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Citation	British Journal of Dermatology, 155(5), 1048-1052 https://doi.org/10.1111/j.1365-2133.2006.07443.x
Issue Date	2006-11
Doc URL	https://hdl.handle.net/2115/30232
Rights	The definitive version is available at www.blackwell-synergy.com
Type	journal article
File Information	BJD155-5.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

British Journal of Dermatology
Manuscript No. BJD-2005-01478 Revised Version
Case Report

Childhood epidermolysis bullosa acquisita with autoantibodies against the non-collagenous 1 and 2 domains of type VII collagen: a case report and a review of the literature

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Short title: Childhood EBA

Key words: EBA, epitope, immunoblot, immunofluorescence, inflammatory phenotype

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Summary

Epidermolysis bullosa acquisita (EBA) is an acquired subepidermal bullous disease characterized by IgG autoantibodies to type VII collagen, a major component of anchoring fibrils. Most patients with EBA are adult and develop autoantibodies to the non-collagenous 1 (NC-1) domain of type VII collagen. In this report, we describe a 4-year-old Japanese boy presenting pruritic vesicles and tense blisters over his whole body. Immunofluorescence studies revealed linear IgG/C3 deposits along the dermal-epidermal junction of the patient's skin, and circulating IgG autoantibodies mapping to the dermal side of 1M NaCl-split skin. By immunoblotting analysis using dermal extracts as a substrate, the patient's IgG antibodies labeled a 290 kD protein corresponding to type VII collagen. Immunoblotting studies using recombinant proteins demonstrated that the patient's circulating autoantibodies recognized not only the NC-1 but also the non-collagenous 2 (NC-2) domain of type VII procollagen.

Review of the previously reported cases and the present case suggested that EBA cases with autoantibodies to regions other than the NC-1 domain are all children younger than 10 years of age with clinical features of an inflammatory phenotype.

Introduction

Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering skin disease which has circulating IgG autoantibodies to type VII collagen, a major component of anchoring fibrils[1], composed of three identical alpha chains. Each chain consists of a long central triple-helical collagenous domain flanked by a large amino-terminal 145 kD non-collagenous 1 (or NC1) domain and a smaller carboxyl-terminal 20 kD non-collagenous 2 (NC2) domain. Type VII procollagen molecules form anti-parallel dimers that are stabilized by disulfide bonding at the carboxyl terminus, and a portion of the NC2 domain is removed by specific proteolytic cleavage to yield the mature type VII collagen. Several dimers aggregate laterally to form the unique cross-banded structure, i.e. anchoring fibrils, which comprise anti-parallel dimers and contain NC1 domains at both ends, locating in the lamina densa and forming semi-circular loops visible by the electron microscope [2].

Two distinct phenotypes of EBA have been described; the classical non-inflammatory type and the inflammatory type. In a large number of the adults with clinically classical type of EBA, it has previously been recognized that autoantibodies are directed against epitopes within the NC1 domain, whereas no immunoreactivity with the NC2 or the triple helical domain was detected[3]. However, recently, in five children with the inflammatory subtype of EBA, novel variants with reactivity to the NC2 or/and the triple helical domains have been reported[4-6]. These findings indicated that some distinct immunopathological differences between childhood EBA and those occurred in adulthood could be present.

In this report, we describe a case of EBA in a 4-year-old Japanese boy and have reviewed previous reported cases of childhood EBA.

Case Report

A 4-year-old Japanese boy, generally in good health, developed painful oral blisters and erosions. A few weeks later, widespread pruritic vesicles and tense blisters appeared over almost his entire body, but especially on his scrotum and buccal mucosa. The blisters were seen both on erythematous bases and on normal skin, and showed an annular arrangement in some legions (Fig.1). His nails were not affected. He had no family history of any blistering disorders.

General laboratory examinations including full blood count, hepatic and renal function tests, urinalysis and C-reactive protein were within normal limits except for an increased number of eosinophils (13.5 %) in his peripheral blood. Anti-nuclear antibodies were negative. Anti-herpes simplex virus antibodies were not detected, either. Viral culture of vesicular fluid and a Tzanck smear test on a vesicular base were all negative.

