

Autoantibodies to bullous pemphigoid and epidermolysis bullosa acquisita antigens in an infant

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Summary

We describe a 1-year-old boy with multiple tense blisters on the skin, who showed circulating autoantibodies directed to both bullous pemphigoid and epidermolysis bullosa acquisita antigens. The patient's serum IgG antibodies bound to the 290-kDa epidermolysis bullosa acquisita antigen with immunoblot analysis of human dermal extracts. Immunoblot analysis also demonstrated that the patient's serum autoantibodies were reactive with recombinant NC16a domain of the 180-kDa bullous pemphigoid antigen. This study confirmed the presence of circulating autoantibodies directed to both bullous pemphigoid antigen and epidermolysis bullosa acquisita antigen.

Bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) are distinct autoimmune blistering diseases of the skin. Both diseases are characterized by the presence of circulating or tissue-deposited autoantibodies against the basement membrane zone in the skin.¹ Immunochemical and immunoelectron microscopic studies have revealed that the major BP antigens are the 230-kDa and 180-kDa proteins associated with hemidesmosomes,^{2–7} while the EBA antigen is the 290-kDa type VII collagen, the main component of the anchoring fibrils.⁸ Recent molecular biological studies have indicated that the NC16a domain of the 180-kDa BP antigen is the most immunogenic domain.^{9,10} In addition, the NC-1 domain of the 290-kDa type VII collagen has been shown to react with most EBA sera.^{11,12}

Here, we describe a 1-year-old boy with multiple tense bullae mainly distributed on the face and extremities, who demonstrated circulating autoantibodies directed against both the 290-kDa EBA antigen and the recombinant protein encoding NC16a domain of the 180-kDa BP antigen.

Case report

A 16-month-old Japanese boy was referred to us with a 2-week history of recurrent multiple tense bullae on the face, neck, hands and feet. His general condition was otherwise good. Bullae, up to 2 cm in size, were located

on both the normal and erythematous skin (Fig. 1). A few small vesicles were seen on the periphery of erythema. Erosive or ulcerative lesions were also present in the oral mucosa. These bullae healed without leaving any scar formation. There was no family history of bullous disorders. Bacterial and fungal cultures of blister fluid were negative. White blood cell count revealed leukocytosis ($20,200/\text{mm}^3$) with eosinophilia (13%). A skin biopsy specimen showed a subepidermal bulla with eosinophils and lymphocytes in the cavity (Fig. 2). A moderate infiltrate consisting mainly of eosinophils was present in the superficial dermis. Based on the clinical and histological features and on the results of direct and indirect immunofluorescence of normal human skin (see below), a diagnosis of childhood BP was tentatively made. Oral methylprednisolone, in a dosage of 1 mg/kg per day, resulted in improvement of lesions in 3 days. However, his skin lesions recurred when the methylprednisolone dose was reduced to a dosage of 0.6 mg/kg per day. The dosage was thus increased to 1.5 mg/kg per day, and 2 mg/kg per day dapsone was added. His eruption improved rapidly, and the blisters completely healed in 2 weeks. The dosage of methylprednisolone and dapsone was then tapered off, and were finally discontinued after 4 months. The patient has remained free from the disease for over a year.

Direct and indirect immunofluorescence of normal human skin sections were performed as described



Figure 1. Multiple tense bullae arising from normal and erythematous skin on lower leg.

previously.¹³ We also performed immunofluorescence of 1-mol/l NaCl-split normal human skin.¹⁴ Immunoblotting of both epidermal and dermal extracts of EDTA-separated normal human skin was done as described previously.^{5,15,16} Immunoblotting was also performed using fusion protein produced using cDNA encoding N-terminal globular (NC1) domain of type VII collagen. The isolation of cDNA encoding for the NC1 domain of type VII collagen and production of the fusion protein with maltose binding protein were described previously.¹¹ The fusion protein covering the 1080 amino acids of the entire NC1 domain of type VII collagen was purified by amylose resin¹² and subjected to immunoblotting.

We have recently prepared bacterial fusion protein containing the NC16a domain, the most immunogenic region, of the 180-kDa BP antigen.¹⁰ Briefly, cDNA encoding for the NC16a domain was amplified by polymerase-chain reaction using the human keratinocyte cDNA library and specific primers selected from the cDNA sequence reported by Giudice *et al.*,¹⁰ followed by subcloning into the bacterial expression vector pGEX-2T, and inducing fusion protein with the addition of isopropyl- β -thiogalactopyranoside.

