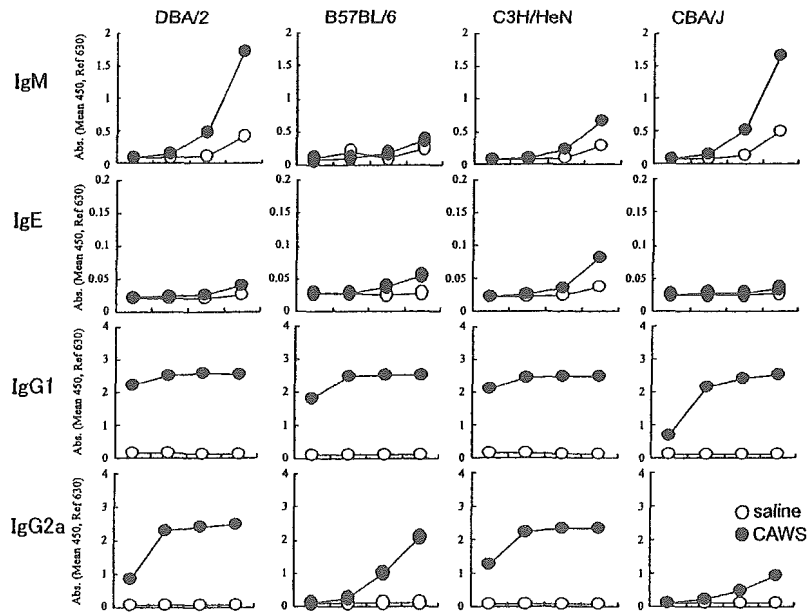

Figure 8. Immunoglobulin subclasses of anti-CAWS antibody in sera from CAWS-administered mice. CAWS (0 or 4mg/mouse) was administered i.p. to DBA/2, C57BL/6, C3H/HeN and CBA/J mice for five consecutive days in the 1st and 5th week. In the 9th week, sera were collected from each mouse. Anti-CAWS immunoglobulin subclasses were measured by ELISA. Color development was stopped after 10 minute.

DISCUSSION

Kawasaki disease is a febrile inflammatory disease that presents with systemic arteritis, and can be fatal particularly in the case of an exacerbation of coronaritis. When CAWS is administered to mice in accordance with the protocol of Murata et al.,[7,8] a Kawasaki-disease-like angiitis is induced at the origin of the coronary arteries. In the present study, strain differences were found to exist with respect to the incidence of the disease induced by CAWS with DBA/2, C3H/HeN and C57BL/6 mice exhibiting sensitivity and CBA/J mice exhibiting resistance. Moreover, histological observation of the sites of coronaritis in DBA/2 mice revealed hypertrophy of the tunica intima and cellular invasion, and the disease occurred with extremely high levels of severity and frequency. DBA/2 mice developed a much more severe coronary arteritis than the other strains. In addition, DBA/2 mice exhibited high mortality during the course of the disease's induction, and based on findings obtained from histological examination of the hearts, the cause of death was suspected to be myocardial infarction attributable to coronary occlusion. Because the difference in sensitivity to CAWS among the mouse strains examined in this study correlates strongly with the diversity of prognoses for Kawasaki disease patients,[19] this model is considered to be effective for elucidating the cause of coronary arteritis associated with Kawasaki disease, analyzing the characteristic condition, and developing more effective treatment methods.

Among patients with Kawasaki disease in the acute stage, cytokines, including IL-1, IL-2, IL-2 receptor, IL-6 and TNF-α, are detected in the serum.[20-23] When the production of cytokines from spleen cells was measured in mice stimulated with CAWS in vitro, a similar trend was demonstrated by the three strains that were sensitive to coronary arteritis induction, namely, DBA/2, C3H/HeN and C57BL/6, and in the case of DBA/2 mice in particular, a prominent CAWS-specific response involving inflammatory cytokines such as IL-6, IFN-γ and TNF-α, was observed, indicating the occurrence of an inflammatory immune response. The levels of inflammatory cytokines produced were high in DBA/2 mice in particular. On the other hand, an increased production of IL-10, which exhibits an immunosuppressive action, was observed in CBA/J mice that exhibited resistance to the occurrence of coronary arteritis. It is interesting to note that these cytokine production patterns resemble those observed in Kawasaki disease patients.

However, as there is one report indicating increased production of IL-4 and IL-10 in patients with Kawasaki disease,[24] dynamics that do not necessarily coincide with the findings of this study are observed. The discrepancies may be related to the stage of the disease (acute, subacute or recovery stage), thus indicating the need for further study of the relationship between cytokine production and the condition of Kawasaki disease.

Analyses have been conducted on the background genes of various diseases, and in the case of Kawasaki disease as well, there is a possibility of some form of involvement by genetic factors.[25,26] During the course of research on genes associated with coronaritis in rodents, numerous analyses have been conducted on the relationship between arteriosclerosis and hyperlipemia, and the IL-10 gene has been reported to have a close relationship with the lesions of coronary arteritis.[27,28] This finding also supports the findings obtained in the present study. In addition, although CBA is a strain derived from DBA, it is quite interesting that there are large differences in the

incidences of coronary arteritis and myocarditis induced by CAWS between the two strains. A survey of the genes involved in mouse coronaritis also suggested the existence of multiple inductive genes and repressor genes. This is a subject that requires detailed analysis.

On the basis of the above results, the activation of lymphocytes, vascular endothelial cells and so forth was prominently induced by means of hypercytokinemia in DBA/2 mice, and the resulting coronaritis promoted the occurrence of chronic myocardial ischemia, which, as a result, was thought to ultimately lead to death caused by fibrosis, infarction and cardiac insufficiency. It is hoped that this model will contribute not only to elucidation of the stage of Kawasaki disease and the associated coronary arteritis, but also to the improvement and development of treatment methods.

ACKNOWLEDGMENTS

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Original Paper

Clinical Efficacy of Intravenous Immunoglobulin for Patients with MPO-ANCA-Associated Rapidly Progressive Glomerulonephritis

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Abstract

Background: To determine whether intravenous immunoglobulin (IVIg) can control disease activity in patients with myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA)-associated rapidly progressive glomerulonephritis (RPGN). *Methods:* Twelve patients with serologically and histologically confirmed MPO-ANCA-associated RPGN (7 men, 5 women; mean age 71 ± 3 years) received IVIg (400 mg/kg/day) alone for 5 days. The effects of IVIg were evaluated by white blood cell counts, serum C-reactive protein levels, Birmingham Vasculitis Activity Score, rate of change in reciprocal creatinine (1/Cre), and plasma tumor necrosis factor-α levels after IVIg administration. Corticosteroids with or without cyclophosphamide were commenced after IVIg. *Results:* After IVIg treatment, a significant decrease was observed in white blood cell count (p < 0.05), C-reactive protein values (p < 0.001), and Birmingham Vasculitis Activity Score (p < 0.001) concomitant with the amelioration of systemic symptoms. The rate of change in 1/Cre significantly improved (p < 0.05). Plasma tumor necrosis

factor-α levels that were significantly elevated in patients before IVIg compared with normal controls (p < 0.0001), rapidly declined after IVIg with a significant reduction (p < 0.05). Three months post-treatment with IVIg, all patients showed improvement of disease without serious infectious complications. *Conclusion:* IVIg is a potential component of remission induction therapy for patients with MPO-ANCA-associated RPGN.

