Serum Levels of Autoantibodies to BP180 Correlate With Disease Activity in Patients With Bullous Pemphigoid

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**Objective:** To investigate the possible correlation of levels of circulating anti-BP180 autoantibodies with disease activity in bullous pemphigoid (BP).

**Design:** Diagnostic study.

**Setting:** Regional referral center at a university dermatology department.

**Patients:** Fifteen patients with typical clinical, histologic, and immunofluorescence findings of BP who had not received prior systemic treatment.

**Interventions:** Initially, 6 consecutive patients with BP were treated with oral doxycycline and niacinamide. Subsequently, 9 consecutive patients with BP received a combination of oral dapsone and prednisolone.

**Main Outcome Measures:** Disease activity, serum levels of autoantibodies to BP180, and titers of anti–basement membrane zone autoantibodies were assayed before initiation of treatment and 4 and 8 weeks later. Reactivity to BP180 was analyzed by enzyme-linked immunosorbent assay using a recombinant form of BP180 NC16A. Titers of anti–basement membrane zone autoantibodies were assayed by indirect immunofluorescence on 1-mol/L sodium chloride–split human skin.

**Results:** In both treatment groups, disease activity correlated with serum levels of autoantibodies to BP180 NC16A ($P = .004$ [dapsone-prednisolone] and .007 [doxycycline-niacinamide]). No correlation was seen between disease activity and indirect immunofluorescence reactivity ($P = .18$ and .16, respectively). In patients receiving dapsone plus prednisolone, the dose of corticosteroids necessary to suppress new blister formation correlated with anti-BP180 reactivity ($P = .002$).

**Conclusions:** In contrast to indirect immunofluorescence reactivity that reflects reactivity to both BP180 and BP230, serum levels of autoantibodies to BP180 correlate with disease activity in BP. Assaying reactivity to BP180 should be a helpful guide for the therapeutic management of patients with this disease. Our results underline the pathogenic relevance of autoantibodies to human BP180.


Bullous pemphigoid (BP) is a subepidermal blistering disease of the elderly that is characterized by linear deposits of IgG and/or C3 at the basement membrane zone (BMZ). Circulating autoantibodies label the epidermal side of 1-mol/L sodium chloride–split human skin and are directed against 2 hemidesmosomal antigens designated BP180 and BP230. BP230 is restricted to the intracellular hemidesmosomal plaque, and thus, autoantibodies to this antigen are thought not to be involved in the initiation of the disease. BP180, on the other hand, is a transmembrane glycoprotein with a long, collagenous extracellular tail domain that spans the lamina lucida of the BMZ. A noncollagenous stretch of the BP180 ectodomain (designated NC16A) has recently been shown to harbor several major antigenic sites recognized by BP serum samples. Using the NC16A domain as the antigenic target, a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) for the detection of autoantibodies to BP180 has recently been developed. In a passive transfer animal model, it has been demonstrated that antibodies to the murine homolog of BP180 NC16A induce a BP-like disease in neonatal mice. These data suggest that antibodies to human BP180 may play a key role in the pathogenesis of human BP.

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Thus far, no clinical or immunologic indicators have been identified that would aid in predicting the clinical course in patients with BP. Although it is clear that BP is an autoantibody-mediated disease, the titers of circulating autoantibodies detected by indirect immunofluorescence do not parallel the clinical activity in BP. This is thought to be due to the...
PATIENTS AND METHODS

PATIENTS

The study included 15 patients treated at the Department of Dermatology, University of Würzburg, Würzburg, Germany, between April 1997 and May 1998. All patients had widespread bullous disease, and no systemic treatment had been initiated. Direct immunofluorescence of perilesional skin revealed linear deposits of IgG and/or C3 along the BMZ in all patients. By indirect immunofluorescence on 1-mol/L sodium chloride–split normal human skin, all sera exclusively stained the epidermal side of the artificial split. Titers ranged from 1:10 to 1:640. Reactivity against recombinant BP180 NC16A was positive by immunoblot and ELISA in all patients. Between April 1997 and September 1997, 6 consecutive patients with BP were treated with a combination of doxycycline and niacinamide. Between October 1997 and May 1998, 9 consecutive patients with BP received a combination of dapsone and prednisolone. Nine patients were included in the dapsone-prednisolone group and 6 in the doxycycline-niacinamide group. In the dapsone-prednisolone group, 4 patients were men and 5 were women. The median age was 71 years (range, 30-90 years), and the mean (±SD) age was 69 ± 13 years. The doxycycline-niacinamide group consisted of 3 men and 3 women between 68 and 92 years of age. The median age was 77 years, and the mean (±SD) age was 79 ± 10 years.

