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Differentiating Anti-Lamina Lucida and Anti-Sublamina Densa Anti-BMZ Antibodies by Indirect Immunofluorescence on 1.0 M Sodium Chloride-Separated Skin

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Sixty-one bullous disease sera containing IgG anti-BMZ antibodies were examined by indirect immunofluorescence on intact skin and skin separated through the lamina lucida by incubation in 1.0 M NaCl. All sera produced an indistinguishable pattern of linear immunofluorescence on intact skin at dilutions of 1:10 or higher. On separated skin, antibodies bound to either the epidermal (epidermal pattern), dermal (dermal pattern), or epidermal and dermal (combined pattern) sides of the separation. The binding patterns were consistent on separated skin from several donors and titers of anti-basement membrane zone antibodies on separated skin were comparable to those on intact skin. Sera from 3 patients with herpes gestationis (HG), 36 patients with bullous pemphigoid (BP), and 1 patient with clinical and histologic features of epidermolysis bullosa acquisita (EBA) showed an epidermal pattern. Sera from 9 patients with BP showed a combined pattern and sera from 6 patients with EBA and 6 patients with clinical and histologic features of BP showed a dermal pattern. Indirect immunoelectron microscopy of selected sera showed antibodies producing the epidermal and combined patterns were anti-lamina lucida antibodies and those producing the dermal pattern were anti-sublamina densa antibodies. These results show indirect immunofluorescence on separated skin is a dependable method for differentiating bullous disease anti-lamina lucida and anti-sublamina densa antibodies and that differentiating between the antibodies is essential for accurate diagnosis in some patients. The results also suggest BP anti-lamina lucida antibodies may have more than one antigenic specificity.

Circulating and tissue-deposited IgG antibodies to the cutaneous basement membrane zone (BMZ) are characteristic of patients with bullous pemphigoid (BP), cicatricial pemphigoid (CP), herpes gestationis (HG), and epidermolysis bullosa acquisita (EBA) [1–8]. By immunoelectron microscopy, anti-BMZ antibodies can be divided into two groups based on their ultrastructural binding sites. In BP, CP, and HG the antibodies bind to the lamina lucida (anti-lamina lucida antibodies) and in EBA, they bind along the lower edge or just below the lamina densa (anti-sublamina densa antibodies) [5–12]. Anti-lamina lucida and anti-sublamina densa antibodies cannot be reliably distinguished using standard direct and indirect immunofluorescence procedures and substrates because of their identical staining patterns.

Due to the limited availability of immunoelectron microscopy, the ultrastructural binding sites of anti-BMZ antibodies are seldom defined in bullous diseases, and differential diagnosis is based primarily on the clinical and histologic features of the diseases. These features may be misleading, especially in patients with BP, CP, and EBA in which features considered characteristic of one disease may be present in another. Since BP and CP are characterized by anti-lamina lucida antibodies and EBA by anti-sublamina densa antibodies, precise localization of anti-BMZ antibody binding sites could be of value in differential diagnosis. Furthermore, routine or more frequent determination of antibody binding sites could provide a better understanding of the clinical, pathologic, and immunoultrastructural correlations in all bullous diseases associated with anti-BMZ antibodies.

In search of an alternative to immunoelectron microscopy for differentiating between anti-lamina lucida and anti-sublamina densa antibodies, we have investigated indirect immunofluorescence using NaCl-treated human skin as substrate. One molar NaCl has previously been shown to separate the BMZ through the lamina lucida leaving BP antigen on the epidermal side and components of the lamina densa, type IV collagen, on the dermal side [13]. Using this substrate, we examined sera containing anti-BMZ antibodies from 61 patients thought to have BP, HG, or EBA based on clinical, histologic, and routine immunohistologic criteria. The results showed that anti-lamina lucida antibodies from patients with BP and HG bound to the epidermal or both epidermal and dermal sides of separated skin while anti-sublamina densa antibodies from patients with EBA bound only to the dermal side. Of interest was the finding that 6 patients thought to have BP and 1 patient thought to have EBA by routine clinical, histologic, and direct immunofluorescence criteria had anti-sublamina densa and anti-lamina lucida antibodies, respectively. In addition, 9 BP sera had anti-lamina lucida antibodies which consistently bound to both epidermal and dermal sides of separated skin while 36 BP sera had anti-lamina lucida antibodies which consistently bound only to the epidermal side.