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Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated rapidly progressive glomerulonephritis (RPGN), which occurs in Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) [1], leads to renal failure through systemic vasculitis and diffuse crescentic glomerulonephritis. Since crescent formation has features of delayed-type hypersensitivity and is accompanied by the presence of T cells, macrophages, and fibrin in the glomerular lesion [2], high-dose corticosteroids and cyclophosphamide (CYC) are standard treatment for ANCA-associated RPGN; however, such immunosuppressive therapy is often complicated by severe infection in elderly patients [3]. Therefore, to induce early remission of

the disease including renal insufficiency and to avoid fatal side effects, it is important to establish a therapeutic regimen that can maintain the immune potency of such patients.

Intravenous immunoglobulin (IVIg) has been advocated as a safe and effective treatment for other immune-mediated diseases, such as Kawasaki disease, idiopathic thrombocytopenic purpura, Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy. European investigators have recently shown that IVIg is clinically useful and safe when administered in conjunction with immunosuppressive drugs, helps suppress disease activity for at least 1 year, and consequently reduces the total dose of immunosuppressive agents in patients with ANCA-associated vasculitis, mainly WG with or without RPGN [4, 5]. Accumulating evidence suggests that it works in multiple phases of immune response; neutralization of circulating pathogenic antibodies, Fc receptor modulation and blockade, or suppression of antibody-dependent cellular toxicity, natural killer cell function, autoantibody production, and complement activation [6]. In addition, Guillain-Barré syndrome patients who received IVIg showed clinical recovery in parallel with reduction in serum levels of tumor necrosis factor-alpha (TNF-α), suggesting an important role of IVIg in inhibiting cytokine activity [7].

Here we report a study evaluating the effectiveness of IVIg as an initial treatment, preceding corticosteroids with or without CYC, in 12 patients with MPO-ANCA-associated RPGN. Since it remains unclear whether IVIg is independently effective [8], we investigated the potential immunomodulatory effect unique to IVIg by comparing renal function, clinical score (Birmingham Vasculitis Activity Score, BVAS), and circulating TNF-α levels before and after IVIg treatment. Since MPO-ANCA-associated RPGN is more common than those related to PR3-ANCA in Asia, which clearly contrasts with the incidence in Western countries, we were able to recruit sufficient numbers of patients with MPO-ANCA-associated RPGN to examine the results statistically. This is the first report of the effects of IVIg in MPO-ANCA-specific RPGN patients.

Patients and Methods

Patients

Twelve consecutive patients with MPO-ANCA-associated RPGN (7 men and 5 women; mean age 72 years; range 57–83 years), who were admitted to the Nephrology Department of Kyoto University Hospital and Kitano Hospital between January 2001 and February 2003, were enrolled in this study (table 1). All patients were diagnosed as having MPA because of elevated serum MPO-ANCA as well as characteristic pathology observed in the renal biopsy specimen before treatment. In all patients, the disease was confirmed based on the definition of MPA described by the Chapel Hill Consensus Conference [9]. Renal involvement was seen in all patients. Patient with rapid aggravation of renal dysfunction with more than 30% rise in serum creatinine (Cre) levels were defined as having RPGN. All except 1 (patient No. 2) were newly diagnosed patients who had been transferred from other hospitals due to the onset of RPGN. Patient 2 had previously demonstrated MPO-ANCA-associated RPGN and recovered after 6 years of treatment with prednisolone and CYC. He again developed fever, arthralgia, and myalgia with elevation of white blood cells (WBC), C-reactive protein (CRP), Cre (33% rise) and MPO-ANCA levels, leading to a diagnosis of MPA recurrence. All patients provided written informed consent for renal biopsy as well as treatment according to the protocol. The hospital Ethical Committee approved the study design.

Histological Evaluation

All renal biopsy specimens showed MPA. Hematoxylin and eosin, periodic acid-Schiff, periodic acid silver-methenamine, Masson trichrome, and elastica van Gieson stain were performed. Direct immunofluorescence studies were performed using frozen sections of renal tissue. Histological activity was assessed as follows: active crescent formation (%) = number of glomeruli with cellular and fibrocellular crescent formation/number of glomeruli without global sclerosis × 100. Each biopsy specimen was scored by two pathologists independently. If there was disagreement between the scores, patients were re-examined by the team to determine a final diagnosis by consensus.

Treatment Protocols

After serum MPO-ANCA, WBC, CRP, renal biopsy, and BVAS were all evaluated to establish a definite diagnosis and activity grading, IVIg was administered intravenously as an initial treatment for 5 consecutive days (400 mg/kg/day). Patients 1–10 received freeze-dried sulfonated human normal immunoglobulin (Kenketsu Venilon-I, Teijin Co., Ltd, Tokyo, Japan), and patients 11 and 12 received freeze-dried polyethylene glycol-treated human normal immunoglobulin (Kenketsu Glovenin-I, Nihon Pharmaceutical Co., Ltd, Tokyo, Japan). Both preparations were free from IgG aggregations that can form in prepared solutions containing sucrose. During IVIg treatment, none of the patients received any other immunosuppressive agents, any blood transfusions, or any intravascular volume repletion treatment. Following the post-IVIg treatment evaluation of clinical scores and laboratory data, all patients received immunosuppressive treatment with oral corticosteroids with or without CYC (table 2). Oral corticosteroids (prednisolone 0–1.0 mg/kg/day) were administered dependent on disease severity and patient age. Methylprednisolone pulse and oral CYC (25–50 mg/day) were also administered to 3 of 12 and 8 of 12 patients, respectively.

Assessment of Disease Activity

Complete blood count and serum markers such as CRP, Cre, and MPO-ANCA were evaluated at the onset of disease, before, and immediately after (mean 6.3 days, range 0–17 days) IVIg, and 1 and 3 months after IVIg. Disease activity was assessed by BVAS before and immediately after IVIg and 1 and 3 months after IVIg. BVAS consists of 59 predefined items derived from clinical, radio-

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Yonemoto/Fukatsu/Kita/Suzuki/Muso

Table 1. Characteristics of the 12 patients (M/F = 7/5)

Pa-tient	Age	Sex	Data before treatment								
			WBC/μl	CRP mg/l	Cre μmol/l	MPO-ANCA, EU	Active crescents, %	BVAS	extrarenal manifestations	pulmonary involvement	latent and antibiotics resistant infections
1	82	F	7,000	80	283	239	81	19	S, F, E		HBV carrier
2	75	M	9,600	178	106	435	0	23	S, F, A, C, N		MAC, K. pneumoniae
3	61	F	8,100	43	126	244	71	15	S, F		
4	82	M	9,400	104	417	159	71	14	S, A		
5	64	F	12,100	154	737	276	81	19	S, F, L	infiltrate	
6	59	M	10,200	139	389	140	90	15	S, F		Aspergillus
7	83	F	4,700	1	258	617	60	20	S, F, Ab		
8	82	F	14,700	113	210	306	38	21	S, F, N		
9	57	M	10,700	99	357	980	64	19	S, A, N		
10	62	M	10,100	101	732	370	80	25	S, L, N	nodules	
11	75	M	9,300	60	1,012	82	33	27	S, E, L	infiltrate	HBV carrier, MRSA, P. aeruginosa
12	67	M	11,900	68	401	1,740	78	19	S, L	hemoptysis	MRSA
Mean	71		9,820	95	419	466	62	20			
Reference range			3,500–9,100	<3	<106	<20		0			

WBC = White blood cell count; CRP = C-reactive protein; Cre = serum creatinine; MPO-ANCA = myeloperoxidase antineutrophil antibody; BVAS = Birmingham Vasculitis Activity Score; S = systemic symptom (malaise, myalgia, weight loss); F = fever; A = arthralgia; C = cutaneous; E = ear-nose-throat; L = lung; Ab = abdomen; K = kidney; N = neuropathy; HBV = hepatitis B virus; MAC = Mycobacterium avium complex; K. pneumoniae = Klebsiella pneumoniae; MRSA = methicillin-resistant Staphylococcus aureus; P. aeruginosa = Pseudomonas aeruginosa.

