

IgA Anti-p200 Pemphigoid

Katarzyna Wozniak, MD; Takashi Hashimoto, MD; Shunpei Fukuda, MD; Bungo Ohyama, MD; Norito Ishii, MD; Hiroshi Koga, MD; Teruki Dainichi, MD; Cezary Kowalewski, MD

Background: Anti-p200 pemphigoid is a rare autoimmune subepidermal blistering disorder. Clinically, it may resemble bullous pemphigoid, linear IgA bullous dermatosis, or dermatitis herpetiformis. Immunologically, anti-p200 pemphigoid is characterized by the development of IgG antibodies directed against a basement membrane zone protein with a molecular weight of 200 kDa.

Observations: We report the first case, to our knowledge, of anti-p200 pemphigoid associated with IgA antibodies and having clinical features resembling pemphigus herpetiformis or dermatitis herpetiformis localized on traumatized areas. Histopathological examination of lesional skin showed dermal-epidermal separation and microabscesses composed of neutrophils in the dermal papillae. Direct immunofluorescence disclosed the presence exclusively of linear in vivo-bound IgA along the basement membrane zone. With the use of laser scanning confocal microscopy, in vivo-bound IgA was localized above

collagen type IV and colocalized with laminin 332. Indirect immunofluorescence showed circulating IgA antibodies against basement membrane zone at a titer of 1:160 that reacted with the floor of an artificial blister of salt-split skin. Western immunoblot analysis using dermal extract confirmed the reactivity of circulating IgA antibodies with the 200-kDa antigen corresponding to laminin γ 1; however, immunoblotting using recombinant protein of 107 amino acid C-terminus of laminin γ 1 was negative for circulating IgA antibodies. Immunoelectron microscopy disclosed the reactivity of circulating IgA autoantibodies within the lower lamina lucida.

Conclusion: To the best of our knowledge, this is the first case fulfilling the immunopathological criteria for anti-p200 pemphigoid associated with IgA antibodies and having unusual clinical features.

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Author Affiliations:

Department of Dermatology, Medical University of Warsaw, Warsaw, Poland (Drs Wozniak and Kowalewski); and Department of Dermatology, Kurume University School of Medicine, and Kurume University Institute of Cutaneous Cell Biology, Kurume, Japan (Drs Hashimoto, Fukuda, Ohyama, Ishii, Koga, and Dainichi).

IN 1996, ANTI-P200 PEMPHIGOID was described as a novel autoimmune subepidermal blistering disorder.¹ Approximately 70 cases have been published so far.² Clinically, it may resemble bullous pemphigoid (BP),^{1,2} linear IgA bullous dermatosis (LABD),² or dermatitis herpetiformis (DH).³ Some cases coexisted with psoriasis.³⁻⁵ The etiology of the disease is not known; however, medications may play a role.⁶ Immunologically, anti-p200 pemphigoid is characterized by the development of IgG antibodies directed against a unique basement membrane zone (BMZ) protein with a molecular weight of 200 kDa.^{1,2} Immunoelectron microscopic studies have disclosed that the 200-kDa protein is localized on the lower part of the lamina lucida.⁴ This explains the dermal binding of circulating antibodies on the salt-split skin. Recently, the 200-kDa protein was identified to be a noncollagenous N-linked glycoprotein of the BMZ, although the function of

the protein within the dermal-epidermal junction remains elusive.⁷ Until now, all published cases with anti-p200 pemphigoid were mediated exclusively by IgG antibodies.¹⁻¹² Then, in 2009, Dainichi et al¹³ identified p200 as laminin γ 1 and renamed it *anti-laminin γ 1 pemphigoid*.

Herein, we report the first case, to our knowledge, of anti-p200 pemphigoid associated with IgA antibodies and having clinical features resembling DH localized on traumatized areas, that is, on the dorsal part of the hands and feet, ankles, and buttocks.

