The Basement Membrane Zone: Making the Connection

(1st Edition, version 1.3, Video series with accompanying text and study guide)

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LTC Eduardo M. Vidal, MD, FAAD
Medical Corps, U.S. Army
Deputy Commander for Clinical Services, Raymond W. Bliss Army Health Clinic
Assistant Professor of Dermatology Uniformed Services University of Health Sciences, Bethesda, Maryland.

Consultants:
Thomas Darling, M.D., Ph.D, Director Research Laboratory Center, Uniformed Services University of Health Sciences, Bethesda, Maryland.

Leonard Sperling, M.D., Chair, Department of Dermatology, Uniformed Services University of Health Sciences, Bethesda, Maryland.

COL George Turiansky, M.D., Medical Corps, U.S. Army, Deputy Director, National Capital Consortium Graduate Medical Education, Bethesda, Maryland.

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PREFACE

I have never read the preface of a book in its entirety, until I started writing this text. Prior to compiling this work, simply had no idea of the amount of work and perseverance that was required to publish anything on this scale. I now understand why authors thank everyone in their immediate family, and everyone else they can think of. Having this newfound appreciation for the effort involved and the support that others have offered me on this endeavor, I will now start reading the forewords of all the books I read.

In every field, there are examples of pivotal moments where someone makes a discovery that leads to profound change. When connections are made between observable facts and consequence. When mysteries are explained, puzzles are solved, and questions are answered. In many cases, the answer was right there in front of us all the time. It is the ability to make the connection, between seemingly unrelated observations or facts that divides extraordinary and pedestrian. I call this phenomenon, "making the connection."

I recall sitting in the lecture hall during dermatology residency listening to basic science lectures. One random fact and association after another paraded through a slide presentation, with the intent to cover all the material we would need to pass our boards. Comprehension was not the goal. The object of this draconian cognitive experience was to drill facts and associations into the deep recesses of our minds so that we could recall these random bits of information on multiple-choice examinations some years later. Well, it worked – somewhat. On my examinations, I was able to pick the right answers, because I could recall seemingly random facts from sheer repetition.

There are those who believe the residency experience is a rite of passage. We all suffered through the countless hours of memorization and study. A few did not fully understand all of the lectures, but we all knew enough to pass the exams. To some with the "rite of passage" mindset, the end justifies the means. The rationale being that one acquires knowledge through repetition and clinical experience. I believe there is a better way.

The basic sciences are crucial. In this work, I set about to explain relevancy and place the material in a context that makes the information meaningful and vital. From my point of view, basic science is not a rite of passage; it is an essential part of the core dermatology curriculum. The focus of this work is on the basic science of the basement membrane, not on the clinical diagnosis and management of basement membrane disorders. The intended audience for this work includes medical students, basic science researchers who wish to get an introduction to the basement membrane zone, dermatology residents, dermatologists, and dermatology educators.

The included DVD facilitates comprehension by presenting a three-dimensional visualization of the epidermal basement membrane zone and its components. The goal of the presentation is a clear understanding of the basement membrane zone - as we

understand it to date. Hence, true fidelity of in-vivo structure is neither required, nor ideal. The molecules are stylized depictions designed to illustrate important concepts and facilitate retention of key facts. Currently, the exact three-dimensional structure of every basement membrane zone molecule is unknown. We have focused on key structural features such as unique molecular architecture, binding sites, and known locations within the basement membrane zone when designing the molecules. Structural domain nomenclature for each molecule has been simplified whenever possible to focus on important functional aspects of the molecules. Copies of all key animation sequences are available on the DVD, in both QuickTime and Audio Video Interleave (AVI) format for royalty-free use in personal lectures and presentations.

Study notes are bundled as an extra with the DVD. These notes are available as a Portable Document Format (PDF) file, for viewing on portable digital media devices, or for printing at home. Despite the flood of digital media alternatives to printed textbooks available to dermatology residents, printed texts, or “handheld” notes are preferred by most. In a recent research study conducted by a private consulting firm, on behalf of a “smart pen” manufacturer, 87% of today’s workers who utilize digital information devices make use of handwritten notes. Surveys conducted on college students indicate that at least 75% of students prefer printed textbooks over e-books.[6]

ACKNOWLEDGEMENTS

I would like to thank my wife and son, who inspire me to do more. I was fortunate to have my mentors as consultants in this endeavor. They provided the support and encouragement that I needed. Drs. George Turiansky, Tom Darling, and Leonard Sperling, are my dermatology role models, and I owe them a tremendous debt. Past (and current) mentors to whom I am forever grateful include: Drs. Larry Bray, Don Roach, Ronald Wolff, Stephen Goldberg, Arnon Krongrad, George Keough, and Scott Norton.

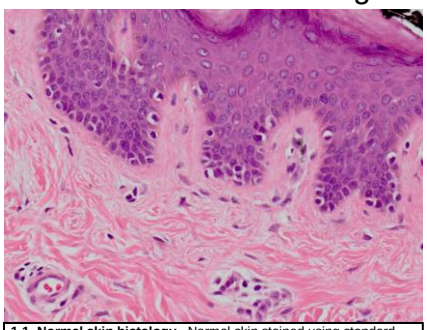
The American Academy of Dermatology's Sulzberger Institute provided me with the grant that made this work possible.

STRUCTURE

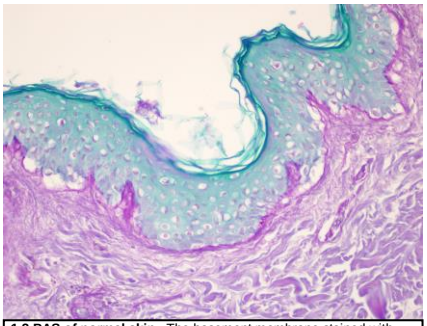
Integument: in-ˈte-gyeə-mənt (n). an enveloping layer (skin or membrane) of an organism...[7]

The skin is a highly specialized and dynamic structure. Its beauty matched only by its wondrous complexity. Its integrity is maintained by a specialized structural complex that anchors the overlying epidermis to the dermal matrix below known as the basement membrane zone. We will explore the basement membrane zone, and make the connection between structure, function, and clinical relevance.

The basement membrane zone is the area of adherence between the basal layer of epidermal keratinocytes and the dermis directly underneath. The basement membrane zone has an approximate surface area of 11-20 ft^2 (1-2m^2), which is roughly equivalent to the surface area of human skin. [8-14] The term "basement membrane" actually refers to the structure seen using light microscopy.[15,16] With conventional light microscopy, one cannot appreciate the complexity of the basement membrane's structure. With routine staining (Figure 1.1), the basement membrane appears to be little more than a barely perceptible thin undulating line beneath the epidermis.[17] Standard hematoxylin and eosin (H&E) stains, impart a pink color to collagen and dermal tissues and do not routinely stain the basement membrane adequately. Special stains such as Periodic acid-schiff stain (Figure 1.2) that imparts a reddish-purple color to glycogen, mucopolysaccharides, and mucin, are used to identify the basement membrane more clearly.[16,18]



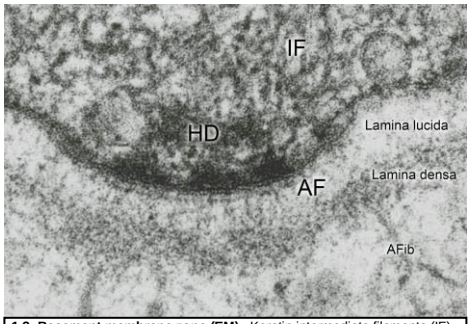
1.1 Normal skin histology. Normal skin stained using standard hematoxylin and eosin (H&E) staining.



1.2 PAS of normal skin. The basement membrane stained with periodic acid-schiff stain.

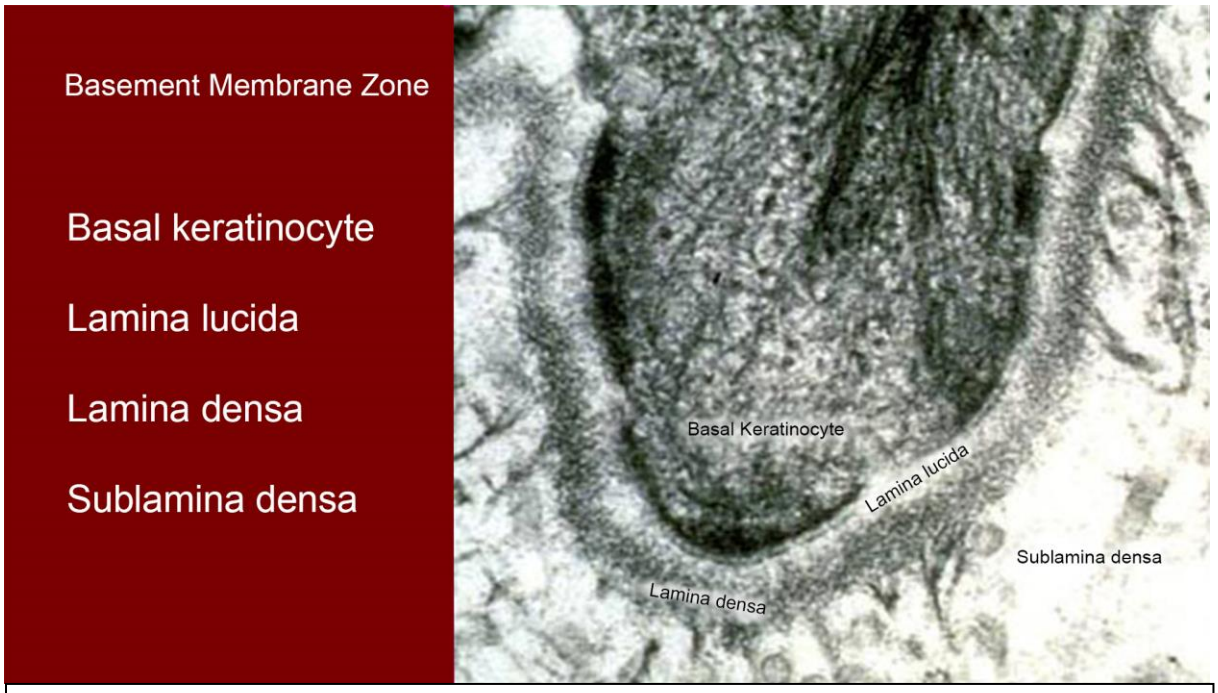
Despite the relative ease with which we can identify the basement membrane on Periodic acid-schiff staining, with an average thickness of 50 nm (lamina densa)[16] the limits of resolution are beyond that of the light microscope. However, with electron microscopy, we can see a great deal of detail - down to 2 nm resolution with 2D images (Figure 1.3).[19] From George Odland's[20] identification of the basement membrane in 1958, to Robert Briggaman and Clayton Wheeler's 1975 comprehensive review,[18] these early electron microscopic studies have formed the basis for our current understanding of the basement membrane. Much of the nomenclature used in Briggaman and Wheeler's review is still in use today.[21]

At the basal keratinocyte level, we can identify structures known as hemidesmosomes and keratin intermediate filaments (Figure 1.3). Beneath the basal keratinocyte is a large sheet-like matrix upon which these basal keratinocytes rest. This extracellular matrix is the known as the basal lamina. It is composed of both the lamina lucida and the lamina densa. Within the lamina lucida, we can see thin strand-like structures known as anchoring filaments. Beneath the lamina lucida and lamina densa (aka basal lamina), is the sublamina densa (superficial papillary dermis). With the sublamina densa we can see loop-like structures called anchoring fibrils.



1.3 Basement membrane zone (EM). Keratin intermediate filaments (IF) insert into hemidesmosomes (HD). Anchoring filaments (AF). Anchoring fibrils (AFib).

Based on the electron microscopic appearance of the basement membrane zone, this zone is divided into four distinct layers (Figure 1.4). These four distinct layers are the: (1) the basal keratinocyte layer, (2) the lamina lucida, (3) the lamina densa, and (4) the sublamina densa (superficial papillary dermis). Collectively, these layers make up the "basement membrane zone".