Table 2. Treatment and outcomes after IVIg treatment

Patient	Initial immunosuppressive treatment just after IVIg				Treatment after 3 months			
	mPSL pulse	PSL dosage mg/kg/day	CYC dosage mg/kg/day	dialysis	PSL dosage mg/kg/day	CYC dosage mg/kg/day	dialysis	Cre μmol/l
1	–	0.3	0.7	–	0.3	0	–	124
2	–	0.7	0.4	–	0.3	0.4	–	88
3	–	1.0	1.0	–	0.5	1.1	–	92
4	–	0.5	–	HD	0.7	–	–[1]	308
5	–	1.0	1.0	–	0.6	1.1	–	204
6	1 g, 3 days	0.7	0.8	–	0.3	0	–	177
7	–	0.5	1.3	–	0.3	0	–	203
8	–	0.6	–	–	0.4	–	–	87
9	1 g, 3 days	0.7	0.7	–	0.2	1.7	–	141
10	–	0.8	0.8	–	0.4	0.8	–	353
11	–	0	–	HD	0.5	–	HD	723[2]
12	0.5 g, 3 days	0.5	–	–	0.5	–	–	131
Mean		0.6	0.8[3]		0.4	0.6[3]		173[4]

mPSL pulse = Methylprednisolone pulse therapy; PSL = prednisolone; CYC = cyclophosphamide; Cre = serum creatinine; HD = hemodialysis.

[1] Cessation of HD; [2] Cre level before a hemodialysis; [3] mean CYC dose of 8 patients; [4] patient 11 was excluded.

logic, and laboratory evaluations in 9 organs systems. Each organ system carries a weight (ranging from 0 to 12), and an item is positively scored if the investigator considers it present and caused by active vasculitis. The maximal score is 63 with higher scores indicating more active disease [10].

Evaluation of the Progression Rate of Renal Dysfunction: Rate of Change in 1/Cre

To determine whether rapid progression of renal failure was occurring in these patients, the rate of change in reciprocal Cre (1/Cre) levels (dl/mg/day) was compared before and after IVIg treatment [11]. Briefly, Cre levels (mg/dl) were evaluated at five time points as follows: the first visit to the primary care physician with initial symptoms (Cre1 at time 1 [T1]), admission to the hospital (Cre2 at time 2 [T2]), transfer to the nephrology unit (Cre3 at time 3 [T3]), just before IVIg treatment (Cre4 at time 4 [T4]), and after IVIg treatment without receiving other immunosuppressive treatment (Cre5 at time 5 [T5]). The unit of time was 1 day. The largest value among (1/Cre4-1/Cre1)/(T4-T1), (1/Cre3-1/Cre1)/(T3-T1), and (1/Cre2-1/Cre1)/(T2-T1) was regarded as a rate of change in 1/Cre before IVIg and compared with (1/Cre5-1/Cre4)/(T5-T4). Only patient 11 was excluded from this evaluation because he was already undergoing permanent hemodialysis before IVIg because of rapidly deteriorating renal function.

Measurement of Plasma Cytokines

Venous blood samples were drawn from patients before and after IVIg, and before initiating immunosuppressive therapy. Plasma samples were stored at −80°C until use. Plasma samples were available for 9 patients, in whom cytokine levels were compared before and after IVIg. According to the manufacturer's instructions, the following cytokines were measured: TNF-α, interleukin (IL)-6, IL-8, IL-1β using Human Cytokine UltraSensitive ELISA kit (Biosource International, Camarillo, Calif., USA). Absolute values of these cytokines were also measured using the blood samples from 12 normal controls, and compared with those of the 9 patients. An average of +2 standard deviations (SD) for each cytokine level in the 12 normal controls was considered the upper limit of the normal range.

Statistical Analysis

The significance of differences between pre- and post-IVIg values of clinical laboratory data was assessed by paired Student's t test using StatView II software (version 5.0 for Macintosh; SAS Institute Inc., Cary, N.C., USA). To compare the cytokine levels of normal controls to the pre-IVIg cytokine levels of patients, unpaired t test was used. Fischer's exact test was performed to compare BVAS and laboratory data before IVIg with those after 1 and 3 months. A p value <0.05 was considered significant. All data were expressed as mean ± SEM.

Results

Clinical and Pathological Features before IVIg Treatment

Demographic and clinical characteristics and renal histological findings of 12 patients enrolled in this study are summarized in table 1. All patients were clinically diagnosed as having RPGN with micro- or macroscopic hematuria and rapidly worsening renal function. The mean Cre value was 419 μmol/l (range 106–1,012) just before IVIg treatment. The mean BVAS was 20 (range 14–27) before treatment. Laboratory tests demonstrated increased levels of WBC (mean 9,820/μl; range 4,700–14,700), CRP (mean 90 mg/l; range 1.0–178; reference <3), and MPO-ANCA level (mean 466 EU; range 82–1,740; reference <20). Crescentic glomerulonephritis with or without systemic features of MPA was present in all patients. Mean percentage of active crescent formation was 62%. Direct immunofluorescence study demonstrated pauci-immune deposition (scant depositions of immunoglobulins) in all patients.

Clinical Responses

(1) The change in WBC count and CRP value: Total WBC counts were 9,820 ± 740/μl before IVIg and decreased to 7,960 ± 870/μl after IVIg; the pre- and post-treatment levels were significantly different (p < 0.01). A significant decrease was also observed in neutrophil, lymphocyte, and eosinophil differential counts: the decrease in neutrophils was the most significant (pre-IVIg 7,950 ± 740/μl; post-IVIg 6,010 ± 800; p < 0.001). Mean CRP value was 97 mg/l (range 5–178) at the onset of vasculitis, and 95 mg/l (range 1–178) just before IVIg treatment. Following IVIg, the mean CRP value decreased significantly to 57 mg/l (range 1–124) (p < 0.001; fig. 1a).

(2) Rapid effect on renal function: The mean Cre level was 89 μmol/l (range 44–124) at the onset of disease, but increased to 365 μmol/l (range 106–737) in 62 ± 14 days (range 22–185) just before IVIg (fig. 1b). As a sensitive method of detecting rapid changes in renal dysfunction, we calculated the rate of change in 1/Cre before and after IVIg as shown previously [11]. The rate of change in 1/Cre was −0.041 ± 0.020 dl/mg/day before IVIg and increased to 0.007 ± 0.004 dl/mg/day after IVIg (p < 0.05).

