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ANTI-BASEMENT MEMBRANE ANTIBODIES IN SERA FROM PATIENTS WITHOUT BULLOUS PEMPHIGOID

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Abstract. Sera from 208 individuals were examined for anti-basement membrane (anti-BM) antibodies by means of an indirect immunofluorescence technique. In sera from patients with bullous pemphigoid 19 of 25 had anti-BM antibodies in titres varying from 80 to 32 000. In 2 of 42 sera from patients with psoriasis, anti-BM antibodies were demonstrated (titres 40–640). Of 58 sera from patients with leg ulcers, antibodies were found in 4 (titres 40–320). Antibodies were found in 2 of 43 patients with cardio-vascular disease (titre 80) and in 1 of 40 control persons (titre 40). Serum from one of the patients with leg ulcers caused a punctate staining pattern unlike the tubular pattern seen in all other positive sera.

Key words: Immunofluorescence technic; Antibasement membrane antibodies; Bullous pemphigoid; Non-bullous dermatosis; Contents

In 1965 Beutner et al. (3) described anti-basement membrane antibodies in sera from patients with bullous pemphigoid. This finding has been confirmed by several authors and anti-BM antibodies have been thought to be highly specific for bullous pemphigoid (4, 6, 11). However, antibodies with the same specificity have been demonstrated in a few patients with benign prostatic hypertrophy (1). Moreover, some groups of investigators have been able to demonstrate anti-BM antibodies in low titres in sera from patients with benign mucosal pemphigoid (7, 8, 12), although others have been unable to confirm this finding (2, 9, 10).

In the present paper the specificity of the anti-BM antibodies has been re-evaluated. For this purpose a number of sera from patients with bullous pemphigoid as well as sera from patients with various other diseases and from control persons have been studied.

MATERIAL AND METHODS

A total of 208 sera from patients and controls were examined. Twenty-five patients had active bullous pemphigoid. None had started treatment with corticosteroids at the time of examination. Fifty-eight patients had leg ulcers, 42 had psoriasis of whom 2 had erythroderma. Forty-three patients had cardio-vascular disease (coronary occlusion or cerebral infarction) without skin disease. Forty were healthy technicians, nurses, and physicians.

Each serum sample was examined in dilutions 1:10, 1:20, 1:40, and 1:80 and positive sera were titrated up to the end point. Guinea-pig lip was used as antigen, and, since it is our experience that the basement membrane antigen deteriorates with increasing age, only young animals of 8–10 weeks were used.

Unfixed, 6–8 μm thick cryostat sections were air dried for 15 min, washed in phosphate-buffered saline (PBS) pH 7.2 for 30 min, incubated at 4°C in a moist chamber with serum dilutions for 30 min, washed for 15 min in PBS and then incubated with one drop of diluted conjugate in a moist chamber for 30 min at 4°C. In each experiment a serum was included containing anti-BM antibodies in high titre from a patient with bullous pemphigoid as well as negative controls.

Biopsies from affected and from clinically normal skin were obtained from 23 patients with bullous pemphigoid and examined for in-vivo bound immunoglobulins and complement C3.

Unfixed, 6–8 μm thick cryostat sections were air-dried for 15 min, washed in PBS and incubated with one drop of diluted conjugate in a moist chamber for 30 min at 4°C. Blocking procedures with unconjugated antisera were included in each experiment. Fluorescein isothiocyanate labelled rabbit IgG specific for human gamma, μ and α chains and complement C3 (Dako, Copenhagen) were used.

The material was examined in a Leitz Ortholux fluorescence microscope equipped for transmittent light illumination using an Osram HBO 200 lamp as light source, a KP 490 nm interference filter for primary light selection, a Tiyoda wide-angle dark-field condensor and a 3 mm OG 530 glass filter as barrier filter.

RESULTS

In 28 of 208 sera, anti-BM antibodies could be demonstrated (Table 1). Nineteen of the 25 sera from patients with bullous pemphigoid contained anti-BM antibodies. A typical example is shown in Fig. 1

Table I. *Anti-basement membrane antibodies in sera from healthy controls and patients with psoriasis, leg ulcers, cardiovascular diseases and bullous pemphigoid*

	No. of sera	Sera with anti-BM antibodies
Healthy controls	40	1
Psoriasis	42	2
Leg ulcers	58	4
Cardiovasc. dis.	43	2
Bullous pemphigoid	25	19
Total	208	28

The end-point titre varied from 80 to 32 000 (Table II). No correlation was found between titre and disease activity. One serum from a healthy control individual and 2 patients with psoriasis had anti-BM antibodies in their sera. The latter cases were complicated with exfoliative erythroderma. None of the psoriasis patients without antibodies in their sera had exfoliative erythroderma. Sera from 4 of 58 patients with leg ulcers and from 2 of 43 patients with cardiovascular disease contained anti-BM antibodies (Fig. 2). In these groups no clinical differences could be found between patients with and without serum antibodies. One of the patients with leg ulcers showed a punctate staining pattern of the basement membrane (Fig. 3) unlike the other positive reactions which showed a tubular pattern.

