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## Pemphigus and Pemphigoid as Paradigms of Organ-specific, Autoantibody-mediated Diseases

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The blistering skin diseases pemphigus and pemphigoid can be considered paradigms of antibody-mediated, organ-specific autoimmune diseases. These diseases not only demonstrate mechanisms whereby autoantibodies can mediate tissue damage, but are also examples of how autoantibodies from patients can be used as tools to further our understanding of the molecular structure of normal tissue. In this Perspectives article, I will briefly describe the clinical, histologic, and immunopathologic features of these diseases. I will then discuss in more detail the newer data regarding the pathophysiology of these diseases, as well as the molecules defined by the autoantibodies from these patients.

### *Clinical and histologic features*

The clinical and histologic aspects of pemphigus and pemphigoid are well established (Table I) (1). There are two major types of pemphigus, called pemphigus vulgaris (PV)<sup>1</sup> and pemphigus foliaceus (PF). Patients with PV almost always develop, and often present with, mucous membrane erosions. Skin lesions, which are seen as flaccid blisters or erosions, tend to gradually enlarge at the edges. PV, if left untreated, is almost always fatal. The histology of a PV lesion shows that the blister (or erosion) forms because of separation of epidermal cells from each other (a process called acantholysis) just above the basal cell layer. In contrast to PV patients, PF patients rarely have mucous membrane lesions. They usually present with scaly and crusted skin lesions, which, unlike PV lesions, do not tend to form extensive and enlarging erosions, but are fixed and well demarcated. Fogo selvagem (also called Brazilian PF) is a form of PF that is endemic in rural areas of Brazil and often affects children and young adults (2). The histology of a PF lesion demonstrates blister formation due to acantholysis in the superficial epidermis at the granular layer. With these histologic findings in mind, both major types of pemphigus, PV and PF, can be thought of as diseases of epithelial cell adhesion, with the blister or erosion a result of loss of adhesion at different levels within the stratified squamous epithelium of skin or mucous membrane.

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1. *Abbreviations used in this paper:* BMZ, basement membrane zone; BP, bullous pemphigoid; PF, pemphigus foliaceus; PV, pemphigus vulgaris.

In contrast to the flaccid blisters, erosions, and crusted lesions seen in pemphigus patients, bullous pemphigoid (BP) patients classically present with tense blisters on normal-appearing or erythematous skin. Whereas pemphigus lesions are due to intraepidermal blisters, histology of a BP lesion indicates a subepidermal blister with an infiltrate containing eosinophils in the superficial dermis and at the epidermal basement membrane zone (BMZ). Clinically and histologically similar to BP, herpes gestationis (also called pemphigoid gestationis) is a subepidermal, autoantibody-associated blistering disease that occurs during the second or third trimester of pregnancy, and spontaneously resolves in the months after delivery.

### *Immunopathology*

Over 20 years ago, Beutner and Jordon established that BP and pemphigus were associated with autoantibodies (3). Both direct immunofluorescence of patients' lesional and perilesional skin and indirect immunofluorescence with patients' sera on normal skin substrates, have demonstrated antibody binding to the same areas of skin that are involved with pathologic change in these diseases (Table I). Thus, BP patients have in vivo bound and circulating antibodies against the epidermal BMZ and pemphigus patients have antibodies against the cell surface of epidermal cells. Fogo selvagem patients display the same immunopathology as other pemphigus patients (4).

Direct immunofluorescence of perilesional skin from BP patients has also demonstrated C3 at the BMZ in virtually all cases. In addition, other elements of the classical and alternative pathway of complement activation may be present, and the anti-BMZ antibody is capable of complement fixation by the classical pathway (5). Similarly, in virtually all herpes gestationis patients, C3 (and sometimes IgG) is present at the epidermal BMZ, and the patients have a circulating IgG that is capable of binding the BMZ of normal skin and fixing complement (6, 7).

C3 usually is not present in perilesional skin of pemphigus patients, but may be present in lesional skin (5). Recent in vitro studies have shown the ability of both PV and PF sera, including fogo selvagem sera, to fix complement by the classical pathway (8-10).

