HUMAN SKIN BASEMENT MEMBRANE IN HEALTH AND IN AUTOIMMUNE DISEASES

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1. ABSTRACT

Skin basement membrane zone (BMZ) is an ultrastructurally defined area situated between the outer layer of skin, the epidermis, and the inner layer of skin, the dermis. The major function of skin BMZ is to serve as an adherent connection between the epidermis and the dermis. Heritable skin diseases characterized by genetic mutations that result in defective BMZ protein production, such as functional and dystrophic epidermolysis bullosa, manifest clinically as skin fragility and subepidermal blister formation inducible by minor trauma. Many distinct, yet interconnected BMZ components, have been identified and studied extensively. Some of these essential components, interestingly, were discovered because they were targeted by an autoimmune reaction. As a result of studying the autoimmune diseases, these BMZ components involved in autoimmune reaction, or autoantigens, were isolated by molecular biology techniques. The isolation of these autoantigens, in turn, facilitates our studies of the normal structures and functions of skin BMZ. The skin BMZ can be divided into four ultrastructurally distinct areas: the hemidesmosome/upper lamina lucida, the lower lamina lucida, the lamina densa, and the sub-lamina densa. Well-characterized BMZ components include the hemidesmosome/upper lamina lucida-located bullous pemphigoid antigens (BP230 and BP180), α6β4 integrin, and plectin; the lower lamina lucida-located laminin-1, laminin-5 (previously named kalinin, epiligrin, nicein, BM600), laminin-6 (previously named k-laminin), p105, and entactin/nidogen; the lamina densa-located type IV collagen and perlecan; and the sub-lamina densa-located type VII collagen (epidermolysis bullosa acquisita antigen). Mucosal BMZ also contain identical components as skin BMZ. Autoantibodies targeting skin and/or mucosal BMZ components, like that of genetic mutation of BMZ components, result in a histopathologically defined subepidermal blistering disease, that is, a blister which occurs just below the epidermis/epithelium. Well-characterized autoimmune subepidermal blistering diseases include bullous pemphigoid (BP), linear IgA...
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Table I. Skin BMZ Laminins

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<thead>
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bullous dermatosis, cicatricial pemphigoid (with subsets of patients characterized by autoantibodies targeting either BP antigens or laminins), anti-p105 pemphigoid, and epidermolysis bullosa acquisita. Using a simple method of direct or indirect immunofluorescence testing on a skin section chemically separated at the middle portion of lamina lucida, in combination with immunoelectron microscopy and target antigen determination, accurate diagnoses for various autoimmune subepidermal blistering diseases can be obtained.

2. INTRODUCTION

Skin BMZ is originally defined histologically by a 0.5-1.0 μm-thick band-like structure situated between epithelium and the underlying tissue that is positively stained by periodic acid-Schiff (PAS) stain (1). Subsequently, ultrastructural studies have identified multiple distinct structural components in the BMZ area. Among them, there are hemidesmosome, anchoring filament, anchoring fibril, lamina lucida, and lamina densa (1). Biochemical and molecular biological studies had further revealed the individual proteins that compose these ultrastructural components (1). Interestingly, some of the components were discovered or isolated because they became targets of autoimmune reactions: bullous pemphigoid antigens, type VII collagen, and p105 (2-6). As a result of studying autoimmune diseases, we now have a better understanding of the normal structure and function of skin BMZ. This article will illustrate this point. In addition, well-characterized BMZ components and their functions will be discussed. Moreover, subepidermal blistering diseases characterized by autoantibodies targeting the BMZ components will be reviewed.

3. HUMAN SKIN BASEMENT MEMBRANE STRUCTURE AND FUNCTION

3.1 Skin basement membrane structure:

Figure 1 is a schematic diagram of skin BMZ showing the relative location of various known components. (BP=bullous pemphigoid; Ag=antigen)

Salt-split zone indicates the approximate location of separation when an intact skin is chemically separated by either 1 M NaCl or 20 mM Na-EDTA.