(3) Temporal profiles of pro-inflammatory cytokine levels before and after IVIg: Before IVIg treatment, the plasma TNF-α levels were significantly elevated in patients compared to normal controls (patients, pre-IVIg 4.23 ± 0.92 pg/ml vs. control, 0.23 ± 0.40 pg/ml; p < 0.0001). After IVIg treatment, the plasma TNF-α levels decreased significantly (pre-IVIg 4.23 ± 0.92 pg/ml vs. post-IVIg 2.40 ± 0.53; p < 0.05; fig. 2). Plasma IL-6 levels (pg/ml) were significantly higher before IVIg treatment in patients compared to that in normal controls (pre-IVIg 2.75 ± 4.45 vs. control, 0.00 ± 0.00; p < 0.05). The plasma IL-6 levels decreased on average after IVIg treatment, but the difference did not reach significance (data not shown).
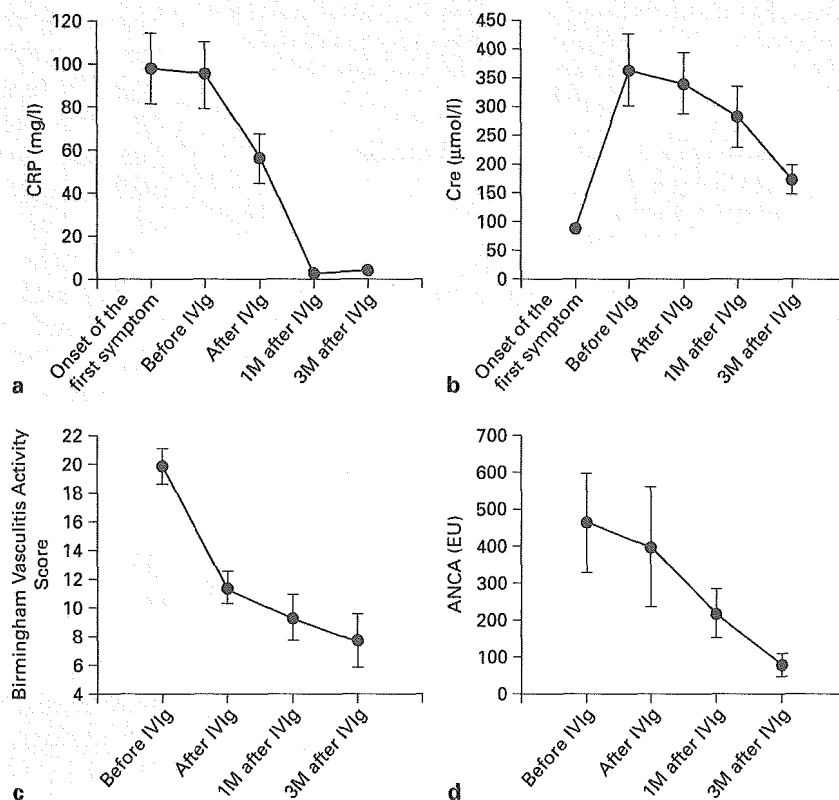
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Yonemoto/Fukatsu/Kita/Suzuki/Muso

Fig. 1. Three-month follow-up of the patients. **a** Serum C-reactive protein (CRP), mg/l (n = 12). **b** Serum creatinine (Cre) level, μmol/l (n = 11). **c** Birmingham Vasculitis Activity Score (n = 12). **d** Anti-neutrophil cytoplasmic antibody (ANCA) (n = 12). M = Months.

The plasma IL-8 levels of patients before IVIg treatment did not significantly differ from that of normal controls or that after IVIg treatment (data not shown). Some patients showed markedly elevated IL-6 (patient No. 1, 6, 8–10) and IL-8 (patient No. 1, 5, 8–10) levels before IVIg treatment, which decreased after IVIg treatment. The plasma IL-1β levels (pg/ml) of patients before IVIg treatment did not significantly differ from that of normal controls or that after IVIg treatment (data not shown).

(4) BVAS: After IVIg treatment, significant reduction was seen in BVAS (pre-IVIg 20 ± 1; post-IVIg 11 ± 1; $p < 0.0001$; fig. 1c). First, systemic symptoms improved; malaise (8 of 12), myalgia (3 of 3), arthralgia/arthritis (2 of 2), and fever (7 of 9). Before IVIg treatment, hematuria and proteinuria were observed in all patients; rapid aggravation of renal dysfunction with more than 30% rise in Cre was also noted in all patients. Lung involvement was seen in 4 patients; 1 showed nodular lesions, 1 showed hemoptysis and the other 2 showed infiltrative lesions. These lesions improved partially following IVIg.
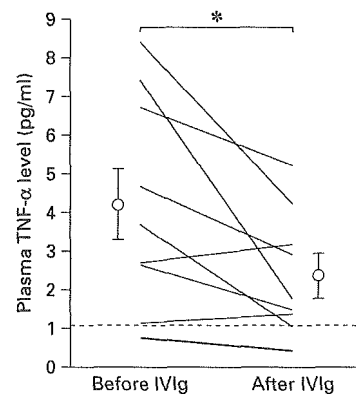

Fig. 2. Plasma TNF-α levels (pg/ml) before and after IVIg (n = 9). The dotted line represents the upper limit of the normal range. * $p < 0.05$ vs. before IVIg.

Immunosuppressive Therapy following IVIg Therapy

Following the 5-day IVIg course, 12 patients were treated as summarized in table 2. After IVIg, 3 patients received steroid pulse treatment and 11 patients received oral steroids with no more than 1.0 mg/kg/day, 6.3 days after IVIg treatment on average (range 0–17). The mean initial dose of oral steroid for 12 patients was 0.6 ± 0.1 mg/kg/day (33.3 ± 4.5 mg/day). Additionally, those who responded inadequately to steroids received CYC unless active infections were concurrent. CYC was administered to 8 patients at a mean dose of 0.8 ± 0.1 mg/kg/day (46.9 ± 3.1 mg/day).

Outcome and 3-Month Follow-Up

There were no disease-related deaths for 3 months after IVIg treatment. Vasculitis recurred 3 months after IVIg treatment in patient 11 who did not receive any immunosuppressive drugs after IVIg treatment because he was a carrier of MRSA and antibiotic-resistant *Pseudomonas aeruginosa*. There was no fatal complication due to infections in any of the patients during the 3-month observation period after treatment.

CRP level continued to decrease and normalized 3 months after IVIg treatment (4.0 ± 2.0 mg/l; p<0.0001 vs. before IVIg; fig. 1a).

As shown in figure 1b, the Cre level began to decrease following IVIg; Cre level was 173 μmol/l (range 88–353; except for patient 11 who was on maintenance hemodialysis) 3 months after IVIg treatment (vs. pre-IVIg; p < 0.0001). Among 3 patients (patient No. 5, 10, and 11) whose Cre level exceeded 700 μmol/l before IVIg treatment, only one patient (No. 11) required chronic hemodialysis within 3 months after IVIg treatment. Although hemodialysis was also required for patient 4 within 1 month after IVIg treatment, he could be withdrawn from hemodialysis shortly thereafter. Collectively, the 3-month renal survival rate was 92% in our 12 patients.