REPORT OF A CASE

A 72-year-old woman with a history of hyperthyroidism controlled with methylthiouracil presented with disseminated annular erythematous plaques that had vesicles on the edge. Skin lesions were localized on the dorsal part of the hands and feet, as well



Figure 1. Clinical characterization of a patient with anti-p200 pemphigoid mediated by IgA antibodies. Annular erythematous lesions localized on traumatized areas: the elbows and wrists (A), the dorsal part of the hands (B), the dorsal and lateral parts of the foot (C), and the buttocks (D).

as on the ankles and buttocks (**Figure 1**). Three weeks earlier, the patient had had herpes simplex infection of the lips. A family history for skin disorders was negative.

A skin biopsy specimen and a blood sample were obtained following signed informed consent. Histopathological analysis of the lesional skin revealed a slight dermal-epidermal separation and microabscesses composed of neutrophils in the dermal papillae (**Figure 2**). Direct immunofluorescence showed the presence exclusively of linear in vivo-bound IgA at the BMZ (**Figure 3**). Laser scan-

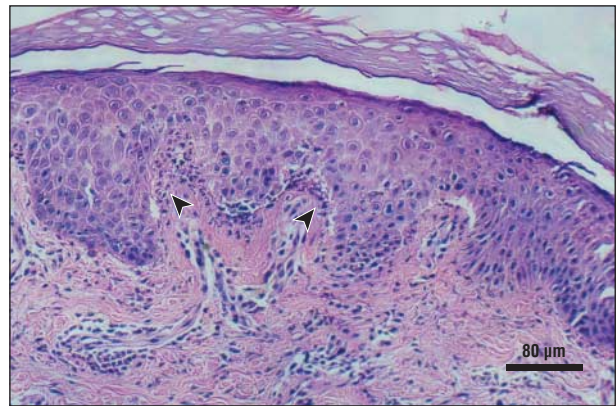


Figure 2. Histological analysis. Hematoxylin-eosin staining of the affected skin showing slight dermal-epidermal separation with microabscesses composed of neutrophils in the dermal papillae (arrowheads).

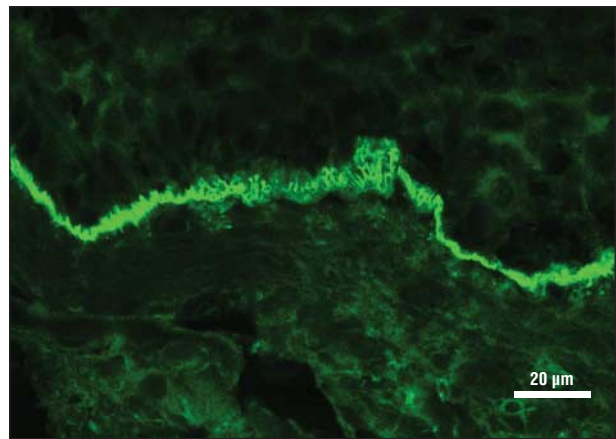


Figure 3. Direct immunofluorescence shows in vivo-bound IgA along the basement membrane zone in perilesional skin.

ning confocal microscopy (LSCM) disclosed that in vivo-bound IgA was localized above collagen type IV and colocalized with laminin 332 (**Figure 4**). Indirect immunofluorescence disclosed circulating IgA anti-BMZ antibodies at a titer of 1:160 that reacted with the floor of an artificial blister created by the salt-split skin technique (**Figure 5A**). Indirect immunofluorescence for IgA antibodies directed against endomysium of the smooth muscles performed on monkey esophagus and enzyme-linked immunosorbent assay (ELISA) with transglutaminase (EUROIMMUNE AG, Lübeck, Germany) were negative. Indirect immunoelectron microscopy disclosed that IgA antibodies deposited to the lower lamina lucida (**Figure 5B**). Western immunoblot analysis using dermal extract showed the reactivity of circulating IgA antibodies with the 200-kDa antigen, suggesting the diagnosis of anti-p200 pemphigoid associated with IgA (**Figure 5C**). However, immunoblotting using recombinant protein of 107 amino acid C-terminus of laminin $\gamma 1$ was negative for IgA antibodies (**Figure 5D**). Different sources of BMZ antigens were used as controls. Immunoblot analysis using epidermal extract, the recombinant proteins of 180-kDa transmembrane hemidesmosomal protein (BP180) (NC16a, C-terminal), and the HaCaT cell line supernatant were negative for both IgG and IgA antibodies in the present case (data not shown).

