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# Differences in Complement-Dependent Chemotactic Activity Generated by Bullous Pemphigoid and Epidermolysis Bullosa Acquisita Immune Complexes: Demonstration by Leukocytic Attachment and Organ Culture Methods

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**Bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) are chronic blistering diseases associated with circulating complement (C)-binding anti-basement membrane zone (BMZ) antibodies and tissue-deposited immune complexes at the BMZ. Experimental evidence supporting a role for C-activating immune complexes in the pathogenesis of dermal inflammation and blisters has been reported in BP but not in EBA. In this study tissue-deposited immune complexes composed of EBA or BP antibodies were tested for generation of C-dependent chemotactic activity and the capacity to cause dermal leukocyte infiltration and dermal-epidermal separation (DES). Chemotactic activity was measured by the leukocyte attachment (LA) method. The capacity of complexes to mediate leukocyte infiltration and DES was examined in vitro using a newly described organ culture method. The results of LA showed immune complexes formed in vivo in EBA skin or in vitro by treating normal human skin with EBA antibodies were significantly more active in mediating C-dependent chemotaxis than complexes in BP skin or those formed with BP antibodies of equivalent or higher C-binding titers. Furthermore EBA antibodies and C caused leukocyte infiltration and DES in organ culture while BP antibodies did not. These results support a role for C-binding anti-BMZ antibodies in the pathogenesis of EBA lesions and demonstrate differences in the capacity of BP and EBA immune complexes to generate C-dependent chemotactic activity. These results suggest factors in addition to C-binding titers are important in the activation of C by BP and EBA immune complexes and suggest chemotactic factors other than those derived from C activation may be important in the recruitment of leukocytes in BP.**

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Abbreviations:
BMZ: basement membrane zone
BP: bullous pemphigoid
BPS: BP sera
C: complement
DES: dermal-epidermal separation
EBA: epidermolysis bullosa acquisita
EBAS: EBA sera
GBSS: Gey's balanced salt solution containing 2% bovine serum albumin
LA: leukocyte attachment
MEM: minimal essential medium containing 5% fetal calf serum
NHS: normal human sera
PBL: peripheral blood leukocyte(s)
PBS: 0.01 M $Na_2HPO_4$, 0.01 M $NaH_2PO_4$, pH 7.2
TCM: tissue culture media

Bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) are chronic blistering diseases characterized by circulating IgG and complement (C)-binding anti-Basement membrane zone (BMZ) antibodies, linear deposits of IgG and C components at the BMZ, subepidermal blisters, and inflammation in the upper dermis [1,2]. These features suggest anti-BMZ antibody-mediated C activation may play a role in the pathogenesis of inflammation and dermal-epidermal separation (DES) in both diseases. Experimental evidence supporting a role for C activation in the pathogenesis of BP has been published but studies in EBA have not been reported [1].

One mechanism whereby C activation may contribute to inflammation is through production of chemotactic factor, mainly C5a and recruitment of leukocytes [3]. In experimental inflammation, C-mediated recruitment of leukocytes is usually associated with an influx of neutrophil granulocytes.

Although C activation may contribute to inflammation in BP, the predominant granulocyte in lesions is usually the eosinophil [4]. This suggests that chemotactic factors other than, or in addition to, those derived from C activation may be responsible for leukocyte recruitment. Recently, we encountered several patients with EBA early in the course of their disease in whom the histology was characterized by a predominance of neutrophils.* Furthermore, these patients had a high incidence of circulating C-binding IgG anti-BMZ antibodies. The histology in these cases suggested that C-dependent recruitment of leukocytes might be involved in inflammation and that there might be differences in the ability of BP and EBA immune complexes to generate C-derived chemotactic factors.

In this study, we have examined that question by comparing the ability of BP and EBA immune complexes deposited in vivo in perilesional skin and formed in vitro with C-binding BP and EBA antibodies to mediate C-dependent leukocyte migration in cryostat skin sections. In addition, we examined the ability of C-binding BP and EBA antibodies to mediate leukocyte migration and DES in human skin organ culture. The results provide support for immune complex and C-mediated inflammation in EBA and suggest EBA immune complexes formed in vivo and in vitro possess greater potential for activating C than do BP immune complexes.