3.1.1 Hemidesmosome:

Situated at the undersurface of basal keratinocytes, hemidesmosome contains half of the structures of a desmosmoe (a structure observed between two adjacent keratinocytes): single intracytoplasmic attachment plaque onto which tonofilament of the basal keratinocytes are attached (1). The BP230 (BPAG1) is located at the intracellular side of the hemidesmososome whereas the BP180 (BPAG2) is a transmembranous protein that contains an intracellular domain, a transmembranous segment, and an extracellular domain that projects into the lamina lucida (7-9). The extracellular domain of BP180 protein contains a collagenous domain interrupted by 16 small non-collagenous domains (9). The largest of these 16 non-collagenous domains, NC16A, is located adjacent to the transmembranous segment. In addition, a member of the integrin family, α₆β₄, and plectin (a cytoskeleton-associated attachment protein), are also located in this area (10-11).

3.1.2 Lamina lucida:

Situated between the hemidesmosome and the lamina densa, the lamina lucida is electron-lucent under electron microscope (1). Nevertheless, fine filamentous structures are observed in this area and are termed anchoring filaments (1). Anchoring filaments extend from the basal keratinocytes’ hemidesmosomes to lamina densa, thus traversing the lamina lucida (1). One of these anchoring filament components is a member of the laminin family, laminin-5 (12-13). The laminin family consists of a group of heterotrimers of various combinations of three chains, α, β, and γ, and are synthesized and secreted by keratinocytes (Table I, 14-16). Laminin-6, previously named k-laminin, is another laminin variant, and its presence is restricted to anchoring filament-containing basement membranes (17). Laminin-6, a heterotrimer of α₁β₁γ₁ chains secreted by epidermal cells, shares structural and immunological similarities with two other lamina lucida-located laminins, laminin-1 (heterotrimer of α₃β₁γ₁) and laminin-5 (heterotrimer of α₂β₁γ₂, previously named kalinin, epiligrin, nicein, BM600) (17). In addition, a 105-kDa protein, termed p105, has recently been identified as a result of studying autoimmune diseases (6). Further biochemical studies indicate that p105 is distinct from the truncated γ₂ chain of laminin-5, which is also approximately 105-kDa in size (18). In addition, the N-terminal amino acids of p105 have been sequenced and shown to have substantial homology with a tumor associated antigen (19). Molecular cloning of p105 is now underway to delineate the structure and function of p105. Another glycoprotein, entactin/nidogen, is also located in this area (20).

3.1.3 Lamina densa:

Lamina densa, named according to its electron-dense appearance under electron microscope, is 35-45 nm-thick (1). This structure is considered to be the true basement membrane by some pathologists (1). The BMZ components that are located in this area include: type IV collagen, perlecan (heparan sulfate proteoglycan), and perhaps also laminin-6 (21-23). Type IV collagen is considered to be the major component in this area (1).
3.1.4 Sub-lamina densa:

Below the lamina densa, there are fibrillar structures that connect the lamina densa onto the dermal plaque-like structures (1). These fibrillar structures have been named anchoring fibrils (1). Type VII collagen is the major component of the anchoring fibril (24). Type VII collagen is a 290-kDa protein synthesized and secreted by both keratinocytes and fibroblasts. The newly synthesized type VII collagen consists of a 145-kDa non-collagenous (NC1) domain at the N-terminus, a 145-kDa central triple-helical collagenous domain, and a short 34-kDa non-collagenous (NC2) domain at its C-terminus (25-26). It appears that either a portion or all of NC2 domain is proteolytically removed before incorporated into the skin BMZ. This is supported by the finding that antibody raised against NC2 domain does not label skin BMZ (27). It is now clear that the triple helical type VII collagen forms anti-parallel dimers, joining two molecules at the C-terminus of the collagenous domains. Thus, the N-terminus-located NC1 domain serves as a binding site to interact with other BMZ components. Besides anchoring fibrils, there are other minor fibers that connect to the area beneath lamina densa: oxytalan, elanin, and elastic fibers (1).