MATERIALS AND METHODS

Immunoreagents

Rabbit anti-laminin serum (specific immunoglobulin concentration = 0.25 mg/ml) was purchased from Bethesda Research Labs, Gaithersburg, Maryland and used at a dilution of 1:100 in 0.15 M NaCl buffered with 0.01 M Na_2HPO_4 and 0.1 M NaH_2PO_4, pH 7.2 (PBS). Fluorescein isothiocyanate (FITC)-conjugated IgG fraction of goat antihuman IgG and antirabbit IgG were purchased from Cappel Laboratories, Cochranville, Pennsylvania. Specific antibody and molar fluorescein to protein ratios of these reagents were 2.1 and 2.6, respectively for

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Abbreviations:
BMZ: basement membrane zone
BP: bullous pemphigoid
CP: cicatricial pemphigoid
EBA: epidermolysis bullosa acquisita
FITC: fluorescein isothiocyanate
HG: herpes gestationis
NHS: normal human serum

antihuman IgG and 3.0 and 2.8 for antirabbit IgG. These conjugates were used at a dilution of 1:10–1:20 in PBS. Bullous pemphigoid anti-lamina lucida and EBA anti-sublamina densa reference sera were obtained from 2 patients diagnosed by direct IgG immunoelectron microscopy. Specificity of these anti-BMZ antibodies for the lamina lucida and sublamina densa zone was confirmed by indirect IgG immunoelectron microscopy at a dilution of 1:10. Titers of IgG anti-BMZ antibodies by indirect immunofluorescence on intact human skin were 1:1280 for BP and 1:320 for EBA. Reference sera were used at a dilution of 1:10 in PBS.

Bullous Disease and Control Sera

Control normal human sera (NHS) and bullous disease sera were obtained from 8 normal volunteers and 61 bullous disease patients with circulating IgG anti-BMZ antibodies at a titer of at least 1:10 by standard indirect immunofluorescence testing. Bullous disease patients were diagnosed as BP (51), HG (3), and EBA (7) by accepted clinical, histologic, and immunohistologic features. Six patients with EBA and 3 with BP were also diagnosed by direct IgG immunoelectron microscopy. Most patients were diagnosed by one of the authors; however, several sera were generous gifts from Drs. E. H. Beutner, L. A. Diaz, S. I. Katz, and W. M. Sams, Jr. All sera were heat-inactivated at 56°C for 30 min and stored frozen at −70°C.

Skin

Strips of adult human skin approximately 2.0 cm wide and 10.0 cm long were obtained from 3 fresh cadavers or surgical patients without skin disease and subcutaneous fat scraped away. Skin was rinsed in PBS and a 1 cm^2 piece was cut from each strip, quick-frozen in liquid N_2, and stored frozen at −70°C. These pieces were used as intact skin.

Sodium Chloride Separation of Skin

The remainder of the skin strips were treated with 1.0 M NaCl using a previously described method [14]. Briefly, each strip was immersed in 50.0 ml of cold (4°C) 1.0 M NaCl in a 50-ml capped polystyrene tube and slowly rotated for 72 h at 4°C. Sodium chloride was exchanged at 24 and 48 h. At the end of 72 h, strips were blotted on filter paper, placed epidermis side up in Petri dishes, and the epidermis gently dislodged from the dermis by lateral traction applied to the skin with a tongue blade. The epidermis was left in place and strips cut into 1.0-cm^2 pieces with a razor blade. Some pieces were quickly frozen in liquid N_2, mounted in Ames O.C.T. compound (Ames Co., Elkhart, Indiana), and stored frozen at −70°C. Several pieces were immediately fixed in 2.5% gluteraldehyde in 0.2 M Soresen's $NaPO_4$ buffer, pH 7.4

Electron Microscopy

Glutaraldehyde-fixed pieces of each of the 3 strips of NaCl-separated skin were embedded and processed for routine electron microscopy according to previously described methods [15].

Indirect Immunofluorescence

Indirect immunofluorescence was performed on intact and separated human skin using established methods [16]. Specimens of separated skin from each of the 3 donors were examined to localize laminin and antigens reactive with the BP and EBA reference sera. All 61 bullous disease sera were examined by IgG indirect immunofluorescence on intact and separated skin from 1 donor at a dilution of 1:10. Thirty-eight of the BP-labeled sera and all HG- and EBA-labeled sera were examined on separated skin from 3 donors at a titer of 1:10. Twenty-one of the BP- and EBA-labeled sera were titered by doubling dilutions with PBS from 1:10 to 1:20,480 and examined on one specimen of separated skin.

Indirect Immunoelectron Microscopy

Indirect immunoelectron microscopy was performed on selected BP- and EBA-labeled sera at a titer of 1:10 using 10–12 μm-thick cryostat sections of frozen intact or separated skin and a previously described peroxidase-antiperoxidase method [6].

