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CLINICAL REPORT

Serum Levels of Autoantibodies to Desmoglein 3 in Patients with Therapy-resistant Pemphigus Vulgaris Successfully Treated with Adjuvant Intravenous Immunoglobulins

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The mainstay of treatment of pemphigus vulgaris is systemic corticosteroids. Intravenous immunoglobulins have been reported as an adjuvant corticosteroid-sparing regimen in recalcitrant pemphigus vulgaris. The purpose of the study was to monitor disease activity, serum levels of autoantibodies and doses of oral corticosteroids in 4 patients with recalcitrant pemphigus vulgaris adjuvantly treated with intravenous immunoglobulins (2 g kg^{-1} monthly). After initiation of intravenous immunoglobulins, all patients showed clinical improvement and a decrease in autoantibody serum levels, as detected by both indirect immunofluorescence microscopy and ELISA. Corticosteroids and immunosuppressants could be reduced and even discontinued in one patient. In 3 patients, intravenous immunoglobulins were discontinued after 12 cycles. Subsequently, new blisters developed and autoantibody levels rose again. After re-initiation of intravenous immunoglobulins, in 2 patients, the condition quickly improved again, along with a decrease in autoantibody serum levels. It is concluded that the administration of intravenous immunoglobulins was associated with a decrease in circulating autoantibodies and clinical improvement in our patients. ***Key words: autoimmunity; bullous disease; desmosome.***

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Pemphigus vulgaris (PV) is a severe autoimmune blistering disease that affects both the mucous membranes and skin. Autoantibodies are directed against desmogleins (dsg) 1 and 3, cadherin-type desmosomal adhesion molecules (1). Levels of autoantibodies have been shown to correlate with disease activity, as detected by indirect immunofluorescence (IF) microscopy on monkey oesophagus or by enzyme-linked immunosorbent assay (ELISA) using recombinant dsg 3 as the target antigen (2, 3). During relapse, levels of autoantibodies detected by ELISA increased earlier than titres measured by indirect IF microscopy. This may reflect a higher sensitivity of the ELISA method compared to indirect IF analysis (4). PV is associated with a high mortality rate when untreated (5), but there is a significant improvement in the prognosis with the advent of systemic corticosteroids (6). However, the use of corticosteroids as long-term treatment may involve serious side effects, such as diabetes mellitus, osteoporosis, depression and opportunistic infections.

High-dose intravenous immunoglobulin (IVIG) therapy has been used in the treatment of a number of inflammatory skin diseases, including toxic epidermal necrolysis (7) and autoimmune diseases such as dermatomyositis (8), bullous pemphigoid (9), pemphigus foliaceus and vulgaris (9–12), linear IgA disease (13) and mucous membrane pemphigoid (14–16). However, the mechanisms of action of immunoglobulins are still poorly understood. Hypotheses on the mode of action include modulatory effects on the immune system, opsonization and down-regulation of the production of autoantibodies (17–19). IVIG preparations consist of about 98% IgG, pooled and purified from a collection of healthy blood donors (1,000 donors are needed to provide 10 g of IVIG). Cold ethanol fractionation, additional viral inactivation procedures and screening of donors for infections as well as normal liver function improved the safety of the products (20, 21). The recommended doses vary, depending on the purpose IVIG is used for. For replacement therapy in patients with immune deficiency diseases, 0.1 to 0.4 mg kg^{-1} body $weight^{-1}$ is used, whereas for treatment of autoimmune disorders, high doses of IVIG, usually 2 g kg^{-1}, are given, either as mono- or as adjuvant therapy. Based on the promising results of IVIG therapy in patients with autoimmune blistering diseases (9–13, 15), we used this treatment modality in 4 patients with PV with recalcitrant disease, suffering from severe side effects of long-term treatment with oral glucocorticosteroids. During and after the administration of IVIG, we carried out close monitoring of disease activity, including assessment of serum levels of autoantibodies to dsg 3.