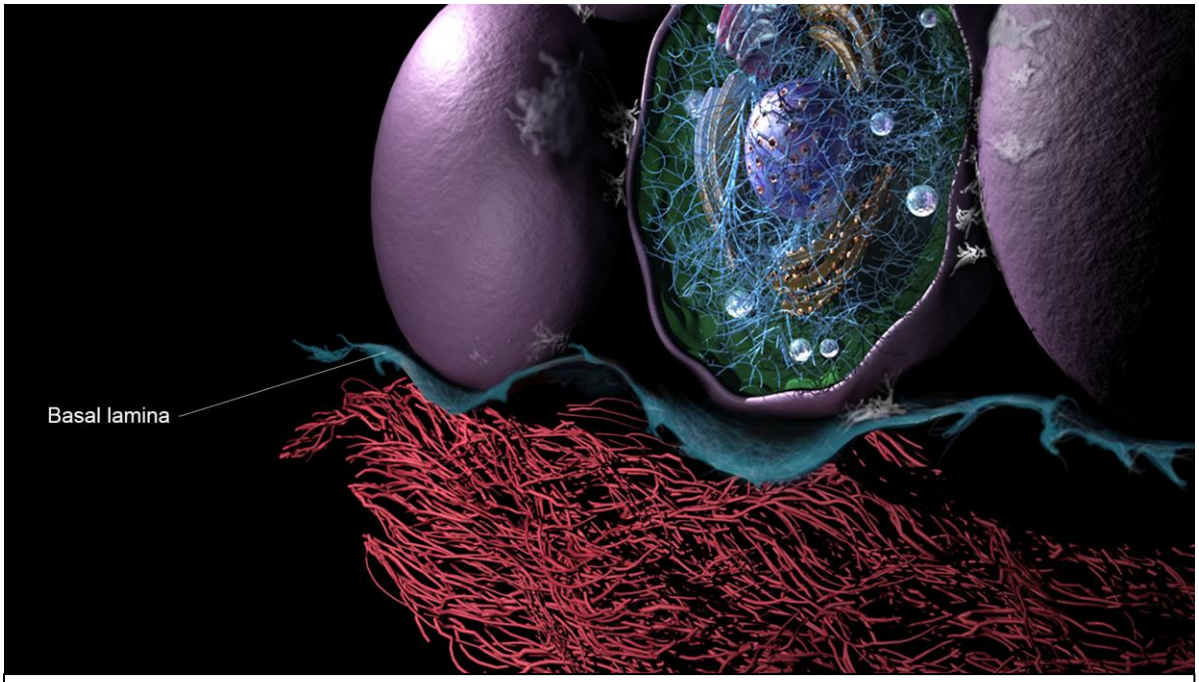


1.4 Four layers of the basement membrane zone. (1) The basal keratinocyte layer, (2) the lamina lucida, (3) the lamina densa, and (4) the sublamina densa (lamina reticularis).

Although most of what we know today is based on these observations, the electron micrographic images studied may not reflect the actual structure of the basement membrane zone in vivo. Our current understanding of the ultrastructural components of the basement membrane is limited by the techniques and processing methods used for immunoelectron microscopy.[21] For example, there is evidence that

the lamina lucida may be an artifact of specimen processing.[22] This alleged artifact is present in most electron microscopic images of skin and is therefore, a valid way to conceptualize the basement membrane zone.

Often, the terms “basement membrane” and “basement membrane zone” are used interchangeably. However, the term “basement membrane” refers to the structure seen on light microscopy (using PAS stain). On scanning electron microscopy, a sheet-like structure appears underneath basal keratinocytes. This dense fabric-like structure is the “basal lamina” (Figure 1.5). The basal lamina seen using scanning electron microscopy is also often referred to as the “basement membrane”. It is more appropriate to view the epidermal basement membrane, not as a distinct structure, as its light microscopic appearance would imply, but, as a zone of adherence between epidermal basal keratinocytes and the extracellular matrix underneath. Hence, we will use the more appropriate term “basement membrane zone” from now on.



1.5 Basal lamina. The basal lamina appears as a sheet-like structure of densely woven fibers on scanning electron microscopy.

Electron microscopy has given us the basic overall structure of the basement membrane zone and its major components. However, the composition and fine structural details of these components have been further refined by scientists using molecular biology techiques. In just a few years, the list of molecules that have been identified within the basement membrane (Table 1.1) has been growing, underscoring the enormous complexity of the BMZ.[23] There are currently at least 13 distinct molecules with important functions within the basement membrane zone.

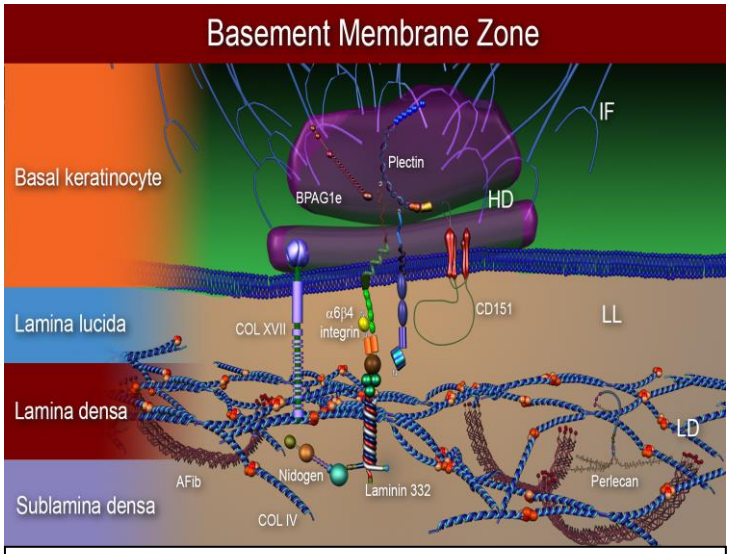
Table 1.1 Basement membrane zone molecules.

Molecule	Basal Keratinocyte	Lamina Lucida	Lamina Densa	Sublamina Densa
Intermediate filaments	✓			
BPAG1*	✓			
Plectin*	✓			
Collagen XVII*	✓	✓		
α6β4 integrin*	✓	✓		
α3β1 integrin	✓	✓		
CD151	✓	✓		
Laminin 332		✓	✓	
Laminin 511		✓	✓	
Laminin 311		✓	✓	
Collagen IV		✓	✓	
Perlecan			✓	
Collagen VII			✓	✓

* Hemidesmosome components.

These discoveries have allowed us to further refine our understanding of this amazing structure. In reality, the distinctions between the layers of the basement membrane zone that are seen in electron microscopy are not as clearly defined at the molecular level. Many molecules extend through several of these layers; however, the four layered basement membrane zone concept helps us organize this complex structure.

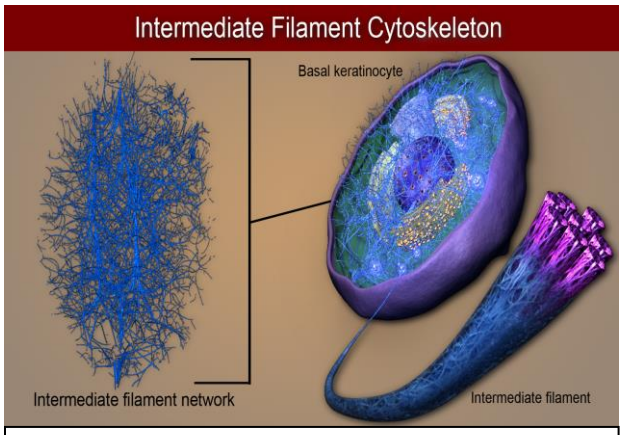
At this point, the overall arrangement of the basement membrane zone begins to take shape (Figure 1.6). The basal keratinocyte is anchored to the basal lamina via the intermediate filaments and hemidesmosomes. Molecules within the basal lamina connect the basal keratinocyte to the basal lamina, seen on scanning microscopy, which in turn anchors the entire basement membrane zone to the underlying collagenous matrix of the superficial papillary dermis. The concept of the basement membrane as a zone of adherence is valid, in that the components span the entire area from the basal keratinocyte to the superficial papillary dermis.



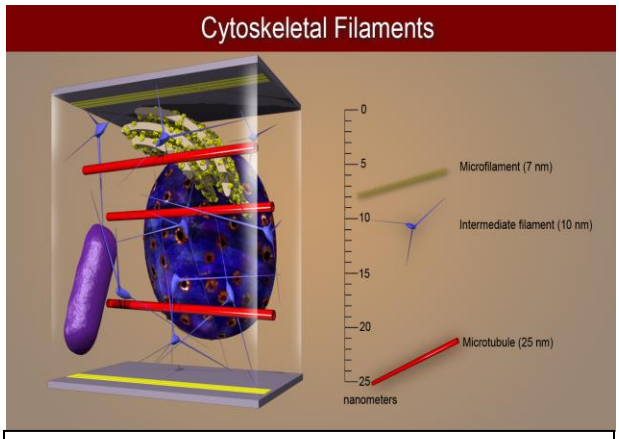
1.6 Basement Membrane zone. The four layers of the basement membrane zone: (1) basal keratinocyte layer, the (2) lamina lucida layer, (3) the lamina densa layer, and (4) the sublamina densa layer. Within the basal keratinocyte, are keratin intermediate filaments (IF), hemidesmosomes (HD), composed of bullous pemphigoid antigen 1 (BPAG1e), plectin, α6β4 integrin, α3β1 integrin, and collagen XVII, and CD151. Within the lamina lucida (LL) are anchoring filaments (AF), composed predominantly of Laminin 332. Also within the lamina lucida are the extracellular portions of collagen XVII, α6β4 integrin, and CD151, and laminins 311 and 511 . Within the lamina densa (LD) are collagen IV, Laminins 332, 311, and 511, nidogen and perlecan. Within the sublamina densa are anchoring fibrils (AFib), composed of collagen VII.

BASAL KERATINOCYTE LAYER

Within each basal keratinocyte is a structural framework known as the cytoskeleton (Figure 1.7). This internal skeleton serves many important functions within the cell. It serves as a structural support, facilitates cell movement, serves as a dynamic scaffold for intracellular transport of organelles and helps anchor cells to their surrounding environment. The cytoskeleton is composed of three main types of filaments: microfilaments, microtubules, and intermediate filaments (Figure 1.8). Intermediate filaments are 10 nanometers in diameter. They are so named, because their diameter is between that of smaller microfilaments and larger microtubules.[24] First visualized in x-ray diffraction analyses, and electron microscopic studies, intermediate filaments were recognized as a distinct cytoskeletal structures by the mid 1970's.[25] Intermediate filaments form a protein scaffold or, internal skeleton for keratinocytes. They form the first link within the basement membrane zone that helps anchor basal keratinocytes to the underlying dermal connective tissues (Figure 1.6).



1.7 Intermediate filament cytoskeleton. Human keratinocyte intermediate filament cytoskeleton.



1.8 Cytoskeletal filaments. There are three main types of filaments: microtubules, intermediate filaments, and microtubules. Intermediate filaments diameter (10 nm), is between that of smaller microfilaments (7 nm) and larger microtubules (25 nm).

There are 6 types of intermediate filaments, sorted based on DNA and amino acid sequence homology (Table 1.2.).[5,26,27] The keratins are the largest subgroup of intermediate filaments.[28] There are two main types of keratins found in epithelial cells: types I and II, known as the acidic and basic keratins respectively.[25,27] The current nomenclature for these keratin proteins, established in 2006,[29] classifies Type I acidic keratins as keratins K9-10, K12-28, and K31-K40, and Type II basic keratins as K1-K8, and K71-86. Type I genes are located on chromosome 17, and type II genes on chromosome 12, with the exception of keratin 18, which is a type I filament, and is located alongside type II genes on chromosome 12.[30]

Table 1.2 **Intermediate filament classification.**

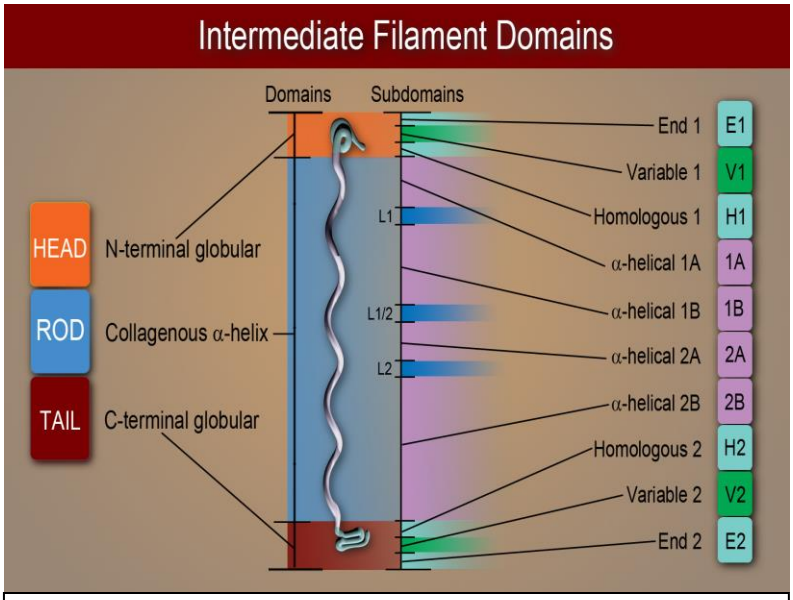
Type	Description
I	Acidic, K9-10, K12-28, and K31-K40
II	Basic, K1-K8, and K71-86
III	Desmin, glial fibrillary acidic protein (GFAP), peripherin, Vimentin
IV	α-internexin, neurofilaments, synemin, syncoilin
V	Laminins
VI	Nestin

The highly regulated process of epidermal keratinization is the result of differential expression of within the epidermis (Table 1.3).[28] Basal keratinocytes express intermediate filament keratins 5 and 14. Suprabasal keratinocytes express keratins 1 and 10, which comprise approximately 85% of total proteins found in fully differentiated keratinocytes.[28] In the upper spinous and granular layers, keratin 2e is produced. Keratin 9 is found in palmoplantar epidermis. Keratins 3 and 12 are seen in corneal epithelium. Keratins 4 and 13 are found in non-cornifiying mucosa. Keratins 6, 16 and 17 are found in hair, nail beds, and hyperproliferative skin states such as during wound healing.

