type 2b. Ann Intern Med 94:362–363, 1981
6. Winkelmann RK: Nerve Endings in Normal and Pathologic Skin: Contributions to the Anatomy of Sensation. Springfield, Illinois, Charles C Thomas, 1960
7. Winkelmann RK, Schmit RW: A simple silver method for nerve axoplasm. Proc Staff Meet Mayo Clin 32:217–222, 1957
8. Winkelmann RK: New methods for the study of nerve endings. Monogr Pathol, No. 10, 1971, pp 112–127
9. Winkelmann RK: Innervation of the skin: notes on a comparison of primate and marsupial nerve endings, Biology of the Skin and Hair Growth. Edited by AG Lyne, BF Short, New York, Elsevier, 1965, pp 171–182
10. Thies W, Cervós-Navarro J: Multiple neurome der haut und schleimhaut, Struktur und Pathologie des Hautnervensystems. Edited by G Lassmann, W Jurecka, G Niebauer. Vienna, Austrian Scientific Society Press, 1977, pp 1–5
11. Winkelmann RK: Quantitative methods in neurohistology. Excerpta Medica International Congress Series No. 289, 1972, pp 937–940
12. Schnitzler L, Simard C, Baudoux C, Lefranc M: Neuromes cutanés et muqueux: avec étude histopathologique et ultrastructurale. Ann Dermatol Syphiligr (Paris) 100:241–260, 1973
13. Breathnach AS: An Atlas of the Ultrastructure of Human Skin: Development, Differentiation, and Post-Natal Features. London, J & A Churchill, 1971
14. Dyck PJ: Inherited neuronal degeneration and atrophy affecting peripheral motor, sensory, and autonomic neurons, Peripheral Neuropathy, vol 2. Edited by PJ Dyck, PK Thomas, EH Lambert. Philadelphia, WB Saunders, 1975, pp 825–867
15. Altmeyer P, Merkel KH: Multiple systematisierte Neurome der Haut und der Schleimhaut. Hautarzt 32:240–244, 1981
16. Jurecka W, Gebhart W, Lassmann H: Ubergang der Nervenfaszikel in diffuse cutane Neurofibrome, Struktur und Pathologie des Hautnervensystems. Edited by G Lassmann, W Jurecka, G Niebauer. Vienna, Austrian Scientific Society Press, 1977, pp 1–4
17. Klingmollür G, Gopel K: Neurofibromatosis von Recklinghausen mit Luse-Korpern, Struktur und Pathologie des Hautnervensystems. Edited by G Lassmann, W Jurecka, G Niebauer. Vienna, Austrian Scientific Society Press, 1977, pp 1–7

0022-202X/82/7905-0312$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 79:312–317, 1982

Vol. 79, No. 5
Printed in U.S.A.

Double Immunofluorescence Microscopy: A Method for Localizing Immune Deposits in Skin Diseases Associated with Linear Basement Membrane Zone Immunofluorescence

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Direct immunofluorescence microscopy has shown that a linear pattern of immunoglobulin and/or complement deposition at the cutaneous basement membrane zone is a characteristic feature in a number of acquired bullous diseases and is occasionally observed in systemic lupus erythematosus. Immunoelectron microscopy has shown the linear pattern of immunofluorescence may be produced by immune deposits located either above the basal lamina (in the lamina lucida) or below the basal lamina (in the upper dermis). Distinguishing between these sites of immune reactant deposition may be of value in differential diagnosis. In this study we report a double immunofluorescent method by which skin biopsies with linear IgG immunofluorescence due to deposits above the basal lamina (bullous pemphigoid) could be distinguished from biopsies with deposits beneath the basal lamina (bullous systemic lupus erythematosus and epidermolysis bullosa acquisita). When skin sections were treated sequentially with rhodamine-labeled anti-human IgG followed by fluorescein-labeled antilamina lucida (pemphigoid) antibody and examined by fluorescence microscopy, the following results were obtained. In biopsies with IgG deposits in the lamina lucida, a single green fluorescent band was observed. In tissues with subbasal lamina deposits, either parallel and contiguous bands of green and yellow-orange fluorescence or a single band of yellow-orange fluorescence was observed. The method is simpler, quicker, and less expensive than immunoelectron microscopy and should be a useful technique for evaluating skin diseases with linear immunofluorescence at the basement membrane zone.

Manuscript received November 30, 1981; accepted for publication March 25, 1982.

Dr. Briggaman is the recipient of a National Institutes of Health Grant, entitled "Utilization of Epidermal Cell Model from Human Skin." Dr. Gammon is the recipient of Dermatology Foundation Grant 5 R01 AM10546-16, entitled "Studies on an in Vitro Model of Bullous Pemphigoid."

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Abbreviations:
BMZ: basement membrane zone
BP: bullous pemphigoid
CP: cicatricial pemphigoid
EBA: epidermolysis bullosa acquisita
FITC: fluorescent isothiocyanate-conjugated
HG: herpes gestationis
NHS: normal human serum
PBS: phosphate-buffered saline
RITC: rhodamine isothiocyanate-conjugated
SLE: systemic lupus erythematosus

Standard direct immunofluorescence microscopy using fluorochrome-labeled antibodies to human immunoglobulins (Ig) and complement (C) components has been of value in the differential diagnosis of skin diseases, particularly the acquired bullous diseases [1]. However, the value of the technique has been limited due to difficulties in distinguishing between diseases associated with similar clinical and histologic features and an identical location, pattern, and composition of immune reactant deposition in skin.