Histopathological examination of skin biopsy specimens from the dorsal side of his foot revealed subepidermal blisters with an inflammatory infiltrate of lymphocytes, neutrophils, and eosinophils in the papillary and superficial reticular dermis. Especially, neutrophils and nuclear dusts were seen at the tips of the edematous dermal papillae (Fig.2).

On direct immunofluorescence (IF) studies, linear deposits of IgG and C3, but no IgA were seen along the dermal-epidermal junction of the patient’s skin biopsy specimen. Indirect IF studies using a 1M NaCl-split normal human skin as a substrate showed IgG deposits on the dermal side of the artificial split at a titer of 1:160 (Fig.3). Circulating autoantibodies to the NC16a domain of 180 kD bullous pemphigoid antigen (BP180) were not detected using a BP 180 enzyme-linked immunosorbent assay (ELISA)

kit (MBL, Naka-ku, Nagoya, Japan). Immunoblot analysis demonstrated that the patient's sera reacted both with a 290 kD protein in dermal extracts and recombinant type VII collagen (Figs. 4a, b). In addition to the 290 kD band, many additional bands were seen in the immunoblot analysis with patient sera using dermal extract as a substrate. These additional bands were thought to reflect reactivity of patient's sera to background, degradation products because, in this immunoblot analysis, we used high concentration sera in order not to overlook 200 kD band in p200 pemphigoid. Epitope analyses with type VII pro-collagen recombinant fragments revealed that the patient's sera recognized both of the NC1 and NC2 domains (Fig.4c).

The boy was treated with oral prednisolone (1.1mg/kg per day) for 2 weeks, which inhibited new blister formation. When the dose of prednisolone was reduced to 0.4mg/kg per day, new blister formation was observed. Oral dapsone 1.5mg/kg per day was added, but on the next day, erythematous macules appeared on his upper trunk. Thus, dapsone was discontinued and the dose of prednisolone was increased to 0.7mg/kg per day. During the next 2 months, the patient kept taking oral prednisolone 0.5mg/kg per day, and the blisters steadily healed, leaving hyper- and hypo-pigmented macules and milia.

Discussion

EBA is a relatively uncommon disease with an incidence about 10 times less than that of bullous pemphigoid (BP). EBA was first defined with following characteristic features: trauma-induced bullae that heal with milia and scar mainly over the joints of the hands, elbows, feet and knees; nail dystrophy; typically adult onset; a negative family history of

epidermolysis bullosa; and exclusion of other bullous diseases on the basis of clinical and laboratory evidence. The definitive features now include histological and immunopathological findings, such as subepidermal blisters; circulating autoantibodies against skin basement membrane zone antigens, binding to the dermal side of NaCl-split skin; IgG/C3 deposits in the anchoring fibril zone; and the presence of anti-type VII collagen antibodies that are identified as a 290 kD band by immunoblot analysis on dermal extracts[1]. Among clinically diagnosed EBA patients, antibodies can be detected by immunoblotting with dermal extracts in approximately 30% of the cases[7], and by indirect IF using NaCl-split skin as a substrate in approximately 50% of the cases[8].

Two distinct phenotypes of EBA have been described, i.e. the classical (non-inflammatory) type and inflammatory type. The classical type presents with marked skin fragility, blisters and erosions at sites of trauma, and healing with scarring and milia. The inflammatory type can mimic almost all other chronic bullous diseases, and its clinical differentiation from BP, cicatricial pemphigoid and linear IgA bullous dermatosis may be difficult[4-6, 9-26]. In some patients, characteristics of both classical and inflammatory phenotypes of EBA have been observed[9], but most patients with EBA in adulthood appear to suffer from the classical type of EBA.

Conversely, patients with childhood EBA (16-years-old or younger) are very rare. To our knowledge, there are 33 cases of childhood EBA including the present case reported in the literature[4-6, 9-26] Among them, six cases were Japanese and the others were non-Japanese including at least five Caucasian and five African. These patients presented mainly with the inflammatory subtype. Some differences exist between EBA in adults and children. In the adult, the occurrence of mucosal involvement was seen in

approximately 50 % of patients, while in the children, mucosal lesions were seen in the vast majority of the reported cases[9, 18]. The adult form of EBA is known to be difficult to treat, requiring high doses of prednisolone, dapsone, immunosuppressive agents, plasmapheresis, etc. In contrast, dapsone and low dose prednisolone are usually effective in treatments for childhood EBA. The prognosis in childhood EBA seems to be much better than adult cases[4-6, 9-26].