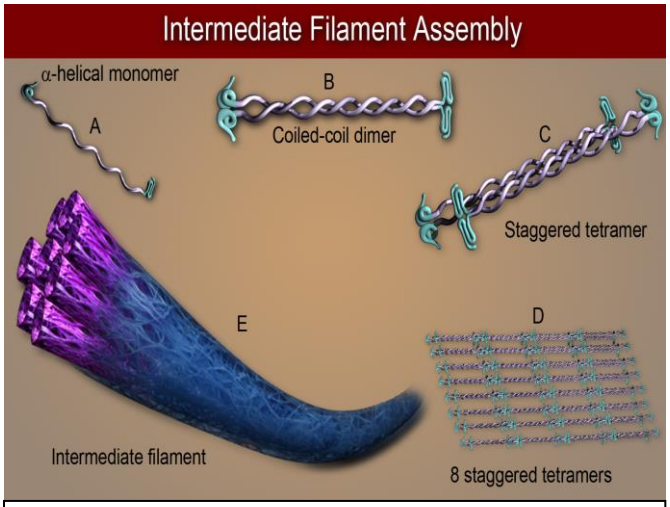
Table 1.3 **Keratin intermediate filament expression patterns.**[26-28,30,31]

Keratin(s)	Expression pattern	Disease association
1	Suprabasal keratinocytes	Ichthyosis hystrix (Curth-Mackin) Palmoplantar keratoderma (striae) Palmoplantar keratoderma (mild ichthyosis) Greither's syndrome
1/10	Suprabasal keratinocytes (stratum spinosa)	Epidermolytic hyperkeratosis
1/16	Suprabasal keratinocytes	Palmoplantar keratoderma (non-epidermolytic)
2e	Stratum spinosa/granulosum	Ichthyosis bullosa of Seimens
3/12	Cornea	Meesmann corneal dystrophy
4/13	Mucosa	Oral white-sponge nevus of Cannon
5	Basal keratinocytes	Dowling-Degos Epidermolysis bullosa simplex (EBS) with migratory circinate erythema. EBS with severe palmoplantar hyperkeratosis
5/14	Basal keratinocytes	EBS-generalized other (formerly Weber-Cockayne, Dowling-Meara, Koebner types) EBS with mottled pigmentation
6a/16	Outer root sheath of hair follicle, nail bed, palmoplantar skin and orogenital mucosa	Pachyonychia congenita (type I)
6b/17	Nail bed, hair follicle, sebaceous glands	Pachyonychia congenita (type II)
6c/16	Suprabasal keratinocytes	Palmoplantar keratoderma, nonepidermolytic (focal)
6/16/17	Hyperproliferative epidermis (e.g. stress, wound healing, dermatoses)	Psoriasis Acute skin inflammation (UV exposure, infection)
8	Bowel, Liver	Chronic pancreatitis Inflammatory bowel disease
8/18	Bowel, liver	Cirrhosis and hepatitis
9	Palmoplantar	Palmoplantar keratoderma (Epidermolytic)
14	Basal keratinocytes	EBS (recessive) Dermatopathia pigmentosa reticularis Naegeli-Franceschetti-Jadassohn syndrome
17	Hair	Steatocystoma multiplex
18	liver	Hepatic artery thrombosis
74	Hair	Woolly hair (autosomal-dominant)
75	Hair	Loose-anagen syndrome Pseudofolliculitis barbae
81/86	Hair	Monilethrix
85	Hair	Ectodermal dysplasia (hair-nail type)

Types I & II intermediate filaments (keratins) are composed of three domains: (1) a central alpha-helical rod domain, (2) an amino-terminal head, and (3) a carboxyl-terminal tail domain (Figure 1.9).[5,25,32,33] Both the head and tail globular domains contain end, variable, and homologous subdomains.[34] The central α-helical domain is approximately 330 amino acids long and is divided into four subdomains (1A, 1B, 2A, and 2B), with three linker sequences.[27] Hydrophobic residues within the central α-helical rod domain positioned at points along the domain, results in self-dimerization into a coiled-coil configuration. The polar ends of the molecule are highly charged. This is the basic structural core of the intermediate filament.



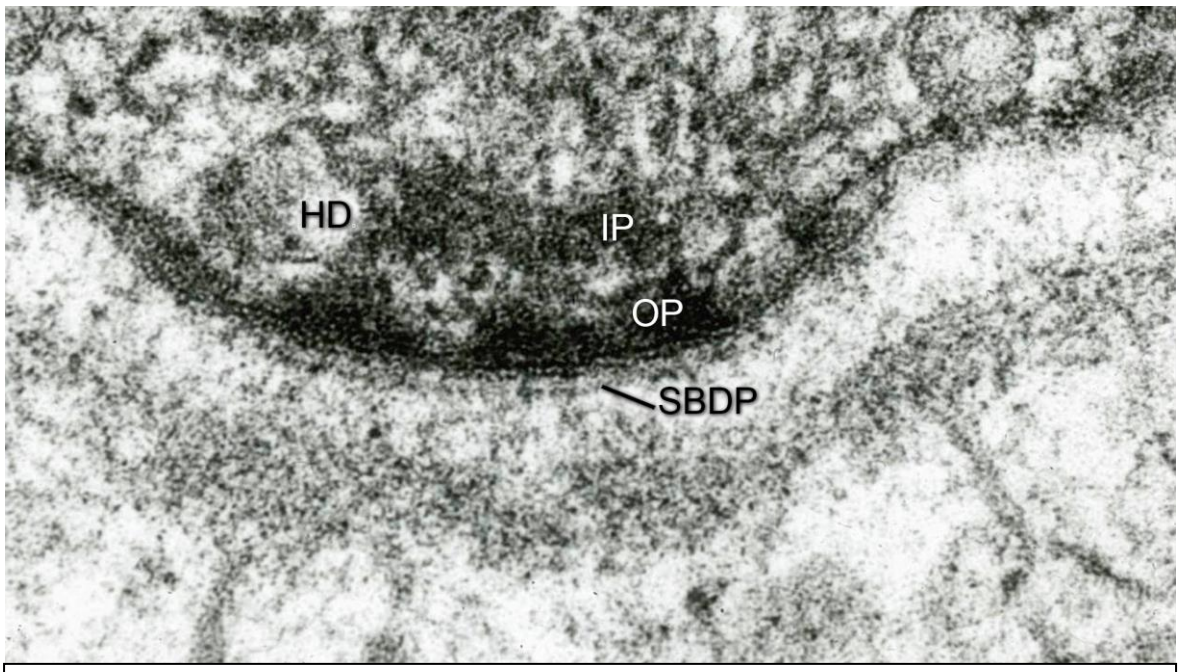
1.9 Intermediate filament domains. Domain structure of intermediate filaments, with a central α-helical coiled rod domain, an amino-terminal head, and a carboxyl-terminal tail. Both the head and tail globular domains contain End, Variable, and Homologous subdomains. The central α-helical domain is divided into four subdomains (1A, 1B, 2A, and 2B), with three linker sequences (L1, L1/2, and L2).



1.10 Intermediate filament assembly. Intermediate filament α-helical monomers (A) self-assemble within the cytoplasm, into dimers (B),[4] using parallel coiled-coil interactions, with both amino-terminal heads on one end, and the carboxyl-terminal tail portions on the other.[5] A staggered tetramer (C) then forms from the union of two dimers. Eight staggered tetramers (D) assemble into a 16 nm transition configuration, which then becomes compacted into the 10 nm fully developed intermediate filament (E).

Intermediate filaments are produced by basal keratinocytes, where they self-assemble from an α-helical monomer, into intermediate filaments containing eight tetramers each (Figure 1.10). Intermediate filament α-helical monomers self-assemble within the cytoplasm, into a dimer,[4] using parallel coiled-coil interactions, with both N-terminal heads on one end, and the C-terminal tail portions on the other.[5] The amino-terminal "head" portion is considered essential for intermediate filament assembly.[33] A staggered tetramer configuration then forms from the union of two dimers. Eight staggered tetramers will then assemble into a 16 nm transition configuration, which then becomes compacted into the 10 nm fully developed intermediate filament. The staggered tetramer configuration, with its overlapping dimers, confers significant tensile strength to the cytoskeleton.

Intermediate filaments attach onto electron dense rivet-like plaques, studded along the ventral surface of a basal keratinocyte cell, called hemidesmosomes (Figure 1.11). These small dense (< 0.5 µm) structures are asymmetrically distributed along the portions of the basal keratinocyte that attach to the basement membrane. High magnification view of a hemidesmosome shows that it appears to be a two plaque type structure[35] - with an inner and an outer plaque. The outer plaque rests on the inside of the plasma membrane, within the cell. Sometimes, an electron dense thin line can be seen underneath hemidesmosomes, outside the cell and adjacent to the plasma membrane. This structure is referred to as the sub-basal dense plate (SBDP).[35,36]



1.11 Hemidesmosome. Electron microscopic image of Hemidesmosome (HD). Hemidesmosomes have an inner plague (IP) and an outer plaque (OP). Sometimes a thin dense line can be found underneath, known as the sub-basal dense plate (SBDP). The inner plaque of hemidesmosomes is composed of BPAG1 and plectin. The outer plaque is composed of α6β4 integrin, collagen XVII.

Since the discovery of the first hemidesmosomal component in the early 1980's,[37,38] our knowledge of the composition of the hemidesmosome has greatly expanded. The hemidesmosome is composed of at least 5 distinict molecules: (1) bullous pemphigoid antigen 1 (BPAG1), (2) plectin, (3) integrins, (4) collagen XVII (aka BPAG2), and (5) CD151.[23,35,36,39] The first two molecules, BPAG1[40] and Plectin, are found within the basal keratinocyte, and have been localized to the inner plaque of the hemidesmosome. The next two molecules, α6β4 integrin, and collagen XVII[40] extend beyond the basal keratinocyte membrane, and into the lamina lucida layer of the basement membrane zone, and hence they are known as transmembrane molecules. These latter two molecules can be found within the outer plaque of the hemidesmosome. The last molecule, CD151, the most recently discovered molecule, is closely associated with α6β4 integrin, and resides at or near the surface of the basal cell plasma membrane.[41,42]

Cytoplasmic portions of CD151 have been identified within and adjacent to hemidesmosomal structures,[41] but is not considered an essential hemidesmosomal structure, as its role is yet to be fully defined.

At least two types of hemidemsmosome have been identified:[36] type I or classical hemidesmosomes, and type II hemidesmosomes (Table 1.4). Type II hemidesmosomes are found in fetal skin and in tissues such as the intestines,[43,44] and contain only α6β4 integrin and plectin.[45-47] Type II hemidesmosomes can be considered immature or developing hemidesmosomes. Type II hemidesmosomes are also seen in the early phases of wound healing within the epidermis.[41] Once BPAG1 and collagen XVII become integrated into type II hemidesmosomes, they become mature type I hemidesmosomes.

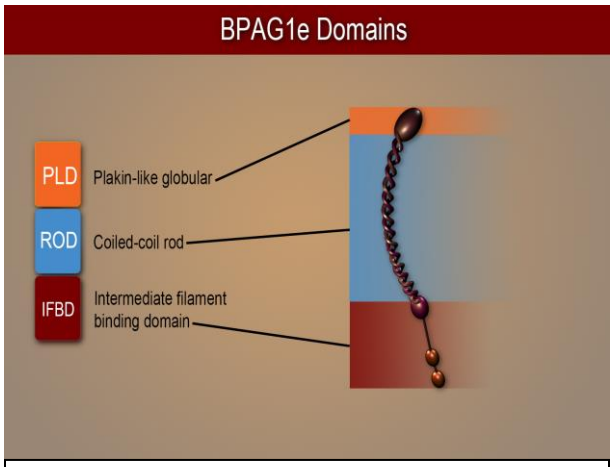
1.4 **Types of Hemidesmosomes.**

Component	Type I (Adult)	Type II (Fetal)
BPAG1	✓	
Plectin	✓	✓
Collagen XVII	✓	
α6β4 integrin	✓	✓

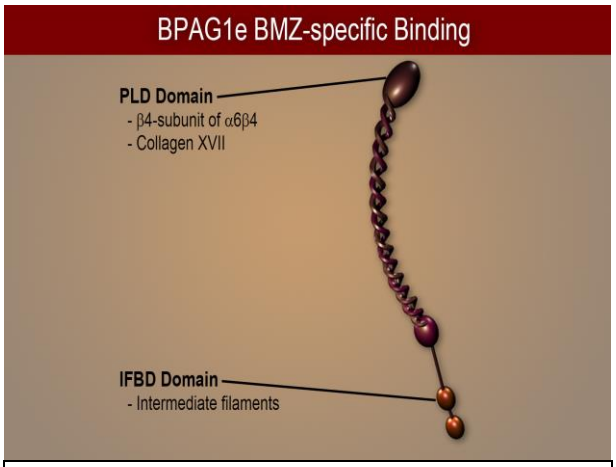
The intermediate filaments attach to BPAG1 and plectin, which compose the hemidesmosome inner plaque.[48] Both plectin and BPAG1 belong to the plakin family of proteins, and share significant sequence homology. Members of the plakin family are known as cytolinkers, as they form vast networks of dynamically linked cytokeratin filaments within cells.[49] These structural proteins are located at adhesion sites in a myriad of different types of cells within mammals. Binding sites near their carboxyl-terminal ends bind intermediate filaments, while sites near their amino-terminal ends target hemidesomosomal outer plaque components and plama membrane binding sites.[50]