Limitations in differential diagnosis by direct immunofluorescence are commonly encountered in diseases associated with a linear pattern of IgG and/or C3 deposition at the cutaneous basement membrane zone (BMZ). This pattern is characteristic of bullous pemphigoid (BP), cicatricial pemphigoid (CP), and

herpes gestationis (HG) but is also observed in most patients with epidermolysis bullosa acquisita (EBA) [2–6]. Although uncommon, the homogeneous band of fluorescence seen in systemic lupus erythematosus (SLE) may appear linear [7–10].

In recent years immunoelectron microscopic studies have shown that the immune reactants in BP, CP, and HG are deposited above the basal lamina in the lamina lucida [11–14]. Similar studies have shown the deposits in SLE and EBA are in the upper dermis just beneath the basal lamina [4–6,15,16]. At the present time only immunoelectron microscopy can reliably determine the precise location of immune reactants in diseases with a linear pattern of immunofluorescence at the BMZ.

Since ultrastructural studies are technically more difficult, require more expensive equipment, are more time consuming, and less available than immunofluorescence, we have developed an immunofluorescence method that allows a relatively rapid and simple means of discriminating among diseases with linear IgG BMZ immunofluorescence due to suprabasal and subbasal immune reactants. In the method, tissue containing linear BMZ deposits of IgG in the lamina lucida (BP) or beneath the basal lamina (EBA, bullous SLE) were treated sequentially with rhodamine isothiocyanate (RITC)-conjugated antihuman IgG and fluorescein isothiocyanate (FITC)-conjugated antibody to the lamina lucida (BP antibody). Tissues were then examined by fluorescence microscopy using fluorescein and fluorescein-rhodamine barrier filters. In tissues with IgG deposits in the lamina lucida a single green fluorescent band was detected. In tissues with subbasal lamina deposits, either parallel linear bands of green and yellow-orange fluorescence or a single band of yellow-orange fluorescence was observed.

MATERIALS AND METHODS

Reagents

Horseradish peroxidase, fluorescein isothiocyanate, and 3,3′-diaminobenzidine tetrahydrochloride were purchased from Sigma Chemical Co., St Louis, Missouri. Osmium tetroxide was purchased from Polysciences, Warrington, Pennsylvania. The following monospecific antisera were purchased from Cappel Labs, Cochranville Pennsylvania: RITC-conjugated goat antihuman IgG; FITC-conjugated goat antisera to human IgG, IgA, IgM, and C3; FITC-conjugated rabbit antigoat IgG and goat antihorseradish peroxidase. Buffers included: 0.15 M NaCl buffered with 0.01 M Na_2HPO_4 and 0.01 M NaH_2PO_4, pH 7.2 (PBS); 0.05 M Tris buffer, pH 7.6, and 0.1 M PO_4 buffer, pH 7.4. Horseradish peroxidase solution was prepared by dissolving 1.0 mg horseradish peroxidase in 20.0 ml PBS. Graham-Karnowsky solution was prepared by dissolving 5.0 mg 3,3′-diaminobenzidine tetrahydrochloride in 25.0 ml Tris buffer and adding 1 drop of H_2O_2 [17]. One percent osmium tetroxide was prepared in 0.1 M PO_4 buffer.

Sera

Normal human serum (NHS) was obtained from a single donor, heat-inactivated at 56°C for 30 min and stored in 1.0 ml aliquots at −20°C. Serum from a patient with BP with an IgG anti-BMZ antibody titer of 1:320 was used as a source of antibody to the lamina lucida (anti-lamina lucida antibody).

Skin

Three- to four-mm punch biopsies of perilesional skin were obtained from each of 4 patients with BP, 4 patients with EBA, and 2 patients with bullous SLE. All patients were diagnosed by established clinical, histologic, immunohistologic, and serologic criteria [6,18,19]. Fresh-frozen human foreskin was obtained following routine circumcision of healthy neonates and quick-frozen in liquid N_2. Two 3-mm biopsies of normal human adult skin were obtained from volunteers and placed in "transport media" for 7 days at 25°C and then quick-frozen in liquid N_2 [20]. Following quick-freezing, all tissues were mounted in OCT compound (Ames Co, Elkhart, Indiana) and stored at −70°C.

FITC-Conjugated Anti-Lamina Lucida Antibody

A crude Ig fraction of BP serum was prepared as follows: 10.0 ml serum was diluted with an equal volume of cold distilled H_2O and slowly mixed with 20.0 ml of a saturated solution of ammonium sulfate (SAS) at 4°C. The mixture was stored overnight at 4°C and the precipitate recovered, washed twice with 25% SAS, redissolved in 0.15 M NaCl and dialyzed 24 hr against 0.85 M NaCl at 4°C. The protein concentration (OD280) of the dialysate was adjusted to 10.0 mg/ml, and 10.0 ml was mixed with an equal volume of 0.5% FITC dissolved in 0.75 M NaH_2CO_3–0.15 M NaCl, pH 9.0. The mixture was stored at 25°C for 4 hr and dialyzed against 0.85 M NaCl for 24 hr at 4°C. A portion of the FITC-conjugated Ig fraction was absorbed with 25% (vol:vol) guinea pig liver powder for 1 hr at 25°C. Final protein concentration and molar F/P ratio of the undiluted material was 5.0 mg/ml and 0.33, respectively [21]. This material diluted 1:4–1:20 in PBS produced a fine linear band of green fluorescence at the BMZ when incubated with cryostat sections of normal human or transport-media-treated skin.