3.2 Skin basement membrane function:

The major function of BMZ is to serve as an adherent connection between the outer layer of epidermis and the inner layer of dermis. The evidence supporting this role of BMZ is observed in several heritable blistering diseases characterized by defective BMZ protein production secondary to mutation of the genes encoding BMZ proteins. Junctional epidermolysis bullosa, a skin disease characterized by either a defective laminin-5, a defective BP180, or a defective β4 integrin, is manifested clinically as skin fragility and subepidermal blister formation in trauma prone skin (28-31). Dystrophic epidermolysis bullosa, another skin disease, characterized by defective type VII collagen synthesis secondary to genetic mutation, is also manifested clinically as skin fragility and subepidermal blister formation in trauma prone skin (32-33).

The adherent role of BMZ is also supported by laboratory evidence. Technical advancement in biochemical and molecular biological methods allow us to study interactions between isolated BMZ components in the well-controlled laboratory environment. Laminin-5, a major lamina lucida component, has been shown to be a preferred ligand for α3β1 and α6β4 integrins in the plasma membrane of basal epithelial cells (34-35). However, it has a low affinity for entactin/nidogen, which serves as a connecting network between laminin-1 and type IV collagen (16,36-37). The high affinity of laminin-1 for entactin/nidogen is due to the fourth EGF-like repeat domain III in its γ1 subclass chain, which is not present in laminin-5 (38-39). Similarly high affinity for entactin/nidogen has been observed in two other laminins not present in skin BMZ, laminin-2 and -4, which also share the γ1 subclass chain (37). Sharing the same γ1 subclass chain, laminin-6 is also likely to have a high affinity for entactin/nidogen. Thus, laminin-5 may bridge epidermal cells to the lower BMZ by linking the integrins on the outer part and forming a complex with laminin-6 on the inner part (40).

Type VII collagen, the major component of anchoring fibril that connects lamina densa to the dermal structure, has a NC1 domain that contains multiple subdomains which share homology with known adhesion molecules, including the fibronectin type III-like repeats and the A domain of von Willebrand factor (41). An eukaryotic expressed recombinant full-length NC1 protein has been shown to exhibit specific interaction with the lamina lucida components, the laminin-5/6 complex, and with the
Figure 2. Indirect immunofluorescence microscopy on chemically separated skin sections showing the autoantibodies from patient with anti-laminin cicatricial pemphigoid (left), patient with BP (middle), and patient with epidermolysis bullosa acquisita (right) labeling the antigenic sites (arrowheads) at dermal floor, epidermal roof, and dermal floor, respectively.

dermal components, fibronectin and type I collagen (41).

Thus, the interactions of various molecules of the BMZ could be envisioned according to the following sequence from the outer part to the inner part of skin: The two basal keratinocyte cell membrane-located integrin family members, \( \alpha_3\beta_1 \) and \( \alpha_6\beta_4 \), serve as ligands for laminin-5 and laminin-5/laminin-1, respectively. Laminin-5 forms a complex with laminin-6. Laminin-1 and laminin-6 both interact with entactin/nidogen, which in turn interacts with type IV collagen. Type IV collagen interacts with type VII collagen (41). Type VII collagen, in addition to interacting with type IV collagen, also interacts with laminin-5/laminin-6 complex on the outer part and with fibronectin and type I collagen on the inner part of skin. By this interconnecting network of molecules within the skin BMZ, an adherent connection between the outer layer, epidermis/epithelium and the inner layer, dermis is established. The interaction of the extracellular domain of BPAg2 with other BMZ components remains to be elucidated. Besides the major function of adherence of epidermis to dermis, minor functions of skin BMZ include structural support, permeability regulation, and embryonic differentiation.

The adherent role of BMZ is further supported by the clinical observation of autoimmune subepidermal blistering diseases, the pathologic conditions resulting from perturbation of BMZ by autoantibodies. We will now illustrate these in the following section.

