Basement membrane zone: Structure, function and pathology

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One of the most important functions of skin is to provide protective barrier, which can be fulfilled only if the skin is intact. Basement membrane (BM) is a complex network of interconnected proteins, which, together with hemidesmosomes, provides a link between the outer epidermal layer and the underlying dermis. Additionally, many of these proteins have been shown to possess signaling properties involved in cell proliferation, keratinocyte migration as well as immunological responses. There are numerous publications discussing the structure and function of BM zone as well as an excellent video reviewing it visually (see references).

Since the last resident review, little has changed as far as the BM zone structure and in the field of autoimmune skin diseases. An open access review paper has been published on the topic of autoimmune subepidermal blistering skin diseases in animals (reference 7 in AISBDs). The best and most updated source of information for genetic mutations affecting the BM zone is the OMIA website (https://omia.org/key_articles/). Additional relevant references and a video link can be found at the end.

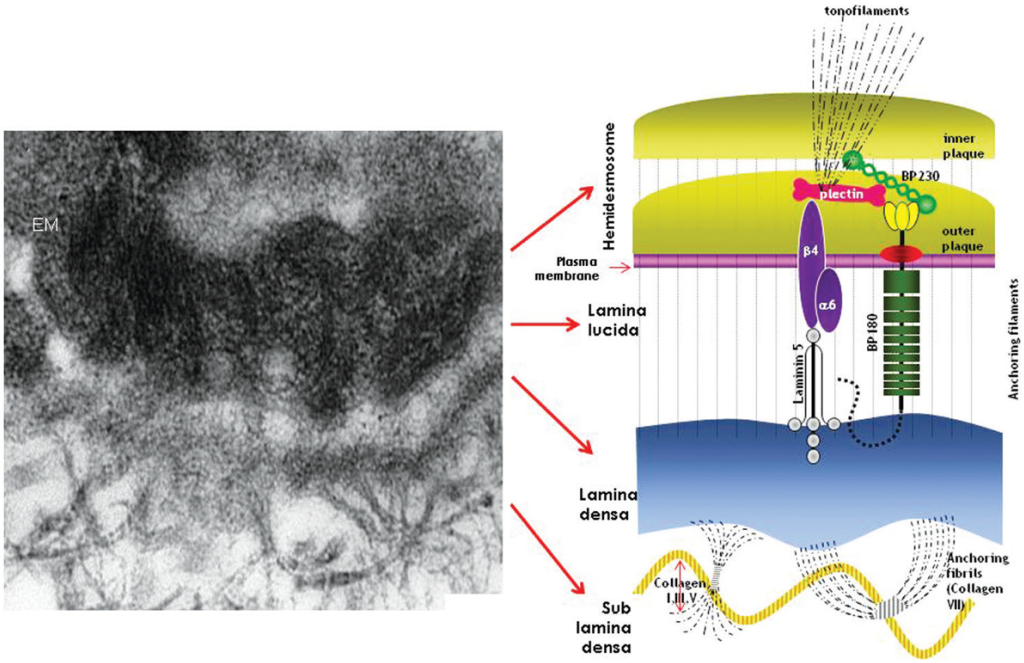
These notes will cover major aspects of the BM zone structure and focus on the diseases affecting this structure. Due to the time constrain, only selected topics of the BM zone structure and pathology will be discussed during the actual lecture.

1. **Basal keratinocyte**
   a. **Intermediate keratin filaments (K5, K14)**
   b. **Hemidesmosome (BPAG1e, plectin, COL XVII, integrin α6β4)**

2. **Lamina lucida**
   a. **Anchoring filaments (laminin 332, COL XVII)**
   b. **Integrin α6β4, CD151**

3. **Lamina densa**
   a. **COL IV**
   b. **Laminin 332, 311, 511**
   c. **Nidogen**
   d. **Perlecan**