The mean BVAS continued to decrease after IVIg treatment (fig. 1c). The mean BVAS was 20 (range 15–27) before IVIg and 11 (range 1–16) immediately after IVIg (p < 0.0001); 9 (range 0–19) 1 month after IVIg (p < 0.0001 vs. pre-IVIg BVAS), and 8 (range 0–22) 3 months after IVIg (p < 0.0001 vs. pre-IVIg BVAS). In particular, urinalysis showed that hematuria and/or proteinuria disappeared in 8 patients, 3 months after IVIg treatment. Systemic symptoms such as body weight loss, and nervous or alimentary tract symptoms also improved at 3 months after IVIg treatment.

The mean MPO-ANCA levels obtained within 8 ± 5 days after IVIg treatment were 401 EU (range 70–990) and those 1 month after IVIg treatment were 218 EU (range 13–640). The titers at these two time points after IVIg treatment were not significantly different from that prior to IVIg treatment (465.7 ± 135.7 EU). The mean MPO-ANCA levels 3 months after IVIg treatment, 78.8 EU (range 0–389), were significantly lower than that prior to IVIg treatment (p < 0.01; fig. 1d).

Adverse Drug Reaction

There were no major side effects observed in patients who received IVIg treatment. Patient 4 experienced transient mild hypertension and edema of the extremities during IVIg infusion, but it subsided when the rate of infusion of IVIg was lowered.

Discussion

The present study was conducted to evaluate the safety and efficacy of IVIg as an initial therapy for patients with MPO-ANCA-associated RPGN. All 12 patients with ANCA-associated RPGN enrolled in this study had experienced rapidly deteriorating renal dysfunction with multiorgan involvement. Administration of IVIg for 5 consecutive days led to partial resolution of inflammatory signs and symptoms in parallel with significant decreases in CRP, TNF-α, and BVAS values as well as cessation of progression in renal dysfunction. No life-threatening infections or side effects developed with our regimens including IVIg in all patients, including those older than 80 (patient No. 1, 4, 7, 8) and those with latent, antibiotic-resistant infections (patient No. 1, 2, 6, 11, 12). Clinical improvement was seen in all patients with IVIg for initial therapy followed by immunosuppressants, none of whom died within 3 months. The 3-month renal and patient survival rates were 92 and 100%, respectively, which were more favorable than those previously reported in MPO-ANCA-positive RPGN patients treated with immunosuppressive agents in Japan: 3-month renal and patient survival rates were about 75 and 85%, respectively [12].

Notably, there was a rapid and significant decrease in neutrophil count following IVIg treatment. Activated neutrophils are known to be involved in vasculitis. During the active phase of Kawasaki disease, circulating activated neutrophils increase in number and secrete excessive amounts of autotoxic mediators such as reactive oxygen species and elastase. In this active phase, neutrophil apoptosis is inhibited, resulting in a prolonged lifespan, which then might contribute to the pathogenesis of the

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vasculitic lesions. High-dose IVIg therapy decreased the number of circulating neutrophils by accelerating their apoptosis in Kawasaki disease and was effective in preventing the development of coronary aneurysm [13]. Similarly, in MPO-ANCA-associated vasculitis, activated neutrophils are involved in renal damage. TNF-α-primed neutrophils undergo accelerated and dysregulated apoptosis, and such apoptotic neutrophils express ANCA antigen on their cell surface in affected organs, where leukocytoclasia can further augment inflammatory injury [14]. Although the precise mechanism by which IVIg affects the apoptosis of neutrophils remains unknown, the rapid decrease of WBC count following IVIg treatment coupled with the significant decrease in CRP suggests accelerated clearance of apoptotic neutrophils by IVIg in patients with MPO-ANCA-associated RPGN in this study.

Our study showed that the plasma TNF-α value significantly decreased following IVIg treatment, suggesting immunomodulatory effect of IVIg. Serum levels of TNF-α were reported to be increased in patients with active vasculitis [15]. In addition, elevation of serum TNF-α was associated with upregulation of TNF-α mRNA at the sites of vasculitis [16]. TNF-α released from activated macrophages following infectious stimuli is known to prime and activate neutrophils. Once activated, neutrophils can attach to the endothelium and further release MPO and reactive oxygen species, ultimately leading to endothelial damage [17, 18]. Consistent with this, Booth et al. [19] recently reported TNF-α blockade with infliximab was effective at inducing remission in 88% of patients with ANCA vasculitis. Their report and our findings suggest that TNF-α may play a key role in ANCA-associated vasculitis. Decrease in the TNF-α value following IVIg suggests that IVIg plays a positive role in disrupting such a vicious inflammatory cycle.

Another possible mechanism of IVIg is that therapeutic concentrations of IgG block Fc receptors on phagocytes and inhibit antibody-dependent cell-mediated cytotoxicity [6] or downregulate the proliferation of activated B and T cells, reducing cytokine production from these immunoeffector cells [20]. Because the latter mechanism requires a substantial time interval, the rapid TNF-α suppression with IVIg observed in this study suggests that IVIg has direct effects on activated macrophages, rather than an effect mediated through T- or B-cell suppression.

IVIg treatment, even without immunosuppressants, has been shown to ameliorate systemic symptoms of active vasculitis [8]. In patients with asthma, IVIg was found to act synergistically with steroids, improved the clinical parameters, and reduced oral corticosteroid requirements and the duration of hospitalization. Such effects are partially mediated by improvement in glucocorticoid-receptor-binding affinity [21]. In the present study, RPGN was significantly improved by a relatively low initial dose of steroid (0.6 mg/kg/day). Our patients, who are relatively old and therefore at higher risk of developing infectious complications after steroid administration, might have benefited from the potential steroid-sparing effect of IVIg.

A major side effect of IVIg is renal dysfunction probably due to hyperosmolarity induced by sucrose contained in immunoglobulin formulations [22]. Therefore, in Europe, such a formulation is used for WG patients without renal involvement, but not for those with renal involvement. For MPA patients with renal involvement, we used immunoglobulin formulation that contained mannitol (Kenketsu Venilon-I) or glucose (Kenketsu Glovenin-I) instead of sucrose because the former two substances are less likely to cause hyperosmolarity. Although we cannot exclude the possibility that intravascular volume repletion with mannitol might have increased tubular flow, there is no convincing evidence for the efficacy of mannitol in RPGN [23]. With our regimen, patients demonstrated improved renal function, supporting the safety of IVIg with mannitol or glucose.

In conclusion, the present study demonstrated the safety and potential efficacy of IVIg as an initial therapy for patients with MPO-ANCA-associated RPGN. Our study is limited by its small size, relatively few severe cases, and non-standardized follow-up protocols. However, our findings suggest that IVIg is potentially effective for treating MPO-ANCA-associated RPGN, either as first-line or adjunctive therapy. Further research into the optimal dose and duration of treatment is required to define the role of IVIg in treatment of MPO-ANCA-associated RPGN.