An unexplained feature of the direct and indirect immunofluorescence of pemphigus antibodies is the pattern of binding within the epidermis. Although PF and PV lesions show blister formation at different levels of the epidermis, in most cases the pattern of PF and PV autoantibody binding is identical, with binding on the surface of cells throughout the epidermis (11). In a few unusual cases, however, PF antibodies that bind only the superficial epidermis have been reported (12, 13). In general, though, PF and PV cannot be distinguished on the basis of their direct or indirect immunofluorescence pattern.

Table I. Clinical, Histologic, Immunopathologic, and Immunochemical Characteristics of Pemphigus and Pemphigoid

	Pemphigus vulgaris	Pemphigus foliaceus	Bullous pemphigoid
Clinical	Mucous membrane and skin erosions, flaccid blisters	Scaly, crusted lesions of skin	Tense blisters on normal and erythematous skin
Histologic	Loss of adhesion with blister in lower epidermis	Loss of adhesion with blister in upper epidermis	Subepidermal blister
Direct immunofluorescence	In vivo bound IgG on cell surface of keratinocytes	Same as in pemphigus vulgaris	In vivo bound C3 and IgG at epidermal basement membrane
Indirect immunofluorescence	Serum IgG binds cell surface of keratinocytes throughout normal epidermis	Same as in pemphigus vulgaris	Serum IgG binds normal epidermal basement membrane
Immunochemical characterization of antigens	130-kD antigen disulfide linked to 85-kD polypeptide	Desmoglein, 160-kD desmosomal glycoprotein	230-kD protein of hemidesmosomes

### Pathogenicity of autoantibodies

There is excellent evidence that pemphigus and BP autoantibodies mediate disease. The mechanisms whereby these autoantibodies are thought to cause blister formation provide excellent examples of different mechanisms whereby autoantibodies can mediate disease.

Initial evidence that pemphigus antibodies cause blister formation came from studies of skin in organ culture. Both PV and PF IgG, without complement, are capable of inducing acantholysis in this organ culture system, at the same level in the epidermis as in actual disease (14, 15). The pathogenicity of both PV and PF antibodies has been confirmed in an in vivo model, in which pemphigus IgG is passively transferred to neonatal mice that subsequently develop blisters and erosions, with acantholysis at the appropriate level of the epidermis (16, 17).

The mechanism whereby these pemphigus antibodies induce acantholysis is an area of active investigation. Organ and cell culture studies have suggested that binding of pemphigus autoantibodies to the keratinocyte cell surface causes release of a protease, which, in turn, causes acantholysis (18–20). The identity of this proteolytic enzyme is in dispute. Some studies suggest that it is plasminogen activator that, once released by keratinocytes, activates plasminogen to plasmin, which, in turn, causes the acantholysis (15). Antibodies to the urokinase-type plasminogen activator were also shown to inhibit development of acantholysis induced by pemphigus IgG in organ culture (21). The in vivo neonatal mouse model of pemphigus was used to confirm that PV antibodies cause an increase in plasminogen activator in the skin; however, dexamethasone inhibited this increase, but did not prevent blister formation, suggesting that plasminogen activator might not be directly involved in blister formation (22).

The role of complement in the induction of blister formation in pemphigus is controversial. Clearly, pemphigus sera are capable of causing acantholysis in skin organ culture and detachment of keratinocytes in cell culture without complement (14, 18, 23). However, complement does enhance pemphigus antibody-mediated detachment of cultured epidermal cells, probably by damage to cell membranes (24, 25). Neonatal mouse studies with passive transfer of PV IgG indicate that complement is not necessary for blisters to occur, but adequate complement levels will enhance blister formation (26). These

studies clearly demonstrate that complement is not required for blister formation in pemphigus, but the fixation of complement by pemphigus IgG might amplify the resulting acantholysis.

Finally, in light of the discussion below of the binding of pemphigus antibodies to desmosomal components, it must be considered that these autoantibodies might interfere directly with cell adhesion.