## MATERIALS AND METHODS

### *Sera and IgG Fractions*

BP sera (BPS) and EBA sera (EBAS) were obtained from patients with active disease. The 4 BPS were obtained from patients with clinical and histologic inflammation. EBAS 1–3 were obtained from patients with clinical and histologic inflammation. All patients were diagnosed by clinical, histologic, immunohistologic, and immunoultrastructural criteria [2]. EBAS were shown by previously reported immunochemical methods to contain antibodies reactive with EBA antigen [5]. IgG fractions of EBAS 1 and 2 were prepared by sequential ion exchange and affinity chromatography on diethylaminoethyl

* Gammon WR, Briggaman RA, Woodley DT: Epidermolysis bullosa acquisita presenting with features of bullous and benign mucous membrane pemphigoid. Submitted for publication.

(DEAE), Sephacel, and protein A Sepharose (Pharmacia, Inc. Piscataway, New Jersey) using established methods [6,7]. IgG fractions were concentrated by ultrafiltration and diluted 10 mg/ml in 0.15 M NaCl buffered with 0.01 M $Na_2HPO_4$, 0.01 M $NaH_2PO_4$, pH 7.2 (PBS). Analysis of fractions by immunodiffusion against sheep antihuman serum and antihuman IgG showed a single line of identity. There were 4 BPS (BPS 1–4), 5 EBAS (EBAS 1–5), and 2 EBA IgG fractions (prepared from EBAS 1 and 2) used in this study. IgG and C-binding titers of anti-BMZ antibodies were determined using normal human skin as substrate and previously described methods [8]. Antibody titers in bullous disease sera, IgG fractions, and normal human control sera are shown in Table I. Normal human sera (NHS) were obtained from healthy adult volunteers. Bullous disease and NHS were heat-inactivated at 56°C for 30 min to destroy C activity. Platelet-poor fresh-frozen NHS from a blood group type Rh-positive donor was prepared as previously described and used as a source of serum complement [6]. All sera and IgG fractions were aliquoted and stored frozen at −70°C.

*Skin*

Perilesional skin was obtained by 4-mm punch biopsy under local 1% Xylocaine anesthesia from 4 BP patients with eosinophilic inflammation and 3 EBA patients with neutrophilic inflammation. Skin was snap-frozen in liquid nitrogen, mounted in O.C.T. compound (Miles Lab. Inc., Naperville, Illinois), and stored frozen at −70°C. Normal human foreskin was obtained fresh from healthy neonates immediately after circumcision, trimmed of excess fat, and mounted on a corkboard. The epidermis and upper portion of the dermis was removed with a Castroviejo keratome set at 0.4 mm. Keratomed skin was rinsed 3 times in minimal essential medium containing 5% calf serum, MEM (Gibco Labs, Grand Island, New York) and immediately used for organ culture studies.

*Peripheral Blood Leukocytes*

Normal human peripheral blood leukocytes (PBL) were obtained by dextran sedimentation of heparinized blood using a previously described method [6]. Cell viability was greater than 98% by trypan blue exclusion. Cells were counted in a hemocytometer and suspended in Geys' balanced salt solution containing 2% bovine serum albumin, GBSS (Flow Labs, McLean, Virginia.) PBL suspensions contained 70–80% granulocytes and 20–30% mononuclear cells.

*Immunofluorescence Methods*

Direct immunofluorescence for IgG and the third component of C (C3) and indirect IgG and C3-binding immunofluorescence was performed using previously reported methods [8]. Immunoreagents purchased from Cappel Labs, Cochranville, Pennsylvania, included fluorescein isothiocyanate (FITC)-conjugated IgG fractions of goat antisera to human IgG and human C3. Molar fluorescein to protein ratios and antibody concentrations were: antihuman IgG (2.8 and 3.0 mg/ml); antihuman C3 (3.0 and 3.4 mg/ml). Conjugates were used at a dilution of 1:10–1:20 in PBS containing 0.2% $NaN_3$.