Immunofluorescent Technique

Standard direct immunofluorescence was performed on all skin biopsies according to the method of Beutner and Nisengard using monospecific FITC-conjugated goat antisera against human IgG, IgA, IgM, and C3 and RITC-conjugated goat antihuman IgG [22]. All FITC-conjugated antisera were used at a dilution of 1:10 in PBS. Specific antibody or protein concentrations and molar fluorescein/protein ratios on undiluted FITC-conjugated antisera were: 6.1 mg/ml and 3.2 for anti-IgG; 3.0 mg/ml and 3.0 for anti-IgA, 1.9 mg/ml and 3.3 for anti-IgM, and 30.0 mg/ml and 2.0 for anti-C3. RITC-conjugated antihuman IgG was used at a dilution of 1:20 in PBS. Protein and rhodomine/protein ratio on the undiluted material was 15 mg/ml and 3.0, respectively. Tissues were examined for fluorescence at 300× and 500× magnification.

Double immunofluorescence was performed as follows: 6-μm thick cryostat sections of skin were cut and air dried on glass slides. Sections were then incubated for 30 min in a humidity chamber at 25°C with a 1:20 dilution of RITC-conjugated goat antihuman IgG. Sections then received a standard 15-min rinse in 3 changes of PBS, excess moisture was removed, and sections incubated 15 min at 25°C with 50% heat-inactivated (56°C × 30 min) NHS diluted in PBS. Following a second 15-min rinse, sections were incubated with 50 μl of a 1:4 dilution of FITC-conjugated anti-lamina lucida antibody, rinsed for 15 min, and coverslipped. Immunofluorescence was interpreted independently by 2 investigators without knowledge of the disease being examined. All immunofluorescence was read at 300× and 500× using a Leitz Orthoplan fluorescence microscope equipped with epi-illumination and appropriate filters. Leitz filter systems used included: (1) for fluorescein fluorescence, excitor filter BP 450–490, barrier filter LP 515, and beam splitter P KP 510; (2) for rhodomine, exicter filter BP 530–560, barrier filter LP 580, and beam splitter P KP 580; (3) for combination rhodamine and fluorescein fluorescence, excitor filter BP 450–500, barrier filter LP 515–560, and beam splitter P KP 510.

RESULTS

Standard Direct Immunofluorescence of Biopsies from Patients with BP, EBA, and Bullous SLE

The results of direct immunofluorescence on 10 patients with EBA, BP, and bullous SLE are shown in Table I. There was a linear pattern of IgG staining in all biopsies. C3 was present in all 4 biopsies from patients with BP, the 2 patients with bullous SLE, and in 2/4 biopsies of EBA. In addition, trace deposits of IgA and IgM were present in several biopsies from patients with SLE and BP. By direct immunofluorescence all biopsies showed a homogeneous linear band of IgG at the BMZ. By standard direct immunofluorescence alone, it was not possible to reliably discriminate among biopsies from patients with BP, EBA, and bullous SLE.

Immunoelectron Microscopy

All biopsies from patients with SLE, EBA, and BP were examined by immunoelectron microscopy using a previously described method [6]. Results are shown in Table I and Figs 1–3. Immune deposits were observed in the lamina lucida in all 4 biopsies from patients with BP. Deposits were present in the upper dermis just beneath the basal lamina in the 6 biopsies from patients with SLE and EBA. In these 6 tissues, peroxidase-labeled deposits formed a relatively homogeneous band that was approximately 2–3 times the width of the deposits seen in BP (Fig 1). In the 2 biopsies from SLE skin there were, in addition to sub-basal lamina deposits, less conspicuous globular

TABLE I. *Results of standard direct immunofluorescence, immunoelectron microscopy, and double immunofluorescence in biopsies of SLE, EBA, and BP skin*

BX #	Disease	Standard direct immunofluorescence		Immunoelectron microscopy	Double immunofluorescence
		Pattern	Composition	Location of immune reactant	
1	BP	Linear BMZ	IgG, C3	Lamina lucida	Linear green band
2	BP	Linear BMZ	IgG, C3, IgA	Lamina lucida	Linear green band
3	BP	Linear BMZ	IgG, C3, IgA	Lamina lucida	Linear green band
4	BP	Linear BMZ	IgG, C3	Lamina lucida	Linear green band
5	EBA	Linear BMZ	IgG	Subbasal lamina	Green and yellow-orange band
6	EBA	Linear BMZ	IgG	Subbasal lamina	Yellow-orange band
7	EBA	Linear BMZ	IgG, C3	Subbasal lamina	Green and yellow-orange band
8	EBA	Linear BMZ	IgG, C3	Subbasal lamina	Green and yellow-orange band
9	SLE	Linear BMZ	IgG, C3, IgM	Subbasal lamina	Green and yellow-orange band
10	SLE	Linear BMZ	IgG, C3, IgA	Subbasal lamina	Green and yellow-orange band