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PATIENTS AND METHODS

Patients

Four female patients (36–78 years old) with recalcitrant PV were included in the study. All had been treated with various immunosuppressive agents for more than 2 years. In 2 patients, lesions were found on both oral/hypopharyngeal and genital mucosa: in the third patient on oral/genital mucosa and normal skin and in the fourth on oral mucosa only. Three patients had been treated with methylprednisolone (initially 1 mg kg^{-1} day^{-1}) and azathioprine (2 mg kg^{-1} day^{-1}); doses were tapered according to a predesigned schedule. One patient had received intravenous pulses with dexamethasone (100 mg day^{-1}) and cyclophosphamide (750 mg day^{-1}) on a monthly base following a previously described protocol (22) for a total of 21 months. In all patients, side effects of long-term immunosuppressive therapy, including diabetes, hypertension, depression, osteoporosis, striae, weight gain, hair loss and acne were seen. Patients' characteristics before IVIG therapy are detailed in Table I.

Methods

Direct IF microscopy of perilesional biopsies from oral mucosa demonstrated intercellular deposits of IgG in 4 patients and of C3 in 3 patients. An indirect IF analysis was performed on monkey oesophagus and revealed intercellular staining of IgG in all patients; titres before IVIG was initiated ranged between 40 and 160. Antibodies to dsg 1 and 3 were determined by ELISA (MBL, Nagoya, Japan) (3, 23). The optical density (OD) results were expressed as index values = (OD sample − OD negative control)/(OD positive control − OD negative control) × 100. The cut-off value was set at an index value of 20; all samples were run in duplicate. Before each IVIG cycle, a serum sample was obtained for indirect IF microscopy and ELISA. For better comparison, samples from the same patient, obtained at different stages of the disease, were analysed on the same ELISA plate.

Disease activity was scored according to the number of fresh lesions on mucous membranes/skin developing in the preceding week: 3 = more than 10 fresh lesions; 2 = 1–10 fresh lesions; 1 = no new lesions, further therapy needed. If no new lesions occurred and therapy was discontinued, disease activity was scored as 0. IVIG (Intraglobin® F, Biotest, Dreieich, Germany) was administered at 2 g kg^{-1} on 2 consecutive days every 4 weeks for a total of 12 cycles.

RESULTS

All patients showed clinical improvement within 1 to 3 IVIG cycles (mean 2.5 cycles) and no new lesions developed within 2 to 12 weeks (mean 7 weeks) after initiation of IVIG. Patients' data during IVIG treatment are summarized in Table II.

After one cycle of IVIG, clinical disease activity had decreased in all patients and levels of circulating antibodies had dropped in 3 and increased in one patient. In 2 patients, we were able to reduce methylprednisolone to 16 mg day^{-1} and 12 mg day^{-1}, respectively; in one patient, azathioprine was omitted and methylprednisolone was reduced to 8 mg day^{-1}.

After 6 cycles of IVIG, all the patients were free of lesions and serum levels of autoantibodies to dsg 3, and indirect IF titres were negative or close to the normal level. In one patient, immunosuppressive therapy could be discontinued completely. In 2 patients, methylprednisolone was reduced to 6 mg day^{-1}, and in another patient the intervals between dexamethasone-cyclophosphamide pulses were extended to 2 months. In addition, the intervals between IVIG cycles were increased to 6 weeks in patients 1 and 2 (Table II).

After 12 cycles, IVIG therapy was discontinued in 3 patients (patient 4 has received only 10 treatments as yet). At this time, cyclophosphamide-dexamethasone pulses were changed to dexamethasone pulses without cyclophosphamide in patient 2, methylprednisolone was reduced to 8 mg day^{-1} in patient 3 and no further immunosuppressive therapy was necessary in patient no. 1. However, within 6 to 10 weeks after discontinuation of treatment, all 3 patients developed new blisters

Table I. *Characteristics of four female patients immediately before initiation of intravenous immunoglobulin therapy*