TABLE I. *Anti-BMZ antibody titers in sera and IgG fractions*

| | IgG anti-BMZ antibody titer | C3-binding anti-BMZ antibody titer |
|---|---|---|
| C3 binding BPS | | |
| $BPS_1$ | 1280 | 320 |
| $BPS_2$ | 1280 | 320 |
| $BPS_3$ | 1280 | 640 |
| $BPS_4$ | 2560 | 640 |
| C3 binding EBAS | | |
| $EBAS_1$ | 320 | 160 |
| $EBAS_2$ | 320 | 160 |
| $EBAS_3$ | 160 | 80 |
| Non-C3 binding EBAS | | |
| $EBAS_4$ | 160 | 0 |
| $EBAS_5$ | 80 | 0 |
| C3 binding EBA IgG fractions | | |
| EBA $IgG_1$ | 640 | 320 |
| EBA $IgG_2$ | 320 | 160 |
| Control Sera | | |
| $NHS_1$ | Negative 1:10 | Negative 1:10 |
| $NHS_2$ | Negative 1:10 | Negative 1:10 |

*Leukocyte Attachment Assays*

Direct and indirect leukocyte attachment (LA) assays on cryostat sections of bullous disease skin and normal human skin treated with bullous disease anti-BMZ antibodies were performed using previously described methods [6,9]. Complement-dependent LA assays were performed using PBL suspended in 10% fresh NHS. C-independent assays were performed using PBL suspended in 10% heat-inactivated NHS. All assays were performed in duplicate or triplicate. LA was quantitated by counting the number of PBL attached to the BMZ per mm BMZ. A mean LA was determined by averaging LA values in 4–6 skin sections. Statistical analysis of results was performed by *t*-test of the difference between means of paired samples.

*Organ Culture*

Culture of skin with bullous disease sera was performed using a modification of a previously described method [10]. Pieces of freshly keratomed foreskin (0.4 mm thick) were trimmed to 1.0 × 1.0 $cm^2$ with a razor blade and washed in sterile MEM containing 100 units of penicillin and 100 mg streptomycin sulfate/ml, tissue culture media (TCM). Skin was placed epidermis side up on a 12.0 mm-diameter, 5.0 $\mu$-pore size nitrocellulose disc (Millipore Corp., Bedford, Massachusetts), moistened with TCM and floated on 1.0 ml of TCM containing 25% heat-inactivated BPS, EBAS, NHS, or IgG fraction of EBAS. Cultures were performed in Limbro 35.0-ml tissue culture wells (Flow Laboratories) for 18 h at 32°C in a humidified 5% $CO_2$-air atmosphere. Skin was then removed, washed twice in TCM, and cut into 1 × 10 mm strips with a razor blade. One strip from the center of each piece of skin was quick-frozen in liquid nitrogen and processed for direct IgG and indirect C3-binding immunofluorescence. Remaining strips were cultured separately and in triplicate in 0.5-ml sterile polypropylene tubes (Brinkman Instruments Inc., Westbury, New York) containing 0.5 ml of: (1) $40 \times 10^6$ PBL/ml and 25% fresh NHS in GBSS; (2) $40 \times 10^6$ PBL/ml and 25% heat-inactivated NHS, or (3) 25% fresh NHS serum alone. Tubes were sealed and incubated 4 h at 37°C with gentle end-over-end rotation. Tissue strips were then washed in PBS and either quick-frozen in liquid nitrogen or fixed in formalin. Frozen tissue was processed for direct IgG and C3 immunofluorescence and fixed tissue for routine H & E histology.