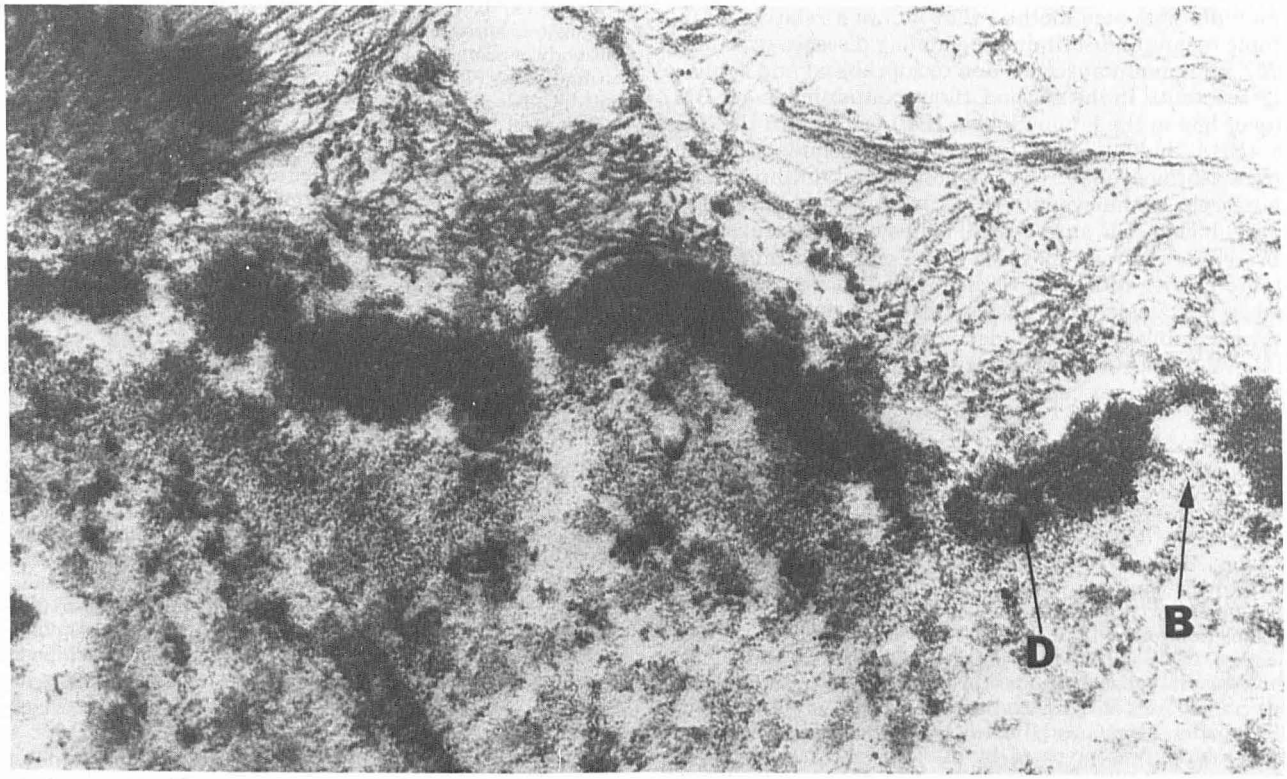

FIG 1. Immunoelectron micrograph of biopsy #1 from a patient with bullous pemphigoid. IgG immune deposits (*D*) are located in the lamina lucida above the basal lamina (*B*). An identical location of IgG deposits were observed in biopsies #1–4. Magnification 5.8×10^4.

deposits distributed randomly in the upper dermis and fine deposits in vessel walls.

Double Immunofluorescence

Both observers interpreted the results of double immunofluorescence independently and agreed on the findings which are shown in Table II. As the table indicates, there were 3 patterns of immunofluorescence in the 10 tissues and the same 3 patterns were observed with both the fluorescein and fluorescein-rhodamine filters. The patterns observed were: (1) a linear green fluorescence in 4 tissues, (2) a linear band composed of contiguous and parallel green and yellow-orange bands in 5 tissues, and (3) a single yellow-orange band in 1 tissue. Using the rhodamine filter, the 6 tisssues (patterns 2 and 3) with yellow-orange fluorescence showed only a single red band. In the 5 tissues showing parallel green and yellow-orange fluorescence, the parallel bands seemed to merge in some areas along the BMZ into a single yellow-orange band. A comparison of the source of tissue with the results of double fluorescence (Table I) showed that the green band pattern was seen only in tissue from patients with BP. The parallel green and yellow-orange band was seen in the 2 patients with SLE and 3 patients with EBA. The single yellow-orange band was seen in a patient with EBA. In all cases, fluorescence was as easily observed and interpreted with the fluorescein filter alone as with the combination fluorescein-rhodamine filter.