4. HUMAN SKIN BASEMENT MEMBRANE AS A TARGET OF AUTOIMMUNE DISEASES

4.1 Techniques for classification of autoimmune subepidermal blistering diseases

Regardless which BMZ antigen is targeted by autoantibodies, the histopathological findings of all autoimmune subepidermal blistering diseases are similar (42). Although the inflammatory cell infiltrate may provide clues in the diagnosis of certain diseases, such as eosinophilic infiltrate in the diagnosis of BP, they are not always reliable and cannot be used as definitive diagnostic criteria. Therefore, more specific techniques are required for accurate diagnoses.

4.1.1 Direct and indirect immunofluorescence studies on chemically separated skin section

As depicted in Figure 1, the skin BMZ can be chemically separated at the middle lamina lucida by either 1 M NaCl or 20 mM Na-EDTA (43). These techniques can be applied to direct immunofluorescence and indirect immunofluorescence in the detection of the relative binding sites of \textit{in vivo}-bound and circulating autoantibodies, respectively. Autoantibodies bound to the epidermal roof of the chemically separated skin section indicate the target antigens are located in the hemidesmosome/upper lamina lucida area, whereas autoantibodies bound to the dermal floor indicate the target antigens are located in the lower lamina lucida, lamina densa, or sub-lamina.
4.1.2 Direct and indirect immunoelectron microscopy

Since immunofluorescence microscopy can only detect the relative location of the autoantibody binding sites (target antigens), more accurate diagnostic techniques are required to delineate the fine location of the target antigens. Immunoelectron microscopy combines ultrastructural definition and immunolocalization of the target antigen, and therefore can provide a more detailed localization of the target antigens (44). The target antigens can be visualized by immunoperoxidase or immunogold techniques. A direct method can be used to detect the binding site of in vivo-bound autoantibodies, whereas an indirect method is used to detect the binding site of circulating autoantibodies. Figure 3 illustrates the immunoperoxidase particles (arrows) localizing the target antigen of the IgG autoantibodies from a patient with epidermolysis bullosa acquisita to the lamina densa and sub-lamina densa areas. (E=epidermis; D=dermis)

4.1.3 Determination of specific target antigens

The most definitive diagnosis can be obtained by determining the specific target antigens of the patients' autoantibodies. The determination of target antigens can be achieved by immunoblotting, immunoprecipitation, or ELISA (2-6,23,45-46). Figure 4 demonstrates the immunoblotting studies showing IgG autoantibodies from a patient with epidermolysis bullosa acquisita (middle lane) that bound to the 290-kDa type VII collagen (arrows), compared to the negative labeling by sera from two individuals who do not have the disease (left and right lanes). The presence of circulating autoantibodies, however, are required to determine the specific target antigens. Thus, the application of these techniques is limited in cases with detectable circulating autoantibodies.

