Recent Progress in Studies on Autoantigens for Various Autoimmune Blistering Skin Diseases

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(Received for publication on August 7, 1995)

Abstract. Autoimmune blistering skin diseases develop separation either between epidermal keratinocytes or dermo-epidermal junction. Recent studies have revealed that the autoantigens for these diseases are components of either the desmosome, the cell adhesion junction between keratinocytes, or the hemidesmosome complex, cell adhesion machinery at the dermo-epidermal junction. Thus, the major pemphigus antigens are desmogleins, one of desmosomal cadherins. Both the 230 kD and 180 kD bullous pemphigoid antigens are present in the hemidesmosome, and epidermal basement membrane zone-specific extracellular matrices, epiligrin and type VII collagen, are detected by sera of cicatricial pemphigoid and epidermolysis bullosa acquisita, respectively. Furthermore, animal model studies using newborn mice have revealed that these autoantibodies are really pathogenic and can induce blister formation by passive transfer into mice. Therefore, these skin diseases seem to be a typical model for various autoimmune diseases, for most of which the role of autoantibodies has not yet been revealed. (Keio J Med 44 (4): 115–123, December 1995)

Key words: autoimmune bullous skin disease, bullous pemphigoid, desmosome, hemidesmosome, pemphigus

Introduction

To establish stable architecture of the epidermis, strong adhesion either between epidermal keratinocytes or at dermo-epidermal junction is critical (Fig 1). It is well known that, among the adhesive junctions between cells, such as the tight junction, the adherence junction, the gap junction and the desmosome, the most important machinery is considered to be the desmosome. As adhesive machinery at the dermo-epidermal junction, the hemidesmosome and the focal contact are known. Dermo-epidermal adhesion is maintained mainly by the hemidesmosome and the structures under the hemidesmosome. Below the hemidesmosome, anchoring filaments are seen in lamina lucida, which connect the epidermis to lamina densa. In the uppermost portion of the dermis anchoring fibrils are seen, which then bind to the interstitial collagen in the dermis. Keratin intermediate filaments insert into attachment plaques of both the desmosome and the hemidesmosome. The intermediate filaments also associate with nuclear envelop via molecules called lamins. Actin microfilaments associate with plasma membrane at the adherens junction and the focal contact.

The patients with autoimmune blistering skin diseases carry autoantibodies reactive with the cell surface of epidermal keratinocytes or the epidermal basement membrane zone (BMZ) which cause detachment of the epidermal keratinocytes or the dermo-epidermal junction. One of the most common type of the disease caused by anti-keratinocyte cell surface antibodies is pemphigus, and another representative disease type is bullous pemphigoid which develops subepidermal blister formation. However, a number of new diseases of distinct entities have recently been identified. The well characterized autoimmune blistering skin diseases, as well as their autoantigens, are summarized in Table 1.

Accumulated evidence has revealed that the antigens for the autoantibodies in these diseases are the components of either the desmosome, the cell adhesion...
Table 1  Classification of Autoimmune Blistering Skin Diseases

<table>
<thead>
<tr>
<th>Diseases with Anti-keratinocyte Cell Surface Antibodies</th>
<th>Antigen Molecules</th>
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<tbody>
<tr>
<td>Classic Pemphigus</td>
<td></td>
</tr>
<tr>
<td>Pemphigus Vulgaris (PV)</td>
<td>130kD Dsg3</td>
</tr>
<tr>
<td>Pemphigus Follicus (PF)</td>
<td>160kD Dsg1</td>
</tr>
<tr>
<td>Paraneoplastic Pemphigus</td>
<td>250kD DPI, BP230, 210kD DPII, 190kD protein, 170kD protein, (130kD Dsg3)</td>
</tr>
<tr>
<td>IgA pemphigus</td>
<td>unknown (Dsc ?)</td>
</tr>
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Diseases with Anti-BMZ Antibodies

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Antigen Molecules</th>
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<tbody>
<tr>
<td>Bullous Pemphigoid</td>
<td>BP230, BP180</td>
</tr>
<tr>
<td>Herpes Gestations</td>
<td>BP180</td>
</tr>
<tr>
<td>Cicatricial Pemphigoid</td>
<td>BP180, BP230</td>
</tr>
<tr>
<td>Epidermolysis Bullosa Acquisita</td>
<td>45kD protein, Epsiligrin</td>
</tr>
<tr>
<td>Linear IgA Bullous Dermatosis</td>
<td>290kD type VII collagen</td>
</tr>
<tr>
<td></td>
<td>97kD protein</td>
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<td></td>
<td>290kD type VII collagen</td>
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Fig 1  Schematic model of adhesive apparatus for keratinocyte-keratinocyte interaction and dermo-epidermal junction in the epidermis.

