Bullous Pemphigoid of Childhood

Autoantibodies Target the Same Epitopes Within the NC16A Domain of BP180 as Autoantibodies in Bullous Pemphigoid of Adulthood

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Background: Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease of the elderly that rarely occurs in children. Most adult BP serum samples react with epitopes within the NC16A domain of BP180, a glycoprotein of the cutaneous basement membrane zone.

Objectives: To characterize the autoimmune response in childhood BP using recombinant forms of BP180 and to determine the subclass distribution of autoantibodies and their correlation with disease activity.

Observations: Serum samples from 2 infants with BP, aged 4 and 5 months, reacted by immunoblot analysis with 4 epitopes clustered within the N-terminal 45 amino acids of the NC16A domain. The same 4 epitopes have previously been shown to be the target in adult BP. Childhood BP antibodies to BP180 NC16A belonged to IgG1, IgG2, IgG3, and IgG4 immunoglobulin subclasses. IgE reactivity was not detected. Serum levels of antibodies targeting BP180 NC16A paralleled disease activity as detected by enzyme-linked immunosorbent assay.

Conclusions: The fine specificity of autoantibodies to BP180 is the same in BP of childhood and adulthood. Childhood BP is a true variant of BP.

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Bullous pemphigoid (BP) is an acquired autoimmune subepidermal blistering disease that usually affects the elderly.1 The disease occurs in children rarely. Approximately 50 cases of childhood BP have been described so far (reviewed in Nemeth et al2 and Rabinowitz and Esterly3). Immunopathologic hallmarks of BP are linear deposits of IgG and C3 along the basement membrane zone (BMZ) as detected by direct immunofluorescence (IF). Indirect IF studies demonstrate the presence of circulating IgG autoantibodies directed against the epidermal side of salt-split skin. These autoantibodies are predominantly of the IgG4 subclass,4 and their titers do not correlate with disease activity.5 Two hemidesmosome-associated proteins, BP230 and BP180, have been identified as targets of autoantibodies in BP.6,7 BP180 is a transmembrane glycoprotein consisting of an intracellular N-terminal portion, a transmembrane region, and a C-terminal ectodomain (Figure 1). Data from a passive-transfer animal model provide support for the hypothesis that anti-BP180 autoantibodies are directly involved in the pathogenesis of BP.8 The extracellular portion of human BP180 harbors 4 major epitopes clustered within the N-terminus of its membrane-proximal noncollagenous (NC) 16A domain.9,10 Recombinant forms of BP180 NC16A, used in immunoblot and enzyme-linked immunosorbent assay (ELISA) systems, were found to be recognized by approximately 90% of serum samples from unselected adults with BP.11-13

A molecular mapping of epitopes targeted by autoantibodies in BP of childhood has not been performed to date. We studied the fine specificity of autoantibodies to BP180 in childhood and adulthood. Childhood BP is a true variant of BP.
MATERIALS AND METHODS

SERUM SAMPLES

Serum samples were obtained from 2 infantile patients with BP at different stages of the disease, reference adults with BP, 2 healthy control subjects, and rabbit 594 and rabbit 136 immunized with recombinant GST-NC16A2-4 and recombinant fusion protein 4575 encompassing 49 amino acids of the C-terminus of BP180, respectively. Monoclonal antibody 123 is directed to the 120-kd soluble ectodomain of BP180.

IF MICROSCOPY

Perilesional skin biopsy samples were stained with fluorescein isothiocyanate–labeled antibodies to human IgG, IgM, IgA, and C3. For indirect IF studies, 1-mol/L salt-split normal human skin was used as a substrate.