DISCUSSION

It is widely appreciated that direct immunofluorescence microscopy is a useful if not essential tool in the complete evaluation of skin diseases associated with cutaneous deposits of Ig and C. Its value in the differential diagnosis of acquired bullous and collagen vascular diseases has been particularly well established and the increasing availability of the test has added considerably to its value in the work-up and management of these illnesses. Direct immunofluorescence is now available in virtually all medical centers, many community hospitals, and, with the development of transport media, has become accessible to all physicians.

In spite of its value, standard direct immunofluorescence does have limitations as a diagnostic procedure. One of its major limitations is the inability to reliably distinguish among diseases associated with a similar pattern and composition of immune

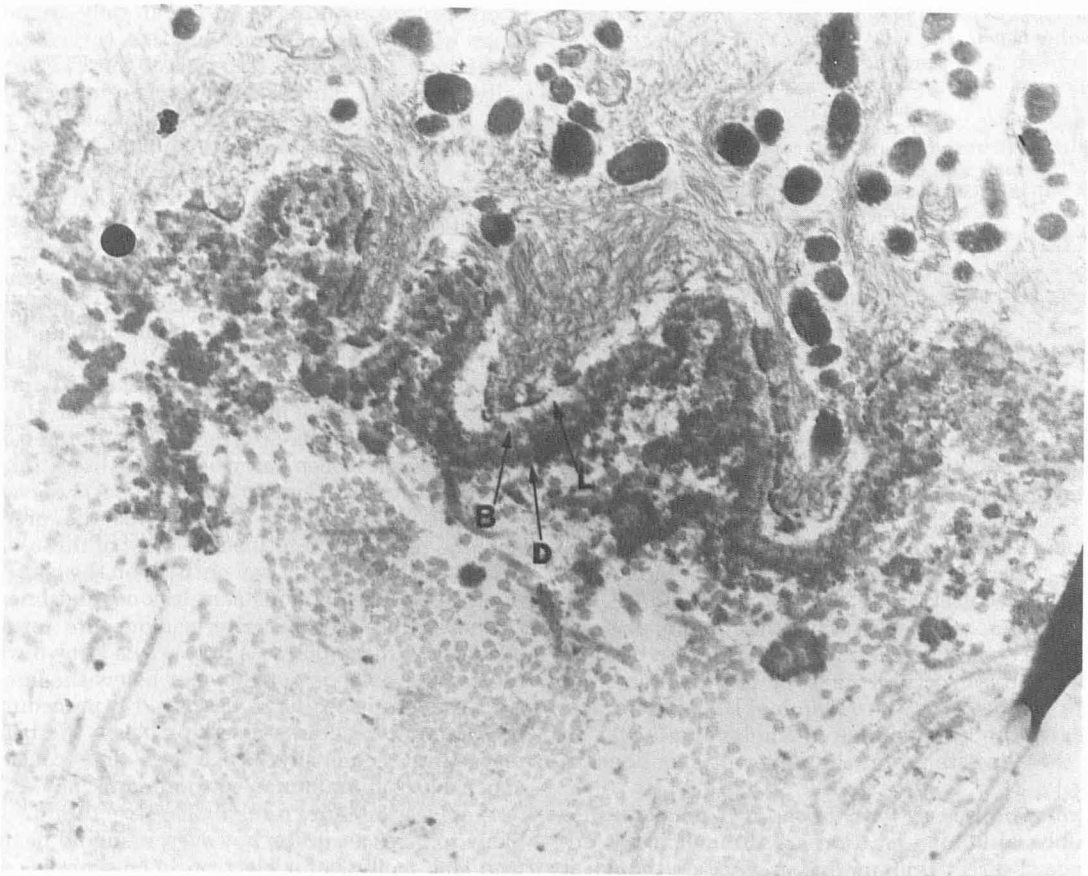

FIG 2. Immunoelectron micrograph of biopsy #9 from a patient with systemic lupus erythematosus. IgG deposits (*D*) are concentrated below the basal lamina (*B*). The lamina lucida (*L*) is relatively free of deposits. An identical location of IgG deposits was observed in biopsies #9 and #10. Magnification 6.5×10^3.