RESULTS

Ultrastructure of NaCl-Separated Skin

Glutaraldehyde-fixed specimens of separated skin from each of 3 donors were examined by electron microscopy to determine the site of separation and preservation of BMZ structures. The results were similar in all 3 specimens. In each, dermis and epidermis were separated primarily through the lamina lucida with recognizable basal cell plasma membrane on the epidermal side and lamina densa on the dermal side. The lamina densa showed some focal thinning and infrequent disruptions but only rarely were fragments of lamina densa adherent to the epidermal side of the separation. The basal cell plasma membrane also showed degenerative changes and interruptions but was only rarely seen attached to the dermal side of the separation. Portions of lamina lucida could be seen adherent to both plasma membrane and lamina densa.

Localization of BMZ Antigens in NaCl-Separated Skin

Frozen specimens of separated skin from each of the 3 donors were examined by indirect immunofluorescence using antibodies to laminin and reference sera to BP and EBA antigens. The

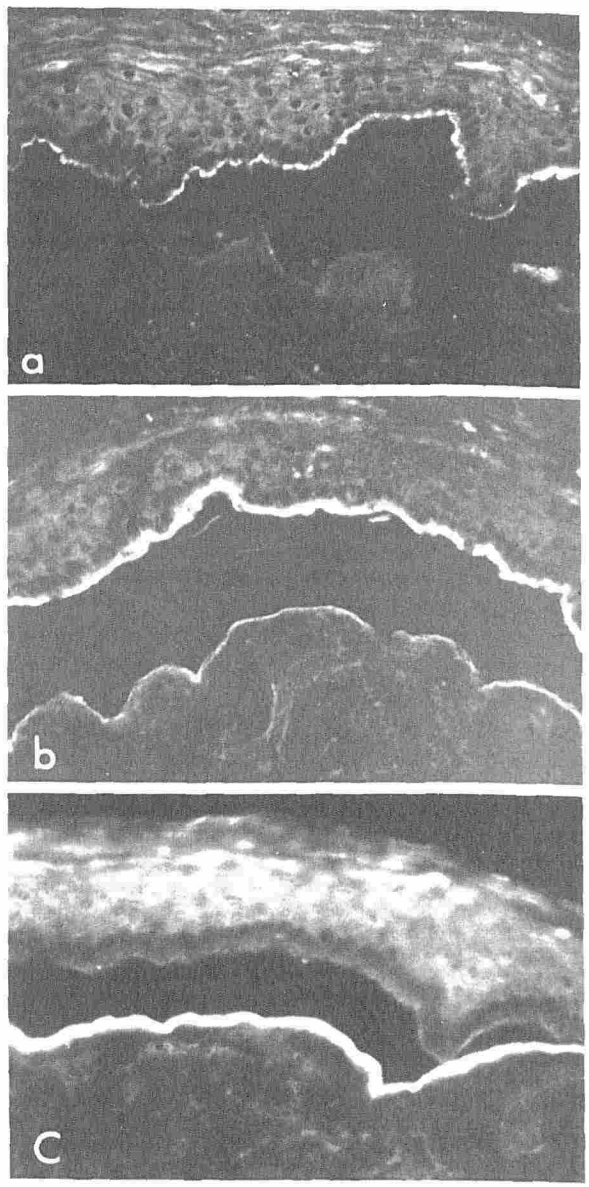

FIG 1. *a*, Epidermal pattern of immunofluorescence on 1.0 M NaCl-separated skin produced by most BP and all HG sera (×250). *b*, Combined epidermal and dermal pattern of immunofluorescence on separated skin produced by a few BP sera (×250). *c*, Dermal pattern of immunofluorescence on separated skin produced by HBA- and BP-labeled anti-sublamina densa antibodies (×400).

results of all 3 specimens were identical. Antilaminin produced a sharp uninterrupted linear band of fluorescence along the dermal edge of the separation with no staining on the epidermal side. Antibody to EBA antigen also produced a sharp, uninterrupted linear band of fluorescence along the dermal edge of the separation which was slightly brighter and wider than that seen with antilaminin but otherwise indistinguishable. Antibody to BP antigen produced a sharp generally continuous band of fluorescence along the epidermal edge of the separation. Occasionally, irregularities and small interruptions in BP staining were seen but in no case was fluorescence seen on the dermis.