PREPARATION OF EXTRACTS, IMMUNOBLOT ANALYSIS, AND IMMUNOADSORPTION PROCEDURES

Preparation of 120-kd soluble ectodomain of BP180 and extracts of cultured human keratinocytes was performed as described previously. Recombinant GST fusion proteins, including GST-NC16A1, GST-NC16A2, GST-NC16A2.5, GST-NC16A3, GST-NC16A1-3, GST-NC16A2-4, GST-NC16A2-5, GST-NC16A1-5, and 4575 (Figure 1), were expressed in Escherichia coli DH5α and affinity purified using glutathione agarose beads (Sigma-Aldrich Corp, St Louis, Mo). Native and recombinant proteins were fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose. Immunoblot analysis with childhood BP and control serum samples was performed as described previously. Bound autoantibodies were visualized enzymatically using peroxidase-conjugated antibodies at the following dilutions: rabbit antihuman polyclonal IgG, 1:15 000 (DAKO, Glostrup, Denmark); sheep antihuman IgE, 1:100; mouse antihuman IgG1 (clone 8c/6-39), 1:1000; antihuman IgG2 (clone HP6014), 1:500; antihuman IgG3 (clone HP6050), 1:200; and antihuman IgG4 (clone HP6023), 1:4000 (all from Binding Site, Birmingham, England). Specificity and sensitivity of the monoclonal antibodies was described previously. Immunoadsorptions were performed using a liquid phase protocol. Briefly, to study reactivity with NC16A region 4, BP serum samples were preadsorbed with fusion protein NC16A1-3 and then analyzed for immunoblot reactivity with fusion protein NC16A2-4. To assay reactivity with NC16A region 5, serum samples were preadsorbed with GST-NC16A2-4 and subsequently tested for reactivity with GST-NC16A2-5.

ELISA

The ELISA using BP180 NC16A as the target antigen was performed as described previously. Briefly, wells were coated with affinity-purified forms of recombinant GST-NC16A1-5 and recombinant GST, respectively. Subsequently, wells were incubated with patient and control serum samples, and bound antibodies were visualized by peroxidase-conjugated rabbit antihuman IgG (DAKO).

A 5-month-old boy (patient 2), born at the 40th week of a normal pregnancy, presented with a 3-week history of

Figure 1. Schematic diagram of bullous pemphigoid (BP) 180 and recombinant forms of the NC16A domain. The BP180 ectodomain spans the lamina lucida (LL) and projects into the lamina densa (LD) of the basement membrane zone. The arrow corresponds to the 120-kd soluble ectodomain of BP180 (LAD-1). TM indicates transmembrane region; HP, hemidesmosomal plaque of the basal keratinocyte (BK); NH2, N-terminus; COOH, C-terminus; and C15, the 15th collagenous domain of BP180. Amino acid residue numbers are shown above the boxes.

Figure 3. By indirect IF, the patient's serum sample contained IgG anti-BMZ antibodies that exclusively bound the epidermal side of 1-mol/L sodium chloride–separated (salt-split) human skin. No circulating IgA and IgM autoantibodies were detected; results of indirect IF of the mother's serum were negative. The infant was treated with methylprednisolone (0.5 mg/kg body weight per day), dapsone (1 mg/kg body weight per day), and vitamin E (600 U/d for prophylaxis of hemolysis). New blisters stopped forming within 4 days of the start of therapy, and lesions cleared completely within 4 weeks. Methylprednisolone therapy was tapered over 4 months. Subsequently, dapsone use was gradually reduced and is currently (7 months after onset of disease) administered at a dose of 0.5 mg/kg body weight daily.

CASE 2

A 5-month-old boy (patient 2), born at the 40th week of a normal pregnancy, presented with a 3-week history of

unaffected. The patient's past and family histories were otherwise unremarkable; the infant was fully breastfed, and she had not received any medication before the bullous eruption occurred. Histological examination of a perilesional skin biopsy sample revealed eosinophilic spongiosis and subepidermal eosinophilic infiltrates. Direct IF microscopy of a perilesional skin biopsy sample showed linear IgG and C3 but no IgA or IgM deposits along the BMZ (Figure 3). By indirect IF, the patient’s serum sample contained IgG anti-BMZ antibodies that exclusively bound the epidermal side of 1-mol/L sodium chloride–separated (salt-split) human skin. No circulating IgA and IgM autoantibodies were detected; results of indirect IF of the mother’s serum were negative. The infant was treated with methylprednisolone (0.5 mg/kg body weight per day), dapsone (1 mg/kg body weight per day), and vitamin E (600 U/d for prophylaxis of hemolysis). New blisters stopped forming within 4 days of the start of therapy, and lesions cleared completely within 4 weeks. Methylprednisolone therapy was tapered over 4 months. Subsequently, dapsone use was gradually reduced and is currently (7 months after onset of disease) administered at a dose of 0.5 mg/kg body weight daily.
multiple tense bullae on itching erythematous plaques that had first appeared on the hands and feet and then spread to the shins, forearms, ventral trunk, and face (Figure 2, right). Mucous membranes were not involved. The patient’s past and family histories were otherwise unremarkable; he was fully breastfed and had not received any medication before development of the eruption. A lesional biopsy sample from the trunk demonstrated a subepidermal blister and infiltration of eosinophils in the upper dermis (Figure 4). Direct IF of perilesional skin showed strong linear deposits of C3, weaker deposits of IgG, and faint staining for IgA and IgM along the BMZ. Indirect IF of 1-mol/L salt-split human skin demonstrated the presence of circulating IgG autoantibodies that exclusively reacted with the epidermal side of the split (Figure 5). No circulating IgA or IgM autoantibodies were detected. After initiation of treatment with prednisolone (0.5 mg/kg body weight per day) and dapsone (1 mg/kg body weight per day), no new blisters developed, and within 2 months all lesions cleared completely. Use of prednisolone first and dapsone subsequently was tapered over 3 months. To date, the patient has been followed up for 14 months and has not been taking any medication for the past 10 months, without recurrence of blisters.