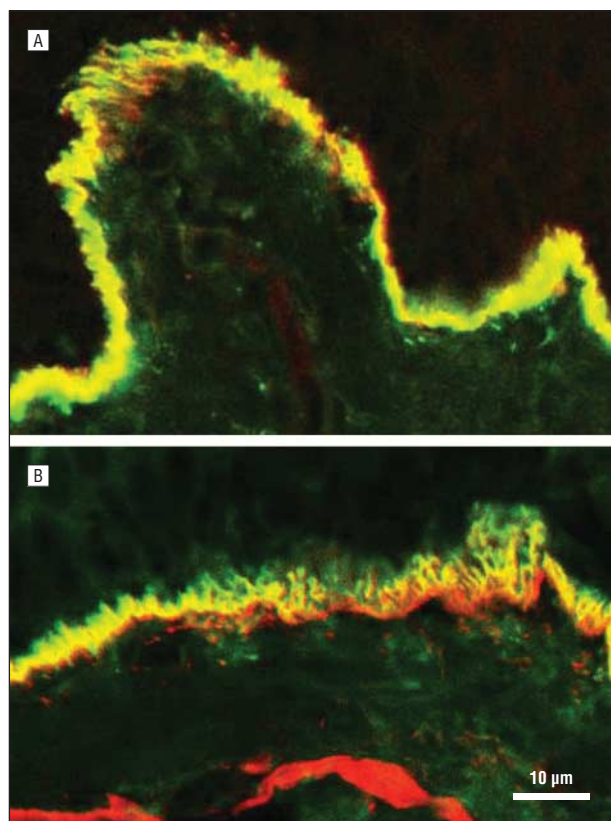


Figure 4. Laser scanning confocal microscopy. A yellow reaction along the basement membrane zone due to overlap of in vivo-bound IgA (green) and laminin 332 (red) (A) and in vivo-bound IgA (green) (B) is localized above collagen type IV (red, lower portion of the image in part B).

A combination of dapsone, 100 mg daily, and prednisone, 30 mg daily, led to the gradual healing of erosions without scarring and milia formation. However, each time the treatment was tapered, skin lesions reappeared. The patient has been in remission without skin lesions for 3 years while taking dapsone, 25 mg, twice a week.

COMMENT

Approximately 70 cases of anti-p200 pemphigoid mediated by IgG antibodies have been reported in the literature so far.¹⁻¹² Herein, we describe the first case, to our knowledge, of anti-p200 pemphigoid associated exclusively with IgA antibodies. The patient presented with erythematous plaques forming an annular arrangement with vesicles on the edge and resembling DH or annular erythema. However, skin lesions were localized on traumatized areas, that is, on the dorsal part of the hands and feet, as well as on the ankles and buttocks, which is observed in a course of mechanobullous disease, such as epidermolysis bullosa acquisita (EBA).