	Patients			
	1	2	3	4
Age (years)	74	63	38	51
Disease duration (months)	38	20	22	18
Medication	MP 16 mg A 150 mg D/C4[a]	D/C 4[a]	MP 24 mg A 150 mg	MP 20 mg A 175 mg
Disease activity[b]	3	3	3	3
Indirect IF titre[c]	40	40	80	160
Autoantibodies to dsg 1[d]	Neg.	Neg.	Neg.	Neg.
Autoantibodies to dsg 3[d]	98	81	120	250

[a]D/C 4: dexamethasone-cyclophosphamide pulses every 4 weeks.
[b]Disease activity score: 3 = more than 10 new lesions; 2 = 1 to 10 new lesions; 1 = no new lesions; 0 = no new lesions and no further therapy needed.
[c]Titre obtained by indirect immunofluorescence (IF) microscopy on monkey oesophagus.
[d]Serum levels of autoantibodies against desmogleins (dsg) 1 and 3, as determined by enzyme-linked immunosorbent assay using recombinant dsg 1 and 3, expressed as index values.
MP: methylprednisolone; A: azathioprine.

Table II. *Patients' data during intravenous immunoglobulin (IVIG) therapy*

	Patients			
	1	2	3	4
Total number of IVIG cycles	16	18	16	5
Number of IVIG cycles until no new lesions occurred	2	3	3	1
Weeks until no new lesions occurred	8	12	9	2
After 1 cycle of IVIG:				
Disease activity score[d]	1	2	2	1
Medication	MP 8 mg A 150 mg	D/C 4[a]	MP 16 mg A 150 mg	MP 12 mg A 175 mg
Indirect IF titre[b]	40	80	Neg.	20
Level of autoantibodies to dsg 3[c]	220	60	45	80
After 3 cycles of IVIG:				
Disease activity score[d]	1	1	2	1
Medication	MP 4 mg A 150 mg	D/C 4[a]	MP 8 mg A 150 mg	MP 8 mg A 175 mg
Indirect IF titre[b]	10	40	40	Neg.
Level of autoantibodies to dsg 3[c]	127	69	65	Neg.
After 6 cycles of IVIG:				
Disease activity score[d]	0	1	1	1
Medication	None	D/C 8[a]	MP 6 mg A 150 mg	MP 6 mg A 175 mg
Indirect IF titre[b]	Neg.	40	10	Neg.
Level of autoantibodies to dsg 3[c]	Neg.	Neg.	Neg.	Neg.

[a]D/C 4/8: dexamethasone-cyclophosphamide pulses every 4 (8) weeks.
[b]Titre of indirect immunofluorescence (IF) microscopy on monkey oesophagus.
[c]Serum levels of autoantibodies against desmogleins (dsg) 1 and 3 determined by enzyme-linked immunosorbent assay.
[d]Disease activity score. See footnote to Table I for both c and d.
MP: methylprednisolone; A: azathioprine.

Table III. *Patients' data after withdrawal of intravenous immunoglobulins (IVIG)*

	Patients		
	1	2	3
Findings after discontinuation of IVIG			
Number of IVIG cycles before discontinuation	12	12	12
Weeks until new lesions occurred	3	3	2
Status at 6 weeks after discontinuation of IVIG:			
Disease activity score[c]	3	2	3
Medication	MP 36 mg A 150 mg	D/C 4[a]	MP 6 mg A 150 mg
Indirect IF titre[b]	160	40	320
Level of autoantibodies to dsg 3[d]	75	18	162
Present status:			
Number of IVIG cycles until no new lesions occurred	1	2	4
Total number of IVIG cycles	17	20	18
Disease activity score[c]	1	1	2
Medication	MP 8 mg A 150 mg	D	MP 32 mg A 150 mg
Indirect IF titre[b]	40	10	160
Level of autoantibodies to dsg 3[d]	61	Neg	365

[a]D/C 4: dexamethasone-cyclophosphamide pulses every 4 weeks.
[b]Titre determined by indirect immunofluorescence microscopy on monkey oesophagus. [c]Disease activity score.
[d]Levels of autoantibodies against desmoglein (dsg) 3 as determined by ELISA. See footnote to Table I for both [c] and [d].
D: dexamethasone pulse therapy; MP: methylprednisolone; A: azathioprine.

