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IMMUNOPATHOLOGY OF BULLOUS PEMPHIGOID

BASEMENT MEMBRANE DEPOSITION OF IgE, ALTERNATE PATHWAY COMPONENTS AND FIBRIN

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SUMMARY

Sixteen patients with bullous pemphigoid were examined using direct and indirect immunofluorescent techniques with antisera specific for C1q, C4, C3, C5, C3 proactivator, properdin, fibrinogen, IgA, IgM, IgG and IgE. The results of these studies are consistent with the activation of complement via the classical (antibody–C1q) sequence as well as via the alternate pathway. Fibrinogen and/or fibrin derivatives were demonstrated on the basement membrane of ten of fifteen patients tested and IgE basement membrane staining was found in four individuals.

INTRODUCTION

Bullous pemphigoid, a chronic subepidermal bullous disease, occurs predominantly in elderly individuals. Direct immunofluorescent studies have demonstrated immunoglobulins and C3 on the skin basement membrane in these patients (Jordon, Beutner & Witebsky, 1971; Beutner, Chorzelski & Jordon, 1970; Chorzelski, Jablonska & Blaszczyk, 1968). Approximately 80% of these patients have circulating antibasement membrane antibodies, the majority of which fix complement (Jordon, Sams & Beutner, 1969).

In addition to the classical complement sequence, recent work has confirmed the existence of an alternate pathway of complement activation involving the properdin system (Sandberg *et al.*, 1970; Sandberg *et al.*, 1971; Götze & Müller-Eberhard, 1971). In addition to immunoglobulins IgE, IgA and IgG4, substances such as *E. coli*, endotoxin, lipopolysaccharides, zymosan and dextran, which are not capable of fixing complement via the classical sequence, activate the alternate pathway (Götze & Müller-Eberhard, 1971; Pensky *et al.*, 1968; Ishizaka, Sian & Ishizaka, 1972).

We have now examined the biopsy specimens of sixteen bullous pemphigoid patients with a variety of antisera directed against immunoglobulins, complement components including several of the alternate pathway components and fibrinogen. This study demonstrates that properdin, C3 proactivator (C3PA) and/or C3 activator (C3A), in addition to

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immunoglobulins and late C components, are deposited on the skin basement membranes of these patients. Fibrinogen and/or fibrin products are also present in the majority of patients. The study is consistent with the hypothesis that complement activation takes place in bullous pemphigoid via both the classical and alternate pathways.

MATERIALS AND METHODS

Sixteen bullous pemphigoid patients were studied. The diagnosis was established by physical examination and histological evidence of a subepidermal bullous disease. All patients were found to have IgG deposited along the skin basement membrane by direct immunofluorescent examination of skin biopsies. Indirect immunofluorescence, using homologous skin as a substrate, demonstrated antibasement membrane antibodies in all sixteen patients' sera. The titres ranged between 1 : 4 and 1 : 320.

Biopsies were taken of apparently normal skin adjacent to areas of blister formation. They were immediately 'snap' frozen and stored at −70°C until studied.

Sera were obtained from seven patients and allowed to clot at room temperature for 1 hr. The blood was then centrifuged and the sera stored at −70°C. The third component of complement (C3) and total haemolytic complement (CH_{50}) were determined by the routine laboratory. The serum was also tested by immunoelectrophoresis using an anti-C3PA antisera.

Direct and indirect immunofluorescent studies were performed on biopsy specimens using the following antisera: an antihuman C1q antiserum was raised in a rabbit by injecting C1q prepared according to the methods of Yonemasu & Stroud (1971). Rabbit antihuman C3, horse antihuman C4, and a fluoresceinated rabbit antihuman fibrinogen were purchased from Hyland Laboratories. Rabbit antihuman C5 and C3PA antisera were a gift of Dr Hans Müller-Eberhard (Nillson & Müller-Eberhard, 1965; Götze & Müller-Eberhard, 1971). A rabbit antihuman properdin antisera was a gift of Dr Jack Boyer (Pensky *et al.*, 1968). Rabbit antihuman IgA, IgM and IgG were prepared in this laboratory as previously described (Tourville *et al.*, 1969; Bienenstock & Tomasi, 1968). An antihuman IgE antisera was raised in a rabbit to an isolated human IgE myeloma protein (P.S.). This antisera, absorbed with whole lyophilized human sera, demonstrated one line in gel double diffusion immunoelectrophoresis when reacted against an IgE standard, but failed to react against normal human serum. This antiserum also showed a line of identity with a commercial anti-IgE antiserum (anti-IgE Fc (N.D.)) when reacted against the IgE standard. An antirabbit globulin antiserum was raised in a goat and an antihorse globulin antiserum was raised in a rabbit. The rabbit antihuman fibrinogen antiserum gave a single β migrating precipitin line on immunoelectrophoresis against plasma but not against whole serum. The goat antirabbit and rabbit antihorse globulin demonstrated, on immunoelectrophoresis, multiple lines in the γ-globulin regions of rabbit and equine sera respectively. All other antisera were demonstrated to be monospecific by gel double diffusion and immunoelectrophoresis. The antisera were conjugated with fluorescein isothiocyanate according to the method of Clark & Sheppard (1963). Unitage assays were performed according to Beutner, Chorzelski & Jordon (1971). Fluorescein protein molar ratios (F/P) are as follows: anti-IgG 3·8; anti-IgA 2·8; anti-IgM 3·8; anti-IgE 4·3; anti-C1q 3·8; anti-C3 3·3; and antifibrinogen 6·7. The horse anti-C4 was used in the indirect immunofluorescent technique with the fluorescein-conjugated rabbit antihorse globulin antisera (F/P 4·2). The rabbit anti-C5, anti-

