—Report on Experiments and Clinical Cases—

Pemphigoid Nodularis: Two Case Studies and Analysis of Autoantibodies before and after the Development of Generalized Blistering

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Abstract

We report 45- and 61-year-old women with generalized prurigo nodularis-like eruption whose clinical, histologic and immunopathologic features were consistent with the diagnosis of pemphigoid nodularis. In one case, nodular lesions preceded the onset of generalized blistering by two years and in the other, no definite blister nor erosion was seen except for some appearing on the soles during the course of the disease. Western immunoblotting of EDTA-separated epidermal extracts revealed that the 230-kD bullous pemphigoid (BP) antigen was recognized by circulating autoantibodies in the patient sera, but the 180-kD BP antigen was not. The 180-kD BP antigen was recognized weakly by immunoblotting of the 180-kD BP antigen NC16a domain fusion protein, which shows high detection sensitivity. These findings suggested that weak reactivity of autoantibodies with either whole or a part of the 180-kD BP antigen molecule in some way accounts for negligible or localized blister-formation in this disorder. However, no particular change was noted in the reactivity with 180-kD BP antigen between the patient serum obtained before and after the development of generalized blistering. It is possible that different factors from the changes in serum reactivity with BP antigens may be involved in initiating generalized blistering.
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Key words: pemphigoid nodularis, BP180, BP230

Introduction

Pemphigoid nodularis is considered to be a variant of bullous pemphigoid (BP). The characteristic eruption is pruritic urticarial papules and hyperkeratotic nodules on the trunk and extremities, coinciding with or preceding the development of generalized blistering[1–3]. The autoantibodies involving the clinical features of pemphigoid nodularis have remained uncertain. To date, immunoprecipitation or immunoblotting has shown IgG autoantibodies to a single antigen of 220-kD/230-kD[4,5] and a variety of antigens including 180-kD and 230-kD antigens (BP 180, BP 230)[6,7]. More specifically Schachter M et al[7] and Kawahara et al[8] have reported that the autoantibody reacts with NC16a domain of BP 180 which is considered to be the major pathogenic epitope in BP. In addition Fujisawa H et al[9] have detected IgA autoantibodies

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against the intracellular domain of desmoglein 1 in the patient serum. However, the fine specificity of these autoantibodies and the mechanism of hyperproliferation accounting for the nodular phenotype as well as that of blister formation appearing in the course of the disease are not known.

The purpose of the present study was to define further details of autoantibodies to BP antigens in pemphigoid nodularis and to evaluate their role in blister formation.

Case Reports

Case 1. A 45-year-old woman with a history of bronchial asthma for the past 10 years, had noticed erythematous plaques, papules and nodules with severe itching on her trunk and extremities. The papulo-nodular lesions were in part hyperkeratotic and hyperpigmented with an excoriated center (**Fig. 1a**). Histologic examination of a skin biopsy specimen from a papular lesion on the forearm revealed serous exudates and inflammatory cells, including eosinophils, neutrophils and lymphocytes, in the dermal papillae and the upper dermis. The epidermis displayed moderate hyperkeratosis and acanthosis, with the lowermost epidermis showing spongiosis. Direct immunofluorescence (IF) of lesional and perilesional skin detected linear IgG and C3. In the indirect IF test, anti-basement membrane zone (BMZ) IgG and IgA antibodies were found on 1M NaCl-separated epidermal layer at a titer of 1 : 160 and 1 : 80, respectively. The serum IgE level was elevated to 1,000 IU/m*l*. Allergen-specific IgE antibody levels to various antigens and intradermal skin test results to common inhalants were normal. The diagnosis of pemphigoid nodularis was made and dapsone and betamethasone at a dose of 50 mg and 0.5 mg daily were administered for 4 months with marked improvement of the eruption. The patient's disease remained under control after the medication was discontinued, but after one year and 9 months, tense bullae, mimicking typical BP both clinically and histologically, developed on the upper extremities, axillae and back (**Fig. 1b**). Betamethasone at a dose of 20 mg was administered daily followed by marked improvement of skin lesions evident from the fourth week.

