Clinical Response of Severe Mechanobullous Epidermolysis Bullosa Acquisita to Combined Treatment With Immunoadsorption and Rituximab (Anti-CD20 Monoclonal Antibodies)

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Background: Epidermolysis bullosa acquisita (EBA) is an autoimmune bullous disorder with mucocutaneous involvement, skin fragility, and tendency to scarring. The mechanobullous form of EBA has a chronic relapsing course and is difficult to treat. We describe herein the therapeutic response of 2 patients with recalcitrant mechanobullous EBA to combined treatment with immunoadsorption and rituximab, an anti-CD20 monoclonal antibody that induces depletion of B cells in vivo.

Observations: Two patients with mechanobullous EBA received combined treatment with immunoadsorption and rituximab, resulting in an almost complete clinical remission in one patient and stable disease in the other patient. In the patient with complete remission, prolonged B-cell depletion and clinical improvement with disappearance of mucocutaneous erosions paralleled the decline in titers of circulating anti– basement membrane zone autoantibodies. In the other patient, combined treatment with immunoadsorption and rituximab reduced the de novo appearance of blisters but did not lead to significant improvement of gingivitis, despite depleted B cells for 6 months that remained at 5% 12 months after the last administration of rituximab, as well as a reduction in autoantibody titers.

Conclusion: The patients’ response suggests that combined treatment with immunoadsorption and rituximab may be a valuable adjuvant treatment regimen for severe mechanobullous EBA, which is in line with recently observed beneficial effects in inflammatory EBA.

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EPIDERMOLYSIS BULLOSA ACQUISITA (EBA) is a chronic subepidermal bullous disease of the skin and mucous membranes characterized by the presence of autoantibodies against type VII collagen, a major component of anchoring fibrils. There is great diversity in the clinical presentation of the disease. At least 5 clinical subsets of EBA can be distinguished: (1) classic mechanobullous presentation with spontaneous or trauma-induced blisters resembling hereditary dystrophic epidermolysis bullosa, (2) bullous pemphigoid–like presentation, (3) mucous membrane pemphigoid–like presentation, (4) Brunsting–Perry pemphigoid–like presentation, and (5) linear IgA bullous dermatosis–like disease. The classic form is especially difficult to treat. This form manifests as mechanobullous noninflammatory disease with an acral distribution and skin fragility over trauma-prone surfaces. The blisters and erosions heal with scarring and milia formation. The vesicles and tense bullae appear on noninflamed or atrophic skin, leading to erosions, crusts, scales, scars, scarring alopecia, cysts, milia, and nail dystrophy. In the more severe form, there can also be loss of nails, sclerosis of the hands and fingers, and esophageal stenosis, reminiscent of recessive dystrophic epidermolysis bullosa.

The treatment of EBA is often a major therapeutic challenge. There are anecdotal reports of successful treatment with cyclosporine, colchicine, high- and low-dose intravenous immunoglobulins, plasmapheresis in conjunction with immunoglobulins, and extracorporeal photochemotherapy. Because of the
rareness of the disease, controlled clinical therapeutic trials have not been performed, to our knowledge.

Immunoadsorption (IA) is used in antibody-mediated autoimmune disorders refractory to immunosuppressive therapy.13-16 It is an effective adjuvant treatment to reduce circulating autoantibodies in patients in whom unacceptably high dosages of glucocorticosteroids are required for initial therapy or in whom the disease activity does not allow for sufficient tapering of the glucocorticosteroid dosage necessary to reduce adverse effects.

Rituximab is a genetically engineered chimeric monoclonal antibody consisting of a human IgG1 constant region with murine light- and heavy-chain variable regions that are specific for CD20, a molecule located on pre-B mature B lymphocytes and in most B-cell neoplasms.17,18 Rituximab was originally designed for the treatment of B-cell neoplasms; it is approved for the first-line treatment of diffuse large B-cell, CD20+, non-Hodgkin lymphoma, in combination with CHOP (cyclophosphamide, doxorubicin, vincristine [Oncovin], and prednisone) or other anthracycline-based chemotherapy regimens. It has also been used to treat various refractory autoimmune diseases, including idiopathic thrombocytopenic purpura,19 autoimmune hemolytic anemia,20 rheumatoid arthritis,21 systemic lupus erythematosus,22,23 myasthenia gravis,24 Wegener granulomatosis,25-27 Sjogren syndrome,28 dermatomyositis,29 and paraneoplastic pemphigus.30-32 Rituximab has been recently approved for the treatment of rheumatoid arthritis by the Food and Drug Administration in combination with methotrexate in patients who are refractory to other disease-modifying antirheumatic drugs, including 1 therapy or more with tumor necrosis factor inhibitors. There are case reports on the effect of rituximab treatment in refractory pemphigus vulgaris and a report on the use of rituximab in a patient with inflammatory bullous pemphigoid–like EBA.33 Herein, we describe 2 patients with mecanobullous EBA who were successfully treated with a combination of IA followed by rituximab.

