Treatment of Refractory Pemphigus Vulgaris With Rituximab (Anti-CD20 Monoclonal Antibody)

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Background: Pemphigus vulgaris (PV) is a severe antibody-mediated autoimmune blistering disease. Because some patients with PV do not enter into remission, despite the use of high-dose corticosteroid therapy and immunosuppressive adjuvant treatments, new effective and safer agents are warranted to treat refractory PV. Rituximab, a monoclonal anti-CD20 antibody, induces depletion of B cells in vivo and has shown efficacy in patients with refractory antibody-mediated autoimmune disorders. We describe herein 3 patients treated with rituximab for severe PV.

Observations: Three patients with refractory PV were treated with rituximab, resulting in a clinical response in all patients, which was complete in 2 patients. A decline in titers of circulating antiepidermis autoantibodies paralleled disease activity, while circulating B cells remained undetectable for 6 to 10 months. Two patients experienced bacterial infection in the weeks following the rituximab course. A clinical relapse occurred in 2 patients, at 6 and 10 months. A second course of rituximab controlled the disease in one of them.

Conclusion: These patients’ response suggests that rituximab may be a valuable treatment for refractory PV and warrants further studies to evaluate the risk-benefit ratio in patients with PV showing resistance to classic therapy.

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EMPHIGUS VULGARIS (PV) IS A severe antibody-mediated autoimmune blistering disease involving skin and mucous membranes. Although the use of systemic corticosteroid therapy has provided a dramatic improvement in the prognosis of PV, the disease is still associated with a mortality of approximately 5%, mostly related to adverse effects caused by the high doses of corticosteroids and other immunosuppressive drugs. Furthermore, a subset of patients never enter into a complete remission, requiring long-term corticosteroid therapy with other immunosuppressive drugs. Therefore, adjuvant treatments such as azathioprine, cyclophosphamide, methotrexate, gold compounds, intravenous immunoglobulins, cyclosporine, mycophenolate mofetil, plasmapheresis, and photopheresis have been tested in randomized controlled trials and in case series of patients with severe PV. However, there is no evidence or clear consensus on the best adjuvant therapy in PV refractory to corticosteroids, and the long-term use of immunosuppressive drugs is limited by their toxicity. Therefore, effective and safer therapies are warranted for the treatment of PV showing resistance to corticosteroid therapy or in patients relapsing during dosage reduction.

Rituximab (Mabthera; Roche, Basel, Switzerland) is a genetically engineered chimeric murine-human anti-CD20 monoclonal antibody (mAb) that targets malignant and nonmalignant mature B cells and induces depletion of B cells in vivo. These effects were the rationale for the successful use of rituximab in the treatment of non–Hodgkin B-cell lymphoma. More recently, rituximab has been used in patients with refractory autoimmune disorders. Therefore, anti-CD20 mAbs have been shown to be effective and well tolerated in patients with refractory idiopathic thrombocytopenic purpura and in children with severe autoimmune hemolytic anemia.

We report herein the results of rituximab therapy in 3 patients presenting with severe forms of PV.

METHODS

PATIENTS

All 3 patients had an unambiguous diagnosis of PV according to the following clinical, histologic, and immunofluorescence criteria: (1)
cutaneous and mucosal superficial erosions, (2) suprabasal clefts with acantholysis, (3) interkeratinocyte IgG and C3 deposits, and (4) serum antibodies against epithelial cell surfaces of rat urinary bladder or monkey esophagus. In addition, antidesmogline 3 antibodies were demonstrated by immunotransfer elometry.

We used the term *complete response* or *complete control* when there was no clinical evidence of disease activity (ie, extent score, 0), regardless of the treatment used. Despite complete control of the disease, the disease activity score sometimes remained positive, depending on the treatment used. We used the term *partial response* or *partial control* when the extent of disease score had decreased and was less than 2.

**CLINICAL END POINTS**

There is no consensus about criteria on which to grade disease severity or to define remission in pemphigus. We used the severity score published by Herbst and Bystryn,2 slightly modified (Table). Briefly, disease severity was graded on a scale of 0 (lack of any lesion without treatment) to 10 (presence of diffuse lesions under high-dosage prednisone therapy and immunosuppressive treatment), based on the sum of the extent of the disease and the intensity of therapy required to control it. Extent of disease was graded as 0 (absence of any pemphigus lesions) to 4 (skin lesions involving >50% of the body surface area and/or ≥6 sites), based on the number of body areas involved with 1 or more lesions; if more appropriate (eg, modification of surface involved in each site), extent was graded according to the total body surface area involved. Intensity of therapy was graded as 0 (no corticosteroid or immunosuppressive therapy) to 6 (association of prednisone therapy at a daily dosage ≥90 mg with an immunosuppressive regimen), based on the dosage and type of medication required to control disease activity.