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1. ANCA 関連血管炎に対する大量 γ グロブリン療法

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動　向

血管炎症候群とは血管壁の炎症を起こす様々な病態を総称している．これらは1994年にChapel Hillで開かれたConsensus Conference on the Nomenclature of Systemic Vasculitisで血管のサイズによる組織学的分類が承認された[1]．血管炎症候群のうち，主に小型の筋性動脈，毛細血管，細静脈に病変を生じる顕微鏡的多発血管炎 microscopic polyangiitis（MPA），Wegener肉芽腫症 Wegener's granulomatosis（WG），アレルギー性肉芽腫性血管炎 Churg-Strauss syndrome（CSS）については，抗好中球細胞質抗体（ANCA）がその病態に関与すると考えられている．ANCAは1982年Daviesらが間接蛍光抗体法によって初めて報告したヒト好中球細胞質に対するIgG型自己抗体である[2]．ANCAの対応抗原は主に好中球細胞質顆粒内に存在するが，proteinase 3（PR3）に対するPR3-ANCAはWGに，myeloperoxidase（MPO）に対するMPO-ANCAはMPA，CSSに多く認められることが広く知られている[3]．

ANCA関連血管炎症候群は血管炎により腎，肺をはじめ皮膚・神経・消化管などの全身臓器症状をきたす重篤な疾患である．年間罹患率は100万人あたり20人以上で[4,5]，年齢とともにその頻度は上昇し，65歳から74歳における年間罹患率は100万人あたり53人であると報告されている．また，近年その発症頻度が増加していることも報告されている[5]．著者らは阪神・淡路大震災の直後から1年後にかけて，神戸地区で高齢者に気道・呼吸器障害を合併した症例の多発を報告し，環境要因による発症の可能性を示唆した[6,7]．

わが国の急速進行性糸球体腎炎（RPGN）調査研究班の報告によるとRPGN症例でANCA陽性者のうちPR3-ANCAに比べMPO-ANCA陽性者が圧倒的に多く，また，この抗体陽性者症例群は高齢者に発症し，生命予後が悪いことが明らかとなった[8,9]．また，RPGNの死因のうち半数は感染症であるとも報告されている[8]．Westmanらも1971年から1993年までの腎障害を有するANCA関連血管炎123例において，3カ月以内の急性期死亡を9%に認め，生存率にかかわる有意な予後要因は年齢と診断時血清クレアチニン値であると報告している[10]．Harperらも1990年から2000年に新規に診断された233例のANCA関連血管炎において，死亡率は治療開始時の腎機能低下（クレアチニン＞4.5mg/dl）と関連したこと，

死因の半数は感染症であることを報告している．彼女らはまた，65歳以上の高齢患者群（n = 115）は65歳以前の患者群（n = 114）と比べ治療開始時により重篤な腎機能障害を示しており，治療反応性ならびに再発率に差はないものの，免疫抑制療法による感染症の有意な増加を認め，生存率は高齢者群で有意に悪く，早期死亡を認めたことを報告した[11]．これらの結果から，特に高齢者においては治療開始期の腎機能低下と治療合併症としての日和見感染症が予後を左右することが明らかであり，現在行われているステロイドをはじめとする免疫抑制療法の限界や副作用を補う治療法としてより安全な治療法の開発が急務であると考えられる．その一つとして，従来，中血管を侵す代表的な血管炎である川崎病で免疫修飾作用による有効性が示されている経静脈的免疫グロブリン大量（IVIg）療法が注目されている．本稿では，ANCA関連血管炎に対する本治療の使用状況とその効果発現機序，さらにその問題点と限界についても最近の報告を含め述べる．

A. 血管炎に対するIVIg療法

自己免疫性疾患において初めてIVIgの有効性が確立された疾患は特発性血小板減少性紫斑病（ITP）であり，それ以来現在までいくつかの自己免疫疾患や炎症性疾患に対して本法の比較試験が試みられてきている（表1）[12,13]．

1. 川崎病に対するIVIg療法

川崎病は4歳以下の乳幼児において中・小動脈を中心に発症する全身性血管炎で，急性期の冠動脈拡張や動脈瘤形成から後に狭窄や閉塞病変へ進行，心筋梗塞や突然死をきたす．その治療には，抗生物質や副腎皮質ステロイドが用いられたが，1984年に古庄らがIVIgの大量療法により冠動脈病変の発生を抑制することを報告した[14]．日本で

表1 効果が比較試験で証明されているIVIgの自己免疫疾患および炎症性疾患の一覧
（文献12より改変）

特発性血小板減少性紫斑病（ITP）
Guillain-Barré 症候群
慢性炎症性脱髄性多発神経根症（CIDP）
重症筋無力症
多発性運動神経疾患
ステロイド抵抗性皮膚筋炎
川崎病
GVHD予防
ANCA関連血管炎*
自己免疫性ぶどう膜炎*
多発性硬化症*

*: 予備的試験で効果が報告されており，比較試験が現在進行中

は400mg/kg/day，5日間の治療が200mg/kg/day，5日間の治療に比べより効果的であることが報告され[15]，米国では1991年にNewburgerらによって2g単回投与の効果が確認された[16]．さらにDurongpisitkulらによってメタアナリシスが行われ，この療法と少量のアスピリン（80mg/kg以下）との併用が最も冠動脈瘤の発生率が低いことが確認された[17]．

2. ANCA関連血管炎に対するIVIg療法

ANCA関連血管炎に対するIVIg療法は1991年Jayneらによりはじめて報告された[18]．その後主に欧州から，腎障害の合併のないWG症例に対する有効性が示されてきた[19-25]（表2）．わが国に発症の多いMPO-ANCA関連血管炎症候群では腎所見が初発症状となることが多くRPGNで発見される例が少なくない．そこで我々はMPO-ANCA関連RPGN12症例において初期治療としてのIVIg療法を行い，IVIg単独療法期間における治療効果判定と，3カ月予後について報告した[26]．ここではその後の追加症例も含め腎障害を有する15人のMPO-ANCA関連腎炎に対するIVIg初期療法と6カ月予後について紹介する．IVIg療法

表2　血管炎に対する IVIg 療法

報告者（年）引用文献	対象患者数	診断（対象患者数）	IVIg投与回数	反応率（%）
Jayne, et al. (1991) [18]	7	WG (4), MPA (2), RV (1)	single	100
Richter, et al. (1995) [19]	9	WG (8), systemic P-ANCA-associated vasculitis (1)	single	55
Jayne, Lockwood, (1993) [20]	26	WG (14), MPA (11), RV (1)	single	100
Finkel, et al. (1994) [21]	3	Parvovirus B19-associated PAN (2), WG (1)	single multiple	100
Richter, et al. (1995) [22]	15	WG (14), systemic P-ANCA-associated vasculitis (1)	1-3	40
Jayne, Lockwood (1996) [23]	6	WG (3), MPA (3)	single	100
Levy, et al. (1999) [24]	10	WG (2), CSV (11), livedo vasculitis (1), other (4)	1-6	60
Jayne, et al. (2000) [25] placebo-cntrolled trial	34	WG (24) MPA (10)　single course of	17 IVIg 17 Placebo	82%IVIg 35%Placebo
Ito-Ihara, et al. (2005) [26]	12	MPO-ANCA-associated RPGN (12)	single (initial therapy)	100

MPA, microscopic polyarteritis; WG, Wegener's granulomatosis; GN, glomerulonephritis;
RV, rheumatoid vasculitis; PAN, polyarteritis nodosa; CSV, Churg-Strauss vasculitis;
AASV, ANCA-associated systemic vasculitis; RPGN, rapidly progressive glomerulonephritis.

