THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 67:683–687, 1976

Vol. 67, No. 6
Printed in U.S.A.

REPORTS

IMMUNOFLUORESCENT LOCALIZATION OF BASEMENT MEMBRANE IN LESIONS OF DERMATITIS HERPETIFORMIS

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Dermatitis herpetiformis (DH) is a blistering disease with a characteristic histology that includes papillary edema, neutrophilic papillary microabscesses, and development of subepidermal blisters. In spite of this pathologic sequence occurring entirely beneath the basement membrane zone, prior studies have indicated that the basement membrane, as defined by periodic acid–Schiff (PAS) or silver stains, lies at the floor of fully formed blisters or is destroyed by the disease process. To more accurately assess its location in primary lesions of DH, the basement membrane was stained using immunofluorescent techniques.

Lesional skin from 5 patients with DH was used as substrate for indirect immunofluorescence with sera from patients with bullous pemphigoid (BP) and fluoresceinated antihuman IgG. The BP-stained basement membrane was attached to the roofs of early blisters, where it would be expected from the pathologic sequence of blister formation. PAS stains of the same or serial sections show the basement membrane to be in the roof or at the floor of the blisters. PAS stains of sections from formalin-fixed lesional skin, on the other hand, show the basement membrane to routinely lie at the blister floor, when not destroyed.

The BP-stained epidermal basement membrane has greater anatomic and functional significance than either the PAS- or silver-stained basement membrane for two reasons: (1) it corresponds to a specific morphologic structure, the lamina lucida, a part of the epidermis, and remains attached to the rest of the epidermis unless destroyed; and (2) it is antigenic, capable of binding with BP antibodies.

Dermatitis herpetiformis (DH) is a chronic, pruritic, blistering disease of uncertain etiology. Its diagnosis and differentiation from other blistering diseases depend on four criteria: clinical, histologic, therapeutic, and immunologic [1–3]. Clinically, it presents as a symmetric, pleomorphic eruption of urticarial plaques, papules, and grouped vesicles preferentially affecting extensor surfaces (elbows, knees, shoulders, sacrum, buttocks), face, and scalp; a prompt response to sulfone or sulfapyridine therapy is characteristic. Histologically, a typical vesicular or urticarial lesion shows subepidermal blisters with an early infiltrate of polymorphonuclear leukocytes and a later admixture of eosinophils; adjacent neutrophilic, papillary microabscesses are common. Immunologically, the vast majority of patients fulfilling all other criteria for DH have in vivo deposition of IgA just beneath the basement membrane zone of normal and perilesional skin; 4% of patients (2/50) in one study required more than one biopsy to demonstrate this finding [4].

From microscopic observations of lesions in different stages of development, one would expect the pathogenetic sequence of blister formation to be (1) nonspecific, mixed-cell, perivascular infiltrate and papillary edema, (2) neutrophilic, papillary microabscess, (3) small, subepidermal blister with a neutrophilic infiltrate, and (4) large, subepidermal blister with an infiltrate of both neutrophils and eosinophils [3,5]. Since the pathology in this proposed schema takes place in the area of the dermal papilla, beneath the basement membrane zone, one might expect the light microscopic basement membrane to be in the roof of the developed blister. Prior studies, however, have reported the microscopic basement membrane as determined by periodic acid–Schiff (PAS) or silver stain to be either attached to the dermal floor of the blister or

Manuscript received March 31, 1976; accepted for publication July 19, 1976.
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Abbreviations:
BP: bullous pemphigoid
DH: dermatitis herpetiformis
IF: immunofluorescence
PAS: periodic acid–Schiff
PBS: phosphate-buffered saline

destroyed [5,6]. The present study was undertaken to more accurately define the location of the basement membrane during blister formation in DH. This was done through the use of bullous pemphigoid (BP) fluorescent antibody localization of the basement membrane [7] in addition to PAS staining of formalin-fixed and frozen-sectioned lesional skin.