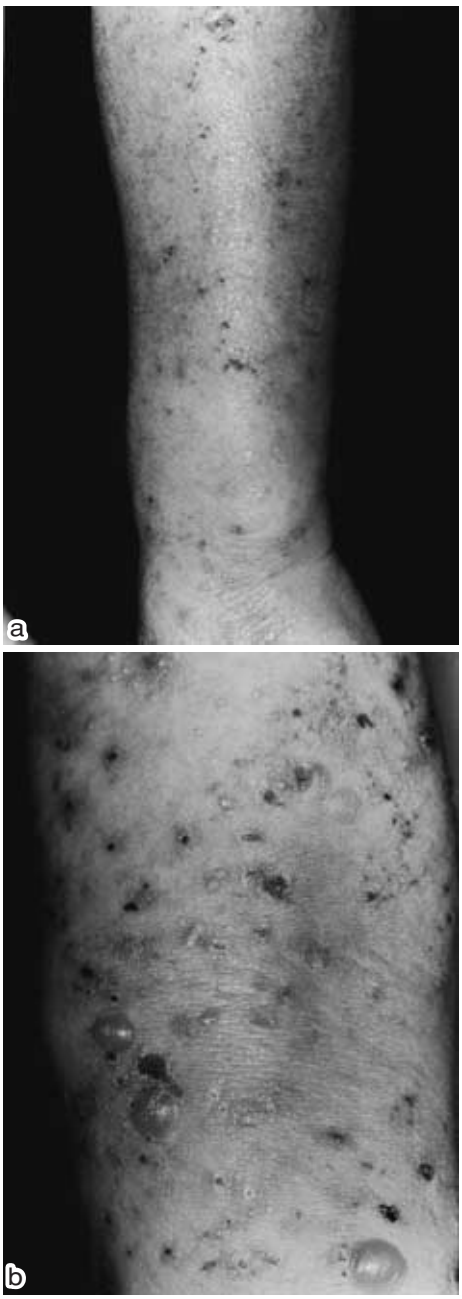

Fig. 1 Case 1. a) papulo-nodular lesions with excoriated surfaces on the forearm. b) Tense bullae developing on the forearm.

Case 2. A 61-year-old woman had a 3-month history of intensely pruritic exanthema. On initial examination, irregular-shaped urticated erythema with some excoriated papules and crusting in a figurate pattern appeared on the trunk and extremities. After 2 months with no improvement with oral antihistamines and topical steroids of high

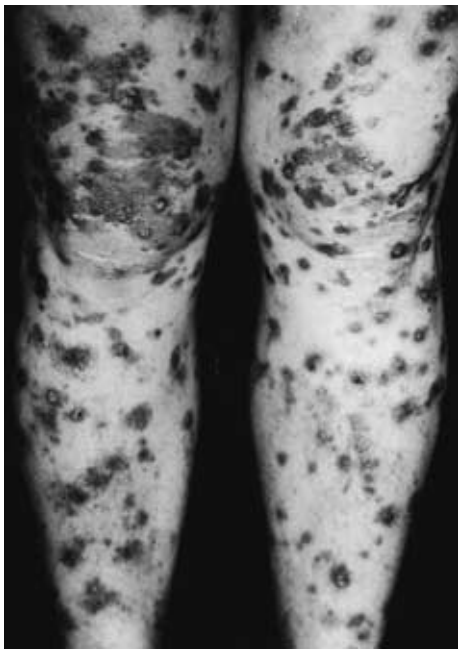

Fig. 2 Case 2. Hyperkeratotic nodules with excoriation on the legs.