の効果は Birmingham Vasculitis Activity Score (BVAS)[27]，血中白血球数（WBC），CRP とともに，IVIg 投与前後の腎機能の変化を 1/クレアチニン（Cre）の単位時間当たりの変化率を施行前，1，3，6カ月時点で測定することで評価した．

15例の患者の年齢は 72 ± 3 歳と高齢，治療前の平均 Cre 値は 4.1mg/dl と腎不全状態のものが多く，腎生検でも 10名に 50% 以上の糸球体に活動性半月体を認めた．MPO-ANCA 値も 389 ± 115EU と高値で，血中白血球数 9900 ± 2200/μl，CRP9.8 ± 1.5mg/dl と高値であった．15例中6例に潜在性ウイルス感染あるいは治療抵抗性感染症の合併を認めた（表3）．

5日間の IVIg 治療後約1週間の時点で CRP，WBC の有意な低下を認めた．腎機能低下を評価するための 1/Cre の変化率も増加し，有意な改善が認められた．白血球分画のうち，好中球の低下が最も有意であった．IVIg による各種炎症性サイトカインの血中における変動をも検討したとこ

ろ TNF-α の有意な低下を認めた（図1）．血管炎活動性を評価する BVAS の低下を認め，臓器別にその有効性を検討したところ全身症状の改善と急速に進行する腎障害の低下が認められた（図2）．

後療法として，比較的低用量の経口ステロイド療法（0.6 ± 0.1mg/kg/day）を行った．3カ月の時点で再発1例，腎死は IVIg 投与以前より血液透析を導入していた1例であったが死亡例はなく，また重篤な感染症の発症はみられなかった．6カ月後の検討では再発による死亡1例，腎死は1例であり6カ月での生存率・腎生存率ともに 93% であった（図3）．

以上の所見は MPO-ANCA 関連血管炎において，初期治療としての IVIg 療法の安全性と臨床効果を確認した．これらの症例では血管炎による腎機能低下を含む多臓器症状を認めたが，IVIg 療法により炎症所見の改善とともに腎機能低下の抑制を認めた．治療前評価で潜在性難治性感染症

表3　患者背景

Patient No.	Age	Sex	Organ Involvement Befor IVIg	Date before treatment				MPO-ANCA (EU)	Active Crescent (%)	Latent of Antibiotios resistant infection
				BVAS	WBC (/μl)	Cre (mg/dl)	CRP (mg/dl)			
1	82	F	S. F, E, L, K	17	10100	3.20	8.00	239	81	HBV
2	75	M	S. F. A, C, L, K, N	25	10100	1.20	17.80	435	0	MAC, K. Pneumoniae
3	77	M	S. F. A, L, K	19	5900	1.20	3.20	25	0	TB, MAC
4	65	M	S. F. A, C, K, N	26	12000	2.00	22.00	80	50	
5	61	F	S. F. K	15	6500	1.42	4.28	244	71	
6	82	M	S, A. K	12	9400	4.27	10.38	159	71	
7	64	F	S. F, L, K	19	12100	8.34	15.35	276	81	
8	83	F	S. F, A, K	15	9300	0.67	7.91	144	29	
9	59	M	S, F, K	15	10800	4.40	13.90	140	90	Aspergillus
10	83	F	S. F, L, K, Ab	24	4800	2.92	0.14	617	60	
11	82	F	S, F, K, N	21	14700	2.38	11.27	306	38	
12	57	M	S, A, L, K, N	21	12500	4.04	9.87	239	64	
13	62	M	S, L, K, N	21	12200	8.23	10.14	370	80	
14	75	M	S, E, L, K	27	6600	11.45	5.98	82	33	HBV, MRSA, P. aerginosa .
15	67	M	S, L, K	19	11900	4.54	6.84	1740	78	MRSA
Mean	72	M/F 9/6		20	9927	4.02	9.80	340	55	

RPGM, rapidly progressive glomerulonephritis; MPA, microscopic polyangiitis; BVAS, Birmingham vasculitis activity score; MPGN, membranoproliferative glomerulonephritis; Anti-GBM GN, anti-glomerular basement membrane glomerulonephritis; HBV, hepatitis B virus; MAC, *Mycobacterium avium* complex; *K. Pneumoniae, Klebshiella Pneumoniae*; TB, Tuberculosis; MRSA, methicillin-resistant *Staphylococcus aureus; P. Aerginosa, Pseudomonas aerginosa.*
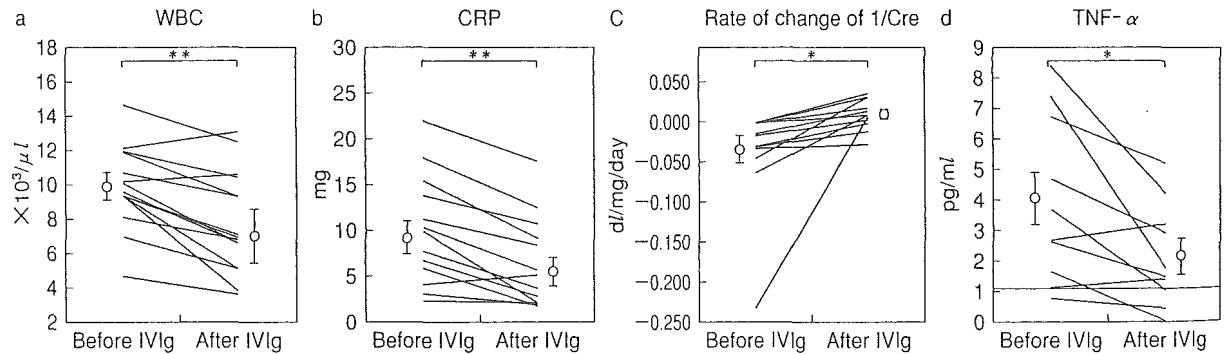

図1　IVIg 療法前後の変化

a: 末梢血白血球数 (/μl), b: C-reactive protein (mg/dl), c: 1/クレアチニン変化率 (dl/mg/day), d: 血漿 Tumor necrosis factor-alpha (pg/ml); *, p < 0.05; **, p < 0.01; Mean ± SEM

を5名に認め，80歳以上の高齢者が5名含まれていたが，経過中重篤な感染症の合併を認めなかった．6カ月生存率・腎生存率93%であり，これらはわが国の MPO-ANCA 関連 RPGN を対象とした報告にみられる，6カ月生存率74.2%，6カ月腎生存率 69.9%と比較し[8]，良好な結果であった．またその機序の一部は炎症性サイトカインの発現抑制を介していることが考えられた．
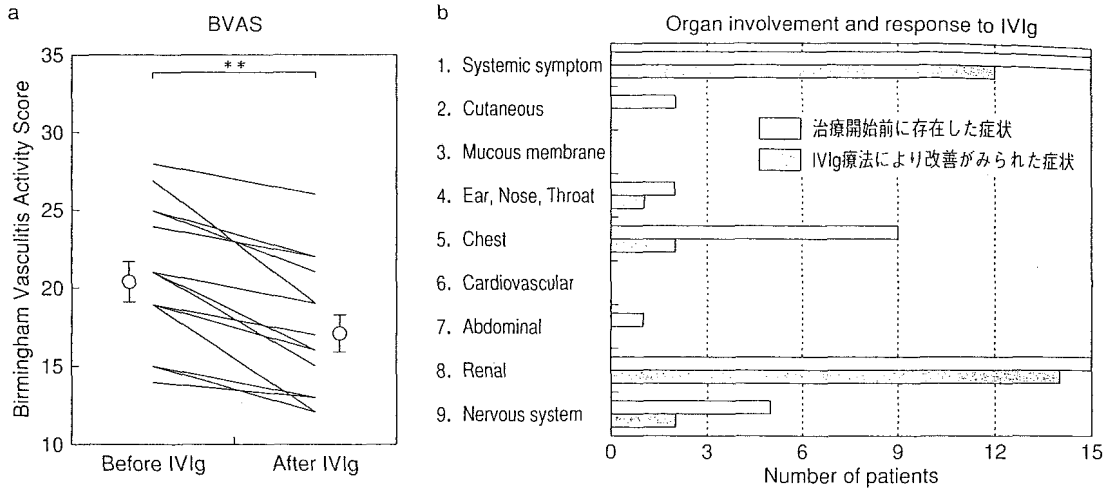
投与された IVIg 製剤の半減期は3カ月といわれており[12]，今後は適切な投与間隔・投与回数