Based on the literature, anti-p200 pemphigoid affects individuals at different ages; however, it usually occurs at a younger age than that observed in patients with BP.¹⁻¹² Anti-p200 pemphigoid does not have specific clinical features, and its clinical appearance is rather heterogeneous.¹⁻¹² Most reported patients presented with disseminated tense blisters and erythematous plaques, similar

to BP.¹⁻⁴ In a few cases, skin lesions resembled LABD² or DH.³ In 2006, Wozniak et al⁶ described a case of anti-p200 pemphigoid mediated by IgG antibodies with unusual erythema gyratum repens-like lesions. In contrast, in one case of anti-p200 pemphigoid, clinical features fulfilled criteria for mechanobullous EBA and exhibited tense blisters localized on traumatized areas, healing with atrophic scars and milia formation, involvement of the oral mucosa, and nail dystrophy.¹⁴ This may be because circulating IgG autoantibodies were directed against both the 200-kDa antigen and the 290-kDa EBA antigen, which was confirmed by immunoblotting in that case.

In our patient, skin lesions were localized on traumatized areas, although without mucous membrane and nail involvement. Because of the uncommon clinical features, our patient was initially suspected to have annular erythema, particularly because the skin lesions were preceded 3 weeks earlier by herpes simplex infection on the lips. Histopathological analysis of lesional skin revealed a slight dermal-epidermal separation and microabscesses composed of neutrophils in the dermal papillae, corresponding to DH. Cases of anti-p200 pemphigoid mediated by IgG antibodies have shown concurrent histological features of different blistering disorders.¹⁵ In most patients, histological analysis disclosed neutrophilic infiltration in the superficial dermis, whereas infiltrates consisted of a mixture of neutrophils and eosinophils in some cases. However, microabscesses in the dermal papillae were observed in only 3 cases. The previous case⁶ of anti-p200 pemphigoid mediated by IgG antibodies presented with a mixed infiltration of eosinophils and neutrophils and with dermal-epidermal separation. Our observations in the present case and the previous case⁶ confirm the histological heterogeneity in anti-p200 pemphigoid.

Whether the type of immunoglobulin (IgA or IgG) involved in the disease influences the histological characterization of anti-p200 pemphigoid remains questionable. We speculate that the cases mediated by IgG antibodies should show predominantly eosinophilic infiltration in the upper dermis, similar to BP, whereas cases mediated by IgA antibodies should show neutrophilic infiltration in the upper dermis and microabscesses in the dermal papillae, resembling LABD and DH, respectively. To elucidate this differentiation, more cases of anti-p200 pemphigoid mediated by IgA antibodies will need to be examined. For now, anti-p200 pemphigoid cannot be distinguished from other subepidermal blistering disorders on the basis of histopathological features alone.

Direct and indirect immunofluorescence in the present case disclosed the in vivo-bound and circulating IgA autoantibodies to BMZ but not to IgG or C3, suggesting a diagnosis of LABD. However, circulating IgA autoantibodies labeled the dermal side of the salt-split skin, which excluded a diagnosis of the lamina lucida type of LABD. Cases in which circulating IgA autoantibodies label the dermal side of the salt-split skin are referred to by some authors¹⁶ as the *sublamina densa type of LABD*. Others¹⁷ have suggested the name *IgA EBA*, especially for cases exhibiting reactivity of the circulating IgA antibodies against the 290-kDa BMZ protein.

Recently, Wozniak et al¹⁸ applied LSCM for the differentiation of autoimmune subepidermal blistering dis-