BPAG1 was one of the first hemidesmosomal components identified[37,38] and has been associated with bullous pemphigoid[51] since the late 1990's. BPAG1 exists in several different isoforms, but it is the 230 kDa epidermal form known as BPAG1e, that is relevant to the epidermal basement membrane zone. However, there is no known genetic disease associated with BPAG1e.[35] A 1993 study[40] using autoantibodies against BPAG1e, demonstrated that the molecule is located inside basal keratinocytes, 40-140 nm from the plasma membrane. We now know it is localized the inner plaque of the hemidesmosome. BPAG1e is approximately 159-186 nm in length[52] and has 3 distinct domains (Figure 1.12), including an amino-terminal plakin-like globular domain, a central coiled-coil rod, and an intermediate filament binding domain carboxyl-terminal end.[35,49,50,53,54] A coiled-coil central rod flanked by two globular ends

represents the overall shape of the molecule. The carboxyl-terminal (tail) region interacts with intermediate filaments (Figure 1.13),[54] while the amino-terminal (head) plakin domain interacts with Collagen XVII and the β4-subunit of α6β4 integrin.[53]

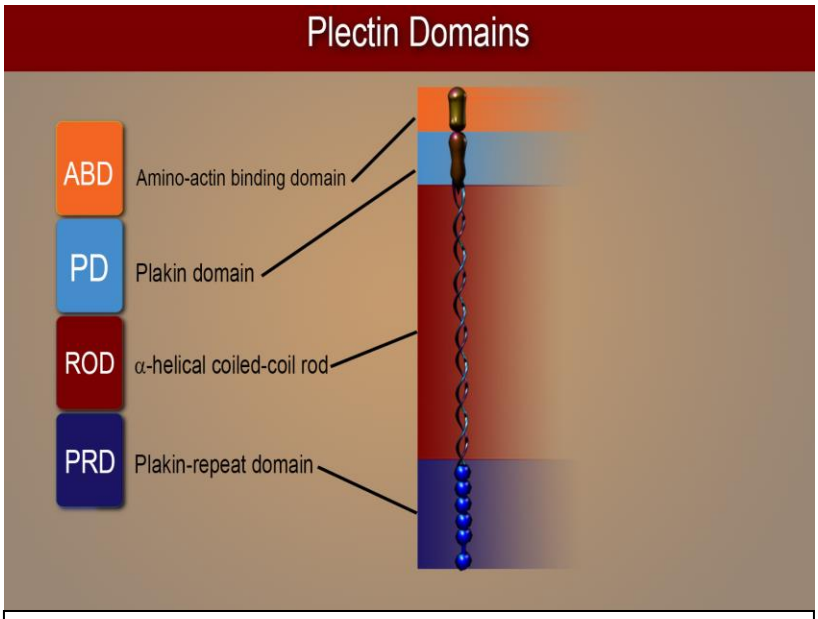


1.12 Bullous Pemphigoid Antigen 1 (BPAG1e) domains.



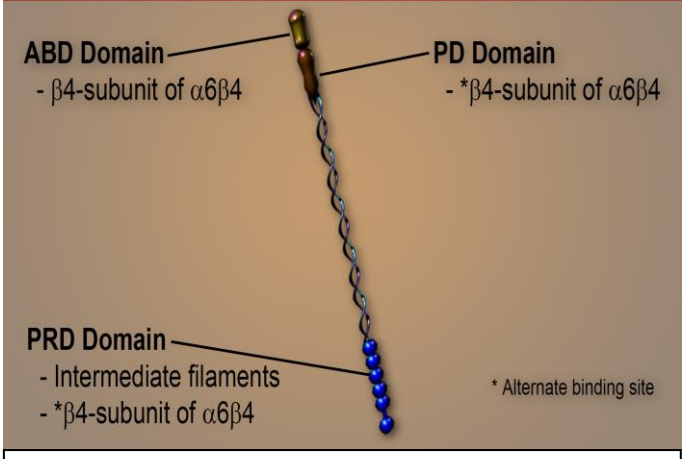
1.13 Bullous pemphigoid antigen 1 (BPAG1e) BMZ-specific binding.

Plectin, like BPAG1e, is a member of the plakin family of proteins. This large protein interacts with all the cytoskeletal proteins: microfilaments, intermediate filaments, and microtubules. Plectin has several isoforms, generated by differential splicing of amino-terminal sequences, which are thought to target different cellular locations. There are two main isoforms in keratinocytes (plectin 1a and 1c). Plectin 1a is the only isoform that binds keratin intermediate filaments to hemidesmosomes.[55]



1.14 Plectin domains.

Plectin is contains a 184-200nm long alpha-helical coiled-coil central rod, with a 2nm diameter, and large (9nm diameter) carboxyl and amino-terminal globular domains at each end, that resembles a dumb-bell[56].[49,57-59] There are four distinct functional domains in a plectin molecule (Figure 1.14), which serve as binding or attachment sites. These include: (1) an amino-terminal actin binding domain, (2) a plakin domain, (3) a coiled-coil α-helical rod domain, (4) and a carboxyl-terminal plakin-repeat-like domain.[49] Plectin binds to intermediate fillaments within the inner plaque, via the R5 subsegment of the plakin-repeat domain (Figure 1.15).[58] The inner and outer hemidesmosomal plaques are linked by the binding of inner membrane proteins, plectin and BPAG1e, to the outer membrane proteins, α6β4 integrin and collagen XVII,[50] thereby stabilizing the hemidesmosome complex[60,61] Binding to α6β4 integrin occurs via the actin-binding domain adjacent to the amino-terminal, which specifically binds the β4-subunit.[62] Alternative binding sites for the β4-subunit of integrin can be found in the plakin domain and the carboxyl-terminal portions of plectin.



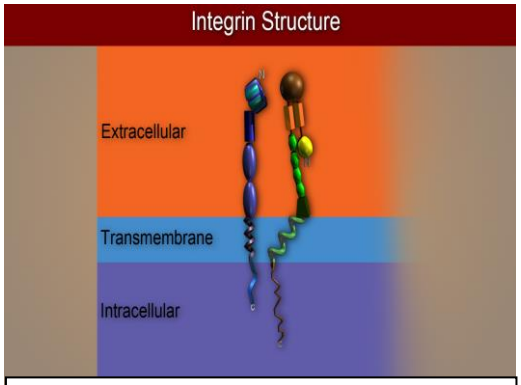
1.15 Plectin BMZ-specific binding. Plectin stabilizes the hemidesmosome by binding both intermediate filaments and outer plaque components (α6β4 integrin and collagen XVII).

Integrins were first described in 1986,[63] and were so named, because they were thought to be an "integral" basement membrane protein, as it linked the cytoskeleton to the extracellular matrix.[64,65] All integrins are transmembrane heterodimeric receptors containing α- and β-subunits that resemble a large "head" on two "legs".[64,65] There are many different integrins, with at least 24 integrins identified in humans, each composed of a unique combination of non-covalently bonded 18 α-subunits and 8 β-subunits.[65,66] These integrins can be grouped based on ligand specifity, such as: laminin-binding (α6β4), collagen-binding (α3β1), leukocyte-binding, and arginine-glycine-aspartic acid-binding or RGD-binding.[66]

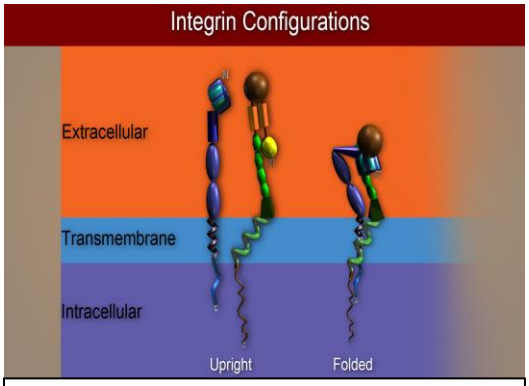
Within the epidermal basement membrane zone are found two main integrins: α6β4 and α3β1.[23] Both of these integrins are receptors for basement membrane zone laminins;[66] however, the α6β4 integrin is unique to the outer plaque of the keratinocyte hemidesmosome, while α3β1 integrin found in many different types of cells[67] - not just keratinocytes - and is seen in other zones of adhesion, such as focal contacts.[23] The two integrins also differ in their function via the binding of laminins. α6β4 integrin and laminin-332 interactions are important in formation of stable adhesion complexes, whereas α3β1 integrin and laminin-332 interactions are important during initial adhesion and migration.[68] The importance of α3β1 integrin may rest on its function as a hemidesmosomal precursor. α3β1 along with CD151 are first two hemidesmosomal molecules identified in the early stages of hemidesmosome development.[41] The

significant interactions of α3β1 integrin are localized with the lamina lucida layer of the basement membrane zone during early hemidesmosome development, and in the lamina densa. It is likely that α3β1 serves as a regulator of basment membrane development, as it is present in pre-hemidesmosomal stages; yet, hemidesmosome formation is known to occur without it.[69] α3β1 integrin is required for the formation of a continuous lamina densa; but, a continuous lamina densa is not needed for hemidesmosome development.[69]

α6β4 integrin, first discovered in the late 1980's,[70] is a heterodimeric transmembrane glycoprotein. It is a two-way signaling molecule that links the keratinocyte cytoskeleton to the extracellular matrix.[42,71] α6β4 integrin is composed of distinct α- and β-subunits. Both the alpha and the beta-subunits contain cytoplasmic, transmembrane, and extracellular domains (Figure 1.16). The extracellular portions are a current source of controversy, as they are believed to exist in at least two configurations (Figure 1.17): a folded or compacted form (~11 nm), or a postulated upright structure (~19 nm).[65] For the sake of simplicity, the upright model will be used. It is important to understand that whether or not integrins adopt a radical conformational change upon ligand binding,[72] or a more sutble change,[73] is still subject to debate.

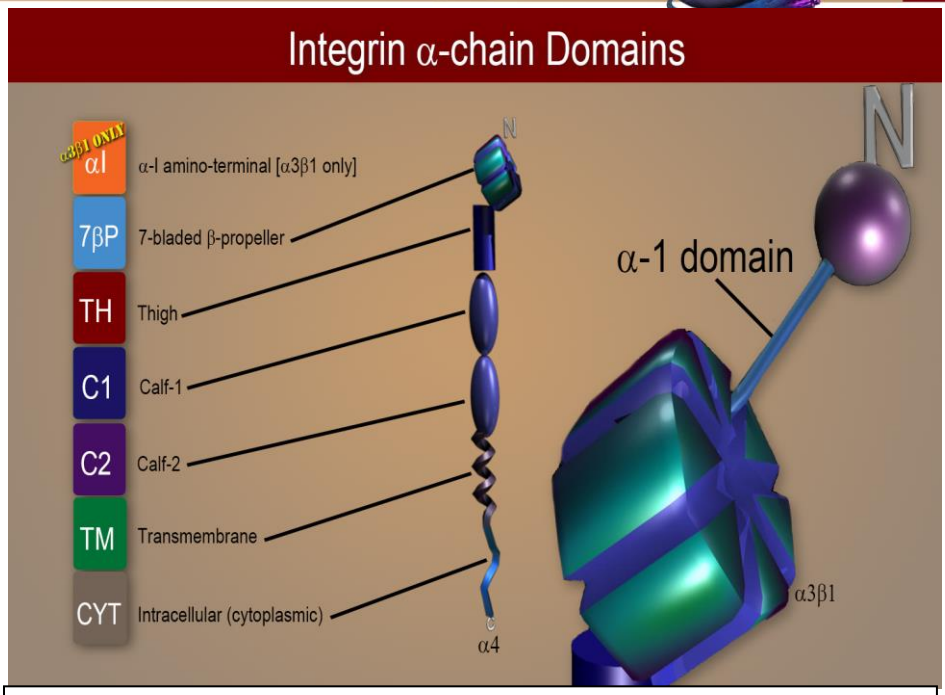


1.16 Integrin structure. α6β4 integrin is composed of two main subunits (α and β-subunits), each of which has cytoplasmic, transmembrane and extracellular domains.



1.17 Integrin configurations. Basement membrane zone integrins are theorized to exist in one of two configurations: (A) a folded or compacted form, or an (B) upltright form.