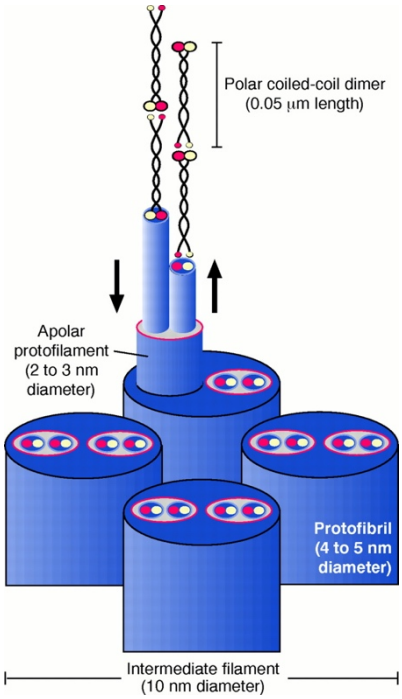
4. **Sublamina densa (COL VII)**



St. John's Institute of Dermatology, London (from: Rao R, *et al*. IJDVL 2012; 78: 692-7)

I. Intermediate keratin filaments (K5, K14)

- Gene*: KRT5, KRT14*
- cytoskeletal protein about 50-58 kDa
- heterodimer (coiled-coil dimer) of one acidic type I (K14) and one basic type II (K5) keratin
- coiled-coil dimers assemble into staggered tetramers → protofilaments → protofibrils → intermediate filaments
- responsible for structural/mechanical integrity of the basal keratinocytes as well as signaling
- binding: plectin, BPAG1
- **diseases:**
  - *congenital:*
    - Epidermolysis bullosa **simplex** (EBS)
      - KRT5 cattle (OMIA 002081-9913)
      - KRT5 Cardigan Welsh Corgi (OMIA:002081-9615)
      - KRT14 feline (OMIA 002281-9685)
  - *autoimmune:*
    - not confirmed in veterinary medicine



Fuchs E, *et al*. Science 23 Jan 1998

II. Plectin

- Gene*: PLEC1*
- dumbbell-shaped protein that belongs to a family of plakins; about 500 kDa
- localized intracellularly, within the inner plaque of the hemidesmosome
- responsible for structural/mechanical integrity of hemidesmosomes, linkage of intracellular cytokeratin proteins as well as scaffold for signaling
- binding: β4-subunit of α6β4 integrin, intermediate filaments, COL XVII

- **diseases:**
  - *congenital:*
    - Epidermolysis bullosa **simplex** (EBS)
      - Eurasier dog (OMIA 002080-9615)
      - Horse (one foal with signs of EBS and laminitis with an absent plectin staining)

  - *autoimmune:*
    - not confirmed in veterinary medicine

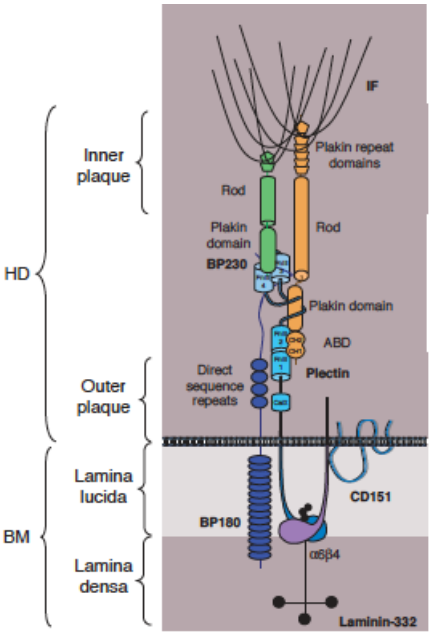
III. BPAG1e (synonyms: BP230, dystonin)