Anti-BM antibodies of the IgG class was the most frequent finding. Three sera (one from a healthy control, one from a patient with psoriasis,

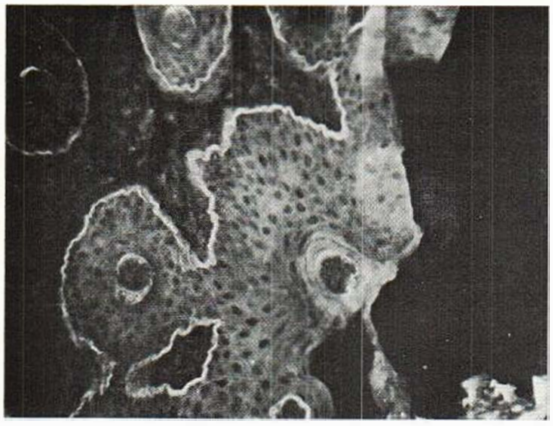

Fig. 1. Indirect immunofluorescence staining of guinea pig lip with a 1:500 dilution of serum from a patient with bullous pemphigoid.

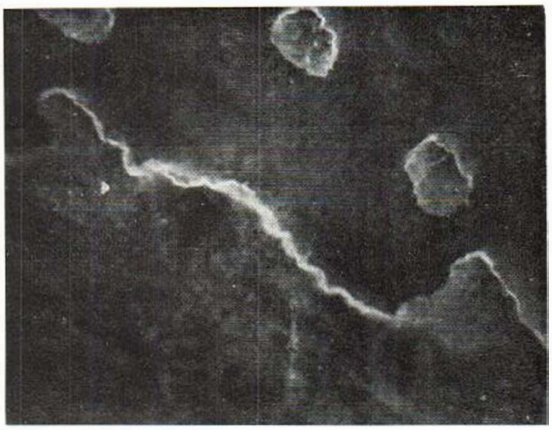

Fig. 2. Indirect immunofluorescence staining of guinea pig lip with a 1:80 dilution of serum from a patient with leg ulcer.

and one from a patient with leg ulcers) contained IgA antibodies. One of these (from the patient with psoriasis) contained IgG antibodies as well. Anti-BM antibodies of the IgM class were not found.

A pro-zone phenomenon was demonstrated in positive sera from patients with, as well as without, bullous pemphigoid.

Approximately 25% of the sera caused peripheral cytoplasmic staining of the basal-cell layer of epidermis (Fig. 4). This phenomenon could be demonstrated in serum dilutions up to 1:160. It was seen in sera from normal persons as well as sera from

Table II. *The end-point titre of the anti-basement membrane antibodies in sera from patients with bullous pemphigoid, psoriasis, leg ulcers and cardiovascular diseases*

The parentheses refer to the fact that one patient had antibodies of both IgG and IgA class

Titre	Bullous pemphigoid	Psoriasis	Leg ulcers	Card. vasc. diseases	Normals
40		(1)	1		1
80	1		1	2	
160	3				
320	4	1	2		
540	1	1			
1 000					
2 000	2				
4 000	3				
8 000	2				
16 000	2				
32 000	1				
Total	19	2 (3)	4	2	1

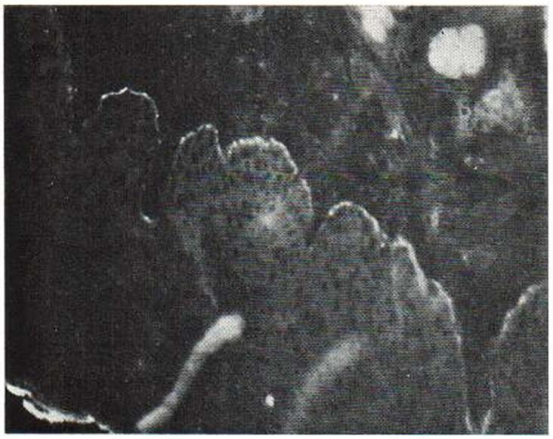

Fig. 3. Indirect immunofluorescence staining of guinea pig lip with serum from a patient with leg ulcer. Punctate staining.

patients with leg ulcers, psoriasis, cardiovascular disease, and bullous pemphigoid.