properdin and anti-C3PA antisera were employed in the indirect immunofluorescent technique using a fluorescein-conjugated goat antirabbit antisera (F/P 4·7).

Skin biopsies from normal volunteers and various inflammatory skin diseases as previously reported served as negative controls for the rabbit antihuman properdin and C3PA antisera (Provost & Tomasi, 1973).

Twenty skin biopsies taken from normal individuals and from patients with urticaria, erythema multiforme, psoriasis and epidermolysis simplex skin diseases served as negative controls for the rabbit antihuman fibrinogen and IgE antisera. Twenty additional skin biopsies from autopsied normal skin, vasculitis, atopic dermatitis, dermatitis herpetiformis, failed to demonstrate IgE basement membrane deposition.

Skin biopsies from twenty-five patients with systemic lupus erythematosus (SLE), examined by direct immunofluorescence, served as positive controls for these antisera. All antisera gave positive staining along the skin basement membrane in at least two or more SLE patients. Five SLE patients were found to have properdin staining. Three SLE patients with

TABLE 1. Immunofluorescent staining of the basement membrane in bullous pemphigoid

Antisera	Total number tested	Number of positive patients
Clq	16	14
C4	15	15
C3	16	16
C5	16	16
IgG	16	16
IgM	16	3
IgA	16	4
IgE	16	4
C3 proactivator	15	13
Properdin	16	14
Fibrinogen	16	10

properdin basement membrane staining were also found to have positive basement membrane staining for C3PA. Two of twelve SLE patients also had granular deposition of fibrinogen or fibrin along the skin basement membrane. Three SLE patients were found to have granular deposits of IgE along the skin basement membrane. This staining could not be blocked by unconjugated rabbit antihuman IgG, IgM, IgA or antiwhole human globulin. Staining, however, could be blocked if the conjugated rabbit antihuman IgE serum was incubated with the IgE myeloma serum. Properdin staining could be blocked by absorbing the properdin antisera with partially purified properdin (Todd, Pillemer & Lepow, 1959). Purified C3PA and C5 were not available for appropriate absorptions.

RESULTS

The results of direct immunofluorescent staining of the skin of bullous pemphigoid patients are presented in Table 1. The staining pattern was similar with all of the antisera used and

consisted of a linear homogenous deposition along the skin basement membrane. Properdin (Fig. 1) and C3PA (Fig. 2) deposition was found on the skin basement membrane in thirteen of fifteen patients tested. Moderately heavy deposition of fibrinogen was found in ten of fifteen patients (Fig. 3).

Four patients had moderate IgE staining of the skin basement membrane (Fig. 4). CH50 and C3 determinations were within normal limits in all of the seven patients examined.

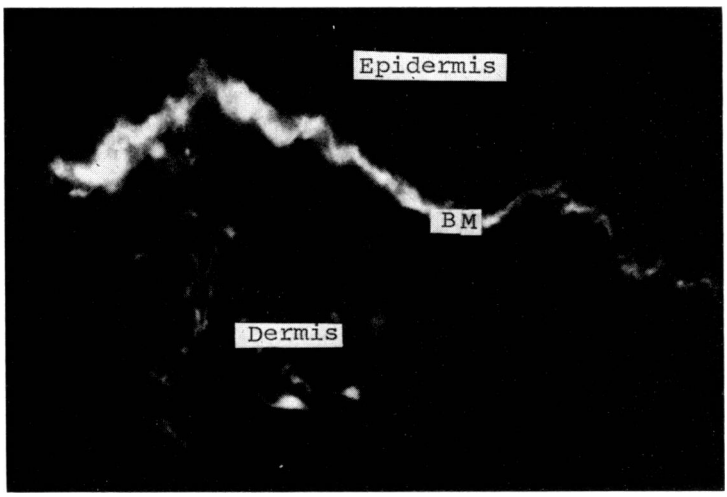

FIG. 1. Heavy staining of properdin along the basement membrane (BM). (Magnification ×43.)