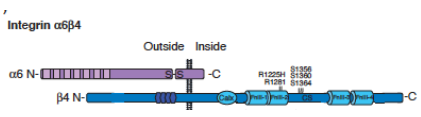
- Gene*: BPAG1 (DST)*
- dumbbell-shaped protein that belongs to a family of plakins; 230 kDa
- localized intracellularly, within the inner plaque of the hemidesmosome
- responsible for structural/mechanical integrity of hemidesmosomes, signaling protein for epidermal migration and cell polarity
- binding: β4-subunit of α6β4 integrin, intermediate filaments, COL XVII
- **diseases:**
  - *congenital:*
    - not confirmed in veterinary medicine

  - *autoimmune:*
    - Bullous pemphigoid: rare dogs
    - Mucous membrane pemphigoid: rare dogs

IV. Integrins (α6β4, α3β1)

- Gene*: ITGA6, IGB4*
- heterodimeric transmembrane protein that belongs to the family of integrins
- α6 is shorter (~120 kDa) with only small intracytoplasmic portion), while β4 is a longer protein (~205 kDa)
- localized intracellularly, within the inner plaque of the hemidesmosome as well as extracellularly (part of lamina lucida)
- responsible for structural/mechanical integrity of hemidesmosomes, homeostasis (adhesion, differentiation, proliferation), signaling and hair growth
- binding: intermediate filaments, BPAG1e, COL XVII, plectin, laminin 332
- **diseases:**
  - *congenital:*
    - **Junctional** epidermolysis bullosa (JEB)
      - ITGB4 cattle (OMIA 001948-9913)
      - ITGB4 sheep (OMIA 001948-9940)
      - ITGA6 cattle (OMIA:002718-9913)



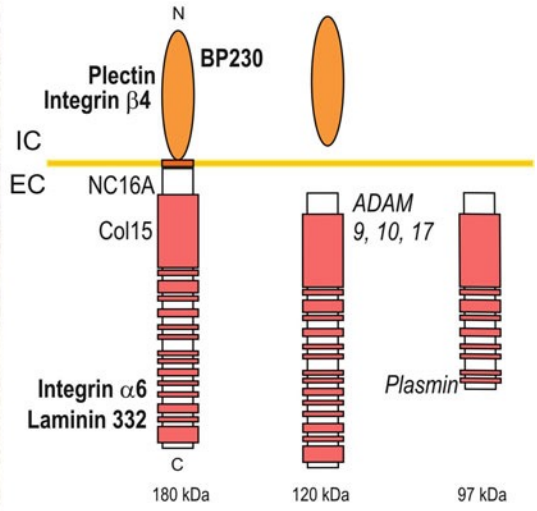


Litjens SHM, et al. Trends in Cell Biology 2006, 7: 376-83
(ITGA6 shorter purple, ITGB4 longer light blue)

- *autoimmune:*
  - not confirmed in veterinary medicine

V. Collagen XVII (synonyms: BP180, BPAG2, the shed (processed) extracellular segment is known as LAD-1)

- Gene*: COL17A1*
- homotrimer [alpha1XVII]$_3$ with non-collagenous globular domain localized in the basal keratinocyte (inner plaque of hemidesmosome) and 15 collagenous domains (Gly-X-Y) interspersed with short non-collagenous domains extracellularly (lamina lucida)
- 180 kDa protein (120 kDA shed domain is the result of a proteolytic cleavage of NC16A domain; which is further altered into a 97 kDa fragment)
- responsible for structural/mechanical integrity of hemidesmosomes, basal cell migration and adhesion, enamel formation
- binding: BPAG1e, plectin, α6β4 integrin, laminin 332
- **diseases:**
  - *congenital:*
    - **<u>Junctional</u>** epidermolysis bullosa (JEB)
      - COL17A1 (two cats) (OMIA:002793-9685)
  - *autoimmune:*
    - Bullous pemphigoid: dog, horse, cat, pig; the major target being NC16A ectodomain of col XVII
    - Mucous membrane pemphigoid: dogs, cat
    - Linear IgA bullous disease: dogs (the antigens is a shorter (120 ka) proteolytically cleaved fragment)