The doxycycline-niacinamide regimen followed a modification of a previously reported protocol.19 Doxycycline, 100 mg orally, was given 2 times daily in combination with niacinamide, 400 mg orally, 3 times daily, and topical 0.5% clobetasol propionate. After cessation of new blister formation and healing of pre-existing lesions, topical treatment was changed to a moisturizing emollient. The mean (±SD) duration of topical clobetasol treatment was 12 ± 8 days for the doxycycline-niacinamide group. Doxycycline dosage was then reduced to 100 mg/d orally and the niacinamide dosage to 600 mg/d orally. Finally, niacinamide and doxycycline were omitted completely. Dapsone was given at 1.0 to 1.5 mg/kg of body weight in combination with prednisolone, 0.5 to 1.0 mg/kg of body weight orally daily (dapsone-prednisolone group). When no new blisters had developed for 1 week, prednisolone was tapered in 10-mg steps to 20 mg/d, then in steps of 5 mg until a dose of 10 mg/d, then in 2.5-mg steps. After discontinuation of use of the corticosteroid, dapsone was reduced by 25 mg every 4 weeks. In both treatment groups, blister fluid was aspirated, and lesions were treated with 0.5% crystal violet solution; all patients received Candida prophylaxis with oral nystatin. Patients in the dapsone-prednisolone group were also treated orally with daily doses of ranitidine, 150 mg, vitamin E, 600 mg, cholecystokinin, 0.025 mg, and calcium tablets, 500 mg.

Patients were seen weekly until lesions had cleared completely and then monthly for 1 year. On each visit, the number of blisters and erosions was recorded. More than 10 blisters and/or erosions corresponded to an activity score of 3, 1 to 10 blisters and/or erosions to a score of 2, and when no blister or erosion was detected, the score was 1. When skin lesions healed completely and no further medical treatment was required to control lesion formation, the score was 0. In addition, blood samples were taken for ELISA and indirect immunofluorescence analysis on each visit.

IMMUNOBLOT

Glutathione S-transferase (GST)–NC16A fusion proteins and recombinant GST were expressed in Escherichia coli.11 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting were performed as described.19 To eliminate reactivity with the GST portion of the fusion protein, serum samples of patients and controls were preadsorbed with bacterial cell lysate containing recombinant GST.11

ENZYME-LINKED IMMUNOSORBENT ASSAY

To determine ELISA reactivity with BP180 NC16A, we used a previously reported protocol.22 Briefly, each well of a standard 96-well microtiter plate was coated with purified recombinant GST-NC16A1-5 or the same amount of recombinant GST. Wells were then incubated with 50 μL of BP or control serum in a 1:100 dilution with assay buffer (phosphate-buffered saline, pH 7.2, containing 1% bovine serum albumin and 0.05% Tween-20; all Sigma, Deisenhofen, Germany). As detection antibody, a horseradish peroxidase–labeled goat anti–human IgG (Kirkegaard and Perry, Gaithersburg, Md), diluted 1:10 000 in assay buffer, was used. After substrate addition, the optical density was measured at 492 nm. Each serum was analyzed in triplicate for reactivity with both GST-NC16A and recombinant GST alone. The mean optical density reading obtained with GST was subtracted from the mean optical density reading for GST-NC16A. To minimize plate-to-plate variability, each ELISA plate included the same 4 internal controls.12

STATISTICAL ANALYSIS

To analyze the correlation between disease activity, titers of anti-BMZ autoantibodies by indirect immunofluorescence, serum reactivity to BP180 NC16A, and prednisolone doses during the first 8 weeks of treatment, the AR(1) Serial Correlation test was applied.20 Analysis was done separately for patients in the dapsone-prednisolone group (n = 9) and doxycycline-niacinamide group (n = 6). P values of .01 or less were considered statistically significant.

