Using light microscopy, it can be observed that the basement membrane separates the epidermis from the dermis. In reality, it actually attaches the epidermis to the dermis by several adhesion molecules when observed with an electron microscope. Humans and animals are known to suffer blistering skin diseases if at least one of these adhesion molecules is impaired. In this section, the normal fine structure of the basement membrane zone is explained and disorders caused by disorganisation of adhesion molecules are reviewed.

1. Normal fine structure of cutaneous basement membrane zone
   Electron microscopic analysis of the epidermal basement membrane zone (BMZ) reveals that it is made up of an interface between keratinocytes and the dermis. At higher magnifications, there are several structures within the BMZ. At the dermal side of the basal keratinocyte, electron dense structures are seen, called hemidesmosomes (HD), which exhibit stud-like structures at the site of the plasma membrane. HDs are subdivided into an inner and outer plaque, with the outer plaque overlapping with the cell membrane.
   An electron lucent area is observed just beneath the plasma membrane of basal keratinocytes, where fine fibrillar materials known as anchoring filaments, traverse the lamina lucida, has been identified. The lamina densa is an electron-dense band-like structure, which runs parallel to the lamina lucida. Anchoring fibrils in a looping structure beneath the lamina densa extend their loops to the dermis to hold the dermal collagen or elastin network. The HD-anchoring filament complex forms a continuous link between the basal keratinocyte intermediate keratin filaments and the BMZ and dermal components.

2. BMZ macromolecules
   2.1. Keratin intermediate filaments
   The basal keratinocyte filaments are composed of cytokeratins 5 and 14. Intermediate filaments are small 50–55 kDa proteins that give shape and structural integrity to basal keratinocytes. The intermediate keratin filaments act as a network through the desmosome and HD for adjacent keratinocytes.

   ![Figure 1: Ultrastructural view of basement membrane zone](image)
   E: epidermis, D: dermis, Arrow: anchoring fibrils

   2.2. Plectin
   The 120 kDa plectin protein is an important molecule in the inner plaque of HDs. Plectin belongs to the plakin family and provides a link between keratin intermediate filaments in basal keratinocytes and HDs. It assists with the attachment of integrin α6β4 and collagen XVII to the outer plaque of HDs.
2.3. Bullous pemphigoid antigen 1 (BPAG1)
BPAG1 is also a member of the plakin family with a molecular weight of 230 kDa in human bullous pemphigoid patients. BPAG1 is expressed in dermal nerves and basal keratinocytes. In the basal keratinocytes, BPAG1 is attached to keratin intermediate filaments, the intracellular domain of collagen XVII (BPAG2) and the cytoplasmic domain of integrin β4.9

2.4. α6β4 integrin
The α6β4 integrin expressed in the HD plaque complex is the chief receptor of laminin-332 in the lamina lucida. A subtype of human junctional epidermolysis bullosa (Pyloric atresia type JEB), it demonstrates the lowest level of expression of all α6β4 integrins.9 The α6β4 integrins also bind to collagen XVII, BPAG1 and BPAG2 in the lamina densa. Collagen VII has been proposed to bind to α6β4 integrin via small proteins, which have been identified as fibulin 2. There have been no reports of clinical cases caused by the genetic dysregulation of α6β4 integrin in canine JEB.

2.5. Laminin-5 (laminin α3β3γ2, laminin-332)
Laminin-332 is the primary component of anchoring fibrils in the lamina lucida and is composed of three chains, α3, β3 and γ2. Laminin-332 plays a crucial role in the BMZ network as one of the main components of adhesion between the epidermis and dermis.1 The long arm of the α3 chain has globular domains (LG1–5) at the C-terminal, however, during processing, the short arm of the γ2 chain and the LG4 and LG5 domains are cleaved by enzymes.1 Laminin-332 attaches to the NC1 domains of type VII collagen in the scaffold of collagen IV by short-arm β2 and γ2 chains, collagen XVII at the lamina lucida/densa by the γ2 chain, and integrin α6 by the LG1–3 domains at the lamina lucida.

2.6. Collagen XVII (BP180, BPAG2)
Type XVII collagen (formerly known as BP180 or BPAG2) is a transmembranous protein with an extracellular domain that spans the entire length of the lamina lucida, and gives stability to anchoring fibrils. Canine BPAG2 has been characterised as the canine counterpart in humans with 85% identity at the deduced amino acid sequence level.13,15 The extracellular domain of BPAG2 contains an NC16A non-collagenous domain just beneath the cell membrane. An NC16A domain, targeted by patient sera with canine bullous pemphigoid.13,15 The intracellular domain of collagen XVII binds to the α6β4 integrin and BPAG1, and the extracellular domain is anchored to the γ2 chain of laminin-332 in the lamina lucida/densa.9

2.7. Collagen IV
The classical type IV collagen in the skin BMZ is composed of a trimer [α1(IV)2α2(IV)] with a C-terminal NC-1 domain adjacent to a flexible 400 nm long interrupted triple helical domain. The heterotrimeric collagen IV molecule can form dimers via its NC-1 domain and tetramers via the N-terminal. The scaffolds of collagen IV bind to the laminin-332 group by the small adhesion molecule, nidogen.9 No skin diseases have been found to be caused by autoimmunity or genetic dysregulation of type IV collagen.

2.8. Collagen VII
Collagen VII is the main component of dermal/epidermal anchoring fibrils. A collagen VII monomer is composed of three identical central triple helical collagenous segments with repeating Gly-X-Y amino acid sequences and a large 145 kDa N-terminal, NC-1, and C-terminal, NC-2 domain at both ends.2 Cleavage of the NC-2 domain occurs by proteolytic enzymes after two monomers form an anti-parallel dimer with central overlap of the NC-2 domain with the outward NC-1 domain. Both NC-1 domains attach to the lamina densa and collagenous domains to make a loop with the dermis and “anchoring fibrils” to entrap dermal collagen fibrils. Then, covalent stabilization of the dimer by disulfide bonds occurs prior to non-staggered lateral association of the dimers to anchoring fibrils.2 Collagen VII binds to laminin-332 and α6β4 integrins by the amino-terminal NC-1 domain in the lamina densa. The NC-1 domain of canine type VII collagen was cloned and sequenced, and found to have 86% identity to the human form.16
3. Diseases caused by the disruption of BMZ molecules

Diagnosis of sub-epidermal blistering diseases is based on light microscopy, electron microscopy, immunofluorescence (IF) with and without salt-split skin (SSS) and immunoblotting. Direct immunofluorescence using SSS distinguishes epidermolysis bullosa acquisita (EBA) from bullous pemphigoid (BP). Perilesional skin incubated in cold 1 M NaCl for 48 h is separates the dermis and epidermis, which effectively places the BP antigen on the epidermal roof and the EBA antigen on the dermal floor.

3.1. Bullous pemphigoid (BP)

BP in a dog was first reported by Kunkle in 1978. Since then, more than 10 dog BP cases have been published, but BP is still an extremely rare autoimmune skin disease in dogs. Animals other than dogs affected by BP include cats, horses and pigs. In dogs, lesions are characterized by erythema, vesicles or bulla, followed by erosions and crust formation in the oral cavity, concave pinnae and lips. There are no reports in patients of organs other than the skin and mucous membranes being affected. The autoantigen of canine BP was demonstrated to be the 180 kDa BPAG2. The extracellular domain of BPAG2, NC16A, is the target antigen of the autoantibody in BP patients. Diagnosis of canine BP is based on clinical signs, sub-epidermal bulla or cleft formation of the dermal-epidermal junction along with eosinophilic and/or neutrophilic infiltration. Direct and indirect IF may reveal IgG deposition at the dermal-epidermal junction. However, we cannot differentiate EBA from BP by an IF test, as both diseases show positive reaction at the BMZ. Direct IF using SSS exhibits fluorescence at the roof of clefts in lesioned skin of BP cases. The 180 kDa proteins may be detected when patient serum and the extracted proteins from dog skin are reacted by immunoblotting. Positive direct IF using SSS enables us to diagnose canine bullous pemphigoid.

3.2. Mucous membrane pemphigoid (MMP)

MMP in dogs was identified by Olivry et al. as a localised variant of BP, characterised by ulcerations predominating in mucosae or at mucocutaneous junctions. Lesions usually occur inside the oral cavity, gingival, buccal mucosa, palate, tongue, nasal planum and periorbital region. A small number of animals are
affected on foot pads and claws. Exanthema includes tense vesicles that evolved rapidly into ulcers and/or erosion.

Histopathology of lesions in mucous membrane demonstrated sub-epithelial vesiculation with a superficial dermal band-like lymphoplasmacytic inflammation. Circulating IgG autoantibodies targeting the BMZ are detected in one third of patients with MMP by indirect IF, and a positive IgG staining result assists in the diagnosis of MMP. Indirect IF using SSS is a more sensitive immunohistochemical method that shows IgG deposition at the epidermal side of the cleft. Olivry proposed the following diagnostic criteria for MMP:

- Clinical examination exhibiting chronic mucosal or predominantly mucocutaneous vesiculation and ulceration with or without scarring.
- Histopathology showing sub-epidermal vesiculation with variable inflammation.
- Immunofluorescence that shows epithelium-fixed immunoglobulins, complement, or circulating autoantibodies binding the BMZ.

Treatment of MMP is managed by immunosuppressive doses of drugs such as prednisolone with or without azathioprine.

3.3. Junctional epidermolysis bullosa (JEB)

Epidermolysis bullosa (EB) is classified into three categories, EB simplex, JEB and dystrophic EB (DEB) as determined by the location of the cleft in BMZ. JEB is reported in dogs to have a lethal and non-lethal subtype, especially in a family of German short haired pointers. JEB is a blistering disease with cleft formation within the lamina lucida of the BMZ. JEB in humans is caused by genetic mutations in several different genes such as *lama3*, *lamb3* and *lame2*, which code for the three chains of laminin-332. In dogs, a French group reported genetic mutations in the *lama3* gene, which codes for the α3 subunit of laminin-332,

Clinical signs of canine JEB present as repeated blisters or vesicle formation followed by erosion and ulcer onset in infantile or very young dogs. After disruption of the vesicle/blister, scar formation and hair loss is visible on the face, concave pinnae and foot pads. Immunofluorescence using antibodies against laminin-332, plectin, collagen XVII and integrin α6β4 may reveal decreased expression of these adhesion molecules. Electron microscopy shows that separation of the dermo-epidermal junction occurs within the lamina lucida with a hypoplastic HD attachment plaque and normal-looking anchoring fibrils.

3.4. Dystrophic epidermolysis bullosa (DEB)

DEB is caused by a mutation in the gene encoding type VII collagen (COL7A1) in neonatal or very young animals. Clinical manifestations of DEB in dogs is caused by the separation of the dermo-epidermal junction at the sub-lamina densa and presents as vesicle formation, erosion and ulcers, and crust formation on the oral mucous membrane, tongue, palate and foot pads.

3.5. Epidermolysis bullosa acquisita (EBA)

EBA is caused by autoantibodies against the NC-1 domain of type VII collagen. To date, two variants of EBA have been reported, a generalised and a localised form.

There have been two independent case reports of generalised EBA in Great Danes, as well as additional 12 cases in various dog breeds. Of these 12 cases, Great Danes made up 50% of the cases, implicating that this breed may have a predilection to a generalised form of EBA. Clinical signs include vesicles or pustules that rupture rapidly and show erosions and/or ulcers on the oral mucous membrane, face, axilla, groin and foot pads. Histopathology revealed sub-epidermal blistering with and without infiltration of neutrophils. Direct IF of lesioned skin shows deposition of IgG at the BMZ, and indirect IF using SSS and patient serum usually stains the bottom of a cleft. Western blotting may recognise a 145 or 290 kDa protein, known to be type VII collagen. These tests aim to differentiate EBA from BP. Olivry proposes the following diagnostic criteria for canine EBA:

- Predominant vesicular and ulcerative disease of the skin
- Microscopic sub-epidermal vesiculation upon histopathological examination of lesional skin biopsy specimens
- Basement membrane-bound autoantibodies and/or circulating autoantibodies targeting the dermal side of SSS by indirect IF
- Circulating autoantibodies targeting collagen VII (ELISA or immunoblotting)
References

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