Indirect IgG Immunofluorescence of Bullous Disease and NHS on Intact and Separated Skin

Each of the 61 bullous disease and 8 NHS was examined at a titer of 1:10 by indirect immunofluorescence on intact and separated skin from 1 donor. All 61 bullous disease sera produced a linear pattern of fluorescence at the BMZ on intact skin. On separated skin bullous disease sera produced a linear pattern of fluorescence either along the epidermal side (epidermal pattern), dermal side (dermal pattern), or both epidermal and dermal sides (combined pattern) of the separation (Fig 1). Of 51 BP-labeled sera, 36 (71%) showed an epidermal pattern, 9 (17%) showed a combined pattern, and 6 (12%) showed a dermal pattern. All 3 HG-labeled sera showed an epidermal pattern. Of 7 EBA-labeled sera, 1 showed an epidermal pattern and 6 showed a dermal pattern. None of the NHS showed fluorescence on intact or separated skin. These results are summarized in Table I. Of interest was the remarkably low background fluorescence observed with separated skin compared to intact skin.

To determine the reproducibility of the staining patterns, 27 sera producing the epidermal pattern (23 labeled BP, 1 labeled EBA, and 3 labeled HG) 9 sera producing the combined pattern, 12 sera producing the dermal pattern (6 labeled BP, 6 labeled EBA), and 8 NHS were examined on separated skin from each of 3 donors. On all 3 specimens, the patterns of fluorescence were consistent for each bullous disease sera and no fluorescence was observed with NHS.

To determine the sensitivity of separated skin as a substrate for detecting anti-BMZ antibodies and further analyze BP-labeled sera producing the combined pattern of fluorescence, 21 BP- and EBA-labeled sera were titered in doubling dilutions from 1:10 to 1:20,480 on intact and separated skin. These results are shown in Table II. Of 5 BP-labeled sera producing the epidermal pattern, titers on separated skin were equal to or 1 dilution greater than on intact skin. Of 4 BP-labeled sera producing the dermal pattern, titers on separated skin were consistently 1–2 dilutions greater than on intact skin. Of 4 EBA-labeled sera producing the dermal pattern, titers were 1–2 dilutions greater on separated skin. These results show separated skin is at least as sensitive a substrate as intact human skin for titering anti-BMZ antibodies. In no case did diluting sera cause a change in pattern from epidermal to dermal or vice versa.

Of 8 sera producing the combined pattern, dermal and epidermal staining antibody titers were the same in 3, higher on epidermis in 4, and higher on dermis in 1. In several cases, the titer of anti-BMZ on either the epidermal or dermal sides of the separation was equal to or greater than titers in intact skin.

TABLE I. *Results of IgG indirect immunofluorescence of bullous disease and control sera on sodium chloride-separated skin*

Serum group[a,b]	Total no. sera	Epidermal pattern	Combined pattern	Dermal pattern
Bullous pemphigoid	51	36 (71%)	9 (17%)	6 (12%)
Herpes gestationis	3	3 (14%)	0	0
Epidermolysis bullosa acquisita	7	1 (14%)	0	6 (86%)
Normal human	8	0	0	0

[a] Sera diluted 1:10 in PBS.

[b] Diagnosis based on clinical, histologic, and routine direct immunofluorescence criteria.

Indirect IgG Immunoelectron Microscopy

To correlate immunofluorescent patterns on separated skin with ultrastructural localization of antibody binding sites, selected sera were examined by indirect IgG immunoelectron microscopy on separated or intact skin. The results are shown in Table III. Two BP and 1 EBA-labeled sera producing an epidermal pattern on separated skin showed IgG deposits on the epidermal side of the separation within the lamina lucida. These sera produced no deposits on the dermis. Two BP sera

TABLE II. *IgG anti-BMZ antibody titers of bullous disease sera on intact and sodium chloride-separated skin*

Disease group[a]	Patterns on separated skin	Serum	Titers		
			Intact skin	Separated skin	
				Epidermal	Dermal
Bullous pemphigoid	Epidermal only	BEI	10,240	20,480	–
		LYN	5,128	10,240	–
		MIL	10,240	10,240	–
		WIL	10,240	20,480	–
		MAR	5,128	10,240	
Bullous pemphigoid	Combined epidermal-dermal	FAL	20	40	40
		MECI	80	320	80
		CAN	40	320	80
		FOR	160	320	320
		WAL	640	320	40
		ROL	10	40	80
		BOL	20	20	20
		WAT	40	40	20
Bullous pemphigoid	Dermal only	DKI	320	–	1280
		DEV	40	–	80
		CONL	160	–	320
		CON	40	–	80
Epidermolysis bullosa acquisita	Dermal	MAN	320	–	1280
		560	80	–	160
		815	80	–	160
		CHO	40	–	80

[a] Diagnosis based on clinical, histologic, and direct immunofluorescence criteria.