Collagen XVII, its binding sites and its shorter fragments, which are the consequence of a proteolytic cleavage (from: Has C, *et al*. Blistering Diseases 2015, page: 67-75)

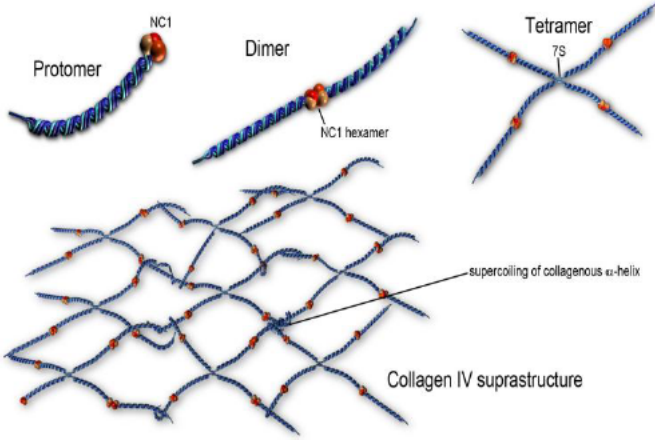
VI. Laminin 332 (old name: laminin-5; synonyms: kalinin, epiligrin)

- Gene*: LAMA3, LAMB3, LAMC2*
- rod-shaped heterotrimer composed of alpha-3, beta-3 and gamma-2 domains organized into a cross-shaped with α3A chain isoform being shorter (truncated; most abundant isoform in the skin)
- extracellular localization within the lamina lucida (anchors into lamina densa)
- responsible for structural/mechanical integrity of the dermo-epidermal junction, embryogenesis, tissue morphogenesis, regulation of proliferation and differentiation, tumorigenesis

- binding: α3A chain: 6β4 and α3β1 integrins; β3 chain: COL VII, laminin 311 and 321; γ2 chain: COL XVII. COL IV, COL VII, perlecan
- **diseases:**
  - *congenital:*
    - **Junctional** epidermolysis bullosa (JEB)
      - LAMA3 Shorthair pointer (OMIA 001677-9615); cross-bred dog (submitted)
      - LAMA3 American saddlebred horse (OMIA 001677-9796)
      - LAMA3 Belgian blue cattle (OMIA 001677-9913)
      - LAMB3 Australian shepherd (OMIA 002269-9615)
      - LAMC2 Belgian, Italian, French draft horses (OMIA 001678-9796)
      - LAMC2 Hereford cattle (OMIA 001678-9913)
      - LAMC2 German black headed mutton sheep (OMIA 001678-9940)
  - *autoimmune:*
    - Mucous membrane pemphigoid: dog, cat
    - Acquired junctional epidermolysis bullosa: dogs with clinical disease reminiscent of epidermolysis bullosa acquisita, but without collagen VII autoreactivity
    - Mixed subepidermal blistering skin disease: dogs with mixed collagen VII and laminin 332 autoreactivity

VII. Collagen IV

- Gene*: COL4A1 to COL4A6 (six different alpha chains)*
- heterotrimer (variety due to six different alpha chains); [alpha-1$(IV)_2$, alpha-2$(IV)_1$] most abundant in basement membrane of different organs, while other alpha chains have more restricted tissue distribution (e.g. glomerulus (alpha-3, 4, 5))
- extracellular localization within the lamina densa, where they are believed to form a tight hexagonal meshwork
- responsible for structural/mechanical integrity of the dermo-epidermal junction by forming a three-dimensional lattice framework (majority of lamina densa), tumorigenesis
- binding: laminin 332, perlecan, nidogen, fibronectin, collagen VII
- **diseases:**
  - *congenital:*
    - COL4A5 Samoyed (Alport syndrome; early onset renal failure) (OMIA 001112-9615)
    - COL4A4 English cocker spaniel (Autosomal recessive hereditary nephropathy (OMIA 000710-9615)
  - *autoimmune:*
    - not recognized in veterinary medicine



