Bullous Pemphigoid Autoantibodies Are Markers of Corneal Epithelial Hemidesmosomes

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Sera from patients with bullous pemphigoid (BP) contain autoantibodies that bind to the BP antigen, which is a component of the epithelial–stromal junction of the cornea. Previous studies, employing direct immunoelectron microscopy (IEM) on perilesional skin of patients have localized the BP antigen to the lamina lucida. On this basis, studies of corneal epithelial–stromal adhesion and wound healing have employed BP antigen as a marker of the lamina lucida of the corneal basement membrane zone (BMZ). The authors used indirect IEM with BP autoantibodies on frozen sections of cornea and found that the majority of the BP antigen is intracellular and is closely associated with the corneal epithelial hemidesmosomes. Only a small amount of BP antigen appears to be extracellular, limited to the portion of the lamina lucida directly beneath individual hemidesmosomes. When rabbit corneal epithelium is extracted and analyzed by Western immunoblotting, BP autoantibodies recognize two polypeptides of molecular weights of 240 and 180 kilodaltons, which comigrate with BP antigens extracted from epidermis. BP autoantibodies are a specific marker of corneal epithelial hemidesmosomes and can be used as a probe to identify and study the role of hemidesmosomes in epithelial–stromal adhesion. Invest Ophthalmol Vis Sci 28:903–907, 1987
Fig. 1. Demonstration of bullous pemphigoid antigen in the basement membrane zone in human corneal epithelium (A) and rabbit corneal epithelium (B) by indirect immunofluorescence. Frozen sections of cornea were incubated first with serum from a patient with bullous pemphigoid at a dilution of 1:20, and then fluorescein-labeled antihuman IgG. Binding of BP antibodies to the basement membrane zone is evident. (250X).

The serum of patients with Bullous pemphigoid (BP) contain autoantibodies against the BP antigen, which is a normal component of the basement membrane zone (BMZ) of cornea and skin. Previous studies, using direct immunoelectron microscopy (IEM) of skin from patients with BP showed that IgG autoantibodies were deposited in the lamina lucida of the BMZ, and it was assumed that the BP antigen was an extracellular protein that could be used as a marker of the lamina lucida of cornea and skin. Recently, however, using indirect IEM with BP antibodies, it was found that in epidermis the major pool of BP antigen is located in the cytoplasmic plaque of the epidermal hemidesmosome (HD), and that the linear fluorescence of the BMZ detected in studies using BP antibodies is due to binding to an intracellular antigen pool and not a lamina lucida antigen.

The authors employed indirect IEM on cornea and demonstrate that the major pool of BP antigen is localized to the cytoplasmic plaque of the corneal epithelial HD. Western blot analysis of rabbit corneal epithelial extracts show that BP autoantibodies recognize two polypeptides of M, 240 and 180 kilodaltons that comigrate with BP antigens extracted from human epidermis, suggesting that BP antigen in cornea and skin are similar. BP autoantibodies can be used as specific markers of corneal epithelial HD in wound healing and cell-matrix studies.

Materials and Methods. Preparation of corneas: Human corneas were obtained from two eye bank globes stored in moist chambers for less than 24 hr. Rabbit corneas were dissected from euthanized New Zealand white rabbits. Procedures used conformed to the ARVO Resolution on the Use of Animals in Research. The corneas were removed from the globe, bisected, embedded in OCT compound (Miles; Naperville, IL.), and 10-μm cryosections were processed by immunofluorescence (IF) and IEM.

Sera: After securing informed consent, serum was obtained from a patient with the typical clinical, histologic, and immunologic features of BP. The IF titer of BP autoantibodies in the serum was 1,280, using monkey esophagus as the tissue substrate. BP serum and normal human sera (NHS) were used at a 1:20 dilution in phosphate-buffered saline (PBS) in all experiments.

Immunofluorescence: IF was performed as previously described, using fluorescein-isothiocyanate labeled goat antihuman IgG (Cappel Worthington, Cochranville, PA). All specimens were examined with a Zeiss fluorescent microscope (Carl Zeiss, New York, NY).