MATERIALS AND METHODS

Patients and biopsies. The study included 5 patients with DH (4 females, 1 male), ranging in age from 15 to 55 years; all satisfactorily met the clinical, histologic, therapeutic, and immunologic criteria described above. Three had punch biopsies of lesional skin sent for routine histology and PAS staining; two had had routine histology in the past and did not have a repeat biopsy sent for fixation and staining. All five had punch biopsies of fresh lesions (less than 24 hr old) which were embedded in O.C.T. embedding compound (Lab-Tek Prod., Napersville, Ill.) and frozen in liquid nitrogen within 20 min. These were then frozen-sectioned at 4 μm and used for indirect immunofluorescence with BP serum; the same sections and/or consecutive sections were stained with PAS. To determine whether prior use of a section for immunofluorescence (IF) affected its PAS staining properties, frozen sections of normal skin from 3 patients with DH and 4 normal controls which had been used as substrate for indirect IF with BP serum prior to being stained with PAS were compared with serial sections that had been stained with PAS directly. Biopsies of normal skin similarly processed were used in direct immunofluorescence for IgA localization.

Immunofluorescence. Direct and indirect IF were done according to previously described techniques [8]. For direct IF, the frozen sections were washed for 5 min in phosphate-buffered saline (PBS), pH 7.4, incubated for 30 min in a moist chamber with fluorescein isothiocyanate-conjugated goat antihuman IgA (Hyland, Costa Mesa, Calif.), washed 3 times for 5 min each in PBS, covered with 50% glycerine in PBS and a coverslip, and viewed under a Leitz Ortholux II, epi-illuminated fluorescent microscope.

For indirect IF, the frozen sections were washed in PBS, incubated in a moist chamber for 30 min with a 1:10 dilution of serum from a patient with BP, washed 5 times for 5 min each in PBS, reincubated in a moist chamber for 30 min with fluorescein isothiocyanate-conjugated goat antihuman IgG (Hyland), washed, and viewed as for direct IF. The F/P molar ratios and specific antibody concentrations for use were 3.3 and 55 μg/ml for IgA, and 3.5 and 25 μg/ml for IgG. Purity and specificity of both conjugates were checked by double diffusion in agar and immunoelectrophoresis. Throughout the study, serum from 1 patient with BP was used to localize the antigenic basement membrane; on lesional skin from 1 patient, however, sera from 2 additional patients with BP were used to verify the constancy of the BP staining. To determine whether antigenicity of the basement membrane was decreased by the pathologic process early in blister formation, BP serum was titered out on serial sections of lesional skin from 2 patients, and the final titer in the area of the blister compared to that of adjacent, nonblistered skin. Normal human sera, a different one for each patient studied, were run as negative controls.

RESULTS

Direct IF. Direct IF on biopsies of all 5 patients showed in vivo deposition of IgA in a speckled pattern just beneath the basement membrane zone.

Indirect IF. Sections of biopsies (fresh lesions from all patients) that were incubated with BP serum and then with fluoresceinated anti-IgG showed positive staining in a bandlike distribution along the basement membrane zone (Fig. 1*a*). In those areas where a small subepidermal blister was seen, moreover, the fluorescent band continued along its roof. With larger blisters, on the other hand, the band was disrupted. In no instance did it localize to the dermal floor of a blister. Identical fluorescent staining was seen with all 3 BP sera in the 1 case so treated (Fig. 2*a*). The titer of the BP serum on the blister roof proved to be the same as that on adjacent nonblistered skin (160). Normal human serum controls run on serial sections of all biopsies showed no fluorescence along the basement membrane zones or in the blisters (Fig. 2*b*).