Table III shows the findings of in-vivo bound immunoglobulins and C3 in biopsies from 23 patients with bullous pemphigoid. In all patients biopsies were obtained from involved skin and, in 15 patients, from clinically normal skin as well. Immunoglobulin and/or C3 were found in all the biopsies from involved as well as from uninvolved skin (Fig. 5). IgG and C3 were by far the most frequent finding though IgM and IgA were also found in several biopsies.

DISCUSSION

Beutner et al. (4) and Burnham & Fine (5) found no anti-BM antibodies in their sera from patients

Table III. *Deposits of immunoglobulins and complement C3 in skin from patients with bullous pemphigoid*

	No. of patients	Deposits demonstrated	IgG	IgM	IgA	C3
Skin lesions	23	23	21	6	6	20
Clinically unaffected skin	15	15	10	1	3	13

without bullous pemphigoid. On the other hand, Ablin (1) found anti-BM antibodies in 3 of 42 patients with benign prostatic hypertrophy, and Dantzig (8), Dabelsteen et al. (7), and Tagami et al. (12) found anti-BM antibodies in a total of 9 patients with benign mucosal pemphigoid. The titres of anti-BM antibodies in sera from patients with prostatic hypertrophy and benign mucosal pemphigoid were low (up to 128). In the present study anti-BM antibodies were found in 9 of 183 control persons and patients without bullous pemphigoid. Most of the titres were low but in 3 cases high titres were found (320 and 640), values which are comparable to those found in sera from patients with bullous pemphigoid.

With one exception the positive sera from patients without bullous pemphigoid caused a tubular staining pattern identical with that seen in bullous pemphigoid.

In this study, some of the sera from patients with bullous pemphigoid showed higher titres than those quoted in the literature (4, 6, 11). On the other hand, only 75% of the sera from the patients with bullous pemphigoid contained anti-BM antibodies,

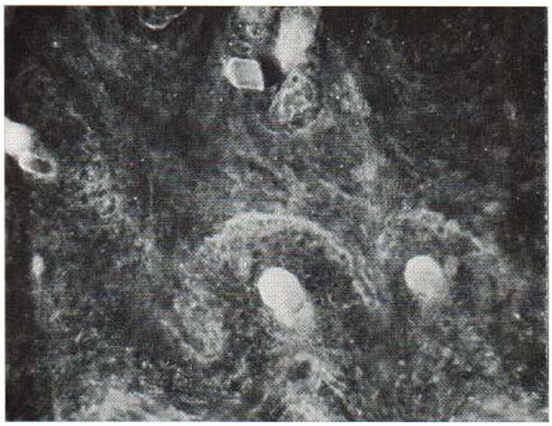

Fig. 4. Indirect immunofluorescence staining of guinea pig lip showing peripheral cytoplasmic staining of the basal-cell layer of epidermis.

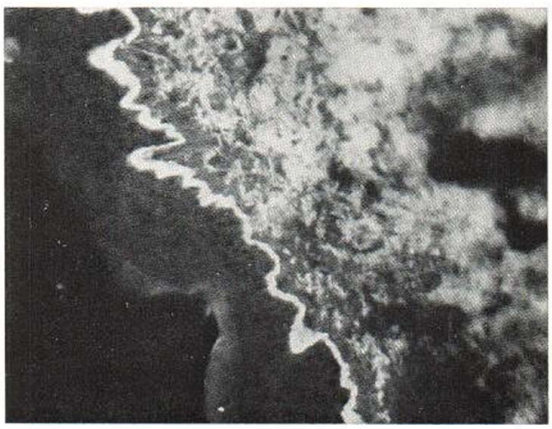

Fig. 5. Deposits of complement C3 in the basement membrane zone of the skin from a patient with bullous pemphigoid.

a percentage which is similar to the findings of others (4). This seems to indicate that the sensitivity of our system is comparable to the techniques used by other authors. Consequently, this study shows that the anti-BM antibodies are not specific for bullous pemphigoid. Our finding of in-vivo bound immunoglobulins and/or C3 in all the biopsies from involved as well as from uninvolved skin of patients with bullous pemphigoid is in agreement with the findings of others (4).

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