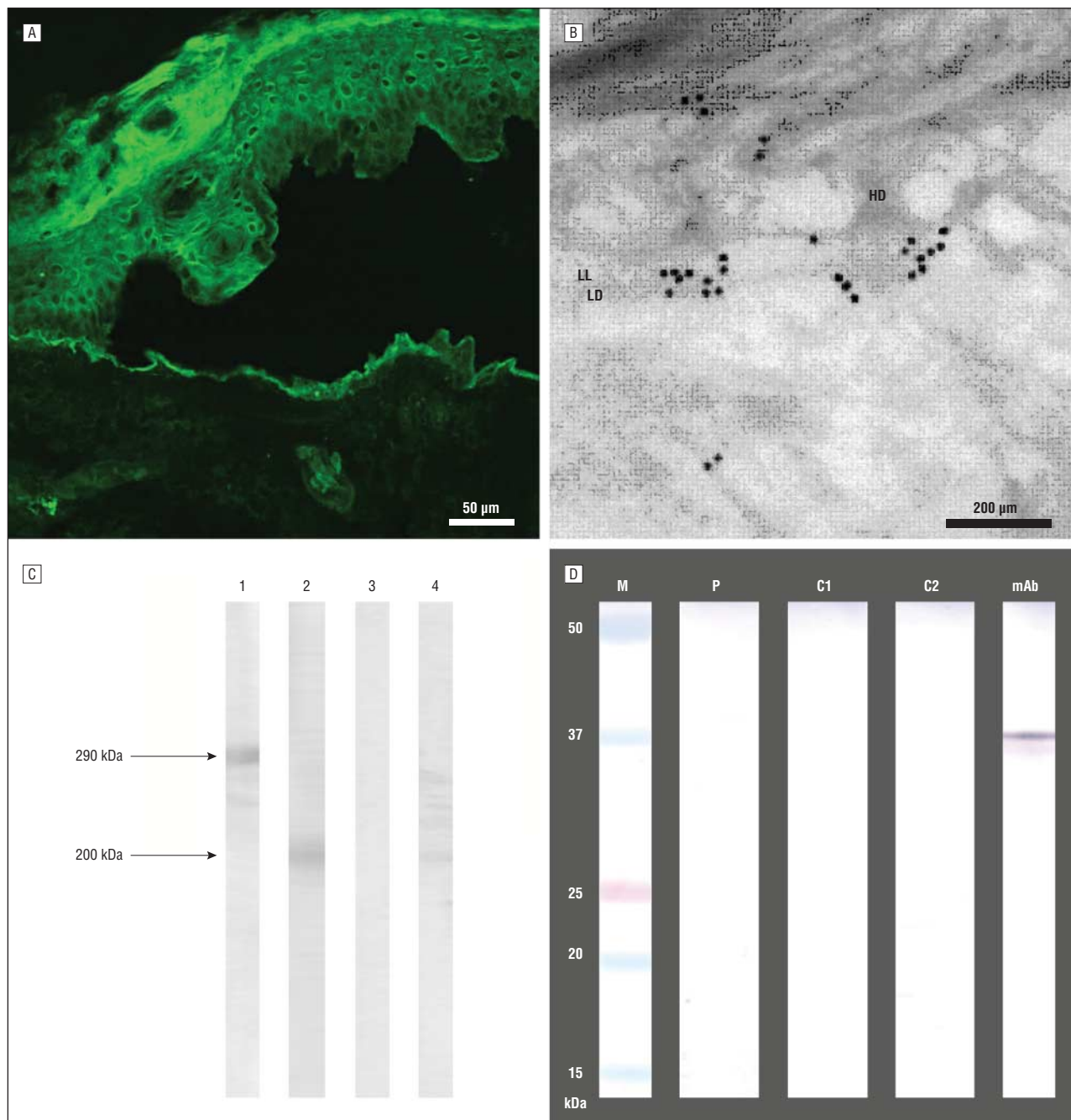


Figure 5. Serological studies. A, Indirect immunofluorescence using salt-split skin shows the presence of circulating IgA antibodies (green staining) bound to the floor of an artificial blister. B, Immunoelectron microscopy shows the presence of circulating IgA autoantibodies at the lamina lucida (LL). LD indicates lamina densa; HD, hemidesmosome. C, Immunoblot analysis using dermal extract discloses circulating IgA antibodies that have reacted with the 200-kDa protein. Line 1 represents the epidermolysis bullosa acquisita control; line 2, p200 control; line 3, the patient's circulating IgG antibodies; and line 4, the patient's circulating IgA antibodies. D, Immunoblot analysis using human laminin γ 1 recombinant protein shows no detection of IgA antibodies against laminin γ 1 protein. M indicates the protein standard marker; P, the patient's serum; C1, control 1 (a healthy man's serum); C2, control 2 (a healthy man's serum); and mAb, monoclonal anti-laminin γ 1 antibody.