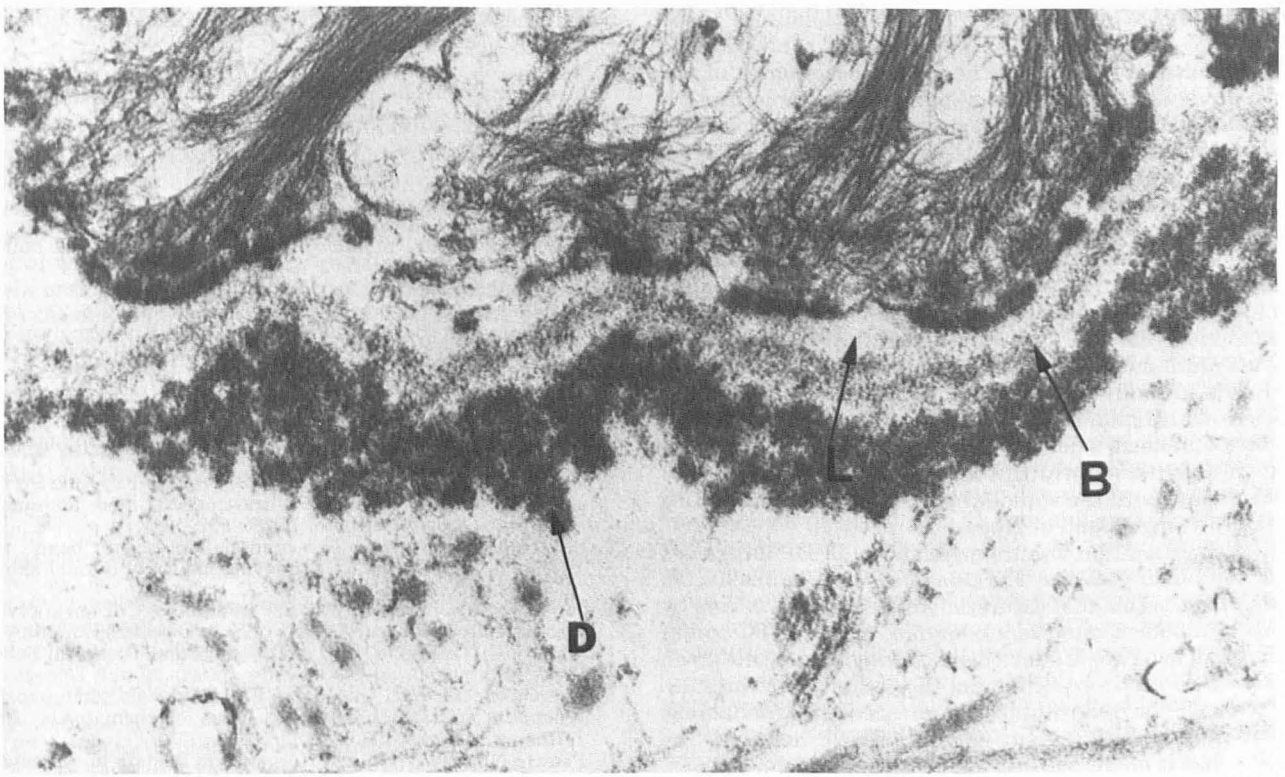

FIG 3. Immunoelectron micrographs of biopsy #5 from a patient with epidermolysis bullosa acquisita. IgG deposits (*D*) are beneath the basal lamina (*B*). The lamina lucida (*L*) is free of deposits. An identical location of IgG deposits was observed in biopsies #5–8. Magnification 4.2×10^4.

TABLE II. *Pattern of double immunofluorescence in ten biopsies from patients with BP, EBA, and bullous SLE*

BX #	Patterns		
	Fluorescein filter	Fluorescein-rhodamine filter	Rhodamine filter
4	Linear green	Linear green	—
5	Linear green, yellow-orange	Linear green, yellow-orange	Linear red
1	Linear yellow-orange	Linear yellow-orange	Linear red

reactant deposition in skin. For example, BP, CP, HG, and EBA characteristically have a linear pattern of IgG and/or C3 deposition. Uncommonly, SLE may show a similar pattern and composition of reactants. In most cases, discrimination among these illnesses can be made on clinical or serologic and perhaps less commonly on histologic features alone; however, many cases lack distinctive serologic and histologic features and clinical and histologic overlaps occasionally occur. Of course, direct immunofluorescence in SLE is usually distinctively thready, stippled, or granular. Therefore, it is only in those very uncommon cases with a "linear-appearing" band that double immunofluorescence might be useful.

Immunoelectron microscopy has shown that among diseases with linear BMZ, IgG, or C3 immunofluorescence there are at least 2 distinctive ultrastructural sites of immune reactant deposition, above and below the basal lamina. BP, CP, and HG have deposits above the basal lamina in the lamina lucida. In EBA and SLE, the deposits are in the upper dermis beneath the basal lamina [2, 3].

Distinctions among diseases with linear IgG deposits in a suprabasal or subbasal lamina location are difficult if not impossible by standard direct immunofluorescence. Although it has been stated that immune deposits in EBA and SLE produce a wider fluorescent band than those in BP, CP, and HG, we and others have experienced difficulties in appreciating this difference particularly in EBA and have had to resort to immunoelectron microscopy to confirm their location [6]. Furthermore, we know of no studies that have documented a difference in width between the fluorescent bands in these diseases. From our experience, it appears that band width is variable and often a function of the plane of sectioning of the tissue. In any event, conclusions about the ultrastructural location of immune reactants based on a subjective impression of band width are not likely to be reliable.

There are several features of the double immunofluorescence technique that merit discussion. First, the method required incubation of skin sections with normal human serum following the initial incubation with RITC-conjugated antihuman IgG. This step was included to saturate the RITC-conjugated antihuman IgG binding sites not occupied by in vivo deposited IgG. It was assumed that had this step been omitted, the RITC-conjugated antihuman IgG might bind the FITC-labeled anti-lamina lucida antibody and either block rhodamine fluorescence or interfere with binding of the FITC-labeled reagent to the lamina lucida. Indeed, when incubation with human serum was omitted (results not shown) the tissues that showed a double fluorescent band with the standard procedure (EBA and SLE) showed only a single band of green fluorescence.