The major epitopes of circulating autoantibodies in adult patients with the classical type of EBA are known to be located within the NC1 domain, but neither in the NC2 nor in the triple helical domains[3]. However in five cases of childhood EBA (four Japanese children and one European child) reported in the literature, autoantibodies have been found to recognize epitopes on the NC2 and/or the triple helical domain over the last years (Table 1) [4-6]. These five patients and the present case were all young children less than 10 years of age, who all showed similar clinical features of the inflammatory subtype of EBA, and complete clearing or a relatively good response to treatments. Interestingly, two of these children also demonstrated autoantibody reactivity towards the 230kD BP antigen (BP230) and the NC16A domain of BP180[4].

Schmidt *et al.*[5] mentioned this phenomenon and referred it as “epitope spreading”. Epitope spreading sometimes occurs in other adult human autoimmune skin disorders, and has been observed in a few cases of EBA, specifically in young children[5]. The molecular events in epitope spreading remain to be elucidated, but it is supposed that the skin damage from autoimmune or inflammatory processes subsequently could induce autoimmunity to a sequestered or closely related antigen or epitope.

Five out of the six EBA patients with autoantibodies to the NC2 and/or

the triple helical domains are Japanese children. Tanaka *et al*.[4] suggested the phenomenon may be unique to a particular ethnic group. To confirm this hypothesis, detailed epitope mappings in bullous dermatosis cases with similar clinical and pathological features will be required, especially in children from different ethnic groups.

Further studies on autoantibodies in larger series of patients with EBA are needed to clarify the correlation between the presence of certain autoantibodies against specific subdomains of type VII collagen and their clinical features, such as classical or inflammatory phenotype, the patient's age of onset, clinical courses, and the prognosis.

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Figure legends

Fig 1. Clinical features. Disseminated, tense vesicles, bullae and erosions with crusts were seen over erythematous plaques on the face (a), arm (b), genital area (c) and the buttocks (d).

Fig 2. Histopathology. A subepidermal blister with an inflmmatory infiltrate of lymphocytes together with eosinophils and neutrophils in the papillary dermis. (Original magnification × 125).

Fig 3. (a) By direct IF staining, linear IgG deposits were seen along the dermal-epidermal junction in the patient's skin.
(b) Indirect IF using 1M NaCl-split human skin as a substrate revealed linear IgG deposits along the dermal side of the artificial split. (･dots: Roof side of the split skin).

Fig 4. (a) Immunoblot analysis of patient's circulating autoantibodies on normal human dermal extracts. *Arrows* indicate the 290kD EBA antigen and 200kD anti-p200 pemphigoid antigen. Control EBA serum (*lane 1*) and patient's serum (*lane 3*) reacted with EBA antigen, whereas control anti-p200 serum (*lane 2*) showed no 290kD band. (b) Immunoblot analysis of patient's circulating autoantibodies on recombinant type VII collagen. *Arrow* indicates the 290kD EBA antigen. Control EBA serum (*lane 1*) and patient's serum (*lane 3*) reacted with EBA antigen, whereas normal control serum (*lane 2*) showed no reactivity. (c) Immunoblot analyses using recombinant fusion proteins of NC1 and NC2 domains of type VII collagen. *Arrows* indicate respective positions of fusion proteins. Both control EBA serum (*lane 1*) and patient's serum (*lane 2*) reacted with NC1 and with

NC2.