potency, hyperkeratotic nodules with an excoriated center developed on the entire body in a symmetrical fashion (**Fig. 2**). No blisters were observed except for some appearing on the erythematous plaque of the soles. Histologic examination of the biopsy specimen from the erythematous lesion of the upper arm revealed eosinophils, neutrophils and lymphocytes accumulating in the lower epidermis and the underlining dermal papillae. Acanthosis and elongation of epidermis with partial parakeratosis were noted. Direct IF studies demonstrated a weak continuous band of IgG and C3 at the dermal-epidermal junction and indirect IF was positive for circulating IgG anti-BMZ antibodies on a 1M NaCl-separated epidermal layer at a dilution of 1 : 320. The diagnosis of pemphigoid nodularis was made and 50 mg of dapsone and 0.5 mg of betamethasone were administered daily, with marked improvement evident from the third week. Abnormal results of the laboratory test included slight eosinophilia (900/u*l*) and the serum IgE and IgA levels were elevated to 1,287 IU/m*l* and 373 mg/d*l*, respectively.

Materials and Methods

Serum Samples

The serum samples were prepared from venous blood obtained from patients 1 and 2, two BP patients who had shown clear reactivity with the 180- and 230-kD proteins in human epidermal extracts and five healthy volunteers.

Immunoblot Analysis

Immunoblotting of extracts of EDTA-separated normal human epidermis was performed as described previously[10].

The recombinant protein containing the BP180 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.[11] Gel-purified cDNA was then subcloned into the bacterial expression vector pGEX-2T (Pharmacia, Uppsala, Sweden). The fusion protein with glutathione-s-transferase was induced by the addition of isopropy1-beta-thiogalactopyranoside and purified on a glutathione sepharose 4B column (Pharmacia).

Immunoblotting using this fusion protein as an antigen source was performed as previously reported[10]. Briefly, purified fusion protein was resolved in Laemmli's sample buffer, and applied to 15% SDS polyacrylamide gel. After transfer to a nitrocellulose membrane, the blots were reacted successively with 1 : 40 diluted serum and 1 : 100 diluted peroxidase-conjugated anti-human IgG antiserum (DAKO, Copenhagen, Denmark), and color was developed using 4-chloro-1-naphthol.

Results

In the immunoblot analysis of epidermal extracts, serum samples of both patient 1 and patient 2 reacted with BP230 without showing apparent reactivity with BP180. The serum samples of the control BP patients reacted with both BP230 and BP180. None of the normal serum samples showed any specific reactivity (**Fig. 3**). In the immunoblot analysis of BP180 NC16a domain fusion protein, the

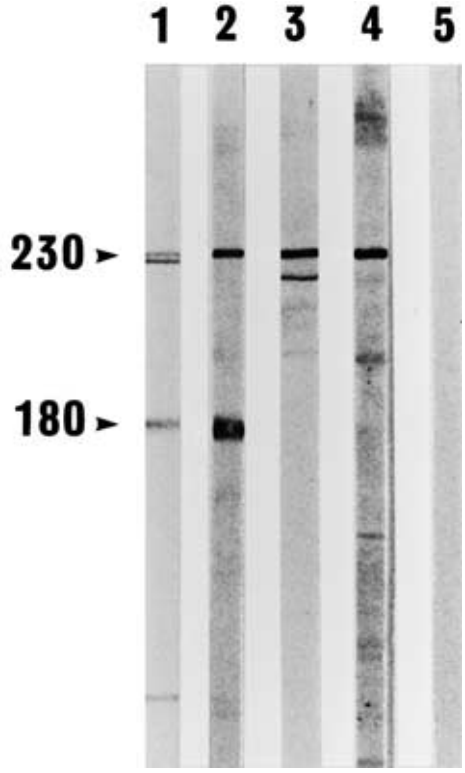

Fig. 3 Immunoblot analysis of normal human epidermal extracts. Lanes 1 and 2: Bullous pemphigoid serum. Reaction with the BP230 and BP180 is indicated by arrowheads on the left. Lanes 3 and 4: Serum from both patients 1 and 2 showing reaction with BP230 without apparent reactivity with BP180. Lane 5: Normal serum.