TABLE III. *Correlation of ultrastructural binding sites and binding patterns of sodium chloride-separated skin*

Group[a]	Patterns on separated skin	No. sera tested	Ultrastructural binding site
Bullous pemphigoid	Epidermal	2	Lamina lucida
	Combined epidermal-dermal	2	Lamina lucida
	Dermal	4	Subbasal lamina
Epidermolysis bullous acquisita	Epidermal	1	Lamina lucida
	Dermal	5	Subbasal lamina

[a] *Diagnosis based on clinical, histologic, and routine immunofluorescence criteria.*

with a combined pattern also showed deposits on the epidermal side of separated skin but in addition showed deposits on that portion of the lamina lucida which had remained attached to the lamina densa (Fig 2). Four BP-labeled sera and 5 EBA sera producing a dermal pattern showed an identical deposition of immunoreactant on and just beneath the lamina densa (Fig 3). No deposits were seen in the lamina lucida; however, occasionally deposits were seen beneath the basal lamina, anchoring fibril complex. These results confirmed the ultrastructural binding sites of anti-BMZ antibodies producing the epidermal, dermal, and combined staining patterns on separated skin and showed that the "EBA serum" with anti-lamina lucida antibodies and the BP-labeled sera with anti-sublamina densa antibodies were from patients who were incorrectly diagnosed.

DISCUSSION

Immunoelectron microscopy is the only method currently available for precisely determining the ultrastructural binding sites of anti-BMZ antibodies. However, it is technically difficult, time-consuming and of limited availability to practicing and academic dermatologists. Because of these limitations, we examined skin separated through the lamina lucida by 1 M NaCl as a substrate for differentiating between anti-lamina lucida and anti-sublamina densa antibodies by indirect immunofluorescence. The results showed that HG and BP anti-lamina lucida antibodies could be reliably and reproducibly differentiated from EBA anti-sublamina densa antibodies by binding patterns on separated skin.

Since anti-lamina lucida antibodies were not available from a patient with classical CP, we cannot be certain they could be differentiated by the method. However, it is likely that CP antibodies would produce a pattern like that seen with BP and HG antibodies since all three are indistinguishable by immunoelectron microscopy [7–12]. The value of the method is, of course, limited by the incidence of anti-BMZ antibodies. In CP the incidence is probably less than 20%; however, in EBA and HG it appears to be at least 50% and greater than 70% in BP [3,4,6,17–21]. In patients without serum antibodies, a double immunofluorescence method has been described for differentiating between in vivo deposited anti-lamina lucida and anti-sublamina densa antibodies [22].

One molar NaCl-separated skin appeared to be at least as sensitive as intact skin for detecting anti-BMZ antibodies. In some cases, end-point titers were 1–2 dilutions greater in separated skin. This apparent increase in sensitivity may have been due to improved exposure of antigen by the separation process and/or the low background fluorescence. The low background fluorescence is presumably due to removal of tissue immunoglobulin during the NaCl incubation. The sensitivity of separated skin also suggests there is little, if any, loss of or alteration in BP or EBA antigens during the separation process.

Since the materials and reagents are available to any immunofluorescence laboratory, the method can be used in the routine evaluation of anti-BMZ antibody binding sites. Once skin is separated, it can be stored at −70°C and in our experience is suitable as substrate for at least 3 months. Although we used electron microscopy to document the site of separation, this can be easily confirmed by immunofluorescence alone using reference bullous disease sera or commercially available antibodies to laminin. The fact that laminin is a lamina lucida constituent and separates to the dermal side of 1 M NaCl-treated skin does raise the possibility that bullous disease anti-lamina lucida antibodies which produce a dermal pattern might be encountered [13,23]. However, this was not observed in our series of 49 bullous disease sera containing anti-lamina lucida antibodies. Antilaminin autoantibodies have not been reported in bullous disease but even if they were, they should be distinguishable by their binding to vascular as well as cutaneous basement membranes [23].