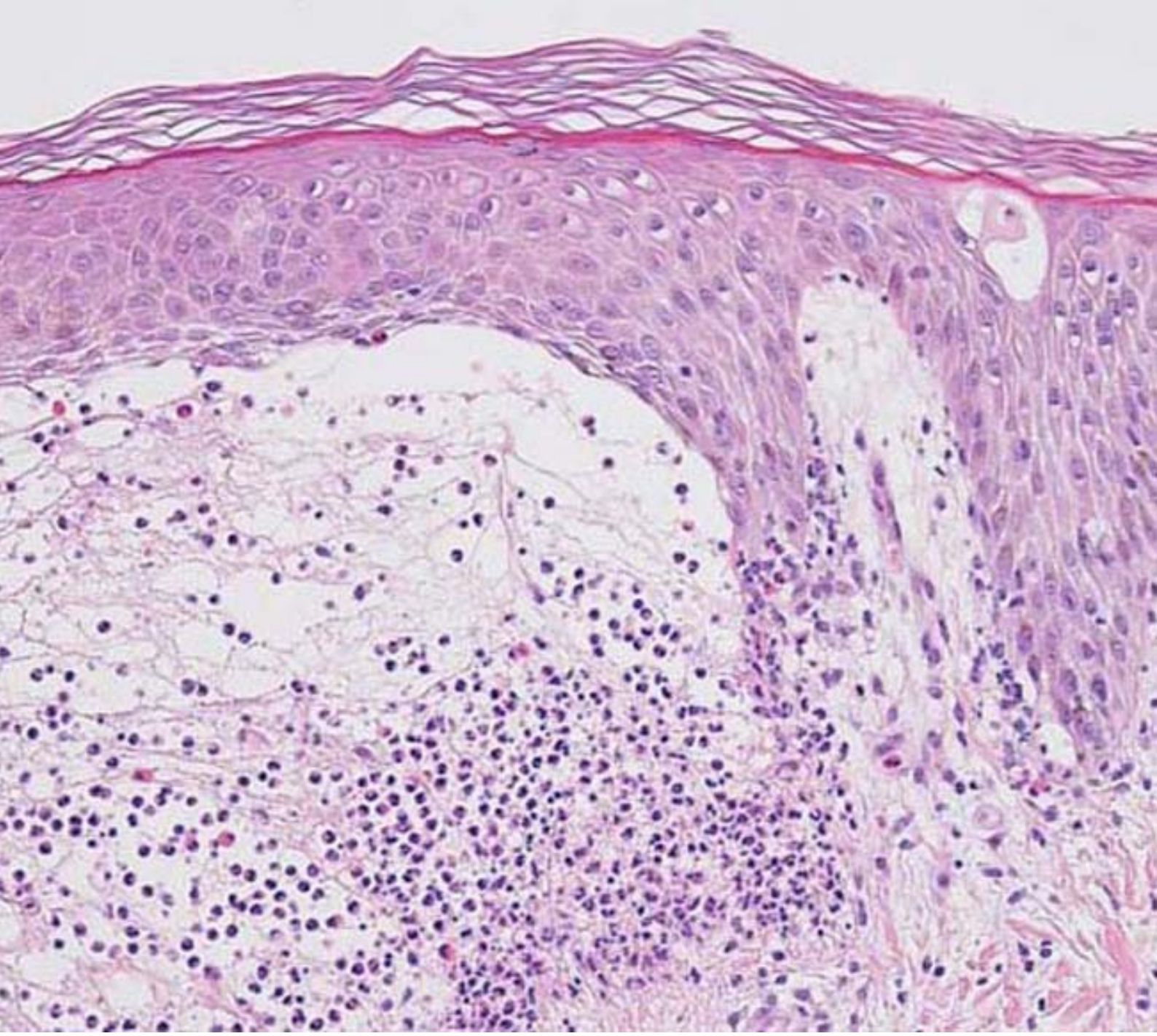
Table 1. Cases with childhood EBA in which epitopes for autoantibodies were detected by immunoblot analysis