## METHODS

### PATIENTS

Both patients had an unambiguous diagnosis of EBA according to the following clinical and laboratory diagnostic criteria: (1) cutaneous and mucosal superficial erosions and skin fragility with a tendency to scarring and cutaneous atrophy, (2) subepidermal loss of adherence as shown by histopathologic findings, (3) indirect immunofluorescence showing linear IgG fluorescence along the dermoepidermal junction and dermal IgG staining on skin substrate that was separated at the lamina lucida by incubation with 1M sodium chloride, and (4) detection of anti–collagen VII antibodies in the serum.

### CLINICAL OUTCOME

To date, there is no consensus about uniform criteria to grade disease severity or to define remission in autoimmune bullous diseases. An autoimmune bullous skin disorder intensity score was established to assess disease activity and therapeutic response in individual patients (M.P., unpublished data, 2006). Briefly, the autoimmune bullous skin disorder intensity score consists of 3 components (skin, oral mucosa, and genital mucosa), representing the most commonly affected areas. Skin involvement was measured according to the type and extent of lesions. This score was designed for monitoring intraindividual therapeutic responses rather than comparing disease activity among individuals. Disease severity of the oral mucosa was graded on a scale from 0 (no disease activity in any of 11 defined sites of the oral mucosa) to 11 (maximum disease activity). Atrophic skin lesions that are typically found in mecanobullous EBA were not counted as lesions.

### TREATMENT REGIMEN

Immunoadsorption was performed according to an established protocol.31 Briefly, IA was performed on 4 consecutive days using commercially available adsorbers (Globaffin; Fresenius Medical Care, Lexington, Mass), representing 1 treatment cycle. Each cycle was followed by a second cycle after a 4-week interval. During the entire IA treatment, patients continued receiving an immunosuppressive medication consisting of glucocorticosteroids and the glucocorticosteroid-sparing adjuvant agent mycophenolate mofetil (1 g 3 times daily).

After IA, rituximab was administered intravenously at a dose of 375 mg/m² in body surface area during 4 to 6 hours once weekly for 4 consecutive weeks. The patient was pretreated with intravenous administration of 100 mg of prednisolone-21-hydrogen succinate, 4 mg of dimethindene maleate, and 30 mg of ranitidine hydrochloride. As an oral antipyretic agent, 500 mg of acetaminophen was given 2 hours before treatment.

### IMMUNOLOGICAL FINDINGS

Both patients showed linear IgG and complement C3 deposits at the dermoepidermal junction of perilesional skin by direct immunofluorescence; anti–collagen VII IgG autoantibodies were detected by immunoblot analysis with recombinant protein of the immunodominant domain NC1 of collagen VII, produced in a baculovirus expression system (Ralf Müller, PhD, unpublished data, 2006). Anti–basement membrane zone antibodies were detected by indirect immunofluorescence analysis of patients’ blood samples using epithelial cell surfaces of monkey esophagus as substrate and sodium split human skin. The count of peripheral blood B cells was investigated by flow cytometric analysis of peripheral blood mononuclear cells using a monoclonal antibody reacting with the pan-B CD19+ differentiation antigen (Department of Oncology and Hematology, University Hospitals of Marburg, Marburg).

### REPORT OF CASES

**PATIENT 1**

A 67-year-old man had been diagnosed as having EBA 4 years previously. He had bullous and erosive lesions of the oral mucosa, esophagus, and nasopharynx. His hands, shanks, and feet showed tense blisters and erosions. On both feet, nail loss had occurred on most of the toes due to postinflammatory scarring (Figure 1A). The lesions on the soles significantly impaired walking. Before IA, he had already undergone several long-term immunosuppressive treatment regimens (Figure 2A). Because of unresponsiveness to these regimens, patient 1 received 2 treatment cycles of IA. After completion of IA,
A 42-year-old man who had been diagnosed as having EBA 9 years previously was admitted to the hospital because of a severe chronic course. He had blisters and skin fragility mainly on his trunk and forearms (Figure 3A). The oral mucosa had also been affected in a previous manifestation. To control the disease, he had undergone multiple immunosuppressive therapies (Figure 2B). Because of recalcitrant EBA, patient 2 received 2 treatment cycles of IA followed by rituximab infusions during 4 weeks and adjuvant immunosuppressive treatment with mycophenolate mofetil (3 g/d). The patient discontinued mycophenolate mofetil treatment after rituximab therapy. When he was seen 34 weeks later, his disease was well controlled, with few crusty erosions and atrophic lesions (Figure 3B). The clinical response was accompanied by a decline in titers of circulating autoantibodies (1:800 before treatment and 1:200 after treatment). Titers were below the detection limit at 34 weeks after rituximab therapy. Complete B-cell depletion persisted for at least 37 weeks (Figure 3C).

