

Relapse-associated autoantibodies to BP180 in a patient with anti-p200 pemphigoid

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Summary

Anti-p200 pemphigoid and bullous pemphigoid (BP) are autoimmune subepidermal blistering diseases characterized by autoantibodies to a 200-kDa dermal antigen (p200) and two hemidesmosomal proteins (BP180 and BP230), respectively. We report a 70-year-old man with haemorrhagic blisters, widespread crusted erosions, and the immunopathological characteristics of anti-p200 pemphigoid. Treatment with doxycycline, topical corticosteroids and immunoadsorption led to rapid clinical remission. However, 19 weeks later, a relapse occurred with generalized itchy urticarial erythema and tense blisters. At this time, both strong dermal and epidermal IgG staining was detected by indirect immunofluorescence microscopy on salt-split skin, and autoantibodies against both p200 and the 16th noncollagenous (NC16A) domain of BP180 were found. Interestingly, the relapse was associated not only with the detection of autoantibodies to a second autoantigen (BP180), but also with an altered clinical phenotype. This case was a unique occasion to directly monitor the emergence of intermolecular epitope spreading during the course of an autoimmune bullous disorder.

Anti-p200 pemphigoid is an autoimmune subepidermal blistering disease associated with autoantibodies to a 200-kDa protein (p200) of the dermal–epidermal junction.^{1,2} Very recently, the laminin γ 1 chain was found to be the target antigen in most patients with this disease.³ Immunopathologically, the diagnosis of anti-p200 pemphigoid is based on the finding by direct immunofluorescence (DIF) microscopy of linear binding of IgG and/or C3 at the basement membrane zone (BMZ) and the detection by indirect immunofluorescence (IIF) microscopy of circulating IgG antibodies that stain the dermal side of salt-split human skin and react with a 200-kDa protein by Western blotting of dermal extracts.^{1,2,4}

Autoantibodies in bullous pemphigoid (BP), the most common subepidermal blistering disorder, target the hemidesmosomal components BP180 (type XVII

collagen) and BP230. The extracellular portion of the 16th noncollagenous (NC16A) domain of BP180 has previously been found to harbour major epitopes targeted by autoantibodies in BP.⁵

Report

A 70-year-old man presented with a 3-week history of partly haemorrhagic blisters and widespread crusted erosions on a background of erythema on the face, neck, arms, hands, thorax and feet (Figs 1a,b) accompanied by severe pruritus.

Histopathological examination of a lesional skin biopsy found a subepidermal blister with a dense infiltrate of predominantly lymphocytes and eosinophils in the upper dermis.

Using DIF microscopy linear deposition of IgG and C3 at the BMZ was seen. Using IIF on human skin split with 1 mol/L NaCl, IgG was seen to bind along the dermal side at a titre of 1 : 640 (Fig. 1c). In addition, faint staining of IgG at the epidermal side was present (Fig. 1c).

