Childhood bullous pemphigoid: Report of a case with characterization of the targeted antigens

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The clinical and immunopathologic features of children with acquired subepidermal blistering disorders show considerable overlap, and their classification frequently requires characterization of the targeted antigens. A 8-month-old boy developed a generalized subepidermal blistering disorder with striking palmoplantar involvement. The patient's serum contained antibodies reacting against the epidermal side of 1 M sodium chloride separated normal human skin. Immunoblotting analysis demonstrated circulating IgG autoantibodies that reacted against a eukaryotic recombinant form of human bullous pemphigoid antigen 180 (BP180). In addition, the patient had circulating IgG autoantibodies that bound a protein of 120 kDa in skin basement membrane zone extracts, that might correspond to the linear IgA bullous disease (LABD) antigen. This study illustrates that a child with clinical and immunopathologic features considered characteristic of childhood bullous pemphigoid (BP) had circulating IgG antibodies that bound to an eukaryotic recombinant form of human BP180, and hence, fulfilled the diagnostic criteria of BP. Review of the literature disclosed only 10 cases of childhood BP, that were characterized on the basis of the targeted antigens. The concomitant presence of circulating IgG autoantibodies against BP180 and a 120 kDa protein may signify either coexistence of autoantibodies with distinct specificities or reflect antigenic cross-reactivity between BP180 and the 120/97 LABD antigen. (J Am Acad Dermatol 1999;40:338-44.)

Bullous pemphigoid (BP) is an acquired subepidermal blistering disease that is characterized by in vivo bound and circulating autoantibodies directed against the basement membrane zone (BMZ) of stratified squamous epithelia. The clinical features of BP include widespread tense blisters, arising on inflamed or normal appearing skin, that are predominantly located on flexural areas.[1] Although BP is usually a disease of the elderly, it may, very rarely, occur in children.[2] Diagnostic features of BP include linear deposition of IgG (and, less frequently, IgA and IgM) and C3 along the dermal-epidermal junction on direct immunofluorescence (IF). By indirect IF, circulating autoantibodies directed against the epidermal side of 1.0 M sodium chloride (NaCl) separated skin may be detected.[3]

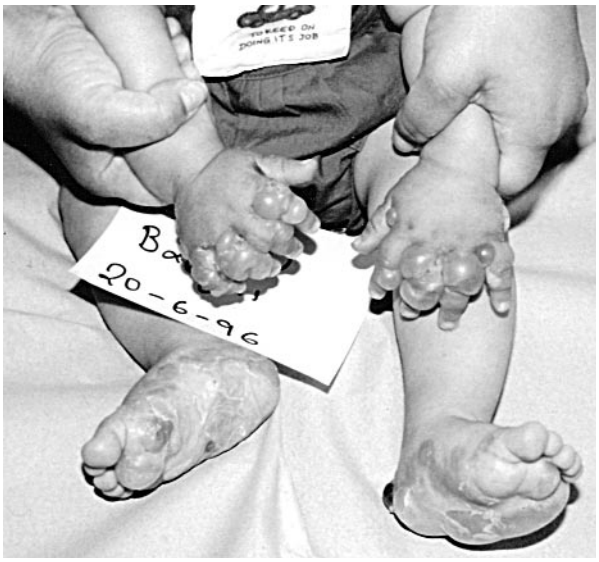

Fig 1. Cheiropompholyx-like, tense blisters of hands characteristic of childhood bullous pemphigoid.