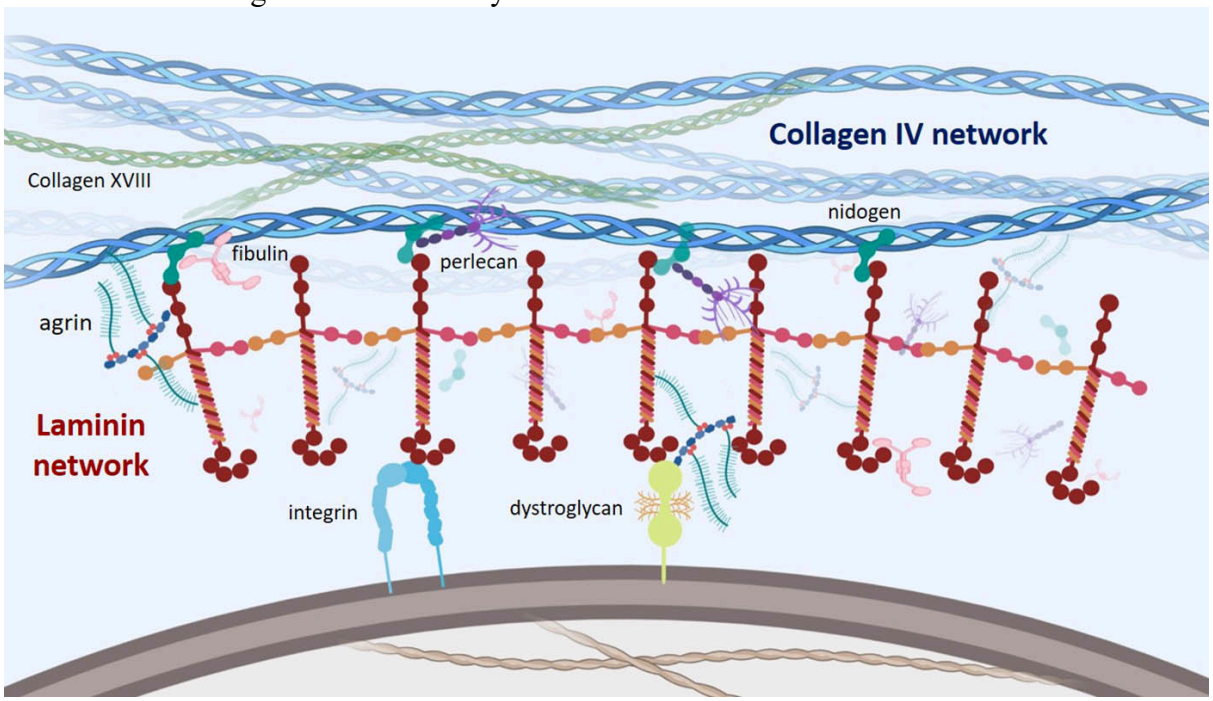
Collagen IV meshwork: AAD Basement membrane zone: Making the connection. 2012

VIII. Nidogen 1 & 2

- Gene*: NID1, NID2*
- sulfated monomeric glycoprotein (= PAS staining of lamina densa)
- dumbbell-shaped molecule within the lamina densa with numerous epidermal growth factor domains
- responsible for structural/mechanical integrity of the dermo-epidermal junction, embryogenesis, tumorigenesis
- binding: COL IV, nidogen, laminin 331
- **diseases:**
  - *congenital:*
    - not recognized in veterinary medicine
  - *autoimmune:*
    - not recognized in veterinary medicine

IX. Perlecan

- Gene*: HSPG2*
- sulfated glycoprotein (= PAS staining of lamina densa)
- responsible for structural/mechanical integrity of the dermo-epidermal junction, epidermal morphogenesis, regulation of angiogenesis and chondrogenesis, cell signaling, growth factor delivery
- binding: laminin 332, COL IV,
- **diseases:**
  - *congenital:*
    - not recognized in veterinary medicine
  - *autoimmune:*
    - no recognized in veterinary medicine