The desmosome is one of the intercellular adhering junctions seen mainly in the epithelial tissues. Inside of the plasma membrane at the desmosome, there are electron dense attachment plaques, where keratin intermediate filaments insert. A number of glycosylated and non-glycosylated proteins have been identified as components of the desmosome. As transmembrane glycoproteins, there are two distinct groups of proteins; ie desmoglein (Dsg)\textsuperscript{10,11} and desmocollin (Dsc),\textsuperscript{12,13} both...
Fig 2 Constituents of the epidermal desmosome. Dsg1: desmoglein 1, PFA; PF antigen, Dsg3: desmoglein 3, PVA; PV antigen, Dsc: desmocollin, DP: desmoplakin, PG: plakoglobin, and IFAP300: intermediate filament associated protein 300.

of which consist of three different isoforms and designated Dsg1–3 and Dsc1–3. These proteins have been shown to be members of cadherin supergene family and are now called desmosomal cadherins. These molecules are considered to play a direct role in the adhesion between keratinocytes via "homophilic binding". As cytoplasmic attachment plaque proteins, desmoplakins (DP) I/II\textsuperscript{14,15} and plakoglobin (PG)\textsuperscript{16} have been best characterized. DP is the most abundant plaque proteins, and several lines of evidence indicated that DP directly binds to keratin intermediate filaments through its C-terminal domain. PG is shown to bind to both Dsg1 and Dsg3, and suggested to regulate the adhesive activity of these transmembrane proteins.\textsuperscript{17} As other desmosomal plaque proteins, band 6 protein,\textsuperscript{18} desmocalmin,\textsuperscript{19} plectin,\textsuperscript{20} intermediate filament associated protein 300 (IFAP300)\textsuperscript{21} and desmoyokin\textsuperscript{22,23} are known. PG and band 6 protein have been shown to be members of "armadillo" supergene family.\textsuperscript{24}

Structures and Constitutional Components of the Hemidesmosome (Fig 3)

Connection between keratin intermediate filaments and the hemidesmosome in basal keratinocytes, anchoring filaments and other macromolecules in lamina lucida,
and interaction between lamina densa and interstitial collagen fibers via anchoring fibrils in papillary dermis form a continuous unit that play an important role in the adhesion at dermo-epidermal junction (Fig 3). The major keratins in basal keratinocytes are keratins 5 and 14. Keratin intermediate filaments insert into electron dense plaques of the hemidesmosome. The hemidesmosome contains the 230 kDa bullous pemphigoid (BP) antigen (BP230) and HD1 as cytoplasmic plaque proteins, both of which are suggested to bind directly to intermediate filaments. At the hemidesmosome, there are two transmembrane proteins, the 180 kDa BP antigen (BP180) and α6β4 integrin. The extracellular domains of BP180 and α6β4 integrin extend into lamina lucida where anchoring filaments are located. Proteins associated with anchoring filaments include kalinin/nicein/epiligrin (also called laminin 5), K-laminin (laminin 6), and 19-DEJ-1 antigen (uncein). Other constituents of lamina lucida include classic of EHS-laminin (laminin 1) and nidogen (entactin). A large part of lamina densa is comprised by type IV collagen, which assembles into lattice network and anchored to the dermis via anchoring fibrils in the sublamina densa region in dermis. Anchoring fibrils consist of laterally associated dimers of type VII collagen. Type VII collagen contains a small non-collagenous domain (NC2) at its C-terminal domain, that forms antiparallel dimer formation. The N-terminal domain of type VII collagen contains a large 145 kDa NC1 domain that binds type IV collagen in lamina densa or anchoring plaques. In this manner, anchoring fibrils exist as series of looping structures and bind to interstitial collagen fibers. If any of these components consisting the adhesive unit are lacking, the adhesion at the dermo-epidermal junction would severely be interfered.

The Diseases with Antibodies against the Keratinocyte Cell Surface

The most common diseases with anti-keratinocyte cell
surface antibodies are classic type of pemphigus, which is divided into two major subtypes: pemphigus vulgaris (PV) and pemphigus foliaceus (PF). Both are characterized by loss of cohesion between epidermal keratinocytes (acantholysis) and by circulating IgG autoantibodies against the keratinocyte cell surface, which bind to keratinocytes in vivo. The autoantigens in pemphigus have been extensively investigated, and shown to be desmosomal glycoproteins. Thus, PF antigen is the 160 kD Dsg1, PV sera react with a 130 kD Dsg3.