There were a total of 34 skin strips treated with 4 C-binding BPS. Twelve were subsequently treated with PBL in fresh NHS, 12 with PBL in inactivated NHS, and 10 with fresh NHS alone. There were 26 strips treated with 3 C3-binding EBAS. Nine were treated with PBL in fresh NHS, 9 with PBL in heat-inactivated NHS, and 8 with fresh NHS alone. There were 16 strips treated with either of the 2 EBA IgG fractions. Six were subsequently treated with PBL in fresh NHS, 4 with PBL in heat-inactivated NHS, and 6 with fresh NHS alone. There were 17 strips of skin treated with either of the 2 non-C-binding EBAS. Six were subsequently treated with PBL in fresh NHS, 6 with PBL in inactivated NHS, and 5 with fresh NHS alone. There were 16 strips treated with control NHS during the initial incubation. Six were subsequently treated with PBL in inactivated NHS, 6 with PBL in fresh NHS, and 4 with fresh NHS alone. Ten to twenty 4 $\mu$m-thick sections from each strip were examined by H & E to evaluate leukocyte infiltration to the BMZ and DES.

## RESULTS

*Direct LA in BP and EBA Skin*

The results of direct LA in BP and EBA skin are shown in Table II. C-dependent LA in each of the 3 EBA biopsies was greater than in any of the 4 BP biopsies. When the average LA for the 3 EBA biopsies (204 ± 24 PBL/mm BMZ) was compared to the average in the 4 BP biopsies (50 ± 5), the difference was significant ($p < 0.001$). A comparison of LA among EBA and BP biopsies showed there was no significant difference within each group. When C-independent LA assays were performed, there was a significant decrease in LA in EBA biopsies (204 to 48) and in BP biopsies (50 to 27). These results suggest the differences in LA between the two groups are largely C-dependent.

*Indirect LA*

Indirect LA was performed simultaneously on cryostat sections of normal human skin treated with each of 4 BPS and 3 EBAS diluted 1:5, 1:10, 1:20, or 1:40. The results comparing the mean LA of three EBAS with 4 BPS are shown in Table

TABLE II. *Direct leukocyte attachment in BP and EBA skin*

| EBA biopsy # | Leukocyte attachment | | BP biopsy # | Leukocyte attachment | |
|---|---|---|---|---|---|
| | W[a]/complement | Wo[b]/complement | | W/complement | Wo/complement |
| 1 | 213 ± 21[c] | 64 ± 6 | 1 | 48 ± 6 | 26 ± 3 |
| 2 | 175 ± 18 | 72 ± 8 | 2 | 32 ± 4 | 18 ± 6 |
| 3 | 225 ± 32 | 49 ± 12 | 3 | 62 ± 2 | 34 ± 4 |
| | | | 4 | 58 ± 9 | 31 ± 4 |
| Average leukocyte attachment[d] | 204 ± 24 | 58 ± 9 | | 50 ± 5 | 27 ± 4 |

[a] W = With.
[b] Wo = Without.
[c] Standard deviation.
[d] Average leukocyte attachment in 3 EBA and 4 BP biopsies.

TABLE III. *Indirect leukocyte attachment using complement binding BP and EBA anti-BMZ antibodies*

| Serum titer | Leukocyte attachment (PBL/mm BMZ) | | | | | |
|---|---|---|---|---|---|---|
| | With complement | | | Without complement | | |
| | EBAS | BPS | (p) | EBAS | BPS | (p) |
| 1:5 | 171 ± 19 | 120 ± 30 | 0.02 | 53 ± 7 | 38 ± 12 | 0.1 |
| 1:10 | 156 ± 8 | 92 ± 22 | 0.001 | 51 ± 12 | 46 ± 8 | 0.1 |
| 1:20 | 133 ± 27 | 75 ± 32 | 0.02 | 38 ± 16 | 42 ± 14 | 0.1 |
| 1:40 | 134 ± 16 | 72 ± 14 | 0.001 | 43 ± 10 | 52 ± 21 | 0.1 |

III. The results of a representative experiment are shown in Fig 1. When the assay was performed in the presence of serum C, all 3 EBAS showed greater LA than any of the 4 BPS at equivalent serum dilutions. When dilutions of EBAS and BPS were adjusted to provide equivalent C-binding anti-BMZ antibody titers, the differences were even greater (results not shown). In the absence of a C source, there was no significant difference in LA mediated by BP or EBA sera at any of the 4 dilutions. These results show significantly greater C-dependent LA by immune complexes prepared in vitro with EBAS even though C-binding anti-BMZ antibody titers were less than those in BPS.