Although the data are not as definitive as those shown with pemphigus antibodies, BP autoantibodies are probably also pathogenic, not directly but through activation of complement and recruitment of inflammatory cells. In vivo studies indicate that BP IgG injected into rabbit corneas causes fixation of IgG and C3 at the corneal BMZ, resulting in inflammation and subepithelial blister formation (27). Other in vivo studies are not as clear. One study indicates that BP IgG injected in guinea pig skin causes subepidermal blister formation, but only in the presence of an intact alternative complement pathway (28). However, another study could not reproduce these findings of blister formation in guinea pig skin with BP antibodies (29). Some of the more convincing studies of the pathogenicity of BP IgG, and the pathophysiology of blister formation, come from in vitro studies in which BP IgG and a source of complement and leukocytes are added to cryostat skin sections (30). All three elements, BP IgG, complement, and neutrophils, are necessary for dermal-epidermal separation to occur. The above studies suggest that BP autoantibodies mediate blister formation through fixation of complement and the resultant inflammation induced at the BMZ. However, as in the case of pemphigus, it must be considered that, because BP antibodies bind hemidesmosomes (see discussion below), organelles presumed to be important in cell attachment to the BMZ, these antibodies might also contribute directly to loss of basal cell attachment to the basement membrane.

### Autoantibodies as tools for diagnosis and understanding the molecular structure of normal tissue

It is becoming increasingly clear, from recent studies of antigens with autoantibodies from patients, that BP, PF, and perhaps PV are autoimmune diseases whose targets are molecules found in hemidesmosomes and desmosomes, adhesion organelles (Table I). This characterization of these antigens has not only resulted in the new hypotheses mentioned above regard-

ing potential pathophysiologic mechanisms in these diseases, but has also given us the ability to diagnose these diseases at a molecular level. Furthermore, the definition of these antigens has contributed to our understanding of the nosology of these diseases by firmly establishing that fogo selvagem is a subgroup of PF and by suggesting a relationship between herpes gestationis and BP.

Pemphigus and BP antigens are normal components of the cell surface and BMZ, respectively, of stratified squamous epithelial tissue. Indirect immunofluorescence studies indicate that pemphigus antigen is detectable in all mammals and birds, whereas BP antigen is detected in all vertebrates (31). Thus, the components defined by these autoantibodies are phylogenetically conserved and, presumably, subserved important tissue-specific functions. Recent technological advances in immunochemical characterization of antigens, as well as the demonstration of these antigens in keratinocyte cell culture, have facilitated their characterization.

Immunoprecipitation analysis of metabolically labeled mouse and human cultured keratinocytes has indicated that PV, but not PF, sera bind a 130-kD glycopeptide that is disulfide-linked to an 85-kD peptide (32). The same PV complex that is synthesized by cultured keratinocytes has also been identified in extracts of normal human epidermis by all of 22 sera from different PV patients but not by PF, normal, and other disease control sera (33).

PF antigen could not be precipitated from cultured cells, but immunoblotting of SDS extracts of normal human epidermis revealed that about one-third of PF sera bound a 160-kD peptide (32, 34). Further studies of nondenaturing extracts of normal human epidermis revealed that all PF sera bind a complex containing the 160-kD peptide (see below) (35). Fogo selvagem sera showed the same antigenic specificity as PF sera, and like PF sera did not bind the PV antigen complex, as synthesized by cultured keratinocytes or extracted from normal epidermis (33–35). These findings established that PV and PF have unique and characteristic antigenic specificities at a molecular level and that fogo selvagem and PF are not only clinically, but also antigenically, related diseases. PV and PF can also be distinguished and diagnosed at a molecular level by identification of the respective antigens.

Recent studies have addressed the question of the relationship of pemphigus antigens to desmosomes, organelles of cell adhesion. Progress in the biochemical characterization of desmosomal proteins and glycoproteins, mostly as obtained from bovine muzzle, has made this question feasible to answer. An immunofluorescence study of mouse epidermal cells in culture suggested that pemphigus (type unspecified) antibodies bound in the same location as desmosomes (36). However, an immunofluorescence study on monkey esophagus with antiovine desmosomal antisera and PV sera demonstrated that the pattern of binding was different (37). In addition, enzyme-linked immunoassays of bovine muzzle desmosome components with PV sera did not show antibody binding (37). These data suggest that PV sera probably do not bind desmosomal components, at least as derived from bovine muzzle. More detailed studies confirmed that PV sera do not bind to peptides extracted from bovine muzzle desmosomes as determined by immunoblotting, but do bind by immunoblotting to a 140-kD protein in desmosomal extracts of bovine tongue (38, 39). Antibodies raised to this 140-kD peptide from bovine tongue bind a 130-kD protein from mouse keratinocytes (perhaps similar