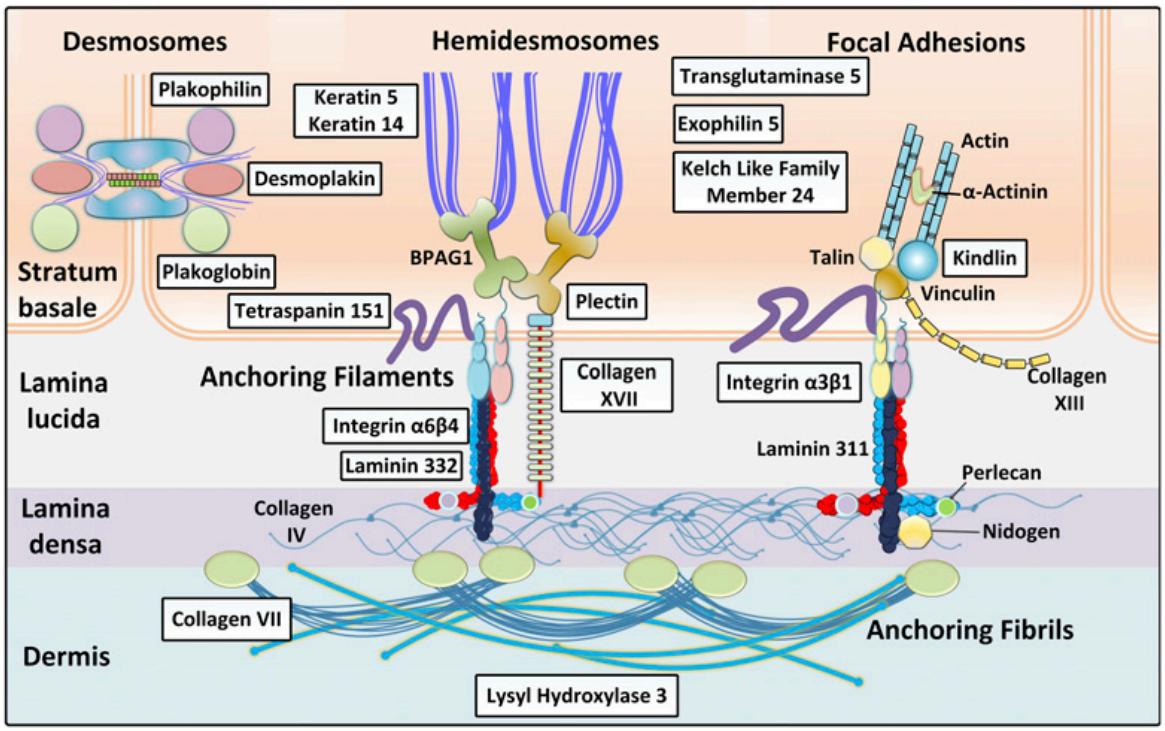


Collagen IV meshwork with perlecan, nidogen and other proteins. Illustration: Fresquet M (University of Manchester, UK) https://sites.manchester.ac.uk/bmbase/

X. Collagen VII

- Gene*: COL7A1*
- large homotrimer [alpha-1 $(VII)_3$] of 290 kDa that forms anchoring fibrils (sublamina densa)
- responsible for structural/mechanical integrity of the dermo-epidermal junction by looping around dermal collagen and thus anchoring the epidermis to the dermis
- binding: COL IV, laminin 331 and 332
- **diseases:**
  - *congenital:*
    - **<u>Dystrophic</u>** epidermolysis bullosa
      - Vorderwald & Rotes Höhenvieh cattle (OMIA 000341-9913)
      - Retriever (mild), Asian shepherd, basset (OMIA 000341-9615)

  - *autoimmune:*
    - Epidermolysis bullosa acquisita: dog (particularly NC1 domain)
    - Type I bullous systemic lupus erythematosus: single dog