### RESULTS

#### IMMUNOBLOTTING OF NATIVE FORMS OF BP180

By immunoblotting, serum samples from the 2 patients with childhood BP (patients 1 and 2) demonstrated weak reactivity with a 180-kd protein of cultured human keratinocytes that was strongly detected by reference control serum samples from adults with BP and by rabbit serum 594 directed to BP180 NC16A2-4. No reactivity of the 2 childhood BP serum samples with BP230 was observed (data not shown). In addition, serum samples from patient 2 reacted with a 120-kd protein present in concentrated keratinocyte culture medium. This immunoreactive band comigrated with LAD-1, the 120-kd soluble fragment of BP180, which reacts with monoclonal antibody 123 and with rabbit serum samples 136 and 594 (Figure 6).

#### IMMUNOBLOTTING OF RECOMBINANT FORMS OF BP180 NC16A

Both childhood BP serum samples, control serum samples from adults with BP, and rabbit serum 594 reacted with...
the full-length BP180 NC16A domain (NC16A1-5) by immunoblotting. Subsequently, immunoblot reactivity of childhood BP serum samples was assayed with different recombinant fragments of BP180 NC16A, each approximately 15 amino acids in length (Figure 7). Both childhood BP serum samples reacted with NC16A1, NC16A2, NC16A3, and the junction of NC16A regions 2 and 3 (NC16A2.5). Immunoadsorption studies, as outlined in the “Materials and Methods” section, demonstrated that the 2 serum samples did not contain antibodies to regions 4 and 5 of NC16A. The serum samples did not react with the fusion protein 4575 representing a C-terminal portion of the BP180 ectodomain, which was recognized by rabbit serum 136 (data not shown).

**IgG SUBCLASS DISTRIBUTION OF ANTI-BP180 NC16A ANTIBODIES**

In the next set of experiments, we characterized the subclass distribution of anti-BP180 NC16A antibodies in the 2 patients. As shown in Figure 8, serum samples from patient 1 contained IgG1, IgG2, IgG3, and IgG4 autoantibodies to this portion of BP180, whereas serum samples from patient 2 demonstrated almost exclusively IgG2 reactivity to this domain. No IgE antibodies to BP180 NC16A were detected in the 2 serum samples.

**ELISA**

Serum samples from both patients with childhood BP were obtained before initiation of treatment and subsequently at different stages of disease. Samples were assayed for reactivity with BP180 NC16A by ELISA and for titers of anti-BMZ antibodies by indirect IF microscopy. Titers of anti-BMZ autoantibodies were 1:320 (patient 1) and 1:160 (patient 2) before treatment was initiated and took longer than 5 months to become negative. In contrast, the NC16A ELISA readings of these serum samples paralleled disease activity and dropped from optical density 0.93 (patient 1) and 0.95 (patient 2) to normal readings (optical density <0.22) within 2 months (patient 1) and 3½ months (patient 2), respectively.