PAS staining. The light microscopic basement membrane as revealed by PAS staining of formalin-fixed fresh lesions was along the dermal floor of the blister in 2 patients and destroyed in 1 patient. On frozen sections, it was more variable, being located on the dermal side in biopsies from 3 patients (Fig. 2*c*) and on the epidermal side in biopsies from 2 patients (Fig. 1*b*); in some areas of several sections it was destroyed. Prior use of the frozen sections for IF had little effect on their PAS staining characteristics, a slightly diminished intensity being the sole noticeable difference in some of the slides when compared with their serial sections stained only with PAS. The results of the PAS staining of frozen sections of lesional skin, moreover, point out the absence of interference by prior IF. The 3 patients with PAS-positive basement membranes at the blister floor are in accord with the findings on formalin-fixed tissue. On the other hand, the PAS-positive basement membrane was seen in the blister roof of 1 frozen section not subjected to IF before PAS staining, thereby obviating the question of interference with PAS staining by prior utilization of the IF technique.

DISCUSSION

That area immediately beneath the basal layer of the epidermis, loosely referred to as the basement membrane zone, is seen by electron microscopy to be divided into four separate structures: (1) basal cell plasma membrane, (2) lamina lucida, (3) basal lamina, and (4) subbasal lamina fibrous elements (anchoring fibrils, dermal microfibrillar bundles, and collagen fibers), which blend into the rest of the papillary dermis [9]. The first three of these structures are produced by the epidermis, the fourth by the dermis [10]. Recent immunoelectron-microscopic investigations have shown that the BP antibasement membrane antibody, identified by