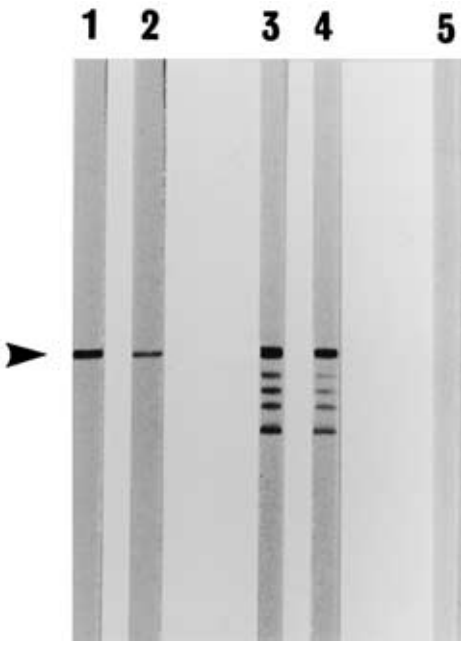

Fig. 4 Immunoblot analysis of the fusion protein of the BP180 NC16a domain. Lanes 1 and 3: Bullous pemphigoid serum. An arrowhead indicates the position of the fusion protein. Lanes 2 and 4: Serum from patients 1 and 2, respectively.

serum samples of patient 1 and patient 2 reacted with the fusion protein, but the reactivity was weaker when compared with that of the control BP samples (**Fig. 4**).

We examined serum samples obtained from patient 1 at two clinical stages: papulo-nodular stage and generalized bullous stage. Both sera showed almost the same reactivity with BP230 of epidermal extracts (end titer of the reactivity; 1 : 80), but no reactivity with BP180 (**Fig. 5**). In contrast, the intensity of reaction with BP180 NC16a domain fusion protein was rather reduced at the bullous stage, when compared with that at the papulo-nodular stage (end titer changed from 1 : 320 to 1 : 80) (**Fig. 6**).

Discussion

Anti-BMZ antibodies in serum from BP patients react with two epidermal antigens, BP180 and BP230[12,13], both of which are localized to the epidermal hemidesmosome[14,15]. Recent studies have shown that BP180 is a transmembranous protein with an extracellular domain consisting of 15 collagen-like domains[11,16] and that the antibodies to BP180 play a crucial role in BP-blister formation[17,18]. In contrast to BP180, BP230 is a protein with a structure similar to desmoplakin and homogeneously distributed in the cytoplasm[19,20]. The role of anti-BP230 antibodies in the pathogenesis of BP is currently unknown.

In the present study using Western immunoblotting of epidermal extracts, we found that the sera from two patients with pemphigoid nodularis were reactive with BP230, but not with BP180. This finding confirmed the findings of previous studies in which immunoprecipitation or