図2　IVIg 前後の疾患活動性の変化
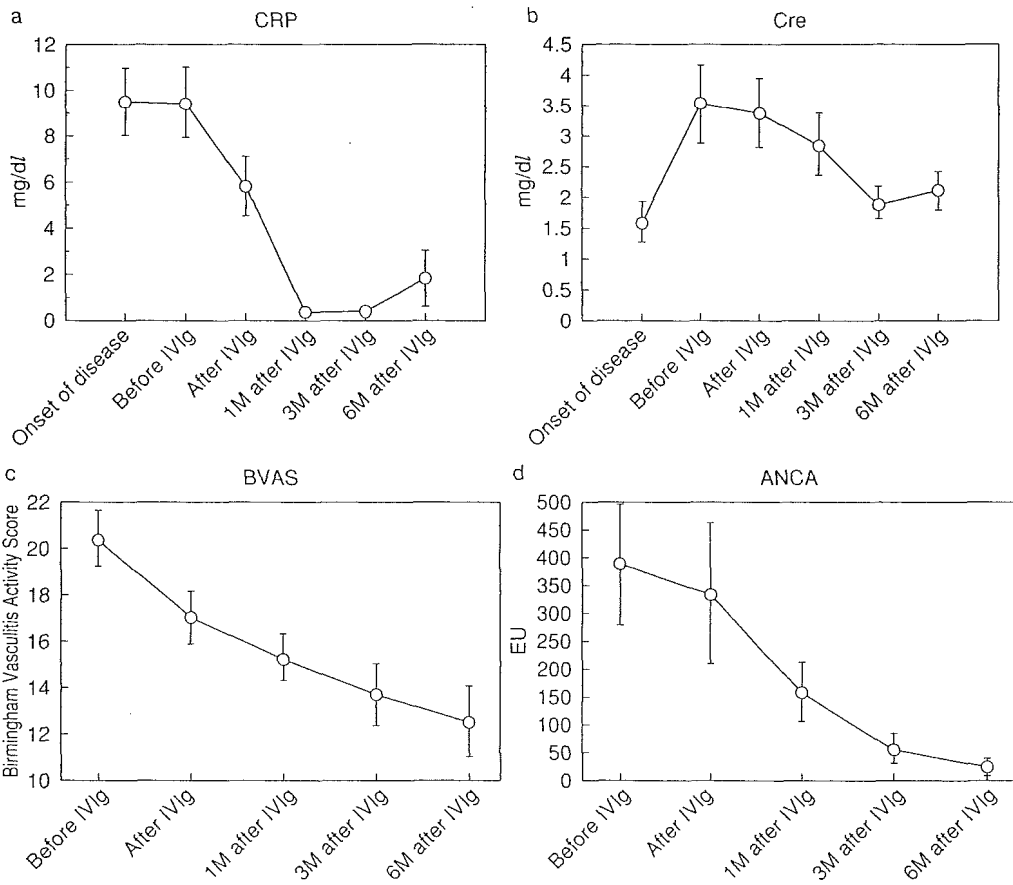a: Birmingham Vasculitis Activity Score; ＊＊, p＜0.01; Mean ± SEM
b: 各臓器別治療反応性

図3　IVIg 療法とその6カ月経過
a: C-reactive protein (mg/dl), b: 血清クレアチニン (mg/dl), c: Birmingham Vasculitis Activity
Score, d: MPO-ANCA (EU); M, month; Mean ± SEM.

の検討が必要であると考えられた．我々の検討においても1例（症例14）は既往に喉頭癌があり気管切開孔より MRSA，多剤耐性緑膿菌の排菌が持続していたため，IVIg療法後のステロイドならびに免疫抑制療法を行っていなかった．IVIg療法により炎症反応低下，全身症状の改善を認めたが3カ月の時点で再発し，少量のステロイドを開始している．

B. 血管炎症候群に対するIVIg療法の効果発現機序の考察

一般にIVIg療法の免疫修飾作用の発現には表4のような機序が考えられている．

1. Fcレセプター（FcR）を介する機序

血管炎における効果についてFcレセプターが関与していることは，川崎病でFcを取り去った F(ab´)2 部分のみの製剤では効果が低いことから

も推測される[28]．また，最近のモデルマウスを用いた報告でITPではIVIgによって抑制性Fcレセプターである FcγRIIB の発現を促進することで，その効果を発揮していることも示された[29]．

2. 抗炎症作用と細胞増殖への作用

炎症性サイトカインのIVIg療法後の減少が種々の自己免疫疾患において報告されており，その機序としてグロブリン製剤に含まれる抗サイトカイン抗体の存在や活性化された末梢単核球・マクロファージからのサイトカイン産生・分泌抑制作用[30]，サイトカインレセプターアンタゴニストの誘導[31]などが報告されている．

TNF-α はANCA関連血管炎症候群において，好中球のプライミングと活性化に重要な役割を果たしており，我々の症例群でIVIg療法後に血中 TNF-α の低下を認めたことは疾患活動性抑制において重要と考えられた．また，ANCA関連血管炎症候群では好中球におけるアポトーシス制御

表4　IVIgの免疫抑制作用（文献 12，13より改変）

Fcレセプターへの作用
　　　　　マクロファージとエフェクター細胞におけるFcレセプターの阻害
　　　　　抗体依存性cytotoxicityの誘導
　　　　　抑制性Fcγ レセプターIIBの誘導
抗炎症作用
　　　　　抗炎症性サイトカインの誘導
　　　　　炎症性サイトカイン抗体の含有
　　　　　内皮細胞活性化の抑制
　　　　　微生物毒素の中和
　　　　　ステロイド使用量の減少作用
細胞増殖への作用
　　　　　アポトーシスの制御
　　　　　リンパ球増殖抑制作用
T cellに対する作用
　　　　　ヘルパーT cellからのサイトカイン産生の制御
　　　　　T cell スーパー抗原の中和
B cell および抗体への作用
　　　　　骨髄B cell repertoiresの制御
　　　　　Fc γ レセプターを介する抑制シグナル伝達
　　　　　抗体産生の選択的抑制および亢進
　　　　　抗イディオタイプ抗体による自己抗体の中和