Another consideration was the availability of BP antigen in BP skin for binding to the FITC-labeled anti-lamina lucida antibody. It is possible that saturation of BP antigen in vivo by anti-BMZ antibodies could block binding of the FITC-conjugated reagent in vitro. Under these circumstances, no green fluorescence would be expected and the tissue would presumably show only the yellow-orange fluorescence of rhodamine. This was not the case in any of our tissues; however, the possibility that it might occur cannot be excluded.

Since it can be assumed that RITC-conjugated antihuman IgG as well as FITC-conjugated anti-lamina lucida antibody bound to BP skin, a question arises as to why only green fluorescence was observed. We can only assume that in those cases where both reagents bind to the same ultrastructural location, the green fluorescence of the FITC conjugate is visually dominant. This assumption is reinforced by the observation that when NHS was omitted from the procedure, only green fluorescence was observed in all tissue even though both fluorescent reagents were apparently bound in skin.

The precise ultrastructural localization of immune reactants in patients with linear IgG BMZ immunofluorescence is of more than academic interest. For example, patients with EBA and bullous SLE may present with bullous lesions clinically and histologically similar to those seen in BP [6,10,23].

In our experience, patients with bullous SLE may present with a clinical picture similar to BP and histologic and direct immunofluorescent findings of acute dermal inflammation with subepidermal blister formation and linear BMZ immunofluorescence [23]. We have recently encountered 2 such patients and others have been described in the literature [10]. Of particular interest are a number of case reports of coexistent BP and SLE [24–27]. In none of these was the precise location of immune reactants identified. In most of these cases coexistence of the 2 diseases was based entirely on the clinical and serologic findings of SLE, bullous skin lesions, and linear BMZ immunofluorescence. These criteria alone are insufficient for the diagnosis of both diseases unless it is known whether immune reactants are deposited above or below the lamina densa.

In this study we have described a procedure for localizing IgG immune reactants above or below the basal lamina. The method is technically easier, less time consuming, and less expensive than immunoelectron microscopy. It, of course, does not replace the more refined ultrastructural technique, nor is it likely to be as accurate; however, it should be of value to those who lack facilities for electron microscopy as a rapid preliminary test for discriminating among diseases with immune deposits above and below the basal lamina. Although we have examined only diseases with IgG deposits in this study, the same method should be applicable to diseases with IgA deposits. The findings that the FITC-conjugated anti-lamina lucida antibody binds to the BMZ of tissues stored in transport media suggest these tissues could also be processed for double immunofluorescence.

The authors would like to express their sincere appreciation to Mrs. Virginia S. Moore for her superb preparation of this manuscript.

REFERENCES

1. Beutner EH, Chorzelski TP, Bean SF (Eds): Immunopathology of the Skin, 2nd ed. New York, Wiley, 1973, pp 3–28
2. Kushniruk W: The immunopathology of epidermolysis bullosa acquisita. Can Med Assoc J 108:1143–1146, 1973
3. Gibbs RB, Minus HR: Epidermolysis bullosa acquisita with electronmicroscopical studies. Arch Dermatol 111:215–220, 1975
4. Benedetto AV, Bergfeld WF, Guirgias M: Epidermolysis bullosa acquisita diagnosed by immunofluorescence and electron microscopy (abstr). J Invest Dermatol 70:221, 1978
5. Neiboer C, Boorsma DM, Woerderman MJ, Kalsbiek GL: Epidermolysis bullosa acquisita—immunofluorescence, electron microscopic and immunoelectron microscopic studies in four patients. Br J Dermatol 102:383–392, 1980
6. Yaoita H, Briggman RA, Lawley TJ, Provost TT, Katz SI: Epidermolysis bullosa acquisita: ultrastructural and immunological studies. J Invest Dermatol 76:288–292, 1981
7. Burnham TK, Fine G: The immunofluorescent "band" test for lupus erythematosis III employing clinically normal skin. Arch Dermatol 103:24–32, 1971
8. Jablonska S, Chorzelski TP: The immunology of lupus erythematosus, Immunological Aspects of Skin Diseases. Edited by L Fry, PP Seah. Lancaster, England, Medical and Technical Publishing Co, 1975, p 66
9. Wierchowiechi WO, Quismorio FP, Frioce GJ: Immunoglobulin deposits in skin in systemic lupus erythematosus. Arthritis Rheum 18:77–82, 1975
10. Pennys NS, Wiley HE III: Herpetiform blisters in systemic lupus erythematosus. Arch Dermatol 115:1427–1428, 1979
11. Holubar K, Wolff K, Konrad K, Beutner EH: Ultrastructural localization of immunoglobulin in bullous pemphigoid skin. J Invest Dermatol 64:220–227, 1975