Discriminating between anti-sublamina densa and anti-lamina lucida antibodies may be more important than previously

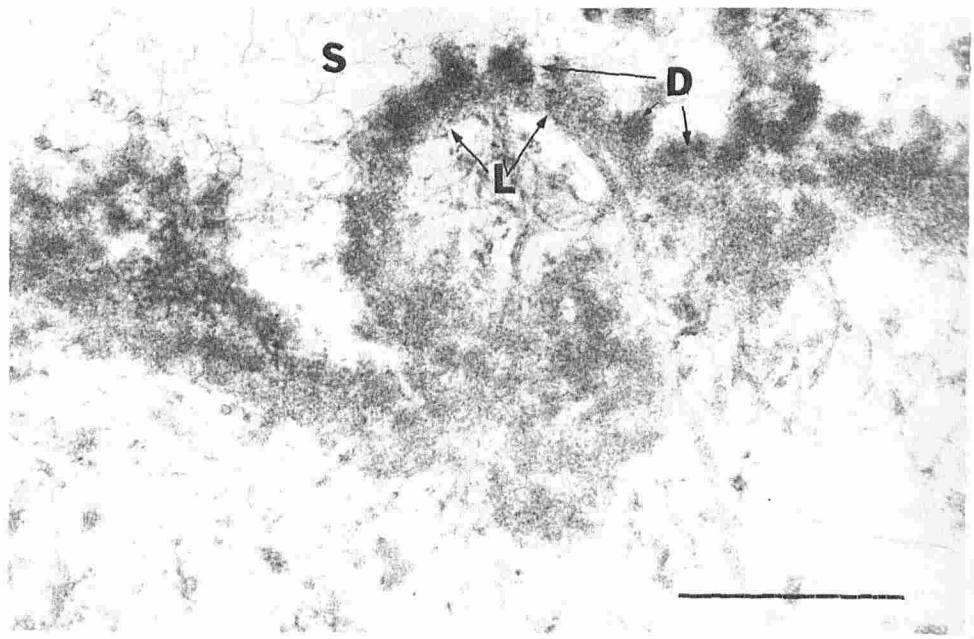

FIG 2. Immunoelectron micrograph showing the dermal side of 1.0 M NaCl-separated skin treated with combined pattern BP serum. Note immune deposits (*D*) on the separation (*S*) side of the lamina densa (*L*). *Bar* = 0.5 μm.

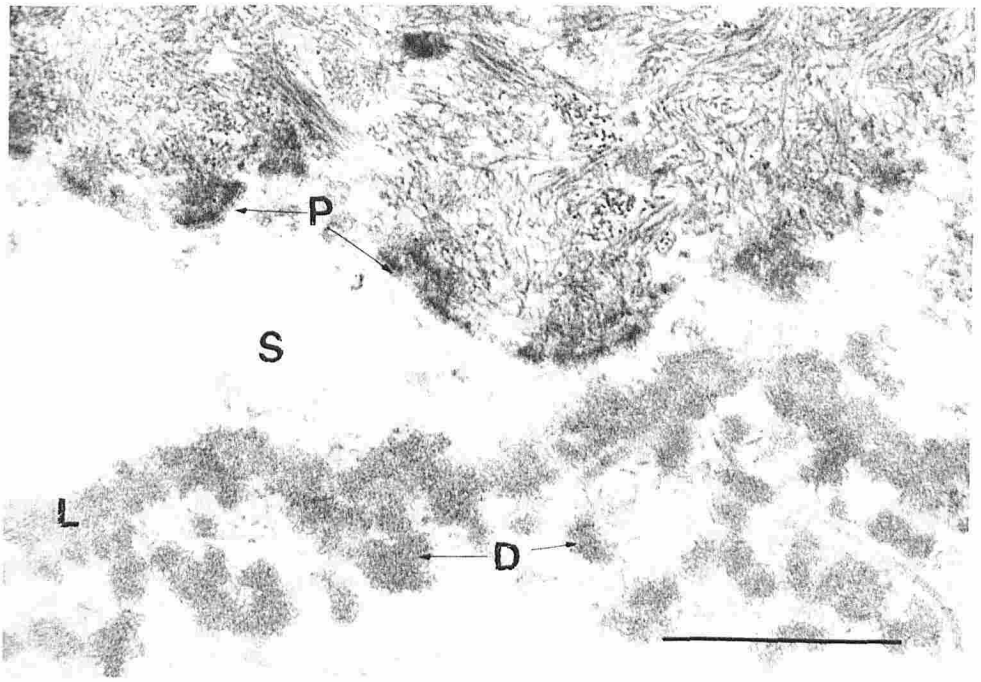

FIG 3. Immunoelectron micrographs showing the BMZ of 1.0 M NaCl-separated skin treated with anti-sublamina densa antibody. Immune deposits (*D*) are present on and just beneath the lamina densa (*L*). The site of separation (*S*) is seen between the lamina densa and the basal cell plasma membrane (*P*). *Bar* = 0.5 μm.