or identical to the 130-kD glycoprotein discussed above) and, as determined by immunogold electron microscopy, bind to the desmosome, but also the keratinocyte cell surface between desmosomes (39). These immunoelectron microscopic findings are similar to those previously reported with PV antibodies bound *in vivo* (40). In summary, then, the relationship of PV antigen to desmosomes is clouded, but the data indicate that PV antigen is not one of the known desmosomal molecules defined by analysis of bovine muzzle desmosomes, but is probably in desmosomes as well as on nondesmosomal cell membranes of keratinocytes.

The current evidence associating PF autoantibodies and a well-characterized component of desmosomes is much clearer. PF autoantibodies bind to desmoglein I, a glycoprotein in the core of desmosomes (35, 38, 41, 42). For example, immunoblots of extracts of normal human epidermis, separated by two-dimensional gel electrophoresis, have demonstrated that PF antibodies and polyclonal, as well as monoclonal, antibodies to desmoglein I bind comigrating polypeptides of ~ 160 kD and pI 5 (41, 42). In addition, PF IgG, affinity purified on the 160-kD PF antigen extracted from normal human epidermis, binds to desmoglein I extracted from bovine muzzle desmosomes (41). Finally, because, as discussed above, only about one-third of PF sera bind to the 160-kD desmoglein I by immunoblotting, a less denaturing procedure of human epidermal extraction was used to characterize PF antigen (35). This procedure demonstrated that all PF sera bind a polypeptide complex containing desmoglein I and that most of these sera bind to a calcium-sensitive epitope on this complex. This finding is of interest in light of the importance of calcium in desmosome formation and suggests that a possible contributing factor in the pathophysiology of blister formation in PF might be direct antibody interference with desmosome assembly.

Just as PF, and possibly PV, autoantibodies bind desmosomal components, BP sera may define components of the hemidesmosome. However, because the molecular composition of hemidesmosomes has not been determined, the association of BP antibodies and hemidesmosomes has been determined by immunoelectron microscopy (43–46). Because BP autoantibodies bind hemidesmosomes, they can be used as tools to define some of their molecular constituents.

The molecular characterization of BP antigen has used the same immunochemical methods discussed above for pemphigus antigens. Immunoprecipitation of extracts of mouse and human cultured keratinocytes has indicated that BP antigen is a 230-kD polypeptide (with a pI of ~ 8), distinct from other known BMZ molecules such as laminin, fibronectin, and type IV collagen (47–49). This 230-kD polypeptide has also been identified by immunoblotting of extracts of normal human epidermis, however immunoprecipitation is a more sensitive method of detection, with 36 out of 37 BP sera precipitating the 230-kD peptide (49–51). Even though the 230-kD antigen is the major BP antigen, there have been recent data suggesting some heterogeneity of this antigen (51, 52). Immunoblotting studies of human epidermal extracts have shown that some BP sera (about 30–50%) bind a 180-kD polypeptide, and even a lesser fraction of BP sera may bind some peptides other than the 230- and 180-kD molecules (51). How all these polypeptides are related is unclear, but one very interesting observation has been that 87% of herpes gestationis sera recognize the same 180-kD polypeptide as do about half of BP

sera (53). These data suggest a close relationship between autoantibodies, which probably mediate disease, in BP and herpes gestationis.

As with other autoantigens, in addition to immunochemical techniques, molecular biologic methods are now being applied to characterize BP antigen (54). Patients' autoantibodies have been used to screen a  $\lambda$ gt11 expression library of cDNA reflecting mRNA extracted from cultured keratinocytes, known to synthesize BP antigen. A 2.1-kb cDNA clone with a 1992-bp open reading frame, encoding a unique peptide sequence of 76 kD, has been isolated. IgG, affinity purified on the peptide encoded by this clone, stained the epidermal BMZ by immunofluorescence, and immunoprecipitated the 230-kD BP antigen. Northern analysis with this clone indicated that the mRNA encoding the BP antigen is 9 kb. These data demonstrate the utility and feasibility of using autoantibodies from patients to isolate cDNA, and ultimately genes, for corresponding antigens, even if little is known about their protein sequence or structure.