Direct immunofluorescence of perilesional skin indicated the linear deposition of IgG and C3 along the dermoepidermal junction (Fig. 3a). Staining for IgM and IgA was negative. Indirect immunofluorescence of normal human skin section detected IgG anti-basement membrane zone antibodies. Indirect immunofluorescence using NaCl-split skin showed

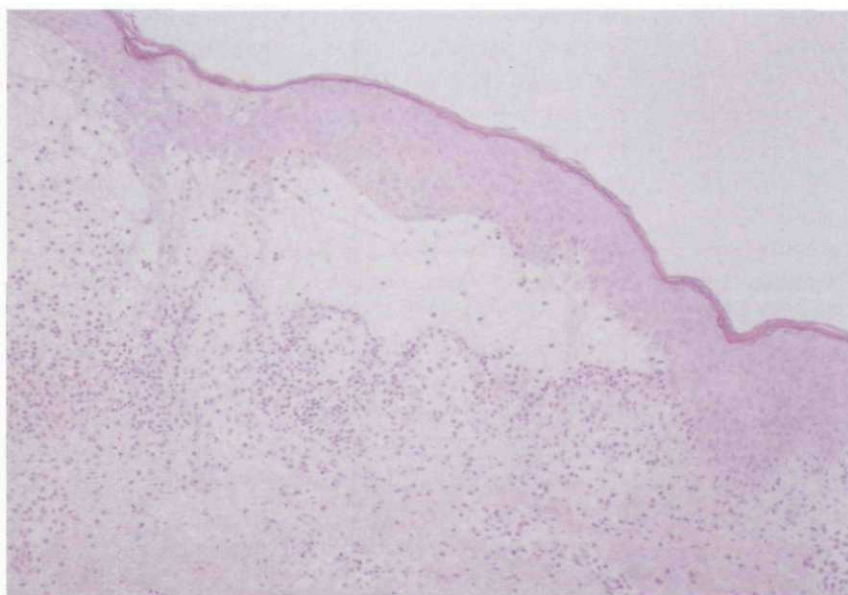


Figure 2. Histological study of vesicular lesion shows a subepidermal bulla with infiltration mainly with eosinophils and lymphocytes in the cavity and the superficial dermis (haematoxylin & eosin stain, $\times 40$).