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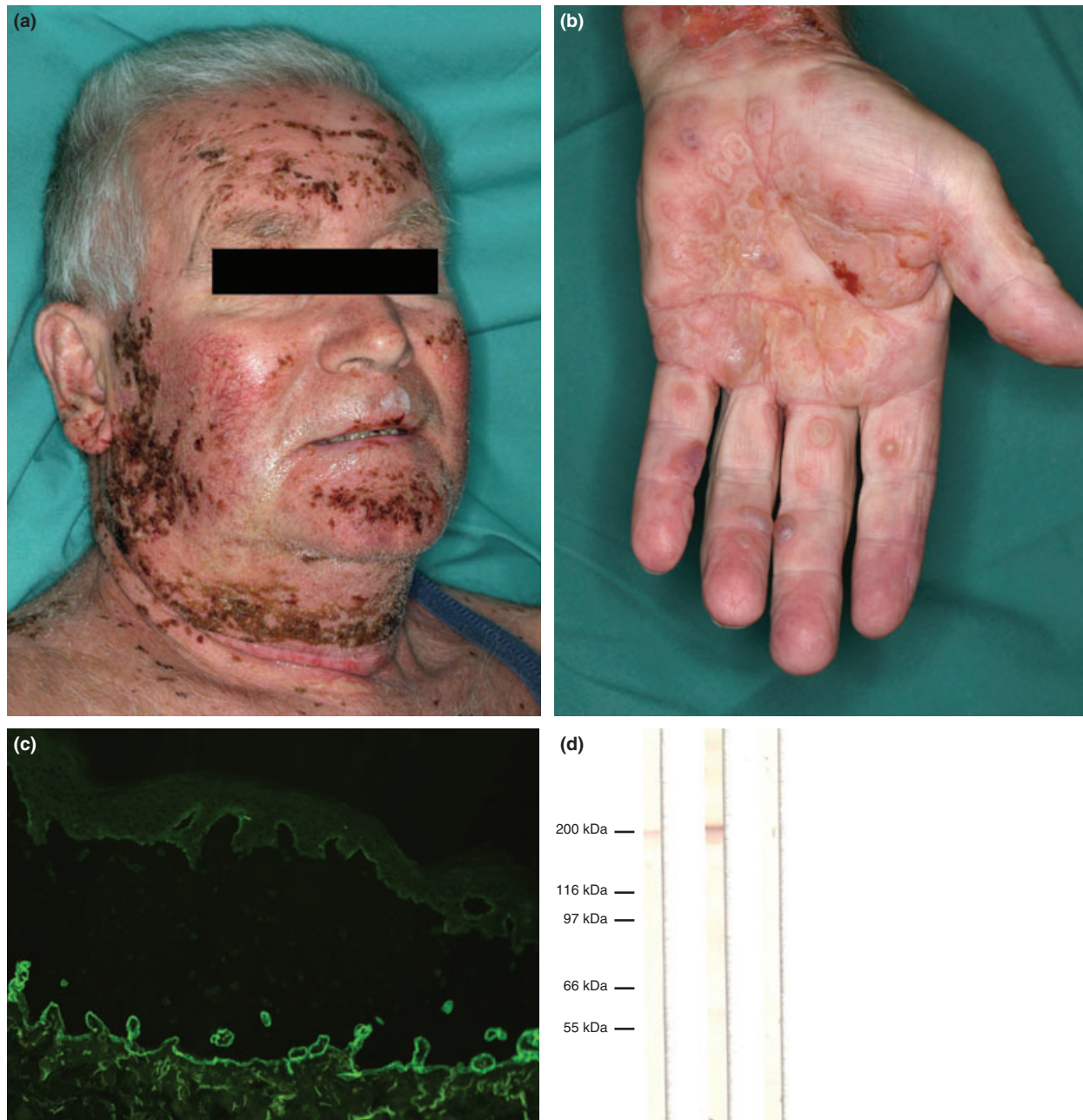


Figure 1 Clinical presentation and autoantibody reactivity at initial presentation. (a) Multiple, partly haemorrhagic crusts and erosions on the face and neck; (b) vesicles and erosions on the palm of the left hand. (c) Indirect immunofluorescence microscopy on salt-split human skin (original magnification $\times 200$). IgG autoantibodies labelled the dermal and, to a much lesser extent, the epidermal side of the artificial split. (d) Immunoblotting using extract of human dermis. The patient's serum (lane 1) and serum of a known patient with anti-p200 pemphigoid (lane 2) reacted with the p200 antigen, whereas normal human serum was unreactive (lane 3). Migration positions of molecular weight markers (kDa) are indicated on the left.

Immunoblotting with human dermal extract found IgG4 antibodies that reacted with a 200-kDa protein (Fig. 1d). Using ELISA, low levels of antibodies to BP230 (19 U/mL, cut-off 10 U/mL) were detected. Immunoblotting

analyses found no reactivity with the soluble ectodomain of BP180 (LAD-1), laminin 332, $\alpha 6 \beta 4$ integrin or type VII collagen, and no autoantibodies to BP180 NC16A. Thus, the diagnosis of anti-p200 pemphigoid was made.

Because of (i) the severity of skin lesions, (ii) the relatively high titres of autoantibodies as detected by IIF microscopy, and (iii) an active duodenal ulcer as seen by gastroscopy accompanied with hematochezia refraining us from employing systemic corticosteroids, immunoadsorption was chosen. Two immunoadsorptions were performed with an interval of 2 days using a tryptophan-linked matrix (Asahi Medical, Tokyo, Japan). In addition, oral doxycycline 200 mg/day and topical clobetasol propionate 0.05% were started.

A rapid clinical response was seen within 2 weeks without adverse events. After 5 weeks, all lesions had cleared leaving hyperpigmented macules. Subsequently, the topical corticosteroid was ceased and doxycycline reduced to 100 mg/day. After 18 weeks of treatment with doxycycline, this medication was also discontinued.

One week later, a relapse occurred, with itchy urticarial papules and plaques developing on the arms and trunk, and tense blisters and oedema on the hands (Fig. 2a). By IIF microscopy, circulating IgG binding to both the dermal (titre 1 : 640) and epidermal side (titre 1 : 640) of NaCl-spilt human skin (Fig. 2b) was seen. Again, IgG antibodies against p200 and BP230 (31 U/mL) were detected by immunoblotting of dermal extracts and ELISA, respectively. In addition, high levels of autoantibodies to BP180 NC16A were also found by ELISA (356 U/mL, cut-off 20 U/mL) (Fig. 3). As the duodenal ulcer had healed in the meantime, treatment with oral prednisolone, initially at 40 mg/day, dapsone 100 mg/day and topical betamethasone 0.1% was started, and the lesions healed within 6 weeks. At follow-up 18 months after his first presentation, the patient remained in clinical remission on prednisolone 10 mg/day and dapsone 100 mg/day. The course of disease and autoantibody reactivity is detailed in Fig. 3.