*Results of Immunofluorescence in Cultured Skin*

Representative strips of skin were examined by direct IgG and indirect C3-binding immunofluorescence following the initial 18-h incubation with bullous disease sera or IgG fractions. Skin strips were again examined following a 4-h incubation with PBL and NHS by direct IgG and C3 immunofluorescence. Following the initial incubation, linear IgG deposits were observed at the BMZ in all sections treated with BPS and EBAS and EBA IgG fractions containing C3-binding anti-BMZ antibodies. IgG deposits were seen in sections treated with only 1 of 2 EBAS containing non-C3-binding anti-BMZ antibodies. By indirect C3-binding immunofluorescence, all sections treated with C3-binding anti-BMZ antibodies showed linear C3 deposits at the BMZ. Following the 4-h incubation with PBL and fresh NHS, sections that had received treatment with C3-binding anti-BMZ antibodies continued to show linear IgG deposits at the BMZ but in addition showed C3 deposits. Sections that received treatment with C3-binding anti-BMZ antibodies and then PBL in heat-inactivated NHS showed IgG but no C3 deposits (Table IV). These results show that both C3-binding EBA and BP anti-BMZ antibodies bind to the BMZ in cultured skin and that these antibodies can mediate C3 deposition during a 4-h incubation with fresh serum.

*Results of Histologic Examination of Cultured Skin*

The results of histologic examination of skin cultured first with antibodies and subsequently with either PBL in fresh NHS or PBL in inactivated NHS are shown in Table V. Most sections cultured with C3-binding EBAS or IgG fractions, PBL, and fresh NHS showed leukocytes (mainly neutrophils) infiltrating the upper dermis and the BMZ (Fig 2). In the majority of these sections, foci of vacuolization or microvesiculation at the BMZ were noted. Foci of microvesiculation varied in length from several hundred microns to over a millimeter along the BMZ. In several sections the epidermis was completely lost and

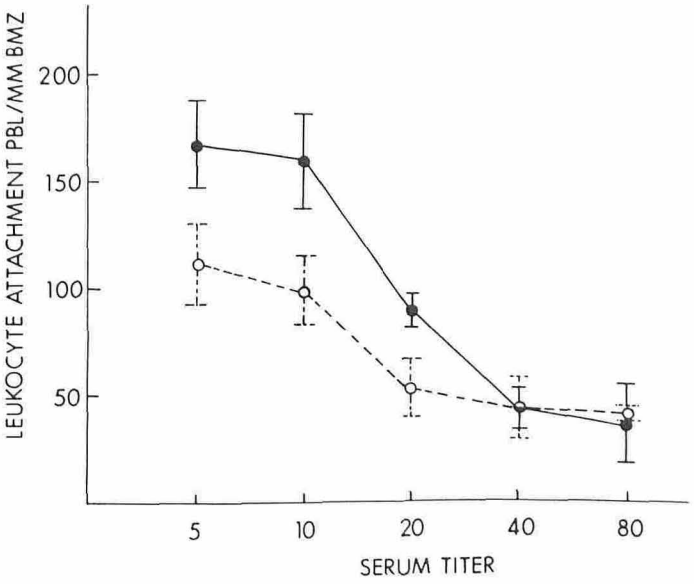


FIG 1. Comparison of LA between $BPS_1$ (O- - -O) and $EBAS_1$ (●——●) at dilutions of 1:5–1:80. C3-binding titers of $BPS_1$ and $EBAS_1$ were 1:320 and 1:160, respectively.

TABLE IV. *Results of immunofluorescence in skin cultured with anti-BMZ antibodies, leukocytes, and NHS*

| Antibody treatment | Serum # | PBL and fresh NHS | | PBL and inactivated NHS | |
|---|---|---|---|---|---|
| | | IgG | C3 | IgG | C3 |
| C3 binding BP sera | $BPS_1$ | + | + | + | − |
| | $BPS_2$ | + | + | + | − |
| | $BPS_3$ | + | + | + | − |
| | $BPS_4$ | + | + | + | − |
| C3 binding EBA sera | $EBAS_1$ | + | + | + | − |
| | $EBAS_2$ | + | + | + | − |
| | $EBAS_3$ | + | + | + | − |
| C3 binding EBA IgG | $EBAS_1$ | + | + | + | − |
| | $EBAS_2$ | + | + | + | − |
| Non-C3 binding EBA sera | $EBAS_4$ | + | − | + | − |
| | $EBAS_5$ | − | − | − | − |
| Controls | $NHS_1$ | ND | ND | ND | ND |
| | $NHS_2$ | ND | ND | ND | ND |