and erosions on mucous membranes and serum levels of autoantibodies rose in patients 1 and 3 (Table III). In the last two patients, methylprednisolone was increased to 1 mg kg^{-1} body $weight^{-1}$ and azathioprine was initiated. In patient 2, the intervals between dexamethasone pulses were reduced from 8 to 4 weeks. Glucocorticosteroid-related side effects, such as diabetes, weight gain and acne, reappeared and IVIG therapy was initiated again in these 3 patients. Within 4 to 8 (mean 6) weeks after restart of IVIG, we found that formation of new lesions ceased and titres of indirect IF microscopy decreased in all patients. Levels of autoantibodies to dsg 3 dropped in patient 1 and became negative in patient 2. In patient 1, methylprednisolone was reduced to 8 mg day^{-1}. To date (mean follow-up 7.5 months), while still on IVIG, these patients are free of new lesions. Patient 3 still shows high serum levels of autoantibodies to dsg 3 and erosions on mucous membranes.

DISCUSSION

The mainstay in the treatment of pemphigus is high-dose corticosteroids in combination with various immunosuppressants. In some patients with recalcitrant disease, these regimens may fail to lead to remission and are associated with severe side effects. In these patients, other treatment modalities with a corticosteroid sparing effect would be helpful. Previously, the successful use of adjuvant high-dose IVIG has been reported in the treatment of severe pemphigus (6, 15). In this prospective study, we treated 4 patients with recalcitrant PV with adjuvant IVIG and monitored disease activity clinically and serologically. IVIG was given in combination with oral methylprednisolone and azathioprine or dexamethasone-cyclophosphamide pulse therapy. Despite previous immunosuppressive treatments, the patients had continued to show high disease activity with multiple erosions on mucous membranes. After adding IVIG to the treatment regimen, we saw a dramatic clinical improvement in all patients within 2 to 6 weeks and a considerable decrease in serum levels of circulating autoantibodies to dsg 1 and 3. Doses of corticosteroids could be reduced and even discontinued in one patient. After discontinuation of IVIG in 3 patients, clinical relapses developed within 2 to 8 weeks along with an increase in serum levels of autoantibodies. IVIG was then restarted and, within 1 to 6 weeks, disease activity and serum levels of autoantibodies decreased again.

Serum levels of autoantibodies had not yet been studied in order to monitor the clinical course under adjuvant therapy of IVIG in PV. Before and during treatment, we therefore measured both disease activity, using a predesigned score, and serum levels of autoantibodies to dsg 1 and 3. Our results demonstrate that IVIG, in addition to improving the skin disease, also affects serum levels of circulating autoantibodies.

In most reports elsewhere (9, 11, 24–26), patients with autoimmune bullous diseases were treated with a single course of high-dose IVIG or shorter series of up to 7 cycles. There are only a few reports on patients treated with a higher number of IVIG cycles (10, 12, 15, 27). Recently, it was suggested that the gradual extension of intervals between IVIG cycles might be more effective in preventing a relapse than prompt discontinuation of IVIG after 6 of 12 cycles. In addition, a continuation of IVIG after the initial control of disease has been recommended (28). We discontinued IVIG after 12 cycles; in 3 patients we extended the intervals to 8 weeks and in one patient to more than 8 weeks. However, in all 3 patients a relapse occurred after discontinuation of IVIG. Following the recent observations (28), extending the interval between IVIG cycles before discontinuing it entirely may have helped in preventing a relapse in our patients. Side effects of IVIG that were observed in our patients were minor and included headache, chills and low-grade fever (max. 38.3°C), which were successfully treated with non-steroidal antiinflammatory drugs.

To summarize, we demonstrated the effect of adjuvant IVIG therapy in 4 patients with severe PV not only with respect to the clinical picture but also regarding serum levels of circulating autoantibodies. So far, experiences with IVIG are based on case reports or small groups of patients. A controlled study should compare side effects and efficacy of IVIG along with other treatment options for severe recalcitrant PV.

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