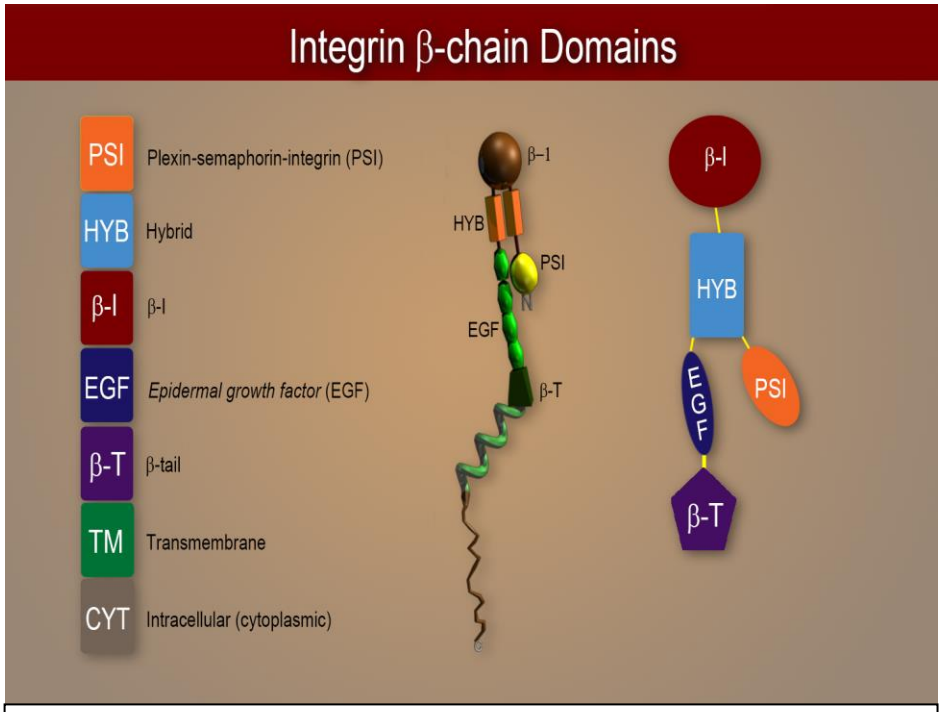
The α-subunit contains a large extra-cellular amino portion, and a relatively smaller, intracellular carboxyl-terminal portion. In contrast, the intracellular portion of the beta-subunit is very large. In fact, the β4-subunit, the largest β-subunit of all the integrins, is responsible for the majority of the intracytoplasmic interactions of this integrin.[61,74,75]



1.18 Integrin α-chain domains. α3β1 contains an additional α-I domain that is inseted between blades 2 and 3 of the 7-bladed β-propeller domain.

The α-chain of α6β4 integin has six domains (Figure 1.18): Extracellularly, (1) a 7 bladed β-propeller domain, (2) a thigh domain, (3) a calf-1 domain, (4) a calf-2 domain; (5) a transmembrane domain, and (6) a cytoplasmic (intracellular) domain. α3β1 has an additional 200 amino-acid containing α-I domain that is seen in only 9 of the 18 integrin mammalian α-chains (a1, α2, α10, α11, α E, αL, αM, αX, and ascidian αHr1).[66] The α-I domain has a ligand binding function, aided by its significant flexibility, and represents a rather recent evolutionary change for integrins, as it is found only in vetebrates.[76] The thigh domain is bounded by two flexible sections found between the β-propeller above, and the calf-1 domain below. These sections allow for conformational changes within the molecule that regulate binding affinity.[65] The cytoplasmic domain also has a significant of flexibility allowing it to conform to a multitude of intracellular protein ligands.

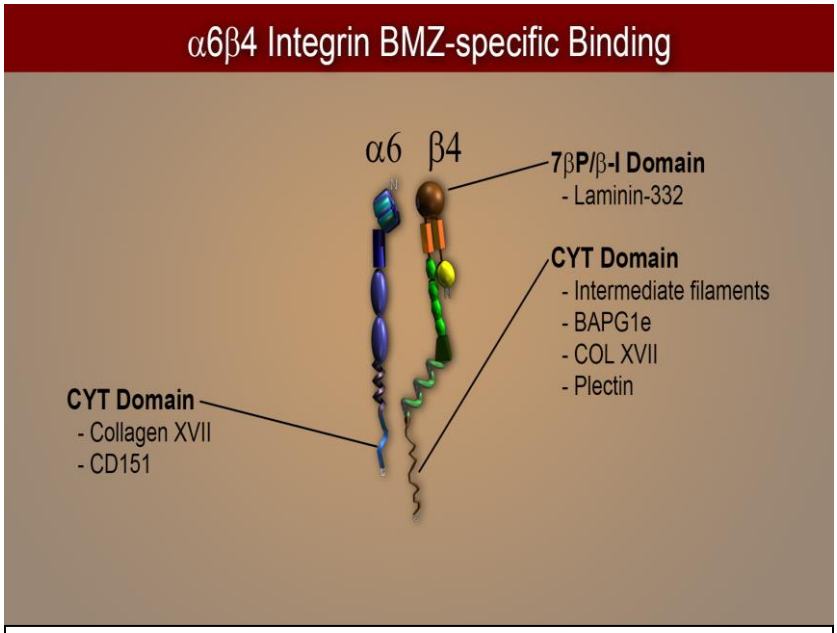
The β-chain of α6β4 integin has seven domains (Figure 1.19): Extracellularly, (1) a plexin-semaphorin-integrin (PSI) domain, (2) a hybrid domain, with an inserted (3) β-I domain, (4) four *epidermal growth factor* (EGF) repeats, and (5) a β-tail domain; (6) a transmembrane domain; and (7) a cytoplasmic (intracellular) domain. The amino-terminal PSI domain contains approximately 50 amino acids, and forms a two-stranded antiparallel β-sheet flanked by two short helices, and contains the leucine to proline amino acid #33 substitution known as the PI allele, which predisposes individuals to arterial thrombotic events (and early death).[77] The β-I domain inserts into the hybrid domain, and is homologous to the α-I domain of the α-chain in α3β1 integrins. The β-I



1.19 Integrin β-chain domains.

domain is believed to have both “open” and “closed” configurations implicating an epitope binding function in integrins, such as α6β4, that lack an α-I domain on their α-chains. Overall, the β-chain is considered more flexible than the a-chain.[65] In contrast to the α-chain’s transmembrane domain, which is perpendicular to the cell membrane’s axis, the transmembrane domain of the β-chain is angled or tilted.[65] Both α- and β-chain tranmembrain domains are structurally equivalent, with interactions between the two transmembrane domains seen with inactivate the integrin molecules, and a separate α- and β-chain arrangement seen when integrins are activate. The intracellular portion of the β4-subunit has four fibronectin type III repeats separated by a connecting segment.

Within the basement membrane zone, α6β4 integrin binds to plectin, BPAG1e, collagen XVII, and laminin-332 (Figure 1.20). α6β4 integrin, or actually its β4-subunit binds to plectin at multiple sites.[61] Within the inner hemidesmosomal plaque, plectin’s “actin binding” and the “plakin” domains bind the cytoplasmic domain of the β4-chain of α6β4 integrin at a site containing four fibronectin type III (FnIII) segments.[61,62,78]

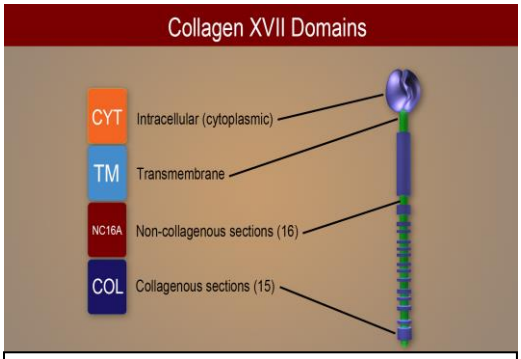


1.20 Integrin BMZ-specific binding.

There is actually a conformational change that occurs in the integrin's β-4 subunit when binding to plectin. In fact, α6β4 integrin[79,80] and its conformational change (when bound to plectin) is required for the assembly of a hemidesmosome,[81] as it appears to open up sites for BPAG1e and collagen XVII interaction.[61] Once bound to plectin, α6β4 integrin interacts with both BPAG1e[82] and collagen XVII[80,83,84] to form a complete hemidesmosome. The α6-subunit is needed for integration of CD151 into hemidesmosomes.[41]

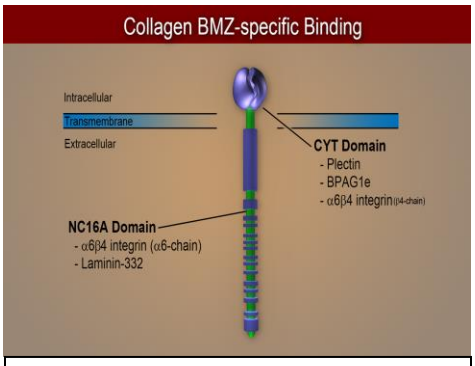
Collagen XVII (BPAG2 is the remaining component of the outer plaque of the hemidesmosome. Collagen XVII is found in the basal keratinocyte, the lamina lucida, and the lamina densa layers of the basement membrane zone.[85] It is a homotrimeric 180 kDa type II transmembrane protein, composed of three collagen alpha-1(XVII) chains.[85-87] Extracellular cleavage of collagen XVII results in two alternate molecules: (1) a 120 kDa soluble ectodomain, referred to as LAD-1, and (2) a 97 kDa fragment, known as LABD97.[85] While the biologic function of these two cleavage products remain unknown, they are both associated with linear IgA bullous dermatosis.

Collagen XVII (159-237 nm) has four domains: (1) a globular amino-terminal intracellular domain (25-35 nm[88]), (2) a short transmembrane domain (~4-7 nm[89]), and extracellularly (130-195 nm[88,90]), a series of (3) 15 collagen subdomains, composed of 15 collagen (Gly-X-Y repeats), separated by (4) 16 noncollagenous (NC16A[86]) subdomains (Figure 1.21).[91] Rotary shadowing images of collagen XVII demonstrate a globular head (25-35 nm), a more rigid central rod-like region (60-70 nm), and a flexible tail (100-130nm).[88] The rigid central rod portion of the extracellular domain contains more collagen, than the tail portion which has noncollagenous (NC16A) segments separating the collagenous segments and imparting greater flexibility.



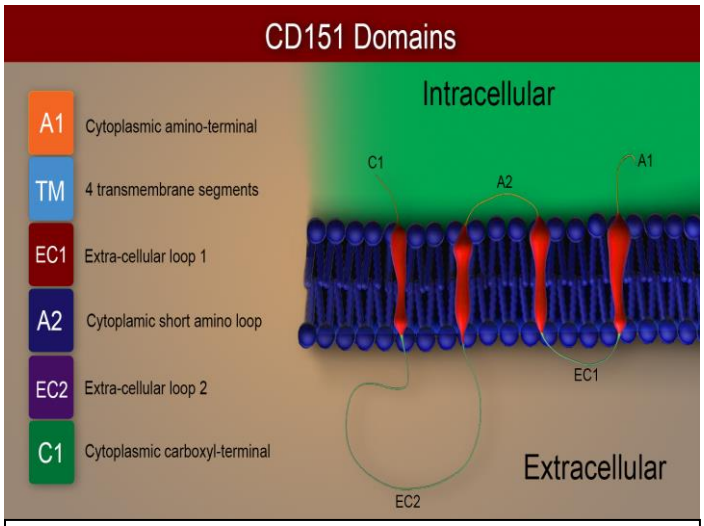
1.21 Collagen XVII domains.

Within the basal keratinocyte collagen XVII binds to α6β4 integrin, plectin, and BPAG1e (Figure 1.22). Intracellularly, collagen XVII binds to the β4-chain of α6β4 integrin,[85,92] and extracellularly the NC16A domain of collagen XVII binds to the α6-chain[91] of α6β4 integrin. Collagen XVII binds to BPAG1e and plectin via their amino-terminal plakin domains.[50] Its extracellular portion spans the length of the lamina lucida and extends into the lamina densa.[85] Extracellularly, collagen XVII binds to the α6-subunit of α6β4 integrin, and interacts with laminin-332 within the lamina lucida, contributing to anchoring filament composition.[85]



1.22 Collagen XVII BMZ-specific binding.

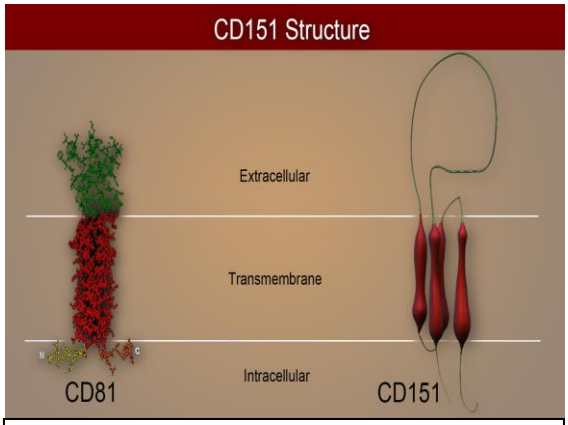
In human epithelia, CD151 (Cluster of Differentiation 151) is a 32 kDa[93] protein found only within the basal cell layer, along the dermal-epidermal interface. CD151 is one of the earliest molecules seen in early embryonic development. CD151 is found in association with integrins, and has been postulated to have a role in the development of hemidesmosomes,[41] as it is seen aggregated and co-located with integrins in pre-hemidesmosomal structures, though more research is needed to define its role. CD151 is a member of the tetraspan superfamily of proteins, which are predominantly cell surface proteins. It interacts with a wide variety of molecules. It associates with α6β4 and α3β1 integrins, forms homodimers with other CD151 molecules, and forms complexes consisting of varied mixures of proteins and tetraspaninins. The ability of tetraspanins to form complexes with each other and a multitude of different proteins has led to the use of the term, 'tetraspanin web' to describe the network of molecular interactions occuring at the cell surface.[94,95] The ability of tetraspanins to form these lateral tetraspanin web distinguishes it from other membrane protieins.[94]



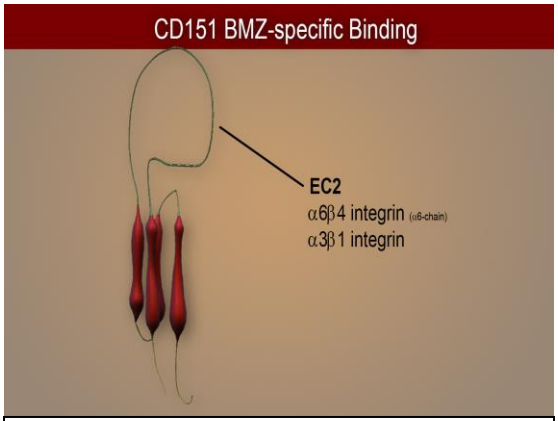
1.23 CD151 domains.