orders. This technique is of special value when circulating antibodies are not detectable or when techniques to characterize target antigens are not available. The LSCM study for our patient revealed *in vivo*-bound IgA located above collagen type IV (the lamina densa marker) and on the same level as laminin 332 (the marker for the lamina lucida-lamina densa border). Such localization of *in vivo*-bound IgA at the BMZ corresponds to its ultrastructural localization on the border of the lamina lu-

cida and the lamina densa and disqualifies the diagnosis of IgA EBA or the classic sublamina densa type of LABD. The same LSCM results were obtained in a previous case⁶ of IgG anti-p200 pemphigoid.

Immunoblotting was performed for the present case using a battery of sources for BMZ antigens, including epidermal and dermal extracts, HaCaT cell supernatant, purified laminin 332, and recombinant proteins of BP180. The IgA and IgG antibodies in this case were negative

for the 290-kDa EBA antigen, LAD-1 (antigen for linear IgA bullous dermatosis), laminin 332, or recombinant proteins of the NC16a domain and the carboxyl-terminal end of BP180. Finally, immunoblotting using the dermal extract disclosed the reactivity of circulating IgA antibodies with the 200-kDa antigen, which additionally reacted to the lower lamina lucida in immunoelectron microscopic studies, similar to the cases^{1,3,8} described previously. However, immunoblotting using the recombinant protein of 107 amino acid C-terminus of laminin γ 1 with circulating IgA antibodies was negative. This may be understandable because, as shown recently,¹⁹ the sensitivity of the ELISA test with the recombinant monomeric C-terminal fragment of human laminin γ 1 is about 70%. This means that serum samples from patients with anti-laminin γ 1 pemphigoid may also recognize epitopes of laminin γ 1 other than hLAMC1-term. Thus, we suggest in this case a final diagnosis of anti-p200 pemphigoid associated with IgA antibodies.

In conclusion, clinical and histological features did not allow us to make a precise diagnosis in the present case, whereas the immunological characterization of the target antigen and the localization of in vivo-bound IgA were similar to those of anti-p200 pemphigoid mediated by IgG.

Previously described cases of anti-p200 pemphigoid mediated by IgG antibodies were treated with various immunosuppressive therapies. Almost all cases required systemic corticosteroids with a combination of dapsone, intravenous immunoglobulins, and cyclosporine or azathioprine.²⁰ A previous case⁶ of anti-p200 pemphigoid mediated by IgG antibodies showed intolerance to dapsone and finally responded well to methotrexate sodium. The present case was treated with systemic corticosteroids in combination with dapsone, which seemed to be particularly effective. However, every time the treatment was discontinued, the skin lesions reappeared. Therefore, the patient is still taking dapsone, 25 mg, twice a week and is in remission. To the best of our knowledge, this is the first case fulfilling the immunopathological criteria for anti-p200 pemphigoid associated with IgA antibodies with unusual clinical features.

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Correspondence: Katarzyna Wozniak, MD, Department of Dermatology, Medical University of Warsaw, Koszykowa 82a St, 02-008 Warszawa, Poland (kwozniak@wum.edu.pl).

Author Contributions: Dr Wozniak had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Wozniak, Hashimoto, and Kowalewski. *Acquisition of data:* Wozniak, Hashimoto, Fukuda, Ishii, Koga, Dainichi, and Kowalewski. *Analysis and interpretation of data:* Wozniak, Hashimoto, Ohyama, Ishii, Dainichi, and Kowalewski. *Drafting of the manuscript:* Wozniak, Hashimoto, Koga, and Kowalewski. *Critical revision of the manuscript for important intellectual content:* Wozniak, Hashimoto, Fukuda, Ohyama, Ishii, Dainichi,

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