Figure 1. Patient 1. Partial clinical response of mechanobullous epidermolysis bullosa acquisita to combined treatment with immunoadsorption (IA) and rituximab. A, The patient had blisters and erosions on his hands, feet, and oral mucosa. B, At 15 weeks after completion of combined treatment with IA and rituximab therapy, marked improvement of the lesions on the dorsal aspect of his hands was notable, while erosions of the oral mucosa remained largely unaffected. C, Data are shown before and at 15 weeks after combined treatment with IA and rituximab therapy. ABSIS indicates autoimmune bullous skin disorder intensity. The light gray arrows each indicate 1 treatment cycle of IA; black arrow, 1 treatment cycle of rituximab consisting of 375 mg/m² body surface area weekly on 4 consecutive weeks.

Epidermolysis bullosa acquisita is a chronic subepidermal blistering disease associated with humoral autoimmunity to type VII collagen, an integral part of anchoring fibrils that are important components of the dermoeidermal junction. The pathogenic relevance of collagen VII–specific autoantibodies in EBA has been recently shown in an animal model. Epidermolysis bullosa acquisita is refractory to many immunosuppressive treatments. At present, no controlled clinical therapeutic studies exist for this disease, to our knowledge. In a recent systematic review of the literature, it was stated that definitive conclusions for the treatment of EBA cannot be drawn. The bullous pemphigoid–like inflammatory presentation of EBA seems to be more responsive to immunosuppression than the classic mechanobullous form, which has been reported to be refractory to systemic corticosteroids, oral azathioprine, methotrexate, and cyclophosphamide. Immunoadsorption has increasingly been used to decrease autoantibody levels in autoimmune disorders refractory to established immunosuppressive agents. Reports on the successful use of IA in systemic lupus erythematosus, Sjögren syndrome, severe bullous pemphigoid, and diseases of the pemphigus group have been published. For patients with severe pemphigus, a treatment protocol was recently published that induced prolonged clinical improvement of mucosal and cutaneous lesions and was accompanied by a dramatic reduction in serum IgG autoantibodies.

Therefore, IA may be an efficient technique to rapidly remove circulating autoantibodies as the pathogenic agent in EBA. In the previous study, the use of an adsorber system (Globaffin) as an adjuvant treatment in 4 patients with pemphigus vulgaris and in 2 patients with pemphigus foliaceus was investigated. The peptide matrix of the adsorber binds to IgG and circulating immune complexes with high affinity...
and with lower affinity to IgA and IgM. Its binding characteristics are similar to those of protein A Sepharose.\textsuperscript{38} In the IA study,\textsuperscript{55} all 6 treated patients tolerated IA well and showed no symptoms of allergic reactions or cardiovascular dysfunction; the adsorber system effectively reduced anti–desmoglein 1 and desmoglein 3–reactive IgG by a mean of 50% to 70% per IA cycle consisting of 4 consecutive IA treatments. In an earlier study by Schmidt et al,\textsuperscript{55} 5 patients with severe pemphigus were treated with IA using \textit{Staphylococcus aureus} protein A columns. The treatment schedule consisted of IA treatment on 3 consecutive days; a fourth IA treatment was given on day 8, followed by up to 19 IA treatments during intervals of 1 to 4 weeks. Protein A IA effectively reduced anti–desmoglein 1 and desmoglein 3 IgG by a mean of 76%, correlating with a good clinical response in all patients.\textsuperscript{55} In a study by Lüftl et al,\textsuperscript{15} a tryptophan-linked polyvinylalcohol adsorber system was applied. Three patients with acute onset and 6 patients with recalcitrant pemphigus received 2 IA treatments each during 3 days, followed by an intravenous prednisolone pulse. The tryptophan-linked polyvinylalcohol adsorber led to a 30% decrease in desmoglein-reactive autoantibodies, which was accompanied by significant clinical improvement. Findings from these studies, as well as other case reports,\textsuperscript{57,58} suggest that IA is an efficacious and safe treatment for severe and therapy-resistant bullous autoimmune disorders.