TABLE V. *Effect of anti-BMZ antibody on leukocyte infiltration and dermal-epidermal separation in skin organ culture*

| Antibody treatment | PBL + fresh serum | | | PBL + inactivated serum | | |
|---|---|---|---|---|---|---|
| | # Specimens examined | LI[a] (% specimens) | DES[b] (% specimens) | # Specimens examined | LI (% specimens) | DES (% specimens) |
| C3 binding BP sera | 12 | 17 | 0 | 12 | 0 | 0 |
| C3 binding EBA sera | 9 | 89 | 67 | 9 | 12 | 0 |
| C3 binding EBA IgG | 6 | 83 | 83 | 5 | 20 | 0 |
| Non-C3 binding EBA sera | 6 | 0 | 0 | 6 | 0 | 0 |
| NHS | 6 | 0 | 0 | 6 | 0 | 0 |

[a] LI = leukocyte infiltration to the BMZ.
[b] DES = Dermal-epidermal separation.

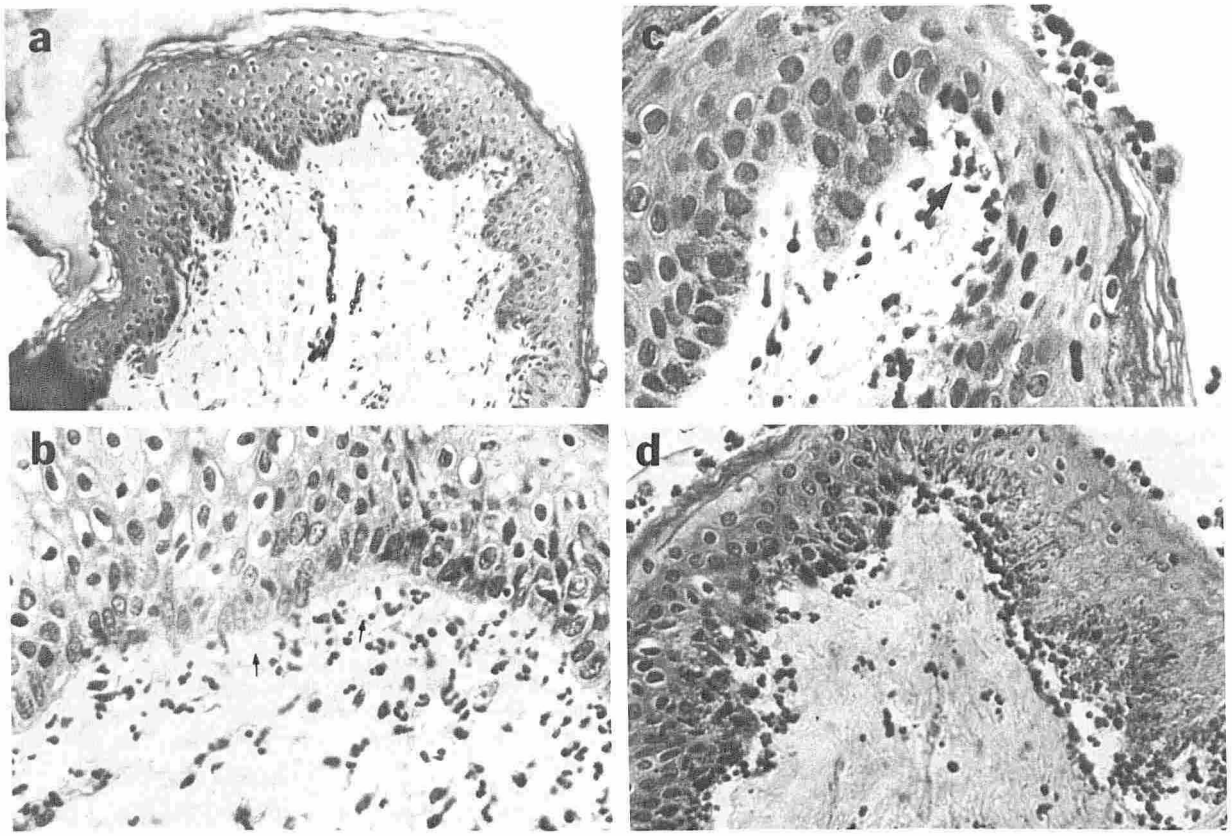


FIG 2. *a*, Histology of skin cultured with C3-binding BPS, PBL, and fresh NHS. No leukocyte infiltration or DES was seen (× 250). *b*, Histology of skin cultured with C3-binding EBAS, PBL, and fresh NHS. Leukocytes are present in the upper dermis and at the BMZ. Multiple foci of dermal vacuolization (*arrows*) are present just beneath the BMZ (× 500). *c*, Culture conditions same as (*b*). Note microvesicle (*arrow*) at BMZ filled with leukocytes (× 400). *d*, Culture conditions same as (*b*). Note almost complete DES and leukocytes infiltrating the BMZ (× 400).

the upper dermis revealed numerous neutrophils. When EBA-treated strips were incubated with PBL and inactivated NHS, there was a significant reduction in the percentage of sections showing leukocyte infiltration and none showed DES. Compared to EBA treated skin, there was little leukocyte infiltration and no DES in skin treated with BPS even though C-binding titers of those sera were greater than those in EBAS. No leukocyte infiltration or DES was seen in skin initially treated with control human sera nor in skin which was not treated with leukocytes.

## DISCUSSION

A role for C-activating anti-BMZ antibodies in the pathogenesis of BP lesions has previously been proposed and supported by a number of observations and experimental studies. These have included: deposits of IgG and C at the BMZ; circulating C-binding anti-BMZ antibodies; evidence for C activation and C5a chemotactic factor in blister fluids; and passive transfer of the immunologic and histologic features of BP