fact that indirect immunofluorescence reveals the presence of antibodies to both BP180 and BP230. Based on the hypothesis that autoantibodies to the BP180 NC16A domain are directly involved in the pathogenesis of BP, we investigated the correlation between serum levels of antibodies to BP180 and disease activity. Fifteen patients with BP were followed up during their therapy, which involved either a combination of dapsone and prednisolone or doxycycline plus niacinamide. During the first 8 weeks of treatment, we monitored disease activity, titers of autoantibodies to the BMZ by indirect immunofluorescence, and serum levels of autoantibodies to BP180 NC16A by ELISA. In contrast to indirect immunofluorescence titers, we observed a direct relation between disease severity and levels of autoantibodies to BP180 NC16A.
After 1 month of treatment, a good response was seen in both the dapsone-prednisolone and the doxycycline-niacinamide groups. Eight of 9 patients in the dapsone-prednisolone group and 5 of 6 in the doxycycline-niacinamide group were free of blisters and erosions. After 8 weeks, however, in 2 patients in the doxycycline-niacinamide group, blisters recurred. One of the 2 patients had deliberately stopped taking the medication. In contrast, all patients in the dapsone-prednisolone group had a complete response after 8 weeks of therapy. In most cases in both groups, indirect immunofluorescence reactivity of IgG anti-BMZ antibodies did not markedly change after 4 and 8 weeks of treatment compared with indirect immunofluorescence titters before initiation of therapy. In 3 patients in the dapsone-prednisolone group, a negative indirect immunofluorescence stain was seen after 8 weeks of treatment, and in the doxycycline-niacinamide group, no patient had a negative stain by this time. In contrast, at the 8-week time point, IgG antibodies to BP180 NC16A were negative in 4 of 9 patients in the dapsone-prednisolone group, and in another 4 patients in this group, NC16A reactivity was just slightly above the cutoff of the assay (optical density, 0.22). After 8 weeks of treatment with doxycycline-niacinamide, on the other hand, 3 patients still had markedly elevated levels of anti-BP180 NC16A antibodies, and the remaining 3 patients had levels close to the cutoff. The results of this analysis are summarized in the Table.

We observed a significant correlation between disease activity and serum levels of antibodies to BP180 NC16A during the first 8 weeks of treatment. This correlation was found for both the dapsone-prednisolone (P = .004) and the doxycycline-niacinamide (P = .007) groups. In contrast, in both groups, no correlation was found between disease activity and titers of anti-BMZ antibodies by indirect immunofluorescence (P = .18 and .16, respectively). The Figure shows a representative example of a patient treated with dapsone-prednisolone. A correlation was also observed between levels of autoantibodies to BP180 NC16A and the prednisolone dose necessary to suppress new blister formation in the dapsone-prednisolone group (P = .002).

The clinical course of BP is heterogenous, and periods of remission may be followed by 1 or more relapses.21 Indirect immunofluorescence titers of anti-BMZ antibodies do not parallel activity of this disease.16,17 Recently, it has been established that most BP serum samples react with the NC16A domain of BP180.12,22,23 The purpose of this study was to test the hypothesis that the level of IgG reactivity to NC16A reflected disease severity in patients with BP. Fifteen consecutive patients were analyzed: 9 patients were treated with dapsone-prednisolone, and 6 patients received doxycycline-niacinamide. Consistent with our hypothesis, disease activity correlated with levels of autoantibodies to BP180 NC16A and the prednisolone dose necessary to suppress new blister formation in the dapsone-prednisolone group (P = .002).

### Table

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>Treatment Group</th>
<th>Disease Activity</th>
<th>BP180 NC16A Reactivity</th>
<th>Anti-BMZ Autoantibodies</th>
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<tr>
<td></td>
<td></td>
<td>0   4  8</td>
<td>0   4  8</td>
<td></td>
</tr>
<tr>
<td>1/M/71 Dapsone-prednisolone</td>
<td>2   1  1</td>
<td>0.541 0.261 0.173</td>
<td>1.20 Negative Negative</td>
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<td>2/F/59 Dapsone-prednisolone</td>
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<td>0.351 0.217 0.158</td>
<td>1.160 Negative 1.80 1.40</td>
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<td>3/F/69 Dapsone-prednisolone</td>
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<td>0.670 0.560 0.256</td>
<td>1.80 Negative Negative</td>
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<td>4/M/77 Dapsone-prednisolone</td>
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<td>1.320 1.160 1.320</td>
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<tr>
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<td>1.640 1.40 1.20</td>
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<tr>
<td>9/F/50 Dapsone-prednisolone</td>
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<td>0.630 0.379 0.266</td>
<td>1.40 1.40 1.20</td>
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<tr>
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<td>1.40 1.40 1.10</td>
<td></td>
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<tr>
<td>13/F/90 Doxycycline-niacinamide</td>
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<td>1.320 1.320 1.320</td>
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<td>15/M/92 Doxycycline-niacinamide</td>
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<td>0.544 0.381 0.266</td>
<td>1.320 1.320 1.320</td>
<td></td>
</tr>
</tbody>
</table>