Nevertheless, it may be difficult to differentiate childhood BP from other subepidermal diseases of childhood, including linear IgA bullous dermatosis

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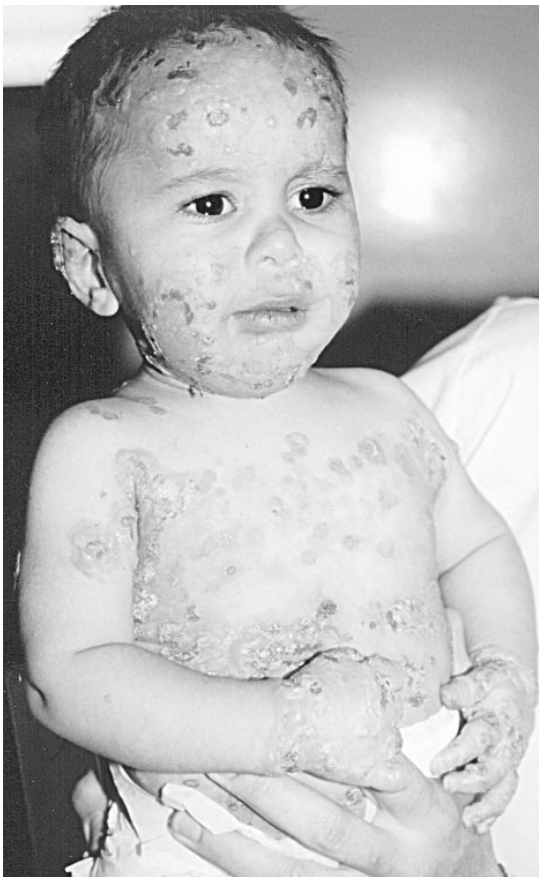

Fig 2. Various sized blisters arising on erythematous base. In some places, new vesicles have appeared at periphery of crusts of older lesions, giving impression of rosettes of blisters.

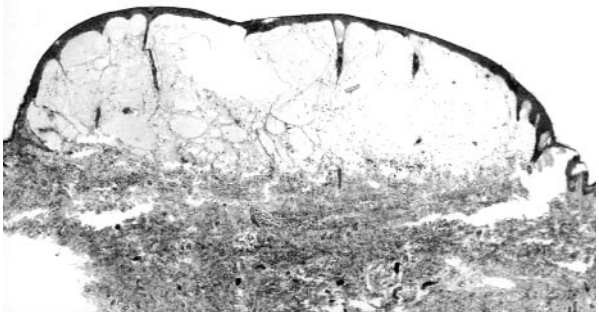

Fig 3. Low-power microphotograph of skin biopsy specimen showing subepidermal blister formation with numerous eosinophils within and at peripheral margins of bulla. (Hematoxylin-eosin stain; original magnification, ×10.)

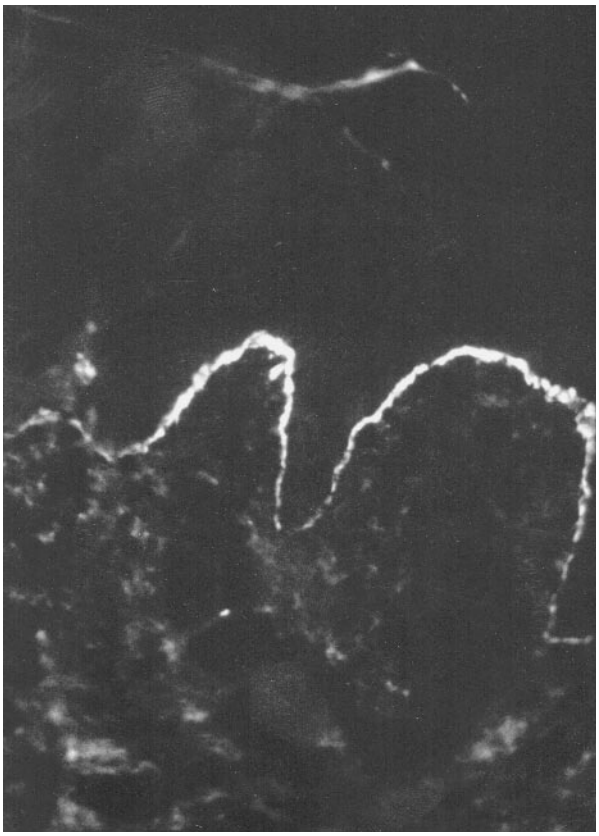

Fig 4. Direct IF microscopy of perilesional skin sample. Linear deposits of IgG along epidermal basement membrane.

(LABD) and epidermolysis bullosa acquisita, because of their clinical, histologic, and immunopathologic overlap.[4,5] LABD appears to be the most frequent autoimmune blistering disease of childhood and is defined on the basis of an exclusive or predominant presence of linear deposits of IgA along the BMZ.[6] Typical clinical findings of LABD include a vesiculobullous eruption distributed over the trunk, with a tendency to form rosettes or clusters.[6] Classification of these subepidermal blistering disorders is facilitated by the characterization of the antigens targeted by the patients' autoantibodies. Immunoblot and immunoprecipitation studies have demonstrated that BP autoantibodies are predominantly directed against 2 proteins of 230 kDa and 180 kDa molecular weight, the BP antigen 230 (BP230, also termed BP antigen 1) and the BP antigen 180 (BP180, also termed BP antigen 2, or type XVII collagen), respectively.[7,8] In contrast, IgA autoantibodies in LABD appear to recognize different antigens, most frequently a 97 kDa and a 285 kDa protein. The 97 kDa protein most likely represents a processed form of a recently described 120 kDa anchoring filament–associated protein.[9,10]

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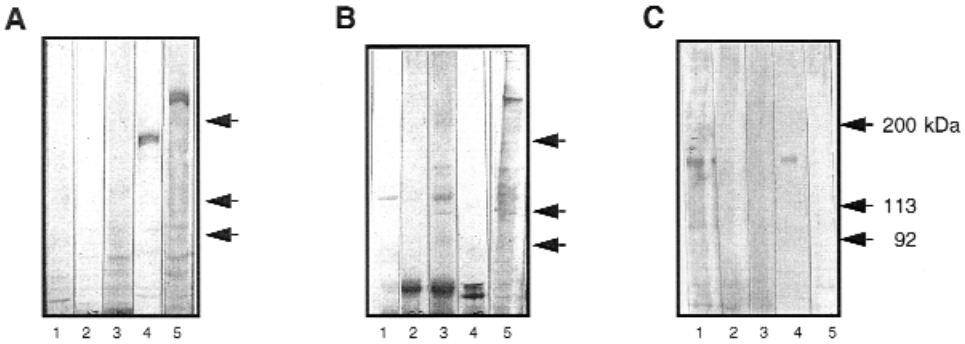

Fig 5. Panel A, Immunoblot of serum samples obtained from patient and control subjects by using extracts of cultured keratinocytes: *lane 1,* IgG reactivity of patient serum (dilution 1:20); *lane 2,* IgA reactivity of patient serum; *lane 3,* IgG reactivity of normal human serum; *lane 4:* IgG reactivity of BP control serum to 180 kDa protein; *lane 5,* IgG reactivity of BP control serum to 230 kDa protein. **Panel B,** Immunoblot of serum samples obtained from patient and control subjects by using dermal extracts: *lane 1,* IgG reactivity of patient serum to 120 kDa protein; *lane 2,* IgA reactivity of patient serum to 120 kDa protein (very weak); *lane 3,* IgA reactivity of LABD control sample to 120 kDa band and against less dense bands; *lane 4:* IgG reactivity of normal human serum sample; *lane 5,* IgG reactivity of epidermolysis bullosa acquisita control serum sample to 290 kDa protein. **Panel C**, Immunoblot of serum samples obtained from patient and control subject by using extracts of COS-7 cells expressing eukaryotic recombinant form of human BP180: *lane 1,* IgG reactivity of patient serum to approximately 160 kDa protein; *lane 2,* IgA reactivity of patient's serum; *lane 3,* IgG reactivity of normal human control sample; *lane 4:* reactivity of mAb 233 to approximately 160 kDa protein (this antibody specifically recognizes ectodomain of BP180); *lane 5,* IgG reactivity of patient serum with extracts of control untransfected COS-7 cells.

We describe a child with a severe subepidermal blistering disorder with features typical for childhood BP. By immunoblot, the patient's serum contained autoantibodies targeting BP180 and a 120 kDa antigen, which may correspond to the LABD antigen.

CASE REPORT

An 8-month-old, previously healthy white child was first seen by his pediatrician for evaluation of multiple tense bullae on the hands and feet (Fig 1). Two weeks later, blisters developed on the trunk, and spread to the face and proximal extremities. Physical examination revealed that the child had widespread vesicles, blisters, and crusted erosions that were frequently distributed in annular and polycyclic patterns (Fig 2). The oral and ocular mucosa were also involved. There was no scarring. The Nikolsky sign was absent.

The biopsy specimen of a blister from the trunk revealed a subepidermal bulla with an intense inflammatory infiltrate, mainly composed of eosinophils and some neutrophils and lymphocytes (Fig 3). The overlying epidermis was intact without necrosis. Direct IF of perilesional skin showed linear deposits of IgG (Fig 4) and C3 along the epidermal BMZ. Staining for IgA and IgM was negative. Indirect IF microscopy on 1 M NaCl separated normal human skin demonstrated that the patient's serum contained circulating IgG autantibodies that exclusively reacted against the epidermal side of the blister at a titer of 1:10. No circulating IgA antibodies were detected.

Despite dapsone therapy (5 mg/kg/day), the patient developed new lesions involving the trunk, extremities, and oral mucosa. He was hospitalized for further evaluation and therapy. The patient was given dexamethasone therapy (3 mg/day) in combination with a 5-day course of intravenous immunoglobulin (5 g/day) with rapid improvement of his eruption. After 7 days, he was discharged from the hospital, and the dexamethasone was substituted by oral prednisone (2 mg/kg/day). However, 2 weeks later the child experienced a relapse of his skin eruption with involvement of the oral mucosa and was readmitted. Cyclosporine therapy was begun at a starting dose of 1 mg/kg, and increased to 5 mg/kg over the following 3 weeks. This resulted in a temporary improvement of the patient's skin lesions, followed by a sudden deterioration. The child developed dyspnea with blistering involving the upper respiratory tract that required intubation. He also had leukocytosis in the magnitude of 40×10^9/L with 33% eosinophils. Cyclosporine was stopped, and pulse glu-

cocorticoid therapy was resumed, resulting in immediate improvement of the child's condition with cessation of new blister formation. Ten days after initiation of pulse glucocorticoid therapy, the patient was discharged from the hospital with further improvement and healing of all lesions with 15 mg prednisone and 20 mg dapsone per day. The prednisone dose was gradually tapered to 10 mg and 2.5 mg every other day, while dapsone was maintained (2.5 mg/kg/day) without recurrence of skin lesions.

MATERIAL AND METHODS

Western immunoblotting (IB)

Equivalent dilutions of patient and control sera were tested against epidermal and skin BMZ extracts by Western immunoblotting (IB). Preparation of keratinocytes extracts with normal human cultured neonatal keratinocytes[11,12] and of dermal extracts has been described in details elsewhere.[12,13] Extracts were subjected to sodium dodecylsulfate-polyacrylamide gel (SDS-PGE), then electrophoretically transferred to nitrocellulose and stained by the peroxydase method.[11,12] Sera were tested at the dilution of 1:10 in washing buffer.

Preparation of human BP180 recombinant proteins

Complementary DNA (cDNA) encoding wild-type BP180 was generated from human keratinocyte RNA by reverse transcriptase-polymerase chain reaction with primers based on the published sequence of human BP180 (GenBank accession number M91669) as decribed in details elsewhere.[14] The full-length human BP180 cDNA was subsequently subcloned using the Not I restriction site in the eukaryotic expression vector pCI-neo (Promega, Madison, Wis) and used for transfection. COS-7 cells were transfected by using the diethylaminoethyl dextran (DEAE-D) method.[15] Forty-eight hours after transfection, the COS-7 cells were lysed with 1% SDS in 25 mmol/L Tris-HCl, pH 7.5, 4 mmol/L EDTA, 100 mmol/L NaCL, 1 mmol/L PMSF, 10 μg/mL leupeptin, and 10 μg/mL soybean trypsin inhibitor. Equal amounts of protein were loaded on a 5% SDS-PGE, separated, and electrophoretically transferred to nitrocellulose sheets. Immunoblotting was performed according to the same procedure as previously mentioned.

RESULTS

By IB that used epidermal extracts, the patient's serum did not show any IgG or IgA reactivity, whereas control BP serum samples blotted a 180 kDa or a 230 kDa protein (Fig 5, *panel A*). By using dermal extracts, the patient's serum was found to contain IgG that immunoblotted a protein of approximately 120 kDa (Fig 5, *panel B*). In addition, weak IgA reactivity with a 120 kDa protein band was also observed. A control LABD serum contained IgA that blotted a protein of similar size and additional, less dense bands of approximately 160 kDa, 110 kDa, and 97 kDa. In addition, a control serum obtained from a patient with epidermolysis bullosa acquisita strongly bound to a 290 kDa band. When extracts of COS-7 cells, that were transiently transfected with cDNA encoding full-length BP180 were used for IB, the patient's serum contained IgG antibodies that strongly recognized a protein of approximately 160 kDa (Fig 5, *panel C*). A protein of a similar electrophoretic mobility was blotted by the mouse IgG1 mAb 233 (provided by Dr K. Owaribe, Department of Molecular Biology, Nagoya University, Nagoya, Japan) directed against the extracellular portion of BP180, whereas the control normal human serum sample did not bind to this band.

DISCUSSION

The childhood form of BP was first proposed as a clinical and histopathologic entity distinct from pemphigus vulgaris by Lever in 1953.[16] In 1970, Bean et al[17] described the first case of a child with a putative diagnosis of BP based on IF. In 1991, Nemeth et al[2] analyzed the features of 33 children with the diagnosis of BP. The patients described were considered to have childhood BP if they fulfilled the following criteria: (1) 18 years of age or younger, with the clinical appearance of tense bullae and the histopathologic finding of subepidermal bulla formation with a variable amount of eosinophils; and (2) direct IF showing linear deposition of IgG or C3 as the major immunoreactant(s) at the BMZ or a positive indirect IF demonstrating circulating IgG autoantibodies that were reactive with the BMZ.[2] However, to date, only a limited number of children with acquired subepidermal blistering disorders have been evaluated in terms of target antigen using both indirect IF on NaCl separated skin and IB or immunoprecipitation studies.[5,18-21] Hence, the diagnosis of BP has been confirmed in only a few cases (Table I).

Our 8-month-old patient had a generalized subepidermal blistering disorder with clinical fea-

Table I. Reported cases of childhood bullous pemphigoid, in which targeted antigens have been characterized by either immunoblotting or immunoprecipitation studies

		Involvement	
Reference	**Age/sex**	**Skin**	**Mucosa**
Saad et al[18]	8 y/F	Vulva	No
Kirtschig et al[5]	6 mo/F	W	No
	4 y/F	W	No
	8 y/F	Vulva	No
	1.5 y/M	W	Yes
	7 y/F	W	No
Nagano et al[19]	3 mo/M	W	No
Wakelin et al[20]	5 y/W	W	Yes
Nyul et al[21]	2.5 y/M	W	Yes
Current case	8 mo/M	W	Yes

W, Widespread; *C*, complement; *Ig*, immunogloblin; *NA*, data not available.
*Linear deposits of immunoreactants along the basement membrane zone on direct and indirect IF microscopy studies.
†In immunoprecipitation studies that use radiolabeled keratinocyte extracts.

tures thought to be characteristic for childhood BP, that is, tense blistering of the hands and feet.[2] He also had linear deposits of IgG and C3 along the epidermal BMZ and circulating IgG autoantibodies reacting against the epidermal side of salt-split skin.

To further define the antigens targeted, immunoblotting studies were performed. Although no reactivity was found with keratinocyte extracts, the patient's serum was shown to contain IgG autoantibodies that reacted against an eukaryotic expressed recombinant form of human BP180, establishing the diagnosis of BP on a molecular level. The failure to detect BP180 by immunoblotting with epidermal extracts is most likely because less BP180 is present in these extracts compared with those obtained from transfected COS-7 cells with high transgene expression. The use of recombinant forms of human BP180 may facilitate the detection of low titer or low affinity antibodies against BP180.

With dermal extracts, the patient's serum was also found to contain IgG (and, weakly, IgA) antibodies directed against a polypeptide of 120 kDa. Although this targeted antigen was not further characterized, it might correspond either to a partial proteolytic product of BP180[22] or to the LABD antigen. Identification of degradation products of both BP180 and of the LABD antigen in dermal extracts is not unusual and depends on the procedure used for antigen preparation as previously described.[22-24] The LABD antigen is a 120/97 kDa anchoring filament–associated protein, thought to be a molecular marker for LABD.[9,23] Because a recent study has provided evidence suggesting antigenic cross-reactivity between BP180 and the LABD antigen,[25,26] it is conceivable that our patient's autoantibodies against BP180 also recognize the LABD antigen. Alternatively, the observed reactivity against both BP180 and a 120 kDa protein may reflect coexistence of circulating autoantibodies of different specificities. Presence of antibodies directed against the BP antigens as well as the LABD antigen has been previously described.[27-30] Darling et al[27] reported a child with features characteristic of LABD, who had IgG antibodies directed against the 2 BP antigens as well as IgA antibodies against the 97 kDa LABD antigen. In addition, Zone et al[29] described several adult patients who possessed IgG as well as IgA antibodies that bound the 97 kDa LABD antigen and the 2 BP antigens.[29] They classified these patients as having "linear IgG/IgA bullous dermatosis." Recent studies indicate that the LABD antigen corresponds to a portion of the extracellular domain of BP180,[31] but it remains unclear if this antigen is a proteolytic product or an isoform of BP180. If these data are confirmed, they would provide an explanation for the clinical, histopathologic, and immunopathologic overlap existing between BP and LABD. However, further

Immunofluorescence			Antigenic reactivity		
DIF*	Indirect IF* on intact skin (titer)	Indirect IF on saline-split skin (titer)	230 kDa	180 kDa	97/120 kDa
C3, IgG	IgG (1:2)	IgG (1:2) epidermal	+(IgG)†	—	—
C3, IgG	IgG	IgG, A epidermal	+(IgG)	—	—
NA	IgG	IgG, A epidermal	—	+(IgG)	—
C3, IgG,M	IgG	IgG epidermal	+(IgG)	—	—
C3, IgG,A,M	IgG, A	IgG, A epidermal	+(IgA)	+(IgG, A)	—
C3, IgG	IgG	IgG epidermal	+(IgG)	—	—
C3, IgG	NA	IgG (1:512) epidermal	—	+(IgG)	—
C3, IgG, M	IgG (1:5)	IgG (1:10) dermal	+(IgG)	+(IgG)	—
C3, IgG	IgG (1:150)	NA	+(IgG)	+(IgG)	—
C3, IgG	NA	IgG (1:10) epidermal	—	recombinant (IgG)	+(IgG, [A])

work is required to understand the exact relation between BP180 and the LABD antigen.

In conclusion, we describe a child with clinical and immunopathologic features characteristic of childhood BP. The observation of the coexistence of autoantibodies reacting against BP180 and a 120 kDa antigen in dermal extracts, which might correspond to the 97/120 kDa LABD antigen, is consistent with recent studies that indicated immunologic cross-reactivity between BP180 and the LABD antigen. These 2 proteins are closely related, LABD antigen being either a proteolytic product of BP180 or an isoform of the BP180 gene.

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