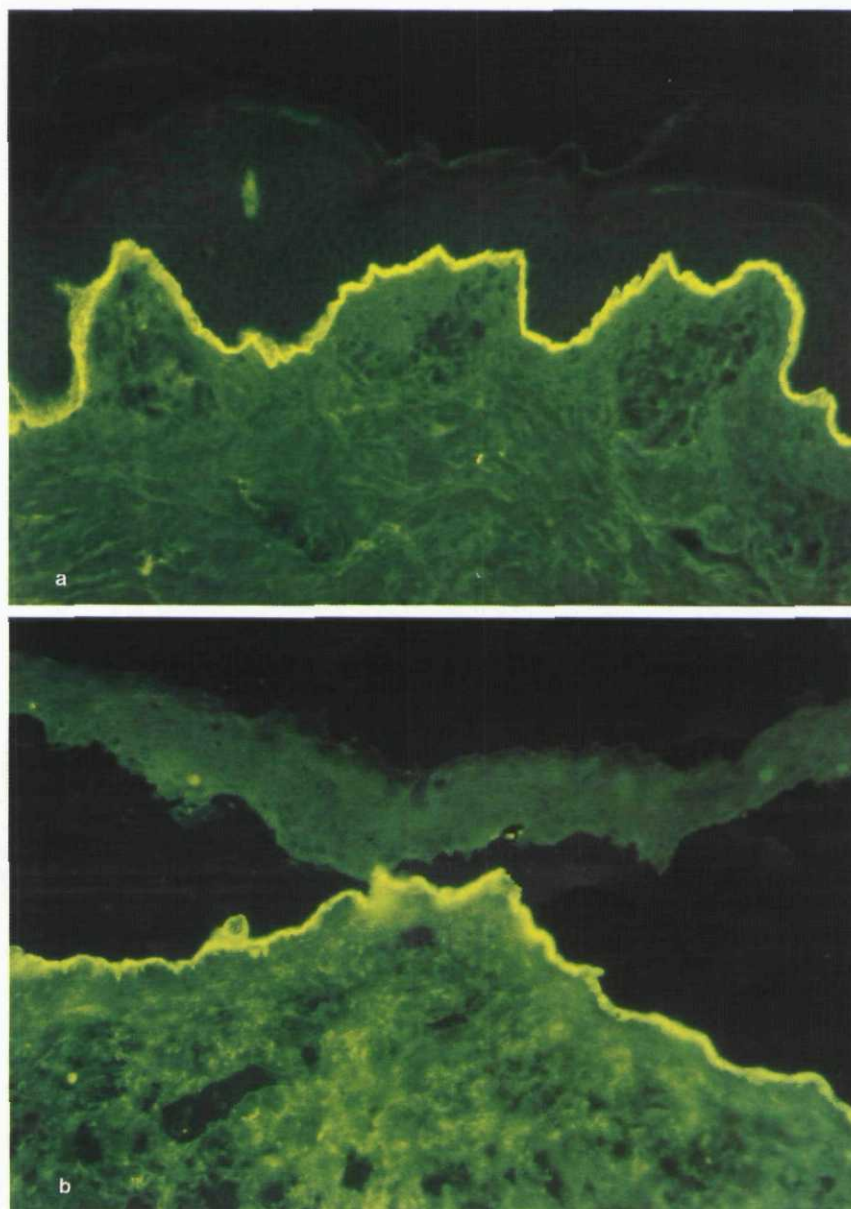


Figure 3. (a) Direct immunofluorescence result of the perilesional skin shows linear labelling along the basement membrane zone ($\times 100$). (b) With direct immunofluorescence of 1-mol/l NaCl-split skin, this patient's serum reacted on the dermal side but not on the epidermal side ($\times 200$).

that the patient's serum bound to the dermal side of the split (Fig. 3b).

Immunoblot analysis of EDTA-separated normal human dermal extracts revealed that this patient's serum reacted with a 290-kDa antigen, which was identical to that detected by control EBA sera (Fig. 4a). However, his serum did not react with the recombinant protein of the NC-1 domain of the EBA antigen, while this recombinant protein was detected by all 10 EBA sera tested, but not by any of 10 normal sera used as negative controls (Fig. 4b).

This patient's serum showed no specific reactivity with immunoblotting of EDTA-separated normal human epidermal extracts (Fig. 5a). However, it recognized clearly

and specifically the recombinant protein of the NC16a domain of the 180-kDa BP antigen (Fig. 5b).

Discussion

We describe a 1-year-old boy who developed multiple bullae on the face and extremities. Immunoblot analysis of the dermal extract revealed that the patient's serum reacted with the 290-kDa EBA antigen. This finding indicates the diagnosis of EBA. However, immunoblot analysis showed that this patient's serum did not react with the recombinant protein of the NC1 domain of the EBA antigen, which has been shown to contain epitopes for most EBA sera.^{11,12} The failure to react with the

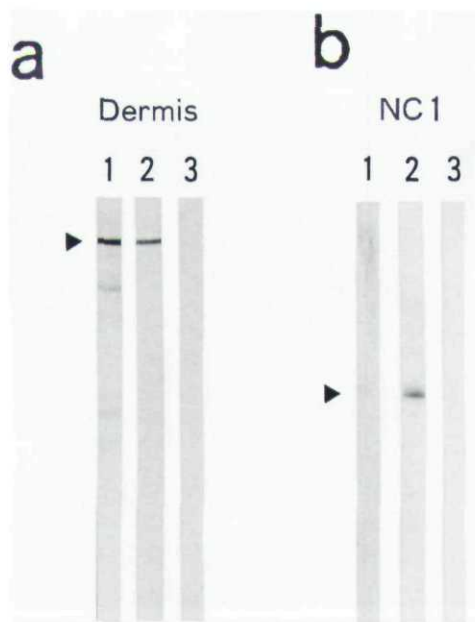


Figure 4. (a) Immunoblotting of EDTA-separated normal human dermal extract. The arrowhead indicates the 290-kDa epidermolysis bullosa acquisita (EBA) antigen. The patient's serum (lane 1) and the control EBA serum (lane 2) reacted with the EBA antigen, whereas normal control serum (lane 3) showed no reactivity. (b) Immunoblotting of the recombinant fusion protein of the NC1 domain of EBA antigen. The arrowhead indicates the position of the fusion protein. This fusion protein was recognized by control EBA serum (lane 2) but not by either the patient's serum (lane 1) or control normal serum (lane 3).