CD151 has 9 domains (Figure 1.23): four transmembrane domains; two extracellular loops, one small (EC1) and one large (EC2), and two short amino-terminal and one carboxyl-terminal end.[41,42] Both the amino- and carboxyl tails are cytoplasmic.[95] While the exact three dimensional structure of CD151 is not yet known, it shares significant homology with another tetraspanin, CD81 whose structure has been solved[1,2] (Figure 1.24).

There are 6 palmitoylation sites in CD151 – 2 in each of its three short intracellular segments. Palmitoylation is the covalent attachment of palmitic fatty acid to the cysteine resides of membrane proteins. Palmitoylation enhances hydrophobicity, and hence the membrane association of molecules, and also been shown to affect cellular signalling, trafficking, and membrane localization of proteins.[96]



1.24 CD151 structure. Hypothetical representation of CD151 based on solved structure of CD81. Three-dimensional model of CD81 (PDB ID: 2AVZ), [1,2] based on Protein Data Bank (PDB) Jmol[3] data.

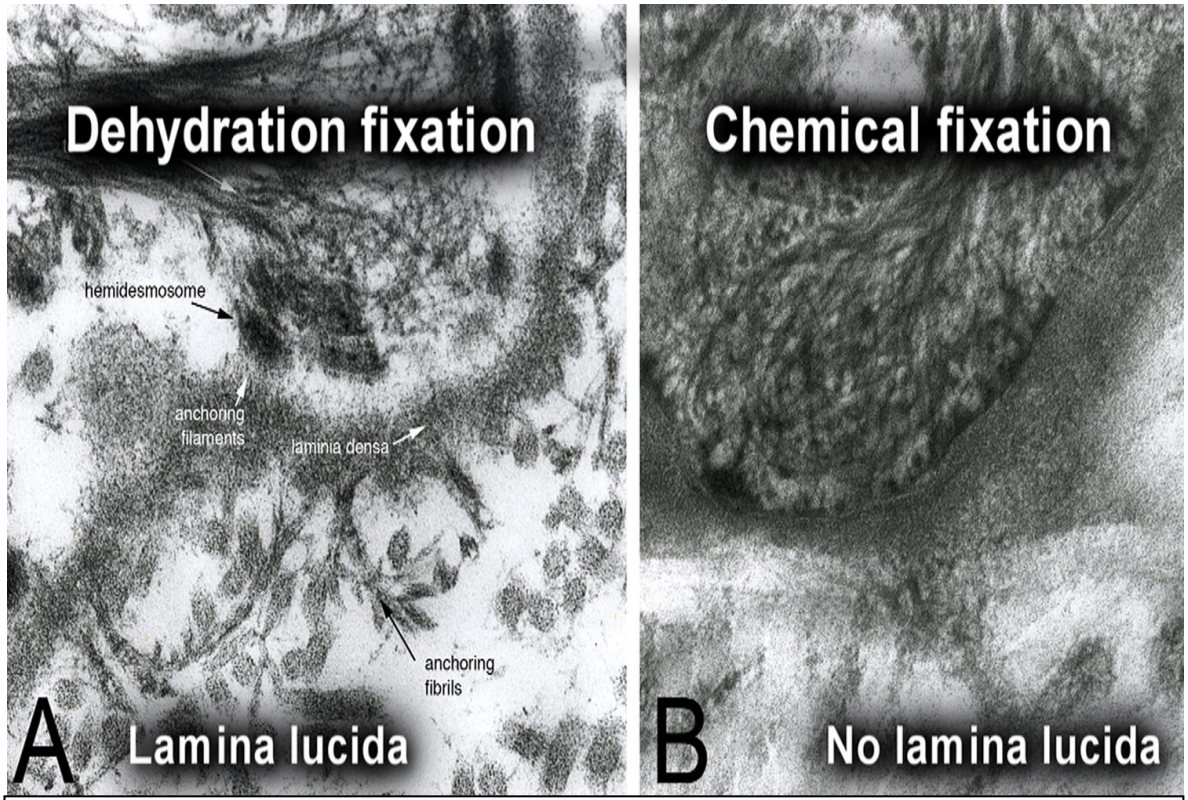


1.25 CD151 BMZ-specific binding.

CD151 interacts with the extracellular α-subunits[97,98] of α6β4 and α3β1 integrins within the lamina lucida, specifically at the basolateral surface of keratinocytes (Figure 1.25).[41] These interactions stabilize α6β4 and α3β1 integrin association with the laminin-332 matrix within the basement membrane. It is the α6-subunit on integrin that has been found to be necessary for the incorporation of CD151 into hemidesmosomal complexes.[41]

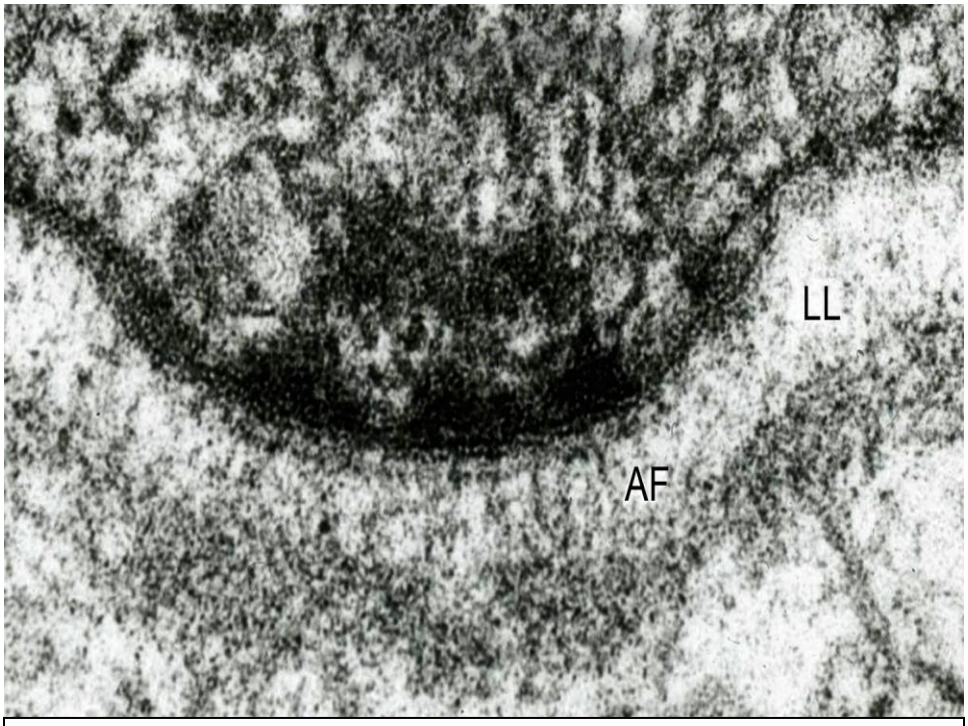
LAMINA LUCIDA LAYER

As discussed in Chapter 1, the structure known as the lamina lucida is believed to be an artifact of processing. Images of the basement membrane zone that demonstrate a lamina lucida were produced by older electron microscopic dehydration techniques for tissue fixation. In 1993, Hipple-Sanwald noted that the main advantage of newer high-pressure freeze substitution electron microscopy techniques, in comparison to convsional chemical fixation (dehydration), is that it allows for greater tissue preservation.[99,100] In a study published by Chang and Inoue in 1994,[22] comparing the electron microscopic appearance of the basement membrane zone prepared during conventional dehydration verses chemical fixation, they discovered that the lamina lucida was only present when dehydration was used for specimen preparation (Figure 1.26). This indicates that the lamina lucida is an artifact, and not a distinct layer within the basement membrane zone. Despite the artifact induced by conventional dehydration techniques, these techniques are still widely used. It therefore is important to remember that the lamina lucida is not present in living basement membrane zones.



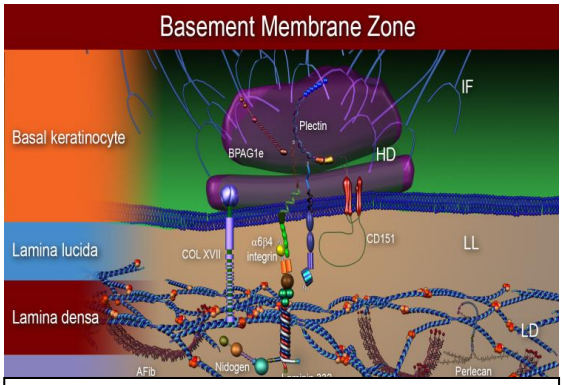
1.26 Lamina Lucida artifact. A. conventional transmission electron microscopy, with dehydration artifact showing lamina densa pulling away from plasma membrane. B. High-pressure freeze substitution electron microscopy image demonstrating absence of lamina lucida and anchoring filaments. Chan FL, Inoue S. Lamina lucida of basement membrane: an artefact. *Microsc Res Tech.* May 1 1994;28(1):48-59.

On electron micrographic images of the basement membrane zone, are ~800nm thin filamentous or threadlike structures seen within the 20-40 nm[101] thick lamina lucida.[102] These structures are called anchoring filaments (Figure 1.27). There composition is somewhat controversial, as several different molecules have been found associated with these filaments, or in the space occupied by these filaments. The list of components includes collagen XVII, α6β4 integrin, the transmembrane molecules found within the hemidesmosome outer plaque, CD151, and two extracellular proteins: (1) laminin 332, and (2) laminin 311.[23] Of these components, laminin 332 is believed to be the main component of anchoring filaments.



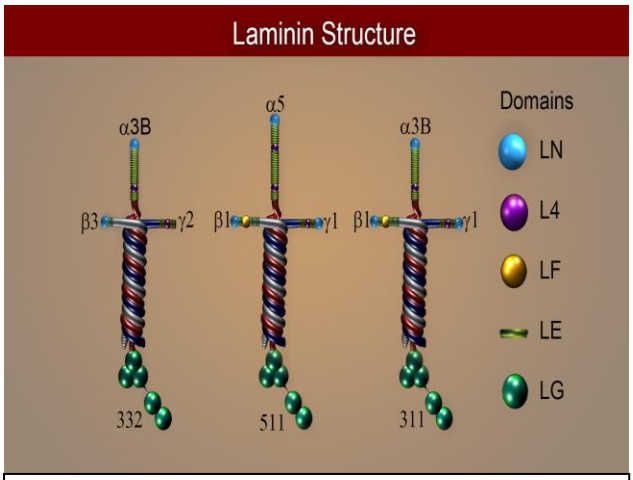
1.27 Anchoring filaments (EM). Within the lamina lucida (LL), are anchoring filaments (AF) composed of collagen XVII, α6β4 integrin, CD151, laminin 332, and laminin 311. Laminin 332 is believed to be the main component.

Within the lamina lucida are the extracellular domains of collagen XVII, α6β4 integrin, α3β1 integrin, and CD151 (Figure 1.6). Collagen XVII is known to traverse the entire lamina lucida[85,103,104] as do α6β4 and α3β1 integrins. The extracellular domain of the α6-subunit interacts with both collagen XVII[105] and CD151.[106]



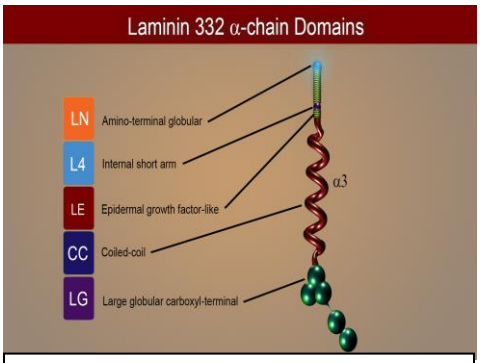
1.6 Basement Membrane zone. The four layers of the basement membrane zone: (1) basal keratinocyte layer, the (2) lamina lucida layer, (3) the lamina densa layer, and (4) the sublamina densa layer. Within the basal keratinocyte, are keratin intermediate filaments (IF), hemidesmosomes (HD), composed of bullous pemphigoid antigen 1 (BPAG1e), plectin, α6β4 integrin, α3β1 integrin, and collagen XVII, and CD151. Within the lamina lucida (LL) are anchoring filaments (AF), composed predominantly of Laminin 332. Also within the lamina lucida are the extracellular portions of collagen XVII, α6β4 integrin, and CD151, and laminins 311 and 511 . Within the lamina densa (LD) are collagen IV, Laminins 332, 311, and 511, nidogen and perlecan. Within the sublamina densa are anchoring fibrils (AFib), composed of collagen VII.