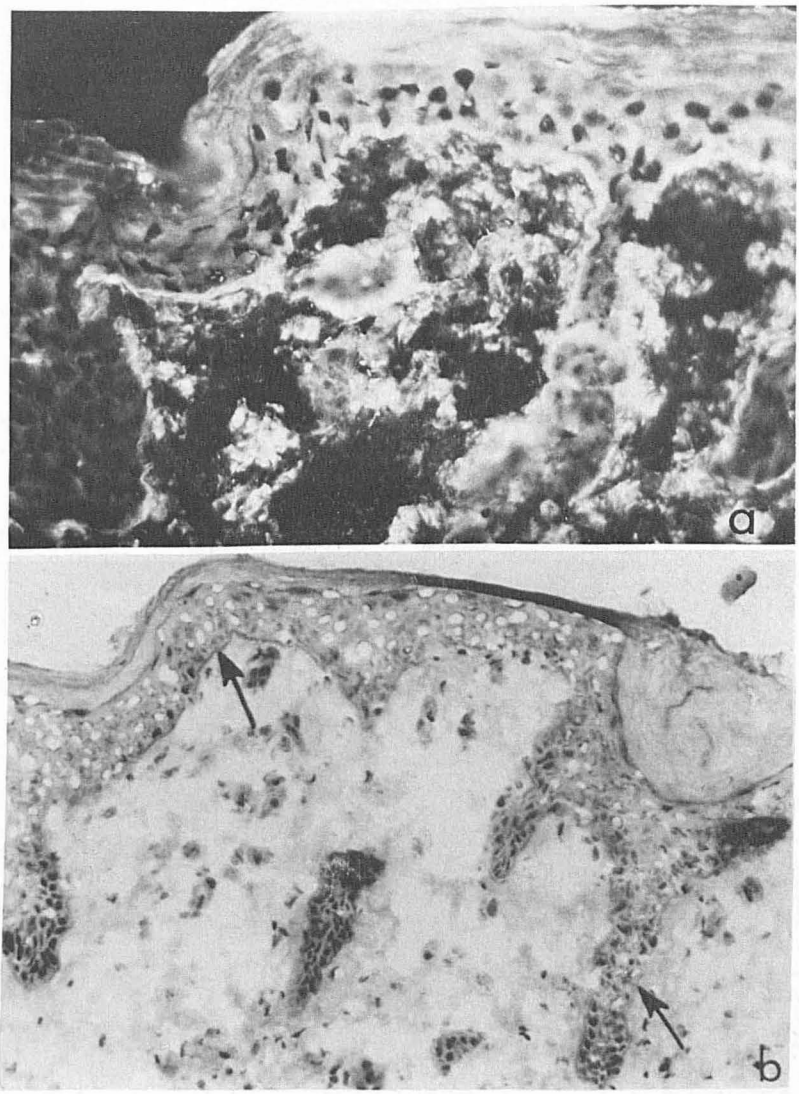
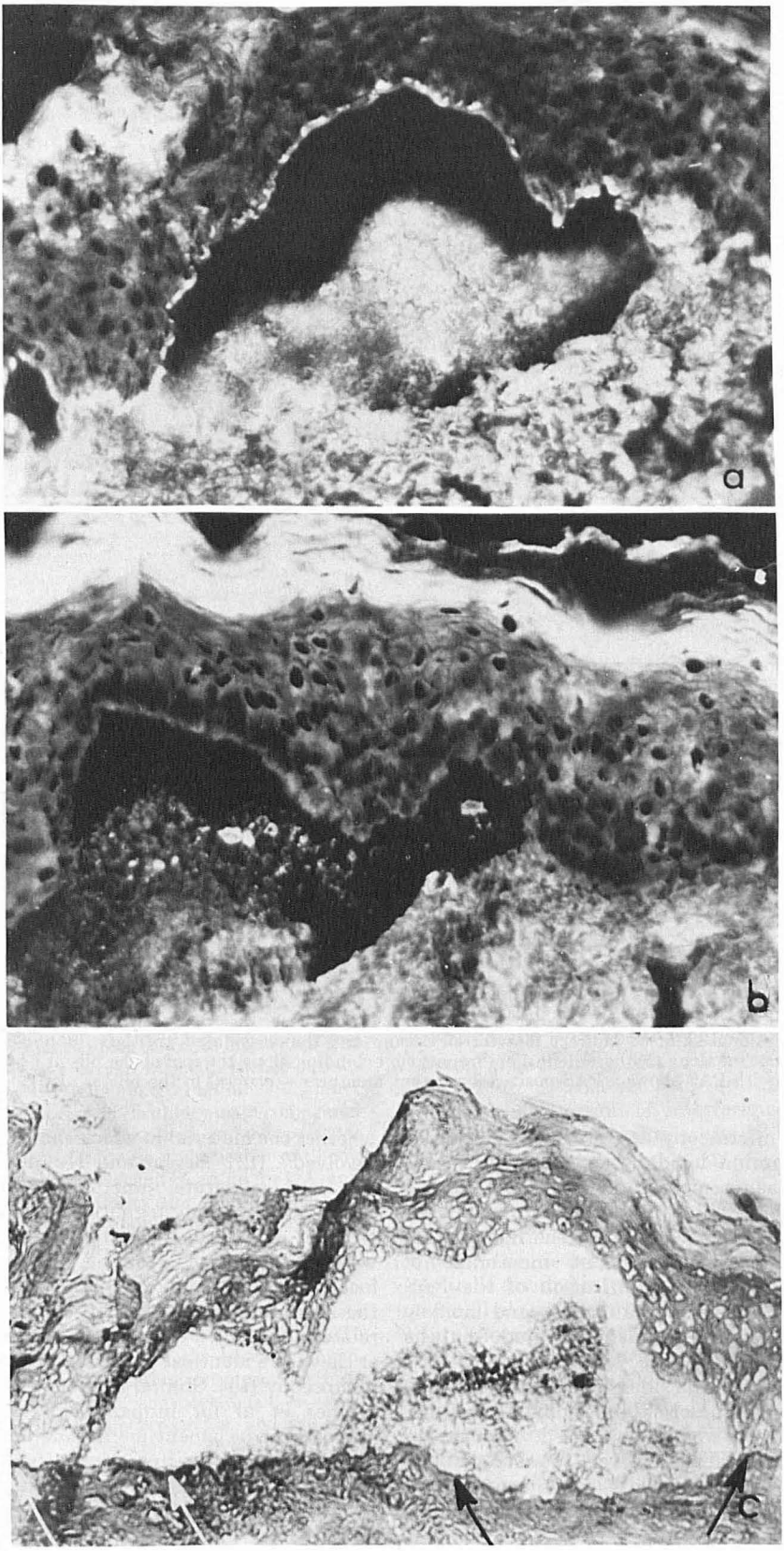