Immunoelectron microscopy: Unfixed 10-μm cryosections of rabbit and human cornea were incubated with a 1:20 dilution of BP serum or NHS for 30 min, washed in PBS and treated with a 1:100 dilution of peroxidase-labeled goat antihuman IgG (Cappel) for 30 min and washed in PBS. Specimens were fixed in 1% glutaraldehyde (Electron Microscopic Sciences, Fort Washington, PA) for 5 min, washed in PBS, treated with 0.05% diaminobenzidine (DAB) in 0.05 M Tris-HCl, pH 7.6 (both from Sigma; St. Louis, MO) for 20 min, fixed in 2.5% glutaraldehyde and prepared for IEM. Ultrathin sections were observed without counterstaining for better visualization of DAB deposition.

Immunoblots: Western immunoblots were performed by the technique of Labib et al. Briefly, the epithelium from the corneas of four rabbit eyes was separated from the stroma by heating at 56°C for 30 sec, cooled on ice, and extracted in 1% SDS and 5% B-mercaptoethanol in the presence of six bacterial proteinase inhibitors, followed by SDS-polyacrylamide gel
Fig. 2. Indirect immunoelectron microscopic localization of bullous pemphigoid antigen in human corneal epithelium. Ten micron cryosections were incubated with a 1:20 dilution of bullous pemphigoid serum, followed by peroxidase-labeled goat antihuman IgG, treated with diaminobenzidine, and processed for electron microscopy. Reaction product is localized to the cytoplasmic plaque of the hemidesmosome (HD). This reaction is specific for HD and is not associated with desmosomes. (S) Stroma; (E) epithelial cell; arrowheads identify the lamina densa.

Results. **Immunofluorescence:** Indirect IF of cryosections of intact human and rabbit cornea demonstrate typical linear staining along the BMZ in both humans and rabbits, confirming previously reported results (Figs. 1A, B).  

**Immunoelectron microscopy:** Indirect IEM of intact human cornea reveals localization of reaction product to the hemidesmosomes (HD) of the basal epithelial cells (Fig. 2). Reaction product is not associated with desmosomes. Higher power immunoelectron micrographs of human cornea (Fig. 3) reveals finer ultrastructure of the BMZ. Densely staining HD are seen and the associated intracellular tonofilaments are faintly visualized. The lamina lucida and lamina densa of the BMZ are seen. There is a suggestion that the...
Fig. 4. Antigens recognized by BP sera in extracts of rabbit cornea (C) and human epidermis (E). Immunoblots were prepared according to the technique of Labib et al. In lane c, polypeptides of molecular weights of 240 and 180 kilodaltons are recognized by BP serum in an extract of rabbit corneal epithelium. No other bands are recognized. Polypeptides of apparently similar molecular weights are recognized in an extract from human epidermis prepared in the same way, suggesting that the BP antigen in cornea and epidermis are similar. Faint bands at other molecular weights represent nonspecific background staining similar to that seen when normal human serum is substituted in place of the BP serum (not shown).

lamina lucida directly adjacent to the HD stains very lightly with reaction product, whereas the lamina lucida not opposed to the HD does not. Control sections, incubated with normal human serum, did not show any specific immune deposits.

Western blot analysis: Extracts of rabbit corneal epithelium and human epidermis were subjected to Western blot analysis, and BP autoantibodies specifically recognized two polypeptide chains: M_r, 240 and 180 kilodaltons in both tissues (Fig. 4).

Discussion. Previous studies of corneal epithelial-stromal adhesion have been conducted and interpreted using BP antigen as a marker of the lamina lucida. However, several recent studies show that in epidermis most of the BP antigen is cytoplasmic, in association with basal cell HD.8,9

Our IEM demonstrates that in rabbit and human cornea, the antigen recognized by autoantibodies from a patient with BP is localized almost exclusively to the HD of the basal cell. The suggestion of faint staining in the lamina lucida directly in apposition to the basal cell HD may correlate with the detection of BP antigen in the lamina lucida that others have observed,5-7 but given the poor resolution of IEM on unfixed frozen sections, precise definition of the distribution of putative intracellular and extracellular antigen pools will require other techniques.