The laminins are important integrin-binding structures within the lamina lucia and lamina densa.[107] The anchoring filaments,[108] for example, are known to be composed predominantly of laminin 332. Laminins are large glycoproteins composed of three chains (α,β and γ) bound by disulfide bonds.[109] There are at least 15 different laminins, comprised of a combination of 5 different α-chains, three β-chains, and three γ-chains. The laminins important to the basement membrane zone are: (1) laminin 332, (2) laminin 511, and (3) laminin 311.[23,35,107] As laminin 332 is the main component of anchoring filaments, it will serve as our prototype laminin.

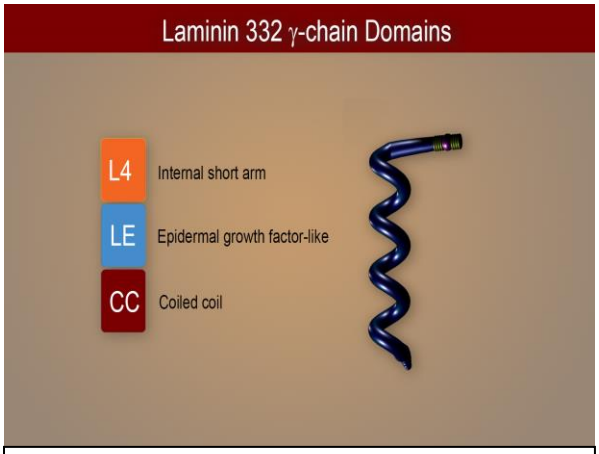


1.28 Laminin structure. The laminins important to the basement membrane zone are: (1) laminin 332, (2) laminin 511, and (3) laminin 311. The molecule resembles a cross, with a large globular base (LG domain). The LG domain is labled G1-G5.

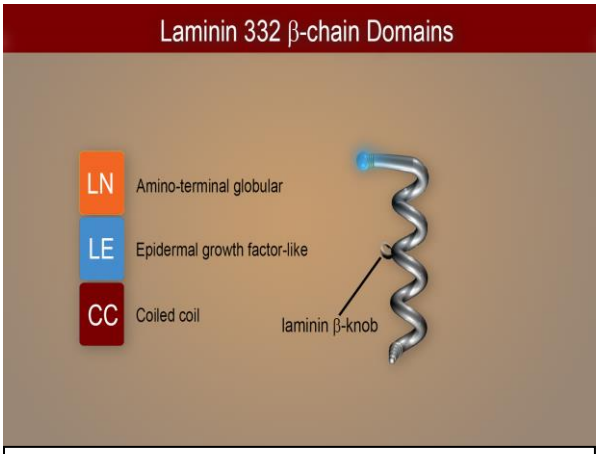
Laminin 332 (410 kDa), composed of three chains, bound by disulfide bonds: an α3-chain (165 kDa), a β3-chain (140 kDa), and a γ2-chain (105 kDa).[110-112] Laminins are secreted, by keratinocytes, as a precursor that assembles together in a long coiled-coil arrangement, that resembles a cross with a large end at its base (Figure 1.28).[23,111] The large globular carboxyl-terminal portion is an extension of the α-chain. The laminin 332 α-chain has five basic domains: (1) an amino-terminal globular LN domain, (2) an L4 internal short arm globular domain, (3) an LE multiple epidermal growth factor (EGF)-like domains, interspersed between the LN and L4 domains, (4) a coiled-coil (CC) domain, and (5) the large globular carboxyl-terminal LG domain (Figure 1.29).[113,114] The LG domain has five separate small globular subdomains, labeled G1 through G5.[107] The β-chain has three domains: (1) an amino-terminal globular LN domain, (2) an LE multiple rod-like epidermal growth factor (EGF) repeats, and (3) a coiled-coil (CC) domain (Figure 1.30).[114] Laminin β-chains contain a short segment of 40 amino acids within the coiled-coil domain, that is incompatible with an α-helix configuration,[112] and loops out, of the coiled-coil region, known as the *laminin β-knob*[115]. The γ-chain has three domains: (1) an LE multiple rod-like epidermal growth factor (EGF) repeats, with (2) an imbedded L4 internal short arm globular domain, and (3) a coiled-coil (CC) domain (Figure 1.31).[114] The γ-chain is responsible for the initial recognition of integrins.[116] The α-helical domains (CC domains) of each of the chains assemble together to form a heterotrimeric "coiled-coil" configuration that is considered essential[117] for laminin to α3β1 integrin binding.[113]



1.29 Laminin 332 α-chain domains.

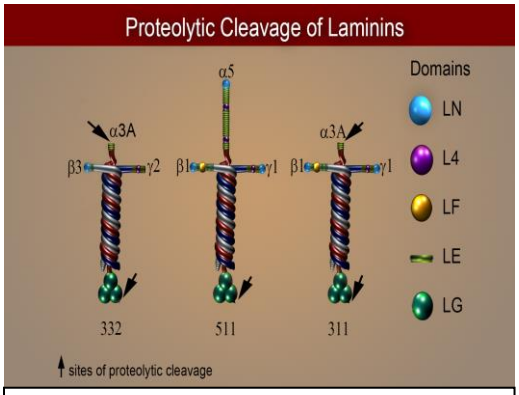


1.30 Laminin 332 β-chain domains.



1.31 Laminin 332 γ-chain domains.

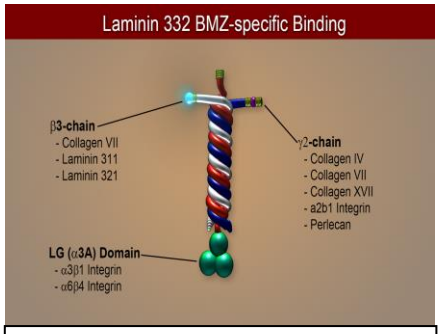
The large globular domain of the α-chain has been theorized by Tisi, et al,[118] as resembling more of a "clover leaf" structure formed by subdomains LG1-LG3. There are approximately 50 amino acid residues separting LG3 and LG4 subunits, in contrast to the only 6-10 resides betwing LG1-LG2 and LG2-LG3 pairings. The short linkers between LG1-LG2, and LG2-LG3 place conformational restrictions on the structure of the LG subdomain. The theorized model for LG1-LG3 subdomains therefore resembles a cloverleaf with a ~9 nm diameter, with an attached tail containing the globular LG4 and LG5 subunits.



1.32 Proteolytic cleavage of laminins.

After secretion, laminins 332 and 311 undergoe proteolytic cleavage leaving a truncated α3A-chain by removing its LN and L4 domains and some of its LE domains, and also undergo cleavage at the link between the LG3 and LG4 sub-domains (Figure 1.32).[109,113] Laminin 511 also undergoes proteolytic cleave at the link between the LG3 and LG4 sub-domains upon secretion. The β3 and γ2-chains also undergoing varing amounts of proteolytic cleavage of there amino-termini by matrix metalloproteases.[109]

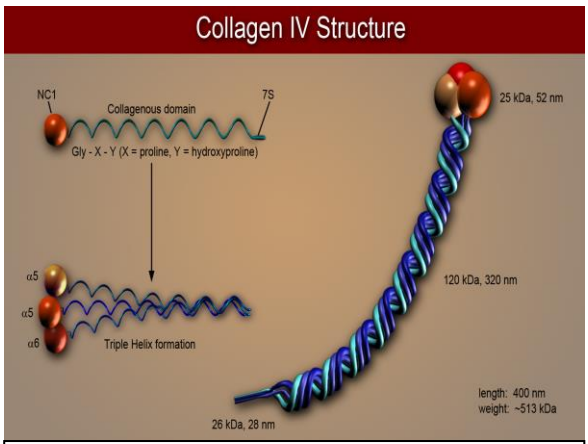
Laminin-332, the major component of lamina lucida anchoring filaments,[108] binds α6β4 and α3β1 integrins [119,120] via its globular domain, laminin 311 and collagen VII[121] along its β-subunit chain, and collagen's IV, VII, and XVII, as well as perlecan molecules along its γ-subunit chain, and may also interact with laminin 511 (Figure 1.33).[120,122] Much like Laminin 332, and Laminin 511 inteact with α3β1 integrin, via its large α-subunit chain globular carboxyl domain.[23]



1.33 Laminin 332 BMZ-specific binding

LAMINA DENSA LAYER

On electron micrographic images of the basement membrane zone, directly beneath the lamina lucida, is the electron dense 40-60 nm[101] zone known as the lamina densa. The threadlike anchoring filaments are seen entering the lamina densa zone, bridging the gap between the hemidesmosome and the lamina densa. Important molecules within the lamina densa include: collagen IV, α6β4 and α3β1 integrin, laminins 332, 311, and 511, nidogen and perlecan.[123] As previously stated, the presence of α3β1 is critical for the development of a continuous lamina densa.[69]

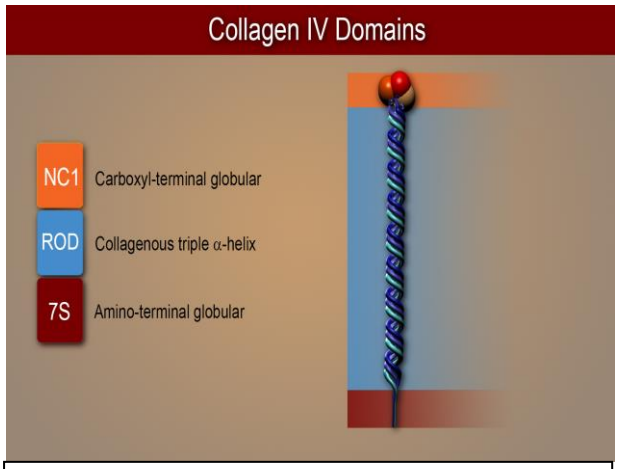


1.34 Collagen IV structure. Collagen IV is made from three 400nm α-ins, which self-assemble into a triple helical structure. The structure of collagen IV is unique, in that it closely resembles that of procollagen, as it retains its globular amino and carboxyl terminal ends. In other types of collagen, these terminal ends are usually removed from the procollagen molecule as soon it exits the cell, by procollagen amino-proteinase and procollagen carboxy-proteinase.

Collagen IV is synthesized in the rough endoplasmic reticulum and secreted via golgi apparatus into the basement membrane. It is made from three 400nm α-chains, which self-assemble into a triple helical structure (Figure 1.34). Recognition motifs in the globular NC1 domain orient the chains into their proper position. There are at least six different types of α-chains for collagen IV (α1-α6).[124,125] The α1α1α2 collagen networks are ubiquitous in the early embryonic stages of life; but are replaced by α5α5α6 collagen networks in adult epidermal basement membrane zones.[126] Each α-chain is composed of collagenous and noncollagenous sequences. Collagenous sequences are composed of glycine –X-Y repeats, with X and Y often composed of proline and hydroxyproline respectively.

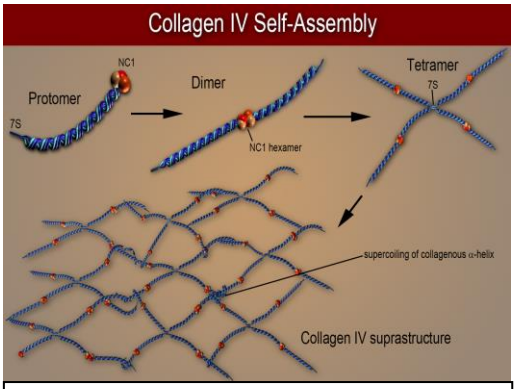
The structure of collagen IV is unique, in that it closely resembles that of procollagen, as it retains its globular amino and carboxyl terminal ends. In other types of collagen, these terminal ends are removed from the procollagen molecule as soon it exits the cell, by procollagen amino-proteinase and procollagen carboxy-proteinase. Collagen IV has been described as resembling a hockey stick,[127] with the 7S portion of the molecule representing the blade of the hockey stick.