4.2 Diseases characterized by autoantibodies against hemidesmosome/upper lamina lucida:

4.2.1 Bullous pemphigoid:

Bullous pemphigoid is the most common and the most thoroughly studied autoimmune subepidermal blistering disease, seen predominantly in the elderly people. The average age of onset is about 65. Mucosal involvement is rare and quite limited. The typical clinical phenotype is a tense blister occurring on the torso and extremities, with a predilection on the flexure areas. The typical histopathologic finding consists of a subepidermal blister with a prominent mast cell and eosinophil infiltrate (42). IgG is deposited linearly at the BMZ of the patient’s peri-lesional skin biopsy specimen. Direct and indirect immunofluorescence microscopy detect in vivo-bound and circulating IgG binding to the epidermal roof of chemically separated skin section (43, Figure 2b). The majority of bullous pemphigoid patients have circulating autoantibodies that recognize a 230-kDa and/or a 180-kDa epidermal protein (BPAg1 and BPAg2 respectively) (2,3). Passive transfer of rabbit antibody raised against the extracellular non-collagenous (NC16A) domain of the mouse BPAg2 to neonatal BALB/c mice induces skin blisters similar to that of human BP blisters (47).
4.2.2 Linear IgA bullous dermatosis:
Linear IgA bullous dermatosis is a clinically distinct subepidermal blistering disease commonly seen in children and young adults (4,42,51). One of the striking clinical features is the grouping of blisters in a circular manner that resembles a “string of pearl” configuration (42). This disease is commonly associated with a significant pruritus. Histopathologically, a subepidermal blister is seen with prominent neutrophilic infiltration mostly at the papillary dermis, the so-called “papillary microabscess”. IgA is linearly deposited at the perilesional BMZ of patients’ skin biopsy specimen. In a minority of patients, circulating IgA autoantibodies that bind to the epidermal side of chemically separated skin section are detected (4,51). Some patients, in addition to IgA, also have IgG class autoantibodies against the skin BMZ (51). Although initially the target antigen of the patients’ IgA autoantibodies was thought to be a unique protein located at the hemidesmosome/upper lamina lucida areas (4), now, it appears that the target antigen is an epitope within the BPAg2 (52). It is not known why the immune system, instead of IgG class, initiates the formation of IgA class autoantibodies to target the same protein.

4.3 Diseases characterized by autoantibodies directed against lower lamina lucida:

4.3.1 Cicatricial pemphigoid with autoantibodies to bullous pemphigoid antigen:
Cicatricial pemphigoid is a putative autoimmune disease characterized by chronic inflammation and scarring at the mucous membranes, especially the oral and ocular mucosae. Sometimes, minimal skin involvement is observed (53). The histologic and direct immunofluorescence findings in cicatricial pemphigoid are quite similar to that of BP (42,53). Within the clinical entity of cicatricial pemphigoid, different subsets can be classified according to the specific target antigens recognized by the patients’ autoantibodies. A subset of patients with cicatricial pemphigoid have autoantibodies recognizing the BP antigens, particularly the BP180 (BPAg2) (53-54). Further studies indicated that the autoantibodies from a subset of cicatricial pemphigoid patients recognized epitopes on BPAg2 that are different from the epitopes recognized by BP patients (54). The cicatricial pemphigoid epitopes are located beneath the lower lamina lucida or lamina densa area, so that direct or indirect immunofluorescence studies performed on chemically separated skin sections detect the in vivo-bound or circulating autoantibodies of this subset of patients at the dermal side. Thus, the location of cicatricial pemphigoid antigenic epitopes is distinct from that of the bullous pemphigoid epitopes which are located close to the hemidesmosome area (54). This deeper location of the cicatricial pemphigoid antigenic epitope may be a factor that influences the scarring nature of the disease. A second subset of cicatricial pemphigoid with clinical disease restricted to ocular mucosa is characterized by the absence of circulating autoantibodies and the presence of in vivo-bound fibrin deposition at the patients’ BMZ (53).

4.3.2 Anti-laminin cicatricial pemphigoid:
The third subset of cicatricial pemphigoid patients were reported to have IgG autoantibodies targeting a lower lamina lucida component epiligrin (55). Subsequently, epiligrin was identified as a member of the laminin family, and was named laminin-5. This protein was also previously known as kalinin, BM600, and nicein (56-57). Laminin-5, a heterotrimer of $\alpha_3$, $\beta_1$, and $\gamma_1$ chains, shares homology with another member of laminin family, laminin-6 (heterotrimer of $\alpha_3$, $\beta_1$, $\gamma_1$), which is also located at the skin BMZ (14,17). Autoantibodies from a subset of patients with cicatricial pemphigoid recognized the $\alpha_5$ chain of laminin-5 and thus also recognized laminin-6 (23,57). As a result, both laminin-6 and laminin-5 become target antigens for this subset of cicatricial pemphigoid (23). Therefore, the appropriate name for this subset of cicatricial pemphigoid should be anti-laminin...
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cicatricial pemphigoid (23). Since both laminin-6 and laminin-5 are localized to the lower lamina lucida areas, autoantibodies from these patients bind to the dermal side of chemically separated skin section (Figure 2a) and localize to the lower lamina lucida by immunoelectron microscopy (23,55).