FIG. 1. DH lesional skin. *a*: Indirect IF with BP serum and fluoresceinated anti-IgG fluorescent staining in a bandlike distribution along the basement membrane zone extending along the roof of the blister (× 170). *b*: A serial section stained with PAS shows a PAS-positive basement membrane (*arrows*) in the blister roof (× 140).

fluorescence microscopy as an IgG, is deposited within the lamina lucida, the area between the basal cell plasma membrane and basal lamina [11]. Numerous studies have been done to identify the ultrastructural equivalent of the light microscopic, PAS-positive basement membrane of human skin. Using a modification of the PAS staining technique on human scalp (and monkey gingiva), Swift and Saxton demonstrated "that the PAS 'basement membrane' of optical microscopy is located on the dermal side of the electron-opaque layer of the dermoepidermal junction in that region which is almost wholly composed of reticulin fibers" [12]. They were, however, "unable to determine whether the periodate labile material is associated with individual reticulin fibers or whether the material in which these fibers are set is involved" [12]. Berger and Hundeiker examined the dermoepidermal junction of normal human skin with a silver impregnation method suitable for electron microscopy. With this technique they were able to show staining of fine microfibrils located in the superficial dermis and attached to the basal lamina; they felt that these fibrils were related to collagen but could not determine whether they were identical to the reticulin fibers of light microscopy [13]. Similar findings were reported by Younes et al for human ectocervix, the light microscopic basement membrane being composed of collagenase-sensitive connective tissue fibers located in the 0.5-μm zone immediately beneath the basal lamina [14].


a
b
c

In the early lesional skin of all 5 patients with DH so studied, the BP-stained basement membrane, presumably lamina lucida, was attached to the epidermal roof of the blisters throughout their evolution from papillary microabscesses to small subepidermal blisters. It was not significantly altered by the pathologic process, as shown by the maintenance of its antigenicity when compared to adjacent intact skin on serial dilutions. In large blisters this BP-stained basement membrane was destroyed, but in no case was it present along the dermal floor of the blister. This is in keeping with the expected pathogenetic sequence of blister formation that begins in the dermal papillae as well as with the electron microscopic disappearance of basal lamina in early lesions [15]. The variable location of the PAS-stained basement membrane in frozen sections of early blisters and its more constant location at the base in sections of formalin-fixed tissue remain to be explained. Were it consistently in the roof, it would indicate a purely dermal process, and in the base, a true dermoepidermal separation. As it is, it might suggest that regardless of where the process begins in relation to the structures of the basement membrane zone, dyshesion occurs at the weakest point, which may vary from patient to patient. An intriguing alternative, however, is that the PAS-positive material at the floor of some DH blisters is not basement membrane at all, but some other substance, such as fibrin. Indeed, direct IF of one section with fluoresceinated antifibrin and subsequent staining of the same section with PAS showed indistinguishable fluorescent and PAS bands along the floor of a blister. In any event, localization of the basement membrane by indirect IF with BP serum would appear to have greater anatomic and functional significance than localization by PAS or silver staining for two reasons: (1) it stains the lamina lucida, which is produced by the epidermal cells and remains attached to them unless destroyed, and (2) it is functionally significant in that it represents the in vitro correlate of an autorecognition phenomenon.

We wish to thank Ms. M. C. Bowling, Ms. S. Wescott, and Ms. M. Duvall for their assistance with the PAS staining, and Mr. H. Schaefer for his help with the photography.

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FIG. 2. DH lesional skin. *a:* Indirect IF with BP serum and fluoresceinated anti-IgG fluorescent staining in a bandlike distribution along the basement membrane zone extending along the blister roof (× 170). *b*: A serial section treated with normal human serum in place of BP serum is negative (× 170). *c*: One of the sections previously used for IF and subsequently stained with PAS shows a PAS-positive basement membrane (*arrows*) in the floor of the blister (× 150).