12. Schaumburg-Lever G, Rule A, Schmidt-Ulrich B, Lever WF: Ultrastructural localization of in vivo bound immunoglobulins in bullous pemphigoid. A preliminary report. J Invest Dermatol 64:47–49, 1975
13. Hönigsmann G, Stingl K, Holubar E, 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
14. 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
15. Schreiner E, Wolff K: Systemic lupus erythematosus: electron microscopic localization of in vivo bound globulin at the dermal-epidermal junction. J Invest Dermatol 55:325–328, 1970
16. Ueki H, Wolff H, Braun-Falco O: Cutaneous localization of human gammaglobulin in lupus erythematosus. An electron microscopical study using the peroxidase-labeled antibody technique. Arch Dermatol Forsch 248:297–314, 1974
17. Graham RC, Karnovsky MJ: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14:291–302, 1966
18. Carlo JR, Gammon WR, Sams WM Jr, Ruddy S: Demonstration of the complement regulating proteins, B_1H in skin biopsies from patients with bullous pemphigoid. J Invest Dermatol 73:551–553, 1979
19. Cohan AS: Preliminary criteris for the classification of systemic lupus erythematosus. Edited by G Rodman. Bull Rheum Dis 21:643–648, 1971
20. Nisengard RJ, Blaszcyk M, Chorzelski T, Beutner E: Immunofluorescence of biopsy specimens. Arch Dermatol 114:1329–1332, 1978
21. Barvie A, Lazarus GS, Barland P: Basement membrane antibody with skin bullae. NY State J Med 76:2127–2132, 1976
22. Beutner EH, Nisengard RJ: Defined immunofluorescence in clinical immunopathology, Immunopathology of the Skin: Labeled Antibody Studies. Edited by EH Beutner, Chorzelski TP, Bean SF, Jordan RE. Straudsburg, Pennsylvania, Dowden, Hutchinson & Ross, 1973, pp 197–247
23. Olansky AJ, Briggaman RA, Gammon WR, Kelly TF, Sams WM Jr: Bullous systemic lupus erythematosus. J Am Acad Dermatol, in press
24. Jordon RE, Muller SE, Hale WL, Beutner EH: Bullous pemphigoid associated with systemic lupus erythematosus. Arch Dermatol 99:17–25, 1969
25. Miller JF, Downham TF, Chapel TA: Coexistent bullous pemphigoid and systemic lupus erythematosus. Cutis 21:368–373, 1978
26. Kumar V, Binder WL, Schotland E, Beutner EH, Chorzelski TP: Coexistence of bullous pemphigoid and systemic lupus erythematosus. Arch Dermatol 114:1187–1190, 1979
27. Jacoby RA, Abraham AA: Bullous dermatosis and systemic lupus erythematosus in a 15 year-old boy. Arch Dermatol 115:1094–1097, 1979

0022-202X/82/7905-0317$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 79:317–321, 1982

Vol. 79, No. 5
Printed in U.S.A.

Fc Receptor-Defined T-Lymphocyte Subpopulations in Patients with Cutaneous T-Cell Lymphoma

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The distribution of peripheral blood T cells bearing Fc receptors for IgG (Tγ) and IgM (Tμ) was determined in 18 patients with cutaneous T-cell lymphoma (Sézary syndrome, mycosis fungoides) and 15 normal controls. The mean percent of Tμ and Tγ cells in the patients with morphologically normal lymphocytes was similar to that of the control subjects. In 13 patients with circulating malignant cells representing 30–100% of their peripheral blood lymphocytes, the mean percentages of Tμ and Tγ were low. In 9 patients the majority of the malignant T cells did not exhibit Fc receptors for IgG or IgM. In some patients, however, high blood lymphocyte counts resulted in normal or high concentrations of all the T-cell subsets. Further, in 4 patients, lymphocytes with the characteristic nuclear abnormalities of "Sézary/mycosis" cells were found in both the Tμ and Tγ, as well as the Tnonγnonμ, subpopulations. The heterogeneity of the Fc receptors on cutaneous T-cell lymphoma lymphocytes suggests that these receptors are not useful as clonal markers in these disorders.

The term cutaneous T-cell lymphoma (CTCL) describes a spectrum of malignant lymphoproliferative disorders primarily involving the skin that includes mycosis fungoides and the Sézary syndrome [1]. The Sézary syndrome is characterized by an exfoliative erythroderma, generalized lymphadenopathy, and circulating abnormal lymphocytes. Approximately 20–40% of patients with mycosis fungoides also have peripheral blood involvement demonstrable by light and electron microscopy [2–4]. The abnormal cell (the CTCL or "Sézary/mycosis" cell) is a lymphocyte of variable size with a high nuclear to cytoplasmic ratio, characteristic convoluted or cerebriform nuclear contours, and often dense nuclear chromatin. These cells usually display properties of T cells in that they form rosettes with sheep red blood cells (SRBC), react with anti-T-cell antisera, and are found in the paracortical areas of involved lymph nodes [5–7].

Recently, subpopulations of T cells have been described which possess receptors for the Fc portion of IgG (Tγ cells) and IgM (Tμ cells) [8–10]. Early studies of isolated subpopulations presented evidence that Tμ cells were able to provide help to polyconal B cell differentiation induced by pokeweed mitogen

Manuscript received June 1, 1981; accepted for publication March 25, 1982.

This research was supported by the Medical Research Service of the Veterans Administation.

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Abbreviations:
AET: 2-aminoethylisothiouronium bromide
CTCL: cutaneous T-cell lymphoma
OXEA: ox red cell-antibody
PWM: pokeweed mitogen
SRBC: sheep red blood cells