* Disease activity was scored according to the number of blisters and/or erosions: 3, more than 10 lesions; 2, 1 to 10 lesions; and 1, no lesions.
† Serum reactivity to BP180 NC16A was assayed by enzyme-linked immunosorbent assay. Samples were analyzed in triplicates, and results are expressed as optical density, measured at 492 nm. SD values ranged from 0.003 to 0.054.
‡ Titters of anti-BMZ autoantibodies were analyzed by indirect immunofluorescence on sodium chloride–split normal human skin.
§ Disease activity, reactivity to BP180 NC16A, and titers of anti-BMZ autoantibodies were determined before treatment was initiated (0) and 4 and 8 weeks later.
¶ Doxycyline, 200 mg/d, plus niacinamide, 1200 mg/d.
# Blister recurred after the patient deliberately stopped taking the medication.
reactivity to BP230 and, to a lesser extent, BP180. This may explain the finding that indirect immunofluorescence reactivity does not correlate with the clinical course of BP, whereas reactivity to BP180 reflects disease severity well.

Serum levels of various other inflammatory mediators, including tumor necrosis factor α, interleukin (IL) 1β, IL-2, soluble IL-2 receptor, IL-5, IL-8, soluble E-selectin, and IgE antibodies to BP230 have been reported to be related to disease intensity in BP. In contrast to antibodies to BP180, which are thought to be directly linked to the pathogenesis of BP, some of these other factors may instead reflect the general inflammatory response in patients with BP.

The pathogenic role of rabbit antibodies to the murine homolog of BP180 NC16A has been demonstrated in a passive transfer mouse model. Since the murine homolog of BP180 lacks the epitopes recognized by autoantibodies in patients with BP, this experimental model is not suitable to test for the pathogenic relevance of antibodies to human BP180. Using organ culture or cryostat sections of normal human skin, autoantibodies from patients with BP induced subepidermal blisters when accompanied by complement activation and infiltration of inflammatory cells. The specificity of autoantibodies in these in vitro models, however, was not further characterized. In the present study, we demonstrate that levels of autoantibodies to human BP180 reflect disease activity and further support the concept that these autoantibodies are pathogenically relevant in patients with this disease.

Levels of BP180 NC16A autoantibodies decreased at a slower rate in patients in the doxycycline-niacinamide group compared with the dapsone-prednisolone group. This difference may be explained by the stronger effect of prednisolone on lymphocyte functions compared with doxycycline-niacinamide. After 8 weeks of treatment, in 2 patients in the doxycycline-niacinamide group, only a slight decrease in levels of antibodies to BP180 NC16A was observed while these patients were free of blisters. To suppress new blister formation, these 2 patients still required the initial dose of doxycycline-niacinamide. This observation suggests that the regimen of doxycycline-niacinamide may exert its effects, at least in part, by modulation of the local inflammatory response in BP lesions. In fact, doxycycline and niacinamide were shown to exhibit their anti-inflammatory actions by interfering with neutrophil and eosinophil functions and, to a lesser extent, by suppression of antibody production. In the dapsone-prednisolone group, we found a direct correlation between the corticosteroid dose necessary to suppress blister formation and serum levels of antibodies to BP180 NC16A. The NC16A ELISA should, therefore, prove to be a helpful tool in guiding treatment of patients with BP, especially in those patients who are free of skin lesions yet still receive systemic treatment.

In summary, this study demonstrates that serum levels of antibodies to BP180, in contrast to indirect immunofluorescence titers of anti-BMZ antibodies, correlate with disease activity in BP. The BP180 NC16A ELISA is, therefore, helpful in guiding treatment decisions in this disease. In addition, our data substantiate the pathogenic relevance of autoantibodies to BP180.