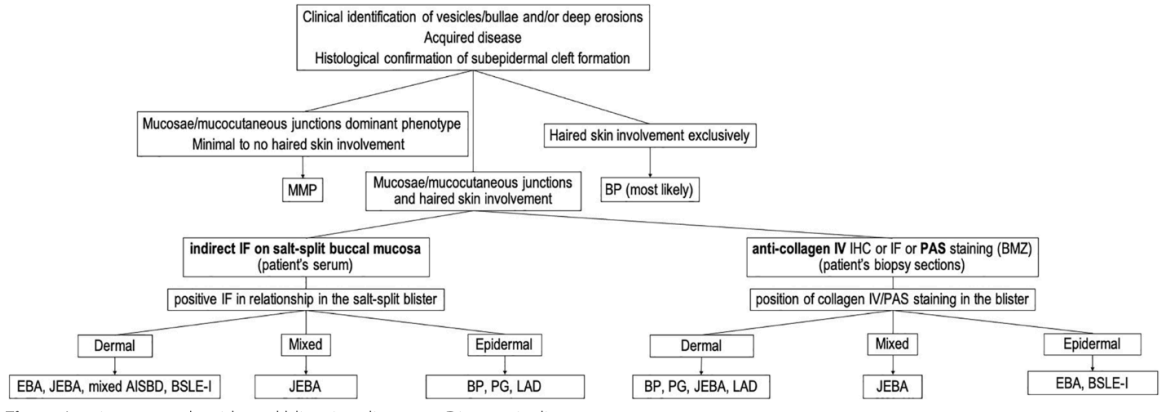


Adhesion structures of the skin. Uitto J. PNAS 2019 116(52): 26147-49

Autoimmune subepidermal blistering skin diseases (Bizikova; publication in progress)

Disease	Percentage of dogs with other AISBDs (n=77)[1]	Breed Predisposition	Age Predisposition	Characteristic Skin Lesions	Characteristic Lesion Distribution	Major Autoantigen	Minor Autoantigen	Level of Split	Histopathology	Other Species
Mucous Membrane Pemphigoid (MMP)	48	German shepherd	middle-aged (median: 6 years)	tense vesicles (rare), deep erosions/ulcers, scarring	mucocutaneous junctions, mucosae	collagen XVII	laminin-332, BP230	lamina lucida	subepidermal vesiculation without or with minimal inflammation (eutrophilic and/or eosinophilic)	human, cat
Epidermolysis Bullosa Acquisita (EBA)	26	Great dane	young (median: 1.2 years)	erythematous macules and papules; tense vesicles; deep erosions/ulcers	haired skin (footpads sloughing common, friction areas) and mucosae / mucocutaneous junctions	collagen VII	nd	sublamina densa	microscopic subepidermal vesiculation with variable degree of predominantly neutrophilic inflammation (intermixed eosinophils may be seen)	human
Bullous Pemphigoid (BP)	10	nd	middle-aged (median: 5 years)	erythematous macules and papules; tense vesicle; deep erosions/ulcers	haired skin predominant (concave pinnae, trunk; footpad sloughing rare) and mucosae / mucocutaneous junctions	collagen XVII	BP230	lamina lucida	microscopic subepidermal vesiculation with variable degree of neutrophilic and/or eosinophilic inflammation	human, cat, horse, pig, (macaque?)
Junctional EBA	6	nd	nd	erythema, vesicles, deep erosions/ulcers	haired skin (footpads sloughing common, friction areas) and mucosae / mucocutaneous junctions	laminin-332	nd	lamina lucida	microscopic subepidermal vesiculation without inflammation or with variable neutrophilic and/or eosinophilic inflammation	human (different nomenclature)
Mixed AISBD	4	nd	nd	erythema, vesicles, deep erosions/ulcers	haired skin predominant (concave pinnae, trunk; footpad sloughing rare) and mucosae / mucocutaneous junctions	collagen VII, laminin-332	nd	lamina lucida*	microscopic subepidermal vesiculation with mixed neutrophilic and eosinophilic inflammation	human (different nomenclature)
Linear IgA Disease (LAD)	3	nd	nd	erythematous macules and papules, deep erosions/ulcers	mucosae and haired skin (ears, nasal planum, footpads)	collagen XVII (secreted)	nd	lamina lucida	microscopic subepidermal vesiculation without or with minimal neutrophilic inflammation	human
Pemphigoid Gestationis	1	nd	nd	vesicles, erosions/ulcers	mucosae and haired skin (ears, nasal planum, areas of nipples, footpads)	nd	nd (BP180 suspected)	lamina lucida	microscopic subepidermal vesiculation	human
Bullous Systemic Lupus Erythematosus	1	nd	nd	erythema, vesicles, deep erosions/ulcers; SLE signs	haired skin (footpads sloughing, friction areas) and mucosae / mucocutaneous junctions	collagen VII	nd	sublamina densa	microscopic subepidermal vesiculation without inflammation or with variable, predominantly neutrophilic inflammation	human