#### *Abnormal immune response*

Although much has been learned regarding the antigens involved in pemphigus and BP and the mechanisms of autoantibody-mediated blister formation, little is known about why patients form the autoantibodies in the first place. However, as reported for other autoimmune diseases recent studies of immune response class II MHC genes in PV patients suggest that there may be specific haplotypes that predispose (or are even necessary) for autoantibody formation against PV antigen.

Serologic analysis of class II HLA-D haplotypes of PV patients has indicated that development of PV is associated with the DR4 and DRw6 alleles (55). However, the same serologically defined haplotypes in the HLA-D region, when examined in more detail by molecular analysis, may display heterogeneity. Such detailed molecular analysis of the HLA-D region of PV patients and controls has been performed by characterization of restriction fragment length polymorphisms generated by sequence variability and by direct DNA sequence determination. These methods have indicated a strong association with PV of certain polymorphisms, or variants, in the HLA-D regions. Specifically, patients with PV and the HLA-DR4 serotype have a specific sequence (corresponding to the Dw10DR $\beta$ I allele) in the region encoding a DR $\beta$  chain in much higher frequency than control DR4 individuals (56, 57). Similarly, serologically defined DRw6 PV patients have a sequence in the DQ region, encoding a DQ $\beta$  chain, associated with disease susceptibility (56, 58).

Why these variant sequences in HLA-D loci are correlated with susceptibility to PV (or for that matter any autoimmune disease) is unclear, but may have to do with the ability of T cells to recognize specific antigens in association with specific class II HLA molecules.

#### *Future directions*

Many questions remain regarding these autoimmune blistering skin diseases, and for most of these the techniques are now available to extend previous findings and to address these issues in the next several years.

If pemphigus and BP are indeed anti-adhesion junction molecule autoimmune diseases, then do these autoantibodies interfere with function of adhesion organelles? What other elements contribute to the pathophysiology of blister forma-

tion? For example, is plasminogen activator the central protease involved in causing blisters? Why do pemphigus and BP affect only stratified squamous epithelia when desmosomes and hemidesmosomes are found in all epithelia? A partial answer to this latter question may be that autoantibodies in these diseases bind to epitopes of these adhesion junction molecules that are only expressed in stratified squamous epithelia (59).

Characterization of the genes for the antigens defined by these autoantibodies will be helpful in many areas, including diagnosis and further characterization of the protein antigens themselves, leading to more detailed knowledge of the biochemistry of desmosomes and hemidesmosomes. In addition, characterization of cDNA for these antigens will make possible the production of antibodies made in animals to various parts of the antigens. These will prove useful to study pathophysiology of disease and for further study of cell adhesion organelles. Expression of parts or all of this cDNA as peptides, as well as synthetic peptides, could be used for studies of the epitopes to which patients make autoantibodies. These peptides could, for example, be used to study idiotype response in different patients with varying degrees and presentations of disease. Synthetic peptides and molecularly engineered antigens could also conceivably be used as a therapy for these diseases by specific antibody absorption techniques.

Finally, at a more basic level, the antigens defined by these diseases are epidermal differentiation molecules. For example, BP antigen is found in and produced by basal cells, but not by more differentiated cells (60). Ultimate characterization of genomic DNA for these antigens will be valuable for the study of the regulation of differentiation in epidermis.

#### *Conclusions*

Pemphigus and BP are models for what can be, and has been, accomplished with autoantibodies from patients in understanding pathogenesis of disease as well as the structure and function of normal tissues. Similar studies have been performed with other tissue-specific, autoantibody-mediated diseases, such as myasthenia gravis and Goodpasture's Syndrome. Even in non-organ-specific autoimmune diseases, such as systemic lupus erythematosus, autoantibodies have been used as tools to define the biochemistry and function of molecules important in cell physiology. These autoantibody-mediated diseases clearly reinforce the general principle that the study of disease also helps us to understand normal tissue.

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