in animals with C-binding BP antibodies [11–16]. Using LA assays, it was previously shown that immune complexes formed in vivo in perilesional BP skin and in vitro by treating normal human skin with C-binding BP antibodies could activate serum C at the BMZ and generate C-derived chemotactic activity, presumably C5a [6,9,17]. Furthermore, it was shown that leukocytes recruited to the BMZ of skin sections could cause BMZ injury and DES [18].

In this study LA assays have shown that EBA immune complexes formed in vivo and in vitro are also capable of activating C and mediating the attachment of leukocytes to the BMZ. A requirement for C in these studies was demonstrated by the significant drop in LA in the absence of fresh serum and when dilutions of EBAS in excess of C-binding titers were used. These studies show that EBA antibodies form functional immune complexes in vitro and more importantly that EBA immune complexes present in vivo are potentially capable of activating C and recruiting leukocytes to the BMZ.

In addition, EBA sera and IgG fractions containing C-binding anti-BMZ antibodies could sensitize viable human skin in vitro and mediate both the C-dependent infiltration of leukocytes to the BMZ and DES in organ culture. Evidence that EBA anti-BMZ antibodies were responsible for C activation was supported by the results of immunofluorescence showing IgG and C3 deposited along the BMZ. A role for antibodies in C activation was further supported by the finding that EBA IgG fractions were just as effective as EBAS in mediating leukocyte infiltration. Leukocyte infiltration to the BMZ was presumably dependent on C-derived chemotactic factors since infiltration required both C-binding EBA antibodies and fresh serum. DES appeared to depend on leukocyte infiltration since it was never observed in sections in which leukocytes had not infiltrated to the BMZ. The mechanism of leukocyte-mediated DES was not determined, but was presumably due to leukocyte-derived proteases, reactive oxygen intermediates, or both. These cells are known to produce or release both sets of mediators when stimulated by immune complexes. Evidence that reactive oxygen intermediates can cause DES has not been reported but there is evidence that leukocyte proteases can [19].