Although there is a clear difference of reactivity between PV and PF sera, several immunoprecipitation or immunoblot studies have shown that the sera of one-third to half of PV patients react with both Dsg1 and Dsg3. The mechanism of this apparent cross-reactivity is present unclear.

It should be interesting to note that autoantibodies against these desmosomal cadherins are really pathogenic, because, if IgG fraction purified from patients' sera injected into neonatal mice, the blisters mimicking patient's skin lesions can be induced in the mice. Using this animal model, Amagai et al have recently shown that antibodies against conformational epitopes on the pemphigus antigens are important in the pathogenesis. They elaborated recombinant proteins of PV and PF antigens with proper conformation using baculovirus expression system, and showed that pathogenic antibodies in patient sera can be completely absorbed with these recombinant proteins. More importantly, this result also indicates that affinity-column using these recombinant proteins may be used for antigen-specific plasmapheresis as an ideal therapy for pemphigus in the future. Furthermore, this treatment may be a paradigm for many other types of autoimmune diseases, and should also be used for the therapy of those diseases.

A new disease entity, paraneoplastic pemphigus (PNP), has recently been identified. Anhalt et al proposed five criteria to define PNP: painful mucosal erosions and polymorphous skin eruptions; the presence of neoplasia; acantholysis, keratinocyte necrosis and vacuolar-interface dermatitis in histology; intercellular deposition of IgG and complement and granular BMZ deposition of complement; and circulating antibodies which bind the cell surface of epidermis and other non-stratifying epithelia and immunoprecipitate a complex of four proteins (250, 230, 210 and 190 kD). It is suggested that the 250 kD and the 210 kD proteins are desmoplakins (DP) I and II, major desmosomal plaque proteins, and the 230 kD protein is the 230 kD BP antigen, but the identity of the 190 kD protein has not yet been established. More recently, another protein with molecular mass of 170 kD has been identified as a new candidate of PNP antigen. This protein was revealed to be a transmembrane protein, suggesting that it may play an important role in the pathogenesis of PNP. We have recently reported that the 130 kD PV antigen may also be involved in the pathogenesis in PNP. However, pathogenic antibodies in PNP have not yet been identified.

Besides these diseases with IgG class anti-cell surface antibodies, a number of cases showing IgA anti-cell surface antibodies have recently been reported. More than 30 cases have been reported in the world literature to date with several different diagnoses, and we proposed the term intercellular IgA vesiculo-pustular dermatosis (IAVPD) for this group of patients. IAVPD is, like PV and PF, divided into two types from histological and immunopathological standpoints. One type shows clinically subcorneal pustular dermatosis (SPD)-like features, showing pustules and IgA deposition in the upper epidermis, and is designated SPD type. The other type, intraepidermal neutrophilic dermatosis (INEN) type, is characterized by atypical vesiculopustular lesions, and shows pustule and IgA deposition in the entire epidermis. Similarly, circulating IgA autoantibodies react with cell surfaces of the keratinocytes in either the upper or entire epidermis, respectively. We have examined autoantigens for IAVPD with immunoblotting of desmosome enriched fraction obtained from bovine snout epidermis, and found that some IAVPD sera reacted with desmocollin, another desmosomal cadherin. However, more studies are necessary for identification of the target antigen(s) in this group of patients.

The Diseases with Antibodies Against the Epidermal BMZ

Bullous pemphigoid (BP) is the most common bullous disease with IgG anti-BMZ antibodies and occur mainly in elder population. BP is characterized clinically by large tense blisters, and histologically by subepidermal bullae with infiltration of eosinophils. In vivo bound and circulating anti-BMZ antibodies detected with immunofluorescence assay are now a hallmark for the diagnosis of BP.

Anti-BMZ antibodies in BP sera react with the BP230 and the BP180. The cDNA for the BP230 was first isolated, and the studies for the cDNA revealed that the BP230 is a cytoplasmic protein with similar structure to desmoplakin. Because autoantibodies can not access to such an intracellular molecule, the role of the anti-BP230 antibodies in the pathogenesis of BP is currently unknown. Subsequently, cDNA for the BP180 was isolated, and this antigen has been shown to be a transmembrane protein of type two orientation and to consist of multiple collagen-like domains in its extracellular region. Recent studies using recombinant proteins have indicated that autoantibodies in BP sera recognize an epitope in the extracellular NC16a domain of the BP180.