Discussion

In this case, we had the unique opportunity to witness the emergence of a new autoantibody response during the course of the disease. Although no serum antibodies to BP180 were present either at the beginning of the disease or during the time of first remission, their appearance was linked to the development of new skin lesions and may have been triggered by the preceding inflammation at the dermal–epidermal junction. At the time of relapse, along with the switch in autoantibody specificity, the clinical presentation changed from the initial, partly haemorrhagic vesicles and widespread crusted erosions to urticarial erythema and tense blisters, closely resembling BP. Similarly, a mixed



Figure 2 Clinical presentation and autoantibody reactivity at the time of relapse, 22 weeks after the initial presentation. (a) Tense blister, erythema and oedema on the left forearm and hand. (b) Indirect immunofluorescence microscopy on salt-split human skin (original magnification $\times 200$). IgG autoantibodies bound strongly to both the dermal and epidermal sides.

clinical phenotype has previously been reported in patients with anti-p200 pemphigoid in whom epitope spreading had occurred. In all of these patients, additional target antigens were already present at the time of first presentation, and included laminin 332, type VII collagen, BP180 or BP230.^{2,6–8}

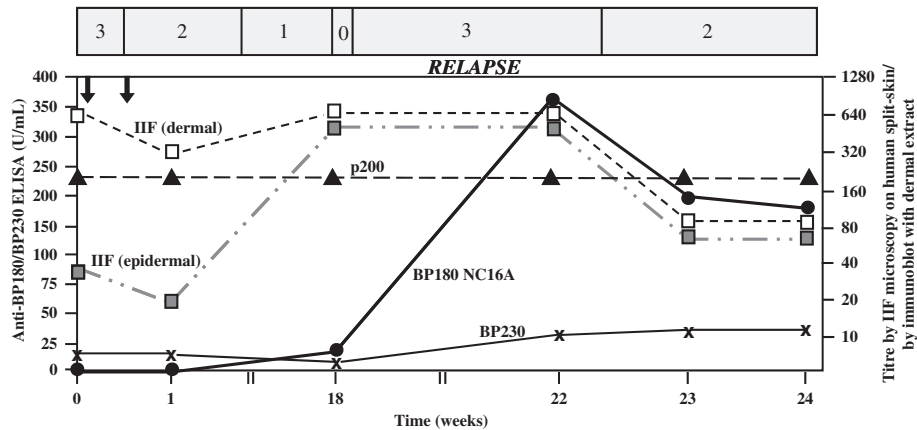


Figure 3 Disease activity and autoantibody reactivity during the course of the disease. Levels of serum autoantibodies as determined by indirect immunofluorescence (IIF) microscopy on salt-split human skin are shown. The clinical score is presented at the top (3 = ≥ 10 lesions at time of evaluation, 2 = 1–10 lesions; 1 = clinical remission but medication required; 0 = complete remission, no medication required).

The pathogenetic role of autoantibodies against BP180 has been confirmed clinically and experimentally.^{5,9,10} In contrast, the pathogenic relevance of autoantibodies in anti-p200 pemphigoid is still unclear. At the time of relapse, for the first time in our patient's disease course, high serum levels of antibodies to BP180 NC16A were detected. These subsequently correlated with disease activity. This finding is compatible with previous observations in patients with BP, in whom serum levels of antibodies to BP180 NC16A paralleled disease activity.¹⁰ Analysis of reactivity to p200 in relation to disease activity during the course of our patient's disease disputes a direct pathogenic role of anti-p200 antibodies. However, pathogenetically relevant autoantibodies in anti-p200 pemphigoid, such as those against BP180 in BP, may be directed to a specific site on p200 and may well correlate with disease activity, even though the overall reactivity to the entire protein, as extracted by the method used in our detection system, does not. Further studies are needed to explore the pathogenic potential of antibodies to p200 in appropriate experimental models.

In summary, we describe the unique emergence of autoantibodies to BP180 during the course of disease in a patient with anti-p200 pemphigoid. The occurrence of anti-BP180 autoantibodies was associated with a severe relapse and a different clinical phenotype compared with the initial presentation. Our observations support the idea that the specificity of circulating autoantibodies is responsible for distinct clinical features of autoimmune subepidermal blistering disorders.

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