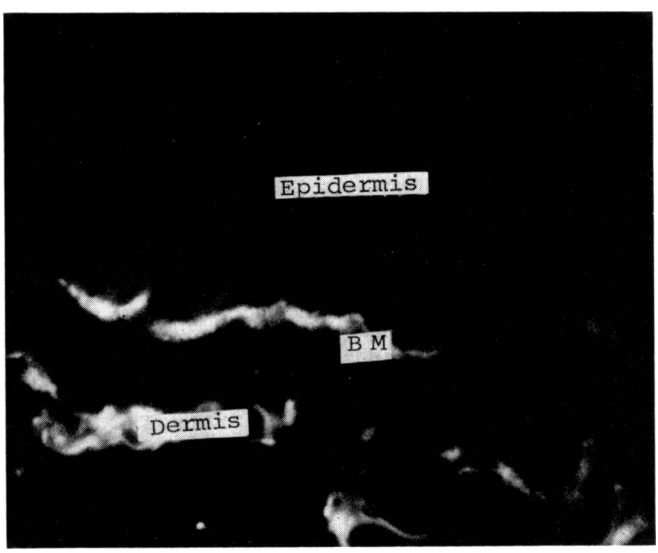

FIG. 2. Basement membrane (BM) deposition of C3 proactivator or C3 activator. (Magnification ×43.)

Immunoelectrophoresis of whole serum employing antihuman C3PA demonstrated the typical fast moving β migrating precipitin line with no evidence of formation of the γ migrating C3PA. Indeed, we have subsequently examined immunoelectrophoretically with antihuman C3PA antisera, the blister fluid from three bullous pemphigoid patients. These fluids were obtained within 24–28 hr of formation and stored at $-70°C$ prior to testing. These fluids show the presence of the γ migrating C3A.

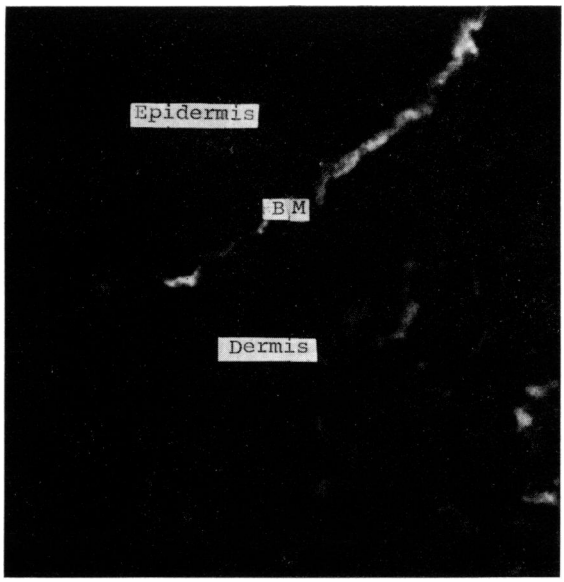

FIG. 3. Linear deposition of fibrinogen and/or fibrin on skin basement membrane (BM). (Magnification ×38.)

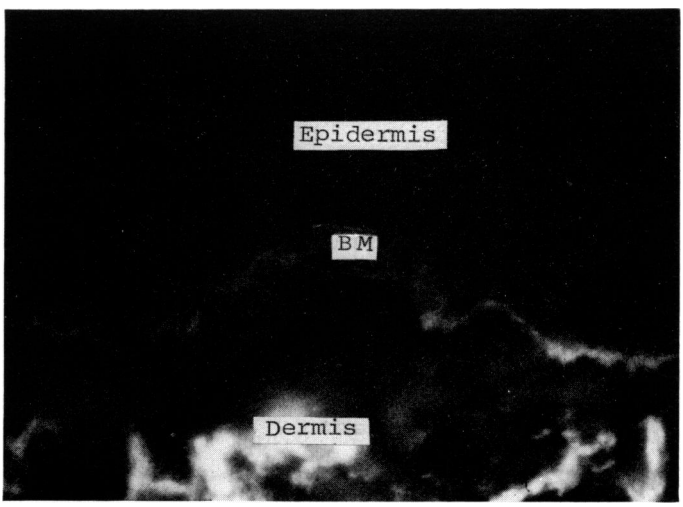

FIG. 4. Linear homogenous deposition of IgE along skin basement membrane BM. (Magnification ×43).