4.3.3 Anti-p105 pemphigoid:
Anti-p105 pemphigoid is a rare IgG-mediated subepidermal blistering disease first reported in 1993 (6), and subsequently, in another patient in 1994 (58). Since the disease is rarely observed, a typical clinical phenotype has not been characterized. The two cases reported thus far showed different clinical phenotype with one case being similar to toxic epidermal necrolysis or pemphigus vulgaris (6,59) and other case being similar to bullous pemphigoid (58). Histopathologically, a subepidermal blister is present predominantly with a neutrophilic infiltration, resembling the histology of linear IgA bullous dermatosis. IgG, and not IgA, is deposited linearly at the BMZ of patient’s perilesional skin biopsy specimen. Direct or indirect immunofluorescence microscopy detect in vivo-bound and circulating IgG binding to the dermal floor of chemically separated skin. These patients also had IgG circulating autoantibodies that recognized a lower lamina lucida-located 105-kDa protein (P105) synthesized and secreted by epidermal keratinocytes and dermal fibroblasts (6).

4.4 Diseases characterized by autoantibodies directed against sub-lamina densa:

4.4.1 Epidermolysis bullosa acquisita:
Epidermolysis bullosa acquisita is an IgG-mediated subepidermal blistering disease with a distinct clinical phenotype. A common clinical feature is chronic inflammation, blister formation, and scarring, predominantly at the extensor surfaces of the skin: elbows, knees, ankles, buttocks, and dorsal aspect of the hands (42). However, generalized blistering formation occurs in a minority of patients. Some patients have severe mucosal lesions. Histopathologically, epidermolysis bullosa acquisita is a subepidermal blister with a mixed inflammatory cell infiltrate (42). A wide band of IgG is deposited linearly at the patient’s skin BMZ when direct immunofluorescence is performed on the perilesional skin biopsy specimen. Direct or indirect immunofluorescence detect IgG autoantibodies that bind to the dermal floor of the chemically separated skin section (43, Figure 2c). Immunoelectron microscopy delineates the IgG binding site to be at the lamina densa and sub-lamina densa area (5, Figure 3). Approximately 50% of the patients have detectable circulating IgG autoantibodies that recognize a 290-kDa skin BMZ protein (5, Figure 4) synthesized and secreted by epidermal keratinocytes and dermal fibroblasts. This 290-kDa epidermolysis bullosa acquisita antigen was subsequently identified as type VII collagen (5). Further studies showed that the patient’s autoantibodies recognized four major immunodominant domains located at the non-collagenous (NC1) domain of type VII collagen, also located at the N-terminus of the protein (60).

5. SUMMARY
Skin BMZ is a complex structure situated between the inner layer of epidermis and the outer layer of dermis. The major function of skin BMZ is adherence of epidermis to dermis. Skin BMZ is composed of many individual yet interconnected components. Disruption of any of these components either due to genetic mutation or as a result of autoantibody attack leads to the loss of adherence of epidermis to dermis and results in the formation of subepidermal blister. Skin BMZ components that have been attacked by autoimmune reactions include the hemidesmosome/upper lamina lucida-located bullous pemphigoid antigens (BP230 and BP180); the lower lamina lucida-located laminin-5 (previously named kalinin, epiligrin, nicein, BM600), laminin-6 (previously named k-laminin), and p105; and the sub-lamina densa-located type VII collagen (epidermolysis bullosa acquisita antigen). Other well-defined skin BMZ components including laminin-1, α6β4 integrin, type IV collagen, perlecain, entactin/nidogen, plcecin, have not been reported as being targeted by autoantibodies in blistering skin diseases. As more components are being discovered and studied, we will get a better understanding of the complex structures and functions of skin BMZ.

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