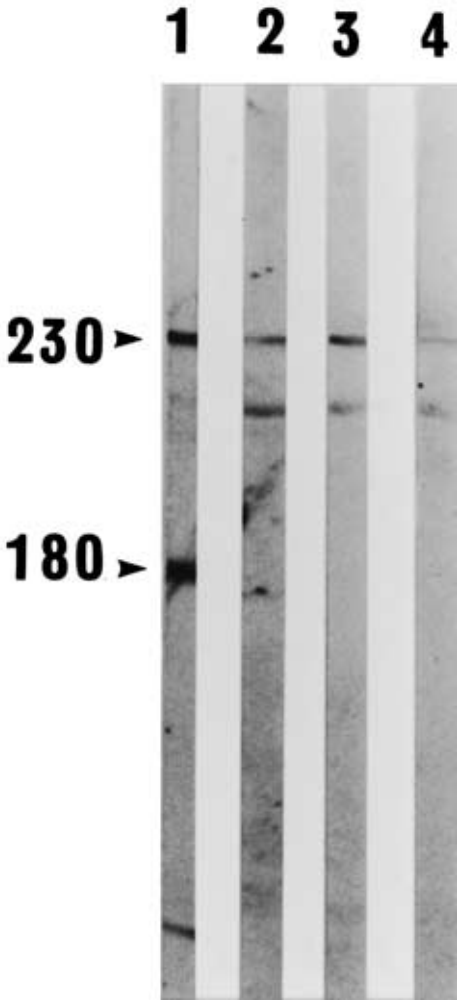

Fig. 5 Immunoblot analysis of normal human epidermal extracts for sera from patient 1 at two clinical stages. Lane 1: Bullous pemphigoid serum. Lanes 2 and 3: Serum from patient 1 at the pemphigoid nodularis stage and the generalized bullous stage, respectively.

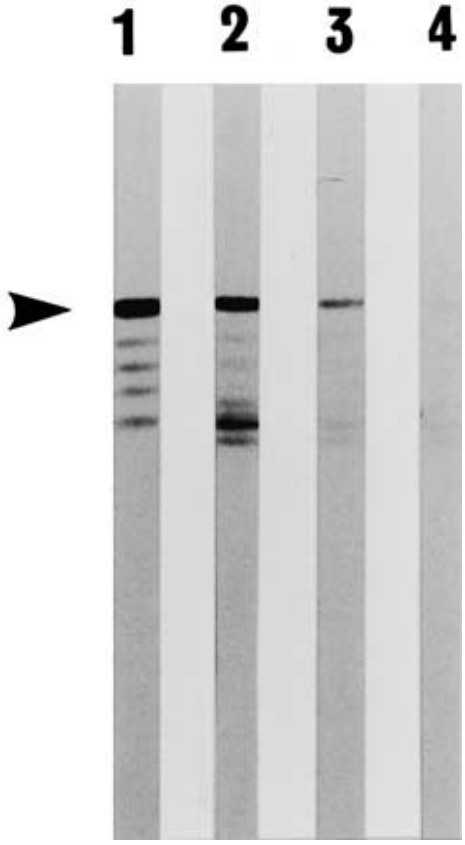

Fig. 6 Immunoblot analysis of the fusion protein of the BP180 NC16a domain for sera from patient 1 at two clinical stages. Lane 1: Bullous pemphigoid serum. An arrowhead indicates the position of the fusion protein. Lanes 2 and 3: Serum from patient 1 at the pemphigoid nodularis stage and the generalized bullous stage , respectively.

immunoblotting has shown a single antigen of 220-kD/230-kD in three patients[4,5]. It is possible that negligible or localized blister-formation in this disorder is in some way related to no reactivity of autoantibodies with BP180. It is interesting to note that two patients with nonbullous variant of BP also shared similar features of immunoprecipitation results[21].

Because the NC16a domain of BP180 is the most immunogenic and pathogenic region in BP, immunoblot technique with the BP180 NC16a domain fusion protein is very sensitive and specific for the detection of anti-BP180 antibodies[22]. Accordingly, we performed immunoblotting of the recombinant protein of BP180 NC16a domain in order to detect BP180 in the patient sera. Using this system, anti-BP180 NC16a domain antibodies were demonstrated successfully in both sera, but the reactivity was relatively weak. This finding confirmed the previous study by Kawahara et al[8] who have analyzed 6 pemphigoid nodularis patients showing faint reactivity with BP180 NC16a domain and indicated the crucial role of autoantibodies with either whole or a part of the BP180 molecule in blister-formation.

However, a controversy exists in the present study. When the patient sera (case 1) obtained before and after the development of generalized blistering

were examined, their reactivity with the NC16a domain of BP180 was not coincident with the process of blister formation. Different factors from the changes in serum reactivity with BP180 may be involved in initiating generalized blistering.

Further studies should be conducted to determine whether or not the present result is a distinctive feature.

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