DISCUSSION

These results suggest that activation of complement in bullous pemphigoid occurs via the classical as well as the alternate pathways. Properdin and C3PA, two proteins of the alternate pathway, were found in moderate to heavy concentrations along the skin basement membrane. All normal as well as a number of biopsies from diseased skin used as controls failed to demonstrate basement membrane deposition of either of these two proteins. There is little doubt, therefore, that staining for these proteins is specific. C3PA and properdin deposition was always found in the presence of both immunoglobulin and early and late complement component deposition. In two instances initial biopsy of normal appearing skin of patients with a recent onset of bullous pemphigoid (i.e. within 3 days) failed to demonstrate C3PA and properdin deposition. Immunoglobulin and complement deposition was weak (1+). Subsequent rebiopsy of normal skin of one of these patients 2 weeks after the onset of bullous pemphigoid demonstrated, in addition to marked immunoglobulin and complement deposition, heavy C3PA and properdin staining.

The presence of early components of complement, i.e. C1 and C4, plus the universal deposits of IgG suggest that complement was also activated via the classical pathway. It should be clearly emphasized that, in these studies, as in most previously reported investigations employing immunofluorescence, only deposition of the various components is demonstrated by the experimental techniques employed. That activation is occurring on the membrane is presumed from the specificity of the morphological data but there is no direct proof that complement or other components are being actively utilized and/or participating in the disease process.

Although it seems likely within the reservations stated above that there is activation of complement in bullous pemphigoid, we were unable to demonstrate a decrease in total haemolytic complement (CH_{50}) or C3 in sera obtained from patients with active disease. However, since the serum complement level reflects a balance between complement synthesis and degradation, it is possible that complement synthetic rates were sufficient to replenish that lost by consumption during active disease.

The failure to demonstrate the γ migrating C3A precipitin band on immunoelectrophoresis of serum, together with the presence of properdin and C3PA on the basement membrane, suggests that complement activation may occur locally at the site of blister formation rather than systemically. The presence of the γ migrating C3A in the blister fluid is also consistent with local activation. Jordon *et al.* (1973) have recently demonstrated decreased levels of individual complement components in bullous pemphigoid blister fluids compared to serum levels and have also reported the presence of the γ migrating C3A in blister fluid. Control inflammatory blisters produced by cantharidin had complement component levels comparable to serum levels and immunoelectrophoresis of the blister fluid demonstrated the β migrating C3PA and no detectable C3A.

Four patients who had properdin and C3PA deposition also demonstrated IgE on the skin basement membrane. These patients had no history of asthma, hay fever, or intestinal disease. Since aggregated IgE can activate the alternate pathway, IgE deposition in these four patients could potentially play a significant role in complement activation in this disease (Ishizaka *et al.*, 1972). Recently, increased serum IgE levels have been found in 70% of bullous pemphigoid patients (Arbesman, Wypych & Reisman, 1973). The serum IgE level in one of our bullous pemphigoid patients who demonstrated IgE basement membrane

deposition was found by these workers to be 10,400 i.u./ml (normal < 400 i.u./ml). Two of the three remaining bullous pemphigoid patients had IgE serum levels of 225 and 280 i.u./ml.

The presence of fibrinogen and/or fibrin on the basement membrane is of interest. Fibrin deposition has been found in the glomeruli of patients with various glomerulopathies and has been postulated to be involved in the pathogenesis of these diseases (McCluskey, 1971). Kaplan, Gigli & Austen (1971) have reported preliminary evidence that antigen–antibody complexes are capable of activating the clotting system via activation of Hageman factor (Factor XII). Another mechanism for fibrin deposition has been reported in rabbits by Zimmerman & Müller-Eberhard (1971). They demonstrated a Hageman factor-independent pathway of coagulation dependent upon activation of the complement system. An alternative explanation for the fibrin deposition is that the inflammatory process and damaged basement membrane components may nonspecifically trigger thrombin formation with the subsequent local deposition of fibrin. Although this possibility cannot be completely excluded, the observation that the 'disease controls' had comparable histological evidence of inflammation and no fibrin deposition, does not support this alternative explanation.

Deposition of alternate pathway components has been demonstrated at the site of the lesion in several human diseases. Thus, bullous pemphigoid joins membrano-proliferative glomerulonephritis (Westburg *et al.*, 1971), acute post-streptococcal glomerulonephritis (Westburg *et al.*, 1971), lupus nephritis (Westburg *et al.* 1971; Rothfield *et al.*, 1972; Provost & Tomasi, 1973), idiopathic focal nephritis and Henoch Schönlein nephritis (Evans *et al.*, 1973), lupus skin disease (Rothfield, 1972; Provost & Tomasi, 1973), herpes gestationis (Provost & Tomasi, 1973), and dermatitis herpetiformis (Provost & Tomasi, 1973), as diseases in which the alternate pathway of complement activation may be of pathological significance.

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