**COMMENT**

We demonstrated that childhood BP serum samples recognize 4 distinct antigenic sites within the NC16A domain of BP180 (Figure 7) that are identical to the epitopes targeted by sera from adults with BP and pemphigoid gestationis.10,19 Our childhood BP serum samples, like those from adults with BP, did not recognize regions 4 and 5 of NC16A. NC16A region 4 seems to be a major epitope targeted by autoantibodies in lichen planus pemphigoides,19 and NC16A region 5 is targeted by some sera in pemphigoid gestationis.19

Tru¨e et al22 recently described a patient with childhood BP whose serum recognized recombinant BP180 and a 120-kd protein from a human dermal extract. The au-
thors suggested that this protein corresponded to the LAD-1 antigen, i.e., a proteolytic product of the BP180 ectodomain\(^{18,23}\) that is targeted by autoantibodies in linear IgA disease and BP of adulthood.\(^ {15,24}\) In the present study, 1 of 2 childhood BP serum samples reacted with this 120-kd protein (Figure 6). As a source for LAD-1, like other investigators,\(^ {17,18}\) we used concentrated medium of cultured keratinocytes. The N-terminus of the 120-kd protein is located within the NC16A domain of BP180 but has not been firmly defined yet. It is thought that the N-terminus is positioned, like that of the 97-kd linear IgA bullous dermatosis antigen (LABD97), at or near 42 amino acids downstream from the transmembrane domain of BP180 (within region 3 of the NC16A domain).\(^ {25,26}\) The lack of reactivity of autoantibodies from patient 1 with LAD-1 might be due to their fine specificity. This serum showed strong reactivity with N-terminal stretches of NC16A that are most likely missing from LAD-1. Serum from patient 2 might, in addition to NC16A, bind to C-terminal sites on BP180, which could account for reactivity of this serum with the LAD-1 protein.

We are also reporting for the first time the subclass distribution of anti-BP180 autoantibodies in children with BP. One of our patients (patient 1) demonstrated strong reactivity of all IgG subclasses with BP180 NC16A, whereas in serum samples from patient 2, reactivity to NC16A was almost exclusively restricted to IgG2 antibodies (Figure 8). In adult BP, IgG4 is the subclass most commonly detected by indirect IF\(^ {21}\) as well as by immunoblotting of epidermal extracts\(^ {27}\) and recombinant BP180 NC16A.\(^ {28}\) However, immunoglobulin subclasses other than IgG4 are also commonly found in serum samples from adults with BP and are thought to be, at least in part, responsible for complement activation in this disease. In fact, complement activation has been shown to be essential for the induction of subepidermal blisters in the passive-transfer mouse model of BP.\(^ {20}\) Our results suggest that there is no major difference in terms of IgG subclass reactivity to BP180 between the childhood and adulthood variants of BP. Although elevated levels of serum IgE are common in adults with BP and reactivity of these IgE antibodies can be directed against the BMZ,\(^ {26}\) we did not detect elevated serum IgE levels or anti–BP180 NC16A antibodies of the IgE class in our patients with childhood BP. Further investigations are necessary to determine whether IgE reactivity against BMZ components is a specific feature of adult BP.

We recently demonstrated that, in contrast to indirect IF titers, levels of antibodies to BP180 NC16A correlate with disease severity in BP of adulthood.\(^ {21}\) Similarly, we now show that in our patients with childhood BP, serum levels of autoantibodies to BP180 NC16A, as determined by ELISA, closely parallel disease activity. In contrast, indirect IF titers of our patients' serum samples decreased with a delay of several months. Reactivity patterns revealed by indirect IF analysis reflect the binding properties of a heterogeneous set of antibodies exhibiting different specificities. This might explain the finding that while indirect IF titers did not closely parallel the course of disease, reactivity to the immunodomi-
nant region of BP180 well reflected disease activity of BP in our 2 patients. The rapid decrease of serum levels of anti–BP180 autoantibodies within 2 and 31/2 months in patients 1 and 2, respectively, was consistent with the IgG half-life of about 3 weeks.

Results of previous studies suggested that mucosal involvement is seen in BP of childhood more often than in BP of adulthood. In addition, it has been reported that in patients younger than 1 year typical clinical findings include blisters on the hands, feet, and face; however, mucous membranes were not affected. Moreover, of the 20 reported cases of childhood BP in which the target antigens were specified, 15 (75%) showed no mucosal involvement (reviewed by Trueb, Edwards, and Arechalde and their colleagues). Therefore, a larger number of well-characterized patients must be analyzed to resolve the issue of whether mucosal involvement is really a characteristic feature of childhood BP.

In conclusion, we demonstrated that autoantibodies in 2 patients with childhood BP reacted with the same 4 distinct epitopes clustered within the N-terminus of BP180 NC16A that are also targeted by sera of adults with BP. Our findings suggest that ELISA and immunoblotting using the NC16A domain as the target antigen are suitable tools for the diagnosis of childhood BP and for monitoring disease activity during treatment.

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