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**TREATMENT REGIMEN**

Rituximab was administered intravenously at a dosage of 375 mg per square of height in meters in 4 to 6 hours once weekly for 4 infusions per course. The day of first infusion was considered day 1.

**BLOOD TESTS**

Antiepidermis antibodies were detected by indirect immunofluorescence analysis of patients’ blood samples using epithelial cell surfaces of rat urinary bladder or monkey esophagus as substrate. The count of peripheral blood B cells was investigated by classic flow cytometry analysis using a monoclonal antibody reacting with the pan-B CD19 differentiation antigen. Serum immunoglobulin levels were determined by nephelometry.

**REPORT OF CASES**

**PATIENT 1**

A 34-year-old woman had been treated for PV since 1989. She showed a clinical response to high-dose oral corticosteroid therapy (1-2 mg per kilogram of body weight per day). However, from 1992 to 2001, the disease flared repeatedly as the corticosteroid dosage was tapered below a threshold of 0.5 to 0.4 mg/kg per day. During the course of her disease, the patient received several adjuvant regimens without satisfactory results: azathioprine, intravenous polyvalent human immunoglobulins, cyclosporine, and mycophenolate mofetil, which were administered in association with prednisone and which did not show any significant corticosteroid-sparing effect; gold compounds, dapsone, and methotrexate were discontinued shortly after being introduced because of adverse effects. In May 2001, a severe relapse occurred involving the oral mucosa, the genitalia, and approximately 10% of the body surface area while the patient was receiving 0.6 mg/kg per day of prednisone. Because the patient was reluctant to increase the corticosteroid dosage, intravenous infusions of cyclophosphamide (500 mg/m² monthly) were initiated. New lesions were still observed 1 month after the third bolus, and disease activity was considered unchanged. At that time, 30% of the body surface area was involved. A rituximab course was administered 6 weeks after the last bolus of cyclophosphamide. A clinical improvement was noticeable from the third week of treatment, and the response was considered maximal (extent score, 1) 4 months after the onset of treatment. At this date, although mucosal involvement was unchanged, skin lesions covered less than 1% of the body surface area (Figure 1).

The overall improvement was considered by the patient and her dermatologists as the best result ever obtained during the past 10 years of her disease course. Serum antiepidermis antibodies had fallen from 1:500 to

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**Pemphigus Activity Score**

<table>
<thead>
<tr>
<th>Score</th>
<th>Extent of Disease†</th>
<th>Intensity of Corticosteroid Therapy</th>
<th>Intensity of Immunosuppressive Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No lesion</td>
<td>None</td>
<td>≤100 mg/d of Azathioprine and cyclophosphamide; or gold, dapsone, mycophenolate mofetil, or cyclosporine at any dosage</td>
</tr>
<tr>
<td>1 to 2</td>
<td>Transient lesions (healing within 48 h)</td>
<td>Prednisone, ≤15 mg/d</td>
<td>100 mg/d of Azathioprine and cyclophosphamide or plasmapheresis</td>
</tr>
<tr>
<td>1</td>
<td>1 Site or 0%-10%</td>
<td>Prednisone, 16-49 mg/d</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2-3 Sites or 11%-30%</td>
<td>Prednisone, 50-89 mg/d</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4-5 Sites or 31%-50%</td>
<td>Prednisone, ≥90 mg/d</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>≥6 Sites or 51%-70%</td>
<td>Prednisone, 100 mg/d</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from Herbst and Bystryn.*2

†Number of the following body areas involved: scalp, face or neck, upper torso, lower torso, arms, legs, oral mucosa, and genitalia. If more appropriate, the assessment of body surface area involved may be used. Percentages are of total body surface area.