realized, particularly in patients with BP, CP, and EBA. In these diseases overlapping clinical, histologic, and immunohistologic features occur. For example, patients with EBA have been described with features considered characteristic of BP [24–26]. These include a generalized inflammatory bullous disease with flexural involvement, spontaneous blisters, healing of some lesions without scarring or milia, and inflammatory subepidermal blisters. Patients have been reported as BP with mechanical fragility of skin, trauma-induced blisters, and cutaneous and mucous membrane lesions that heal with scarring and milia [27–29]. Both EBA and CP are diseases which typically heal with scarring and both may have mucous membrane lesions including lesions of ocular mucous membranes [17,30–32]. It is conceivable that failure to define the ultrastructural localization of anti-BMZ antibodies in these diseases may have led to misdiagnoses and to the proposals that CP and EBA may be the same disease and that BP and CP may evolve into EBA [31,32].

The results of this study showed that misdiagnoses can be made if the ultrastructural localization of anti-BMZ antibodies is not determined. Sera from 6 patients suspected of having BP and 1 suspected of having EBA on the basis of clinical, histologic, and routine immunofluorescence criteria were shown to contain anti-sublamina densa and anti-lamina lucida antibodies, respectively. The patients initially thought to have BP will be discussed in more detail in a separate communication but, in brief, they presented either with clinical features indistinguishable from classic BP or combined features of both BP and EBA. Because these patients have immunoelectron microscopic features indistinguishable from classic EBA, we currently regard them as being a variant of that disease. In the 1 patient with clinical and histologic features of EBA and anti-lamina lucida antibodies the diagnosis is not clear but the localization of antibodies to the lamina lucida suggests a diagnosis of CP. The patient had a scarring, blistering eruption localized to trauma-susceptible sites of skin and oral mucous membranes but without ocular involvement.

In this study, 9 (20%) of 45 BP sera produced a combined pattern of immunofluorescence on separated skin from several donors. These results suggest some BP sera have anti-BMZ specificities different from or in addition to those BP sera producing only epidermal deposits on separated skin. Two of the "combined staining" sera have been examined by Dr. John Stanley, and shown to immunoprecipitate a 220,000 dalton protein identical to that immunoprecipitated by other BP sera (personal communication) [33]. This would suggest that all BP anti-lamina lucida antibodies share an identical specificity and that those producing the combined pattern may have additional specificities.

REFERENCES

1. Jordon RE, Beutner EH, Witebsky ET, Blumental G, Hale WL, Lever WF: Basement zone antibodies in bullous pemphigoid. JAMA 200:91–96, 1967
2. Jordon RE, Triftshauser CT, Schroeter AL: Direct immunofluorescent studies of pemphigus and bullous pemphigoid. Arch Dermatol 103:486–591, 1971
3. Bean SF: Cicatricial pemphigoid. Arch Dermatol 110:552–555, 1974
4. Lawley TJ, Stingl G, Katz SI: Fetal and maternal risk factors in herpes gestationis. Arch Dermatol 114:552–555, 1978
5. Nieboer C, Boorsma DM, Woerdeman MJ, Kalsbeek GL: Epidermolysis bullosa acquisita: immunofluorescence, electron microscopic and immunoelectron microscopic studies in four patients. Br J Dermatol 102:383–392, 1980
6. Yaoita H, Briggaman RA, Lawley TJ, Provost TT, Katz SI: Epidermolysis bullosa acquisita: ultrastructural and immunological studies. J Invest Dermatol 76:288–292, 1981
7. Holubar K, Wolff K, Konrad K, Beutner EH: Ultrastructural localization of immunoglobulins in bullous pemphigoid skin: employment of a new peroxidase-antiperoxidase multistep method. J Invest Dermatol 64:220–227, 1975
8. Schaumburg-Lever G, Rule A, Schmidt-Ullrich B, Lever WF: Ultrastructural localization of in vivo bound immunoglobulins in bullous pemphigoid. A preliminary report. J Invest Dermatol 64:47–49, 1975
9. Hönigsmann H, Stingl G, Holubar K, Wolff-Schreiner E, Konrad K, Wolff K: Autoantibodies and immune complexes in immunodermatoses. Mapping of fine structural binding sites (abstr). J Invest Dermatol 66:263, 1976
10. Albini B, Holubar K, Shu S, Wolff K: Enzyme antibody methods in immunodermatology, Immunopathology of the Skin, 2d ed. Edited by EH Beutner, TP Chorzelski, SF Bean. New York, John Wiley & Sons, 1979, pp 93–95
11. Hönigsmann H, Stingl G, Holubar K, Wolff K: Herpes gestationis: fine structural pattern of immunoglobulin deposits in the skin in vivo. J Invest Dermatol 66:389–392, 1976
12. Harrington CI, Bleehen SS: Herpes gestationis: immunopathological and ultrastructural studies. Br J Dermatol 100:389–399, 1979
13. Woodley D, Sauder D, Talley MJ, Silver M, Grotendorst G, Qwarnstrom E: Localization of basement membrane components after dermal-epidermal junction separation. J Invest Dermatol 81:149–153, 1983
14. Scaletta LJ, Occhino JC, MacCallum DK, Lillie JH: Isolation and