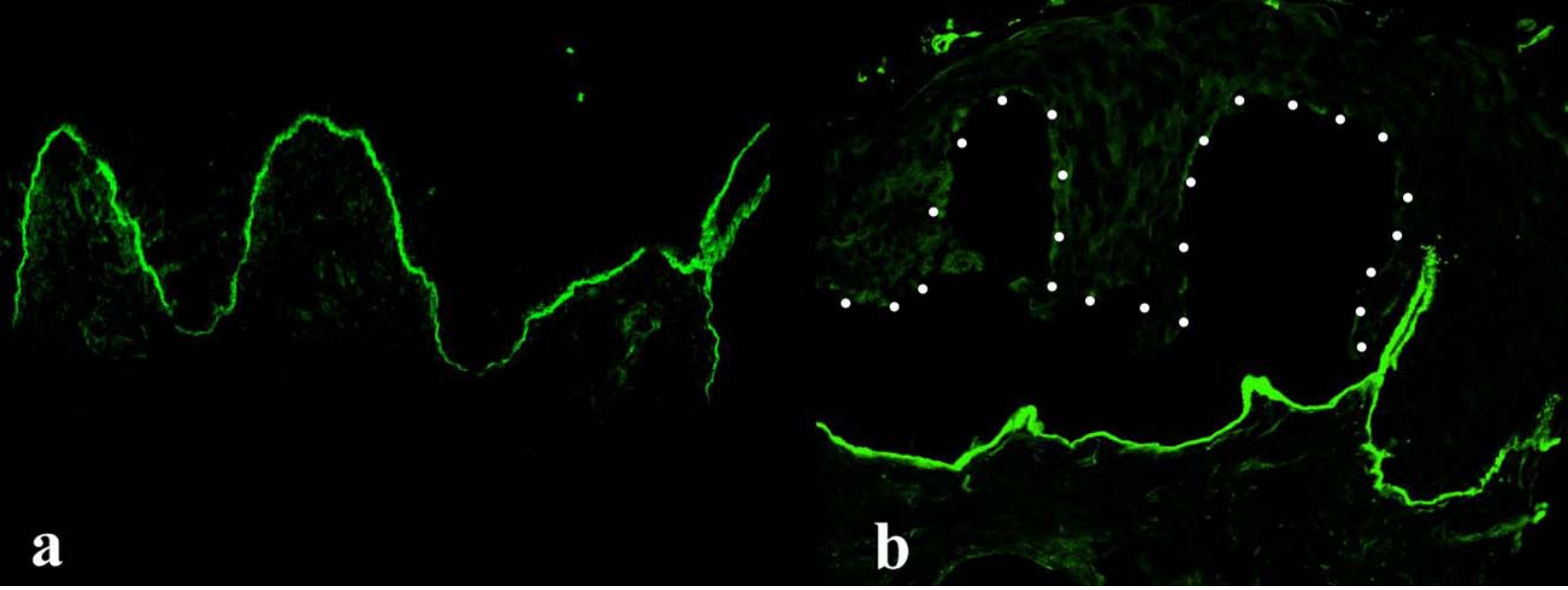
Case	Age/Race/Sex	DIF (BMZ)	IgG deposits in NaCl split skin IIF	Immunoblot on dermal extracts	Immunoblot on epidermal extracts	Recombinant protein immunoblot analysis				NC16a ELISA	Therapy	Reference
						NC1	TH	NC2	rBP180			
1	8/Japanese/F	IgG, IgA, C3	Dermal side	290kD (+)	230kD(+)	-	+	-	ND	-	PSL+DDS	4
2	1/Japanese/M	IgG, C3	Dermal side	290kD (+)	no reactivity	-	+	+	ND	+	Methylprednisolone+DDS	4
3	2/Japanese/F	IgG, C3	Dermal side	290kD (+)	no reactivity	-	+	+	ND	-	PSL	4
4	4/European/F	IgG, C3	Dermal side	290kD (+)	no reactivity	+	+	+	-	ND	PSL+DDS	5
5	5/Japanese/M	IgG, C3	Dermal side	290kD (+)	no reactivity	+	+	+	ND	-	Betamethasone	6
6	4/Japanese/M	IgG, C3	Dermal side	290kD (+)	ND	+	ND	+	ND	-	PSL	Present case