As mentioned above, pemphigus lesion can be pro-
duced in neonatal mice by passive transfer of IgG purified from patient sera. However, BP skin lesion could not be induced by the same method. Recently, sequence comparison of BP180 cDNAs has revealed that the homology around the pathogenic epitope in NC16a domain of the BP180 between human and mouse is very low, and the failure of blister induction in neonatal mice by injection of human IgG is considered to be due to inability of the human IgG to bind to the mouse BP180. Therefore, Liu et al produced rabbit antibodies by immunizing rabbit with mouse-specific peptide, and showed that the resultant rabbit antibodies could induce blister formation. This result strongly suggested the crucial role of anti-BP180 antibodies in the blister formation in BP. Moreover, this study suggests that the affinity-column using this peptide containing the pathogenic epitopes may be used as antigen-specific plasmapheresis, as is suggested in the treatment of pemphigus.

Herpes gestationis (HG) is a rare autoimmune bullous disease of pregnancy and postpartum period, which occurs in less than 1:50,000 pregnancies and is clinically and immunologically reminiscent to BP. HG is characterized immunopathologically by in vivo bound C3 and IgG at the basement membrane zone (BMZ) and by circulating HG factor, which was later shown to be complement-fixing IgG anti-BMZ antibodies. These antibodies are reactive preferentially with the BP180. HG sera also recognize a common epitope in the extra-cellular NC16a domain of the BP180.

Cicatricial pemphigoid (CP) is characterized by almost exclusive involvement of the oral, ocular and other mucous membranes leaving scar formation. Although CP shows distinct lesions, it is occasionally difficult to be distinguished from BP. Titers of the circulating antibodies in CP are in general very low, and difficult to be detected in some cases. Several immunoprecipitation and immunoblot studies have identified two major BP antigens; the BP230 and the BP180. Another study has suggested that BP180 is the major target antigen for IgG or IgA antibodies in a group of this disease. In patients showing exclusively ocular lesions, IgA antibodies have been suggested to react specifically with a 45 kD protein, the nature of which has not been unraveled. In addition, a new group of CP has recently been identified, who showed IgG autoantibodies against epiligrin and is thus designated anti-epiligrin CP. Ultrastructurally, the antigens targeted by IgG anti-BMZ antibodies in patients with this disease have been localized to the lower lamina lucida. Because passive transfer of the high titer of anti-epiligrin rabbit antibodies can induce blister formation in the newborn mice, the autoantibodies in the patients' sera are also considered to play an important role in the pathogenesis. However, the antigen molecules for CP are still controversial and considered to be heterogeneous.

Epidermolysis bullosa acquisita (EBA) showed blisters and erosions after minor trauma, and these lesions leave extensive scar and milia formation. Like BP, EBA also showed in vivo bound and circulating IgG anti-BMZ autoantibodies. However, EBA can be easily distinguished with immunofluorescence of 1.0 M NaCl split normal human skin section which is separated at the level of lamina lucida. Immunelectron microscopic studies showed that EBA antibodies reacted with anchoring fibrils. Both immunoblotting of human dermal extract and immunoprecipitation of radiolabeled cultured keratinocytes showed that the EBA antibodies react with type VII collagen, the major component of anchoring fibrils.

Linear IgA bullous dermatosis (LABD) is characterized by both in vivo bound and circulating IgA anti-BMZ autoantibodies. Immunofluorescence of 1.0 M NaCl split skin section revealed that LABD is divided into two subgroups; lamina lucida type and sublaminar densa type. Immunelectron microscopic studies have also demonstrated that IgA is deposited in the lamina lucida adjacent to the plasma membrane of basal keratinocytes or in the sublaminar densa region. However, antigen molecules for LABD have not been fully elucidated. Several studies have suggested that certain lamina lucida type LABD sera detected the 97 kD protein with immunoblotting of normal human epidermal extracts. Either the 285 kD protein or the 255 kD protein was also reported to be detected by immunoblotting of human dermal extracts. In addition, type VII procollagen, the EBA antigen, has been reported to be detected by IgA anti-sublaminar densa type LABD autoantibodies. These results indicate that LABD is a heterogeneous disease.

Conclusion

Recent biochemical and molecular biological studies have provided us important information on the autoantigens in various autoimmune blistering skin diseases, particularly, on the relationship between those autoantigens and constituent proteins in the desmosome and the hemidesmosome. Because cDNAs coding for the antigens are available, these proteins can be clinically applied for: e.g. enzyme-linked immunosorbent assay for detection of the antibodies as the diagnostic method, and antigen-specific plasmapheresis or B-cell targeting with toxin-conjugated antigen as therapeutic methods. These studies on autoantigens in turn provide us biologically important information to understand the mechanisms of adhesion in both the desmosome and the hemidesmosome.

Acknowledgments: This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (0445-4289), a grant from the Ministry of Health and Welfare of Japan.
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