Collagen IV is a heterotrimeric glycoprotein with three distinct domains (Figure 1.35): (1) an amino-terminal 7S domain (26 kDa, 28 nm); (2) a collagenous triple α-helical ROD-like domain (120 kDa, 320 nm), and (3) a carboxyl-terminal non-collagenous (NC) globular NC-1 domain (25 kDa, 52 nm).[99,125]

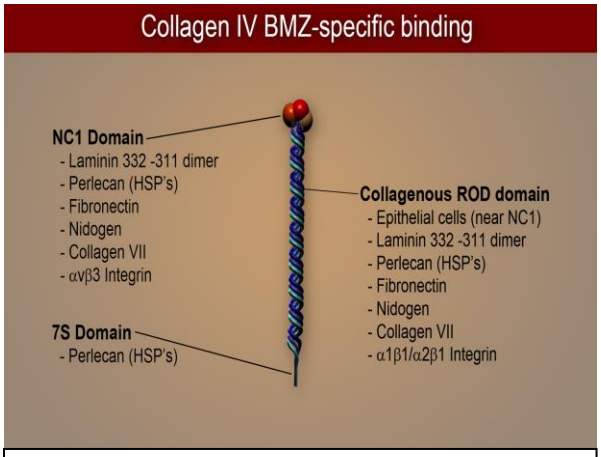


1.35 **Collagen IV domains**

Collagen IV self-assembles into dimers via the carboxyl-terminal end, and tetramers via its amino-terminal ends (Figure 1.36).[125] Collagen IV triple helical molecules bond via NC-1 domains to form a dimer, with a NC-1 hexamer stabilized by disulfide bonding.[128] Adjacent collagen IV dimers then assemble via the 7S or "hockey blade" portion of the molecule to form a tetramer spider connector in a 2-dimensional lattice structure. Short non-collagenous sequences within the triple helical collagenous (ROD) domains provide the molecule with sufficient flexibility to coil around the lattice and interact with NC-1 domains, forming supercoiled structures (Figure 1.36).[125] Complex interactions between these side chains and globular domains form a 3-dimensional lattice structure, which along with laminin chain polymerization forms a dense plaque on electron microscopy, known as the lamina densa.[129,130] Binding of collagen IV to other basement membrane zone molecules is complex (Figure 1.37).[126] Collagen α5α5α6 networks are known to bind epithelial cells, laminins, perlecan, nidogen, integrins, and collagen VII.



1.36 Collgen IV self-assembly. Collagen IV protomers self-assemble into dimers via the NC1 domain, forming an NC1 hexamer. Dimers assemble together via the 7S or "hockey blade" portion of the molecule to form a tetramer spider connector. The collagen IV network is stabilized by supercoiling of collagenous triple helix domains, and their interactions with NC-1 domains. Short non-collagenous sequences within the molecules' rod-like central collagenous domain, provide collagen IV with significant flexibility (supercoiling). The collagen IV suprastructure appears as a dense plaque on electron microscopy, known as the lamina densa.

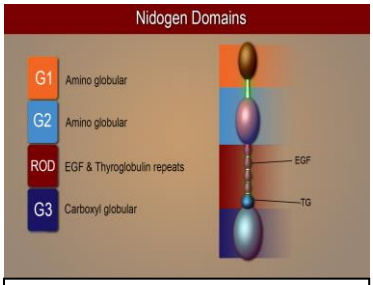


1.37 Collagen IV BMZ-specific binding.

Laminins, known to self-associate into hexagonal networks,[121] also form their own unique mesh-like networks with the basement membrane zone, forming the lamina densa.[20,129] There are several different proposed configurations for laminins within the basement membrane zone. Laminins 332 and 311 are known to ineract via their amino-terminal arm segments to form dimers, which likely interact with collagen IV.[107] Laminin 332 by itself attaches to collagen IV.[121] Dense mesh-like networks formed by laminin 332, and collagen IV, appreciated on electron microscopy as the lamina densa, anchor molecules above and below. At the lamina densa level that we begin to see how the hemidesmosomes attach to the underlying connective tissue matrix of the papillary dermis, through transmembrane proteins such the integrins, and their interactions with collagen XVII, and these mesh-like structures.

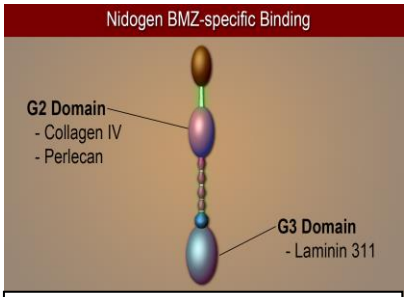
Other molecules found within the lamina densa layer of the basement membrane zone include nidogens and perlecan.[131-134] These molecules play a supportive role in the stabilization of the basement membrane.[135] Despite their ubiquitous presence in epidermal basement membrane zones, they are not absolutely required for formation of the basement membrane zone.[23,132] The concept of molecules such as nidogens and perlecan having a less important role is relatively new,[23] and is based on data[133] demonstrating that in the absence of nidogen, a working basement membrane will still be produced. Similarly, basement membranes are known to form in the abscence of perlecan.[136] However, unlike nidogens, the absence is perlecan is associated with early embryonic death related to loss of basement membrane integrity surrounding embryonic myocardial cells.[132] This underscores the complexity of interactions within the epidermal basement membrane.

All basement membranes contain members of the nidogen family of proteins.[133] There are two members of the mammalian nidogen family, nidogen-1, and nidogen-2. Nidogen was first discovered from a Engelbreth-Holm-Swarm (EHS) mouse tumor basement membrane as a fragment seen on electron microscopy,[137] and named for its ability to aggregate into nest like structures (Latin: *nidus*, “nest”).[138] Nidogens are a group of 150-200 kDa glycoproteins consisting of three globular domains (G1-G3) that somewhat resemble a dumb bell (Figure 1.38). The two amino globular domains (G1,



1.38 Nidogen domains.

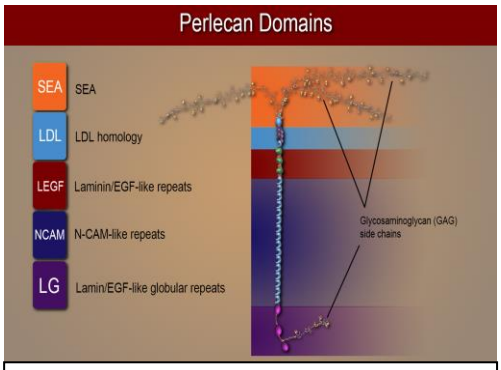
G2) and a carboxyl globular domain (G3) are connected by a rod domain composed of mostly epidermal growth factor (EGF)-like modules and Thyroglobulin (TG)-like modules (Fig 7.1).[139] One of Nidogens' globular domains, designated as G3, binds to the γ-subunit of laminin 311(Figure 1.39). Another globular domain, known as G2, binds to collagen IV, and perlecan.[139] Nidogen helps stabilize the laminin 332-311 dimer as it binds to collagen IV.



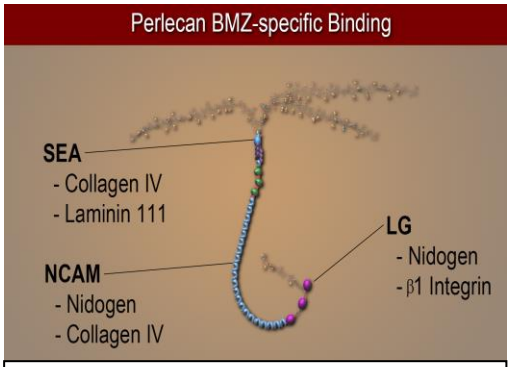
1.39 Nidogen BMZ-specific binding.

Perlecan is a heparin sulfate proteoglycan. Heparin sulfate proteoglycans are large molecules composed of a central 470-kDa protein core, to which glycosaminoglycan chains are covalently bonded. These glycosaminoglycans include chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin, and heparan sulfate. Glycosaminoglycan side-chains are long and negatively charged, and are commonly described as having either a "bottle-brush"[140] or “pearls on a string”[141] appearance. Basement membrane heparin sulfate proteoglycans include perlecan, collagen XVIII and agrin. Perlecan serves as our prototype basement membrane proteoglycan.

Perlecan's central core is divided into 5 domains (Figure 1.40).[142,143] The first domain is an amino-terminal domain that serves as the attachment site for glycosaminoglycans (mostly heparan sulfate), and some chrondroitin sulfate. This first domain contains a sea urchin sperm protein-enterokinase-agrin module, known as SEA. The second domain is homologous to the low-density-lipid (*LDL*) receptor ligand-binding domain. The third domain has three laminin-like domain modules and eight epidermal growth factor (*EGF*)-like repeats. The fourth domain contains N-CAM-like Immunoglobulin repeats and binds to other basement membrane components. The final domain consists of laminin-like globular domains separated by four EGF-like repeats. Perlecan’s SEA, N-CAM, and LG domains bind Nidogen, Collagen IV, and other molecules within the basement membrane zone (Figure 1.41).



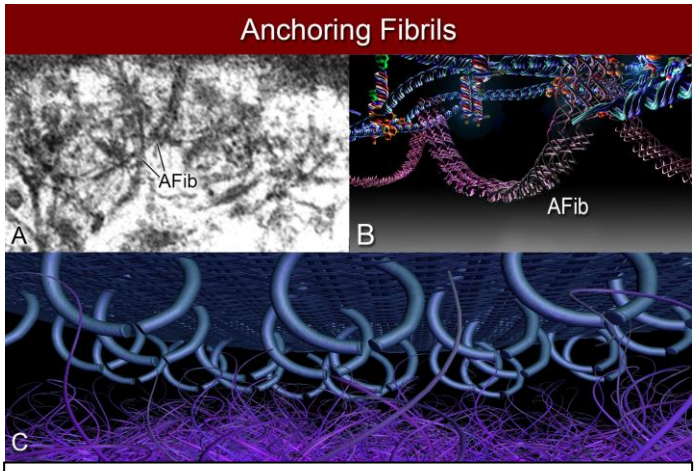
1.40 Perlecan domains.



1.41 Perlecan BMZ-specific binding.

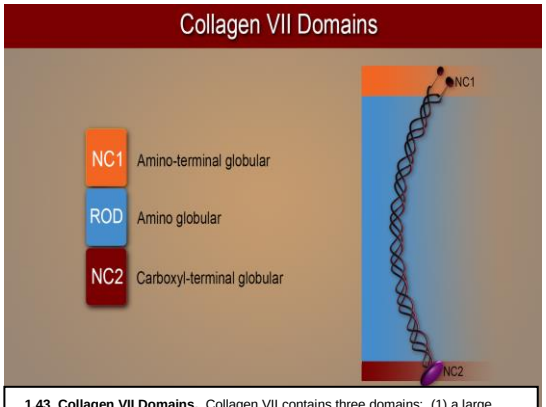
SUBLAMINA DENSA LAYER

Beneath the lamina densa lays the sublamina densa layer or superficial papillary dermis. Directly beneath the lamina densa are bundles of anchoring fibrils (Figure 1.42A,B) These anchoring fibrils form semi-circular loops[144] that resemble Velcro® fasteners (Figure 1.42C). The lamina densa is anchored to the underlying superficial papillary dermis using these anchoring fibrils that weave between the connective tissue matrix of the dermis. The anchoring fibrils are composed of collagen VII.[102,145,146] Collagens I and III are major components of the superficial dermis.

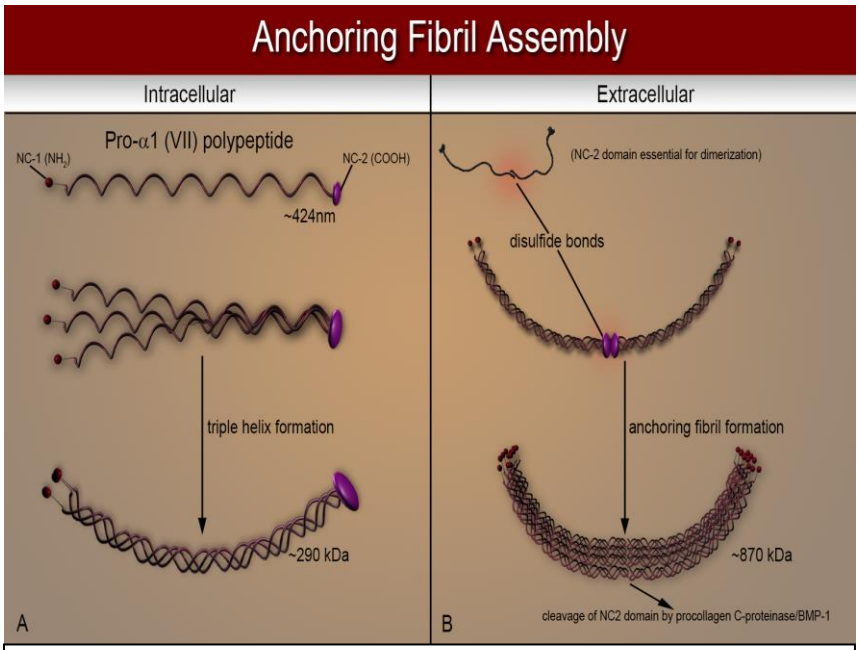


1.42 **Anchoring Fibrils.** (**A**) **and** (**B**) Anchoring fibrils (AFib) beneath the lamina densa, with NC1 amino-terminal ends within lamina densa and collagenous triple α-helix rod-like domain bundles forming Velco®-like loops within superficial papillary dermis. (**C**) Velcro®-type fasteners.