There are several points that may help clarify the discrepancy between the localization of BP antigen to the HD and previous reports of its localization to the lamina lucida. Recent studies8,9 show that disruption of the plasma membrane of the basal cell, either by saponin treatment, freezing, sectioning, or trauma is necessary for the BP IgG to gain access to the intracellular antigen. Initially, direct IEM was performed on perilesional skin from patients.7 This tissue was chosen because the lack of inflammation would allow optimal preservation of ultrastructural morphology. However, the plasma membrane of the basal cell would be intact, and thus there could not be binding of antibodies with intracellularly located HD. These studies generally localized autoantibody deposition to the lamina lucida, and it was assumed that that was the only location of the antigen in situ. Even in these studies, however, some binding of BP antibodies to HD was noted 5-7. In our study, unfixed tissue was sectioned prior to exposure to BP antibody, and the IgG bound strongly with intracellular HD, and perhaps weakly with antigen present in the lamina lucida. Using frozen sections without prefixation does not allow optimal preservation of ultrastructural morphology but does allow access of antibody to cytoplasmic antigens that are destroyed by prefixation.

Secondly, there appears to be more than one antigen reactive with BP antisera. Yamasaki and Nishikawa10 using IEM found that with some BP sera, the IgG autoantibodies bound along the cytoplasmic side of epidermal basal cell membranes in large discontinuous aggregates (HD), whereas when complement fixation was employed, the reaction products were distributed predominantly in the lamina lucida in a continuous fashion. This would imply that a population of complement fixing autoantibodies antibodies bound an antigen in the lamina lucida while another population of noncomplement fixing autoantibodies bound an antigen associated with the intracellular attachment plaque area of the HD. Sera from patients with BP have been tested against human epidermis by immunoblotting and most consistently reacted with protein bands of 240 and 180 kilodaltons.4 However, the 28 BP sera tested recognized up to five different polyepi-
tide chains, implying that different BP patients may have various sets of autoantibodies reactive with epidermal antigens. The BP serum we tested reacted with protein bands of 240 and 180 kilodaltons in rabbit corneal and human epidermal epithelium, implying that by this criteria, the BP antigen is similar in both cornea and epidermis. Similar studies with the less frequently detected autoantibodies in BP sera are being performed. Immunoblots with 24-hr-old eyebank human corneas detected similar bands at 240 and 180 kilodaltons, but several additional bands that were likely proteolytic fragments were also detected (not shown). It should also be noted that although IEM points to the HD as the site of BP antigen, the authors cannot conclude conclusively from these data that the proteins detected by immunoblotting with BP sera are in fact the same HD proteins that are identified by IF and IEM techniques.

Fujikawa et al\(^2\) have reported a detailed analysis of BMZ components in healing rabbit corneal epithelial wounds. When the corneal epithelium was removed by a scrape wound, BP antigen was also removed. The migrating corneal epithelium lacked HD, and by IF the BP antigen was absent. BP antigen reappeared after migration was complete, at which time EM showed that HD had reformed. Their observation that the absence or presence of the antigen (by IF) correlates with the presence or absence of HD (by EM), provides additional correlative evidence for the authors' finding that BP antigen is localized to the HD.

Hemidesmosomes may play an important role in the adherence of stratified squamous epithelium to the underlying stroma. Definition of HD formation, composition, and function may be important for understanding the biology of corneal epithelial adherence and migration. These findings demonstrate that the BP antigen is a component of the HD and suggest that HD formation and presence may be studied by light microscopy using IF with BP sera.

Key words: bullous pemphigoid, hemidesmosomes, immunoelectron microscopy, Western blot, cornea, wound healing

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References


Clearance and Localization of Intravitreal Liposomes in the Aphakic Vitrectomized Eye

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The authors have examined the fate of intravitreally injected liposomes in the aphakic, vitrectomized eye of the rabbit. Liposomes labelled with \(^{125}\)I-p-hydroxybenzimidylphosphatidylethanolamine were eliminated rapidly from the intraocular fluid. Nonetheless, a significant fraction of these liposomes were found to bind to various ocular tissues including the retina, iris, sclera, and cornea. Ultrastructural studies with gold colloid-loaded liposomes revealed that ret-