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1:200 titers while receiving rituximab therapy, and the prednisone daily dosage was tapered down to 0.3 mg/kg by month 5. Thereafter, genital involvement progressively worsened, and new skin lesions occurred at month 6, with an extent score of 3 and a concomitant increase in circulating antiepidermis antibodies to 1:500 and a recovery of circulating B cells, which were previously undetectable. A second rituximab course was administered. Five weeks after the first infusion of this second course, because the disease was still active, prednisone was increased to 1 mg/kg per day with 100 mg/d of azathioprine, achieving partial control in 3 weeks. During both courses, the infusions were well tolerated. Eight weeks after the first course of rituximab, the patient presented with a community-acquired pneumonia, which was successfully treated with oral ampicillin. No adverse effect was observed after the second course. The time course of disease activity, drugs used, titers of circulating antibodies, numbers of circulating B cells, and amounts of IgG and IgM are presented in Figure 2A.

PATIENT 2

A 42-year-old woman was admitted for a severe flare of PV (extent score, 4), comprising approximately 60 blisters covering the whole body and involving oropharyngeal, laryngeal, and genital mucosae. She had been followed up for 5 years for a severe, life-threatening form of PV showing resistance to therapy. Indeed, she previously received many successive immunosuppressive treatments (methotrexate, azathioprine, cyclosporine, mycophenolate mofetil, intravenous immunoglobulins, and extracorporeal photopheresis), which failed to control the disease even in association with high dosages of prednisone ranging from 1 to 2 mg/kg per day. Of note, she also had had several severe infectious complications (staphylococcal septicemia and Pseudomonas aeruginosa hip arthritis). At admission, she had been receiving 1.1 mg/kg per day of prednisone for almost 2 years and 3 g/d of mycophenolate mofetil for 2 years. Cyclosporine (150 mg/d), which was given for 4 years, was withdrawn 6 weeks before the flare, while extracorporeal photopheresis therapy was stopped 3 weeks later.

Cyclosporine was reintroduced, in association with methylprednisolone hemisuccinate boluses (500 mg/d for 3 days), followed by administration of 2 mg/kg of prednisone per day. Sixteen days later, she showed a worsening of her skin condition with new blisters, leading to the introduction of rituximab. Clinical improvement was noticeable from the second week; at week 3, only 3 fresh blisters were observed, with the others healing and the mucosal involvement partially improved (Figure 3). Prednisone dosage was progressively decreased from week 10, while the other treatments remained unchanged. A complete clinical response (extent score, 0) was achieved at month 4 and persisted for 6 months. Several blistering lesions recurred as she was receiving 25 mg/d of prednisone, 150 mg/d of cyclosporine, and 3 g/d of mycophenolate mofetil. At that time, erosions involving the eyes, oropharynx, and genitalia were also noticed (extent score, 4). A new course of rituximab was initiated, while the corticosteroid daily dosage remained the same. Three weeks after the first infusion of this second course, no new blisters had appeared, none were observable on the skin, and mucosal lesions had dramatically improved (extent score, 1).