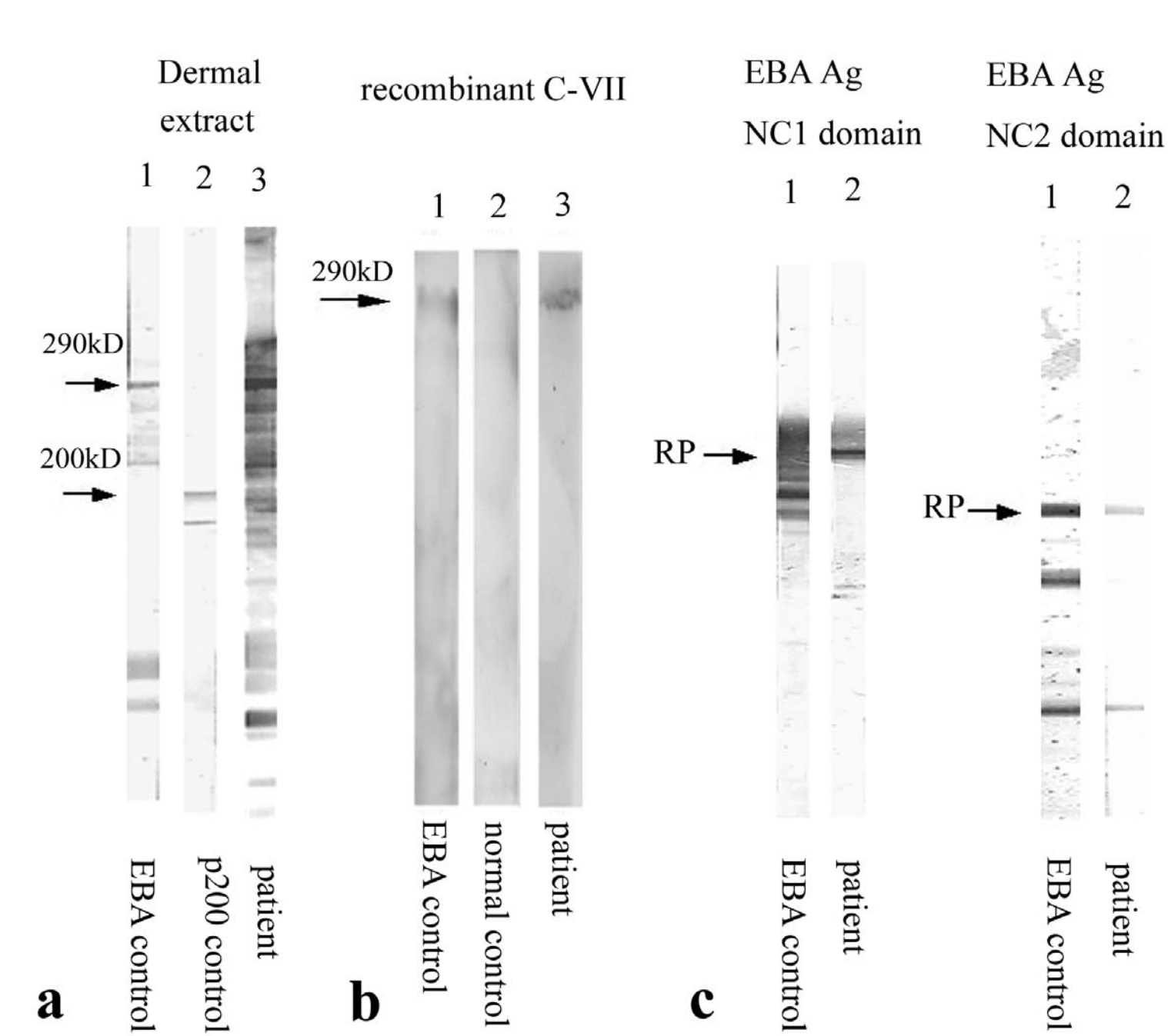
DIF: direct IF, IIF: indirect IF, BMZ: basement membrane zone, TH: triple helical collagenous domain, NC16a: NC16a domain of BP180, rBP180: reconbinant BP180, ND: not done

PSL: prednisolone, DDS: diaminodiphenylsulfone

a
b
c
d




a
b


Dermal extract
1
2
3
290kD
200kD
EBA control
p200 control
patient
a
recombinant C-VII
1
2
3
290kD
EBA control
normal control
patient
b
EBA Ag NC1 domain
1
2
RP
EBA control
patient
c
EBA Ag NC2 domain
1
2
RP
EBA control
patient