NC1 domain may be explained by the fact that this patient's serum recognized conformational epitopes on the NC1 domain, or recognized the epitopes on other domains of the type VII collagen.

Conversely, although our patient's serum showed no specific reactivity in epidermal extracts, immunoblot analysis unexpectedly revealed that the serum reacted with the recombinant protein of the 180-kDa BP antigen NC16a domain, the most immunogenic domain.^{9,10}

The reason for these discrepant results is at present unclear, but one possibility is that the amount of the antibodies against the 180-kDa BP antigen was not enough to permit detection of the obvious positive signals on either immunofluorescence or immunoblotting of epidermal extracts. Our previous study using 100-BP sera showed that, while about 50% BP sera reacted with the 180-kDa BP antigen in epidermal extracts, the recombinant protein of the NC16a domain was recognized by about 85% sera but not by any control sera, demonstrating a very high sensitivity

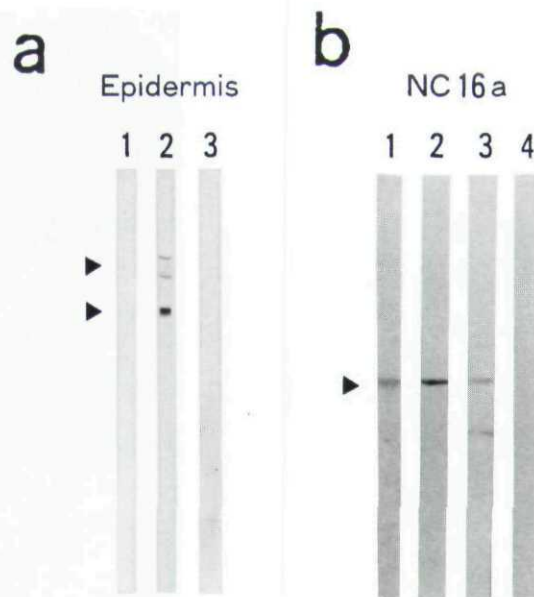


Figure 5. (a) Immunoblotting of EDTA-separated normal human epidermal extract. Upper and lower arrowheads indicate the 230-kDa bullous pemphigoid BP antigen and the 180-kDa (BP) antigen, respectively. Control BP serum (lane 2) reacted to both the BP antigens, while the patient's serum (lane 1) and control normal serum (lane 3) showed no reactivity. (b) Immunoblotting of the fusion protein of the 180-kDa BP antigen NC16a domain. The arrowhead indicates the position of the fusion protein. The patient's serum (lane 1), control BP serum (lane 2) and control herpes gestationis serum (lane 3) reacted clearly with the fusion protein, while normal serum (lane 4) did not.

and specificity of the immunoblot assay of the fusion protein. Such a small quantity of antibodies acting against this domain may be sufficient to induce blister formation in BP.

Based on the findings of the immunofluorescence and immunoblot analyses, we conclude that our patient developed at least two different autoantibodies against the BP and EBA antigens. Most antibodies seem to be operating against the EBA antigen judging from the staining intensity in immunofluorescence of salt-split skin and by immunoblotting of epidermal and dermal extracts. However, this patient's serum reacted with the NC16a domain of the 180-kDa BP antigen but not with the NC1 domain of the EBA antigen. These domains are now considered to play an important role in each disease. Furthermore, this patient responded well to relatively mild treatment and did not show scar formation. These features support a diagnosis of BP. Therefore, at present, the exact role of these antibodies on the pathogenesis in this patient remains unknown.

BP and EBA are distinctly acquired autoimmune bullous diseases that usually affect the elderly. The

incidence of both diseases in childhood is rare. To date, about 40 cases of childhood BP¹⁷ and only six cases of childhood EBA¹⁸ have been reported. The coexistence of these two diseases in the same patient has never been reported. In other autoimmune bullous diseases, such as pemphigus and BP, several reports presented the coexistence of distinct diseases in the same patient at either the clinical or molecular level.^{19,20} Furthermore, recent immunochemical studies have demonstrated further heterogeneity in autoantigens. For example, identification of a novel 125-kDa BP antigen located in the dermis and detection of IgA antibodies against the 290-kDa EBA antigen in linear IgA bullous dermatosis have recently been reported.²¹⁻²³ However, the role of these heterogeneous autoantibodies on the pathogenesis in these autoimmune bullous diseases still remains to be elucidated.

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