The results from the monitoring of circulating antibodies and of the peripheral blood CD19+ lymphocyte counts are reported in Figure 2B. Briefly, CD19+ blood lymphocyte subsets rapidly decreased during administration of rituximab and B lymphocytes were still unde-
We report 3 cases of severe refractory PV treated with rituximab, a chimeric anti-CD20 mAb. All patients described herein exhibited a response following the first rituximab course, with a tapering of the daily dosages of corticosteroids or other immunosuppressive drugs during the following months. Patients 2 and 3 had a complete response 3 months after the onset of rituximab therapy. Such a response had not been achieved for 2 years in these 2 patients while receiving high-dose corticosteroids and various immunosuppressive treatments. Paralleling the clinical improvement, serum titers of pemphigus autoantibodies sharply declined in patient 1 and turned negative in patients 2 and 3. However, a complete response was not achieved in patient 1, and a sec-
ond course of rituximab, 6 months after the first one, was not sufficient to prevent a relapse. Two patients treated with rituximab for a paraneoplastic pemphigus associated with CD20+ follicular B-cell lymphoma have been previously described. In these 2 patients, mucocutaneous lesions rapidly improved during rituximab therapy given to control the lymphoma. More recently, Salopek et al described a woman with a severe refractory form of PV who was successfully treated with rituximab. Originally developed as a therapy targeting B-cell malignancies, humanized anti-CD20 mAb selectively depletes B cells while sparing progenitor cells, providing a rationale to evaluate its therapeutic effect in autoimmune diseases. Promising results have been reported in diseases in which autoantibodies play a critical role, such as idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, myasthenia gravis, and antineutrophil cytoplasmic antibody–positive Wegener granulomatosis. In PV, antidesmoglein 3 autoantibodies play a direct pathogenic role by inhibiting interkeratinocyte desmoglein 3–dependent adhesion. Serum levels of pemphigus autoantibodies strongly correlate with disease activity. In our patients, clinical improvement was noticeable as early as 2 weeks after the first infusion, paralleling the decrease in serum antiepidermis antibodies and peripheral B-cell count. The relapses occurred several months after the end of rituximab treatment, with a concomitant rise in serum antiepidermis antibodies level and a recovery of peripheral blood B cells, as shown in patient 1. This profile suggests that the therapeutic effect of rituximab is related to the induced suppression of autoreactive B-cell clones. Rituximab targets the B-cell differentiation CD20 antigen, which is a 33- to 37-kDa non-glycosylated phosphoprotein highly and specifically expressed by pre-B cells, normal mature B cells, and most malignant B cells, but which is lacking at the surface of plasmocytes and of stem cells. The CD20 antigen is resistant to internalization or shedding from the plasma membrane following ligation. In malignant B cells, rituximab has been shown to mediate cytotoxicity via several mechanisms in vitro, including complement-dependent cytotoxicity, antibody-dependent cytotoxicity, and direct antiproliferative and apoptotic effects. However, the in vivo relevance of these mechanisms is not demonstrated, and little work has been carried out in normal B cells. Considering the data presented herein, and those provided in other autoimmune disorders such as autoimmune hemolytic anemia, it is likely that the mechanism of action of rituximab in pemphigus lies in the transient deletion of autoreactive B cells. In patient 2, the level of serum IgG subclasses paralleled disease activity and pathogenic antibody levels, while IgM subclasses remained stable. As autoreactive B cells produce pathogenic antibodies of IgG subclasses, this may reflect a specific targeting of monoclonal anti-CD20 mAb to autoreactive B cells. Alternatively, it may be hypothesized that long-lived plasma cells, which do not bear CD20 and may account for the normal serum immunoglobulin levels following rituximab therapy, may not produce pathogenic antibodies. On the other hand, the clinical relapse and the rise in autoantibody level occurred in patient 2 while peripheral B cells were still not detectable, possibly suggesting that autoreactive B cells recover earlier in lymph nodes than in the peripheral blood.

The partial response to the second rituximab course observed in patient 1 raises the issue of resistance to rituximab. We did not search for human antichimeric antibodies because of the unavailability of a commercial test; development of such antibodies has been reported in fewer than 1% of patients with non–Hodgkin lymphoma. Loss of CD20 expression by previously CD20+ lymphomas following rituximab therapy has been reported, but this is unlikely to occur in normal B cells. As the dosage of corticosteroid was half the amount during the second course

Figure 3. A, Skin lesions involving the axillary region are observed in patient 2 before beginning rituximab therapy. B, Lesions resolved 6 weeks after beginning rituximab therapy.

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compared with the first, one could hypothesize a synergetic effect between both drugs, accounting for a lesser efficiency when the corticosteroid dosage is lower. Interestingly, recent in vitro data demonstrated synergistic antiproliferative and apoptotic effects between dexamethasone and rituximab on malignant B cells.²⁰

Rituximab has a tolerable adverse-effect profile, and life-threatening events have rarely been reported. Moderate fever and chills may typically occur during the first infusion, as in patient 2, while most patients do not experience further infusion-related toxicity during the subsequent infusions.²⁰,²⁹ Infectious adverse effects are of particular concern as a prolonged period of B-cell depletion might compromise the immune responsiveness to infectious agents. However, serum immunoglobulin levels are usually maintained during the period when no circulating B cells are observed, because plasma cells do not express CD20.³¹ Two patients experienced major infectious episodes: a pneumonia in patient 1 and a relapse of infectious arthritis in patient 2, consistent with reports that rituximab may impair secondary humoral immune responsiveness.³¹ Transient hypogammaglobulinemia may also have favored infection in patient 2. Compared with patients with lymphoma, infectious adverse effects might be more common in patients with autoimmune disease receiving rituximab due to the long history of therapeutic immunosuppression.

To conclude, although systemic corticosteroids remain so far the mainstay of treatment for PV, the results presented herein suggest that rituximab may have a beneficial effect in corticosteroid-resistant and corticopenic PV. However, the effectiveness and tolerance of rituximab in PV should be confirmed by further prospective controlled studies investigating larger series of patients.

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