immunologic identification of basement membrane zone antigens from human skin. Lab Invest 39:1–9, 1978
15. Briggaman RA, Dalldorf FG, Wheeler CE Jr: Formation and origin of basal lamina and anchoring fibrils in adult human skin. J Cell Biol 51:384–395, 1971
16. Beutner EH, Nisengard RJ: Defined immunofluorescence in clinical immunopathology, Immunopathology of the Skin: Labeled Antibody Studies. Edited by EH Beutner, TP Chorzelski, SF Bean, RE Jordon. Stroudsburg, Penna, Dowdon, Hutchinson & Ross, 1973, pp 197–247
17. Griffith MR, Fukuyama K, Tuffanelli D, Silverman S: Immunofluorescent studies in mucous membrane pemphigoid. Arch Dermatol 109:195–199, 1974
18. Rogers RS III, Perry HO, Bean SF, Jordon RE: Immunopathology of cicatricial pemphigoid: studies of complement deposition. J Invest Dermatol 68:39–43, 1977
19. Ahmed AR, Maize JC, Provost TT: Bullous pemphigoid clinical and immunologic follow-up after successful therapy. Arch Dermatol 113:1043–1046, 1977
20. Hodge L, Marsden RA, Black MM, Bhogal B, Corbett MF: Bullous pemphigoid: the frequency of mucosal involvement and concurrent malignancy related to indirect immunofluorescence findings. Br J Dermatol 105:65–69, 1981
21. Dabelsteen E, Ullman S, Thomsen K, Rygaard J: Demonstration of basement membrane autoantibodies in patients with benign mucous membrane pemphigoid. Acta Derm Venereol (Stockh) 54:189–192, 1974
22. Gammon WR, Robinson T, Briggaman RA, Wheeler CE: Double immunofluorescence microscopy: a method for localizing immune deposits in skin diseases associated with lamina basement membrane zone immunofluorescence. J Invest Dermatol 79:312–317, 1982
23. Foidart JM, Bere EW Jr, Yaar M, Rennard SI, Gullino M, Martin GR, Katz SI: Distribution and immunoelectron microscopic localization of laminin, a noncollagenous basement membrane glycoprotein. Lab Invest 42:336–342, 1980
24. Gammon WR, Briggaman RA, Wheeler CE Jr: Epidermolysis bullosa acquisita presenting as an inflammatory bullous disease. J Am Acad Dermatol 7:382–387, 1982
25. Provost TT, Maize JC, Ahmed AR, Strauss JS, Dobson RL: Unusual subepidermal bullous diseases with immunologic features of bullous pemphigoid. Arch Dermatol 115:156–160, 1979
26. Caputo R, Berti E, Monti M: Pemphigoide bulleuse: a type d'epidermolyse bulleure acquise. Les Gournees de Dermatologiques a Paris 114:1127, 1982
27. Person JR, Rogers RS III: Bullous pemphigoid responding to sulfapyridine and the sulfone. Arch Dermatol 113:610–615, 1977
28. Chorzelski TP, Jablonska J, Beutner EH: Pemphigoid, Immunopathology of the Skin: Labelled Antibody Studies. Edited by EH Beutner, TP Chorzelski, SF Bean, RE Jordon. Stroudsburg, Penna, Dowdon, Hutchinson & Ross. 1973, pp 243–255
29. Dahl MGC, Cook LJ: Lesions induced by trauma in pemphigoid. Br J Dermatol 101:469–473, 1979
30. Dantzig P: Circulating antibodies in cicatricial pemphigoid. Arch Dermatol 108:264–266, 1973
31. Palestine RF, Kossard S, Dicken CH: Epidermolysis bullosa acquisita: a heterogeneous disease. J Am Acad Dermatol 5:43–53, 1981
32. Dahl MGC: Epidermolysis bullosa acquisita—a sign of cicatricial pemphigoid: Br J Dermatol 101:475–484, 1979
33. Stanley JR, Hawley-Nelson P, Yuspa SH, Shevach EM, Katz SI: Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia cell. Cell 24:897–903, 1981