Previous authors have demonstrated BP antibody and C binding to the BMZ using a similar tissue culture system; however, to our knowledge, this is the first successful transfer of both histologic and immunologic features of a subepidermal bullous disease in organ culture [10,16]. These studies provide the first experimental evidence supporting a role for C-activating anti-BMZ antibodies in the pathogenesis of EBA lesions.

An unexpected finding in this study was the significantly greater activity of EBA complexes than BP complexes in assays requiring C activation. In the direct and indirect LA assays and in organ culture, immune complexes formed with EBA anti-

bodies were significantly more active in recruiting leukocytes than those formed with BP antibodies. The greater activity of EBA complexes formed in vitro was particularly surprising since titers of C-binding anti-BMZ antibodies in EBAS and IgG fractions were 2–3 doubling dilutions less than those in BPS. These results show that C activation by different anti-BMZ antibodies may vary independent of C-binding titers and suggest that other factors are important in determining the C-activating potential of complexes formed by these antibodies.

In organ culture, BPS containing C-binding anti-BMZ antibody titers as high as 1:640 were relatively ineffective in mediating leukocyte infiltration. This did not appear to be due to the absence of C-binding immune complex formation in tissue since IgG and C3 deposits were observed at the BMZ following incubation of BP treated sections with PBL and fresh NHS. However, it is possible that relative to EBA, fewer BP immune complexes were formed and activated less C. BP complexes are formed within the lamina lucida which is located between the lamina densa (basal lamina) and basal cell plasma membrane. EBA complexes are located in the upper dermis beneath the lamina densa or beneath the lamina densa-anchoring fibril zone [2]. Assuming anti-BMZ antibodies diffused through the dermis to reach their antigen, BP antibodies would have to travel further and pass through the lamina densa. The lamina densa could, as has been suggested, act as a physiochemical barrier which might retard diffusion of BP antibodies and limit their access to antigen [20]. However, greater diffusion distance and permeability barriers would not explain why EBA complexes were more potent in leukocyte recruitment in the indirect LA assay. In that assay, both BP and EBA antibodies should have direct access to their respective antigens in cryostat skin sections. An additional reason for the apparently greater activity of EBA immune complexes may be that there is a higher density of EBA complexes formed at the BMZ. Although the relative amounts of BP and EBA antigen in skin have not been quantitated, immunoultrastructural studies suggest EBA antigens occupy a larger portion of the BMZ. Whereas BP antigen is located within a 20–30 nm-wide lamina lucida, EBA antigen may occupy an area several hundred nanometers wide beneath the lamina densa [2]. Other factors affecting amounts of complexes formed or the amounts of C activated could include the relative amounts of antigens available to form complexes and the properties of the complexes themselves that affect C activation and regulation. It is unlikely that these properties can be determined until the complexes can be prepared in relatively pure form and studied in vitro.

The results of this study may be relevant to understanding the mechanisms whereby inflammation is initiated in BP and EBA and the differences we have observed in the types of inflammation in each disease. Most BP lesions are characterized by a predominance of eosinophils and relatively few neutrophils in the upper dermis [4,21]. In the cases of EBA whose tissues and sera were used in this study, neutrophils were a prominent component of the inflammatory infiltrate and, in some cases, the only granulocyte seen. Since neutrophils are more characteristic of C-mediated inflammation, C-dependent cell recruitment may play a greater role in the development of inflammation in EBA than in BP. The findings in this study would support that hypothesis. In this regard, it is of interest that the only other disease in which we have observed direct LA levels as great as those in EBA skin was in a form of bullous systemic lupus erythematosus which is also characterized by neutrophil-predominent inflammation [22].

It is important to note that the findings in this study may not be relevant to all EBA lesions. Many patients have been described with marked skin fragility and DES unassociated with significant inflammation [23–25]. Most of these patients have been seen relatively late in their disease and in very few have circulating C-binding anti-BMZ antibodies been present. Such cases would suggest that mechanisms other than C-mediated inflammation may be involved in the pathogenesis of EBA lesions.

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