In contrast to the IA protocols by Schmidt et al\textsuperscript{55} and by Frost et al,\textsuperscript{58} we do not favor frequent and successive use of IA. In our view, IA represents an efficacious method that rapidly removes circulating autoantibodies as the pathogenic agent in blistering autoimmune disorders like pemphigus or EBA. However, for sustained remission of the disease, sufficient immunosuppressive treatment after IA is important to prevent rebounding autoantibody synthesis by autoreactive B cells and long-lived plasma cells. To achieve long-term arrest of production of pathogenic antibodies, we administer rituximab according to an established protocol previously used in patients with pemphigus and the inflammatory form of EBA.\textsuperscript{33-41,43,45-49}

Rituximab is administered by slow infusion during several hours. The standard regimen consists of 4 infusions (1 course) of rituximab with a dose of 375 mg/m\textsuperscript{2} at weekly intervals following premedication with an analgesic (such as acetaminophen) and an antihistamine or a corticosteroid. Systemic infusion reactions are frequently observed in patients with lymphoma, probably owing to a high load of abnormal B cells.\textsuperscript{59,60} Approximately 50% of patients treated with rituximab experience infusion-related adverse reactions, including cytokine release syndrome. These are accompanied by hypotension and bronchospasm in about 10% of patients. Severe cytokine release syndrome has been reported to occur mostly in patients with lymphoma.\textsuperscript{60,61} However, a few patients have been described who were treated for indications other than lymphoma. A common feature was development of severe reactions during the first infusion, particularly dyspnoe, hypoxia, or severe bronchospasm.\textsuperscript{59} In autoimmune diseases, these adverse reactions seem to be less of a problem. A prickling sensation in the throat that occurs 30 to 60 minutes after the start of the infusion is a common feature and has been interpreted as penetration of rituximab into the Waldeyer ring.\textsuperscript{21} The absence of normal B cells for several months has not been associated with a significant increase in infectious risk.\textsuperscript{62} Total

Figure 2. Both patients received the following treatment regimens before combined treatment with immunoadsorption (IA) (1 treatment cycle on 4 consecutive days) and rituximab (375 mg/m\textsuperscript{2} in body surface area once weekly for 4 consecutive weeks) (arrows): extracorporeal photopheresis (ECP), dexamethasone sodium phosphate pulse (50-100 mg during 3 days every 5 weeks), cyclophosphamide pulse (500-600 mg/m\textsuperscript{2} every 4 weeks in patient 1 and an unknown dosage in patient 2), cyclosporine (3.5 mg/kg per kg of body weight in patient 1 and 2.8-1.5 mg/kg per kilogram in patient 2), dapsone (100 mg/d), leflunomide (20 mg/d), intravenous immunoglobulins (IVIG) (1-2 g/kg in patient 1 and 2 g/kg in patient 2), methotrexate (25 mg/wk in patient 1 and an unknown dosage in patient 2), azathioprine (1-2 mg/d per kilogram), and mycophenolate mofetil (1-3 g/d). The complete regimens are shown for patient 1 (A) and for patient 2 (B), who also received an unknown dosage of prednisolone.
IgG levels and antitetanus titers are unaffected, whereas IgM levels are reduced to the lower end of the normal range with rituximab therapy.61

In patients with pemphigus treated with rituximab, treatment is generally well tolerated. Most adverse reactions occur during the first infusion and consist of occasional dyspnea, nausea, fever, chills, and hypotension.50 These reactions usually diminish with subsequent infusions and can be well controlled by premedication with acetaminophen and antihistamines. However, case reports exist of patients with pemphigus or other autoimmune bullous diseases that describe serious adverse effects, such as pneumonia, septic arthritis,33 sepsis,40 fatal Pneumocystis carinii pneumonia,59 deep venous thrombosis,50 and hypogammaglobulinemia.43

Based on previous observations in patients with recalcitrant pemphigus vulgaris,32 the suppression of de novo autoantibody production may be critical for a prolonged clinical remission of EBA. In support of this, patient 2 in our study with mecanobullous EBA who was recalcitrant to various immunosuppressive treatments (Figure 2) showed a remarkable clinical response to combined treatment with IA and rituximab. Although reaching complete B-cell depletion, patient 1 did not similarly benefit from the combined treatment, but we believe that both treatments contributed to the patient’s improvement. In the short term, IA decreased the pathologic circulating autoantibodies, and long-term treatment with rituximab led to clinical remission. We believe that the lack of detectable antibodies to collagen VII in patient 2 reflects the excellent clinical response to combined treatment with IA and rituximab and indicates that spontaneous remission was not responsible for the improvement of EBA.

To our knowledge, this is the first publication on successful therapeutic control of the mecanobullous form of EBA by combined treatment with IA and rituximab. It is known from the literature that the NC1 domain of type VII collagen constitutes the major immunodominant epitopes that are targeted by most EBA serum samples.64-66 In recent studies,52,67 the pathogenicity of autoantibodies against the NC1 domain of collagen VII was shown in animal models. Therefore, it is likely that circulating antibodies against the NC1 domain of collagen VII may correlate with the disease activity of patients with EBA. Based on the good response in patient 2 and the stable disease in patient 1, the combination of IA and rituximab treatment may be a novel therapeutic option for patients with severe long-standing refractory EBA.

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