Autoimmune subepidermal blistering skin diseases (canine): Diagnostic diagram
(Bizikova; BMC Vet Res 2023)



Recommended literature:
Basement membrane zone structure:
https://www.aad.org/member/education/residents/bmz

1. Pozzi A, Yurchenco PD, Iozzo RV. The nature and biology of basement membranes. Matric Biol. 2017; 57-58: 1-11. DOI: 10.1016/j.matbio.2016.12.009 (open access)

2. Walko, G., Castañón, M.J. & Wiche, G. Molecular architecture and function of the hemidesmosome. Cell Tissue Res. 2015; 360, 529–544. https://doi.org/10.1007/s00441-015-2216-6 (free access)

Basement membrane zone - genetic diseases:
Variety of case-based articles; a quick search of following databases can provide you with a complete list of human and animal congenital diseases and links to the publications:
1. human diseases: Online Mendelian Inheritance in Man (OMIM): https://www.omim.org
2. animal diseases: Online Mendelian Inheritance in Animals (OMIA): https://omia.org/home/
3. Bardham A, Bruckner-Tuderman L, Chapple ILC, et al. Epidermolysis bullosa. Nat Rev Dis Primers. 2020; 78 https://doi.org/10.1038/s41572-020-0210-0

Basement membrane zone - autoimmune skin diseases
1. Egami S, Yamagami J, Amagai M. Autoimmune bullous skin diseases, pemphigus and pemphigoid. J Allergy Clin Immunol. 2020 Apr;145(4):1031-1047. DOI: 10.1016/j.jaci.2020.02.013. PMID: 32272980.

2. Olivry T, Chan LS. Autoimmune blistering dermatoses in domestic animals. *Clin Dermatol* 2001; 19: 750-760.

3. Olivry T. An autoimmune subepidermal blistering skin disease in a dog? the odds are that it is not bullous pemphigoid. Vet Dermatol 2014; 25: 316-318.

4. Tham HL, Olivry T, Linder KE, *et al*. Mucous membrane pemphigoid in dogs: A retrospective study of 16 new cases. *Vet Dermatol* 2016; 27: 376-e94.

5. Bizikova P, Linder KE, Wofford JA*, et al*. Canine epidermolysis bullosa acquisita: A retrospective study of 20 cases. *Vet Dermatol* 2015; 26: 441-50, e102-3.

6. Olivry T, Bizikova P, Dunston SM*, et al*. Clinical and immunological heterogeneity of canine subepidermal blistering dermatoses with anti-laminin-332 (laminin-5) auto-antibodies. *Vet Dermatol* 2010; 21: 345-357.

7. Bizikova P, Olivry T, Linder K, Rybnicek J. Spontaneous autoimmune subepidermal blistering diseases in animals: a comprehensive review. *BMC Vet Res.* 2023; 19 (1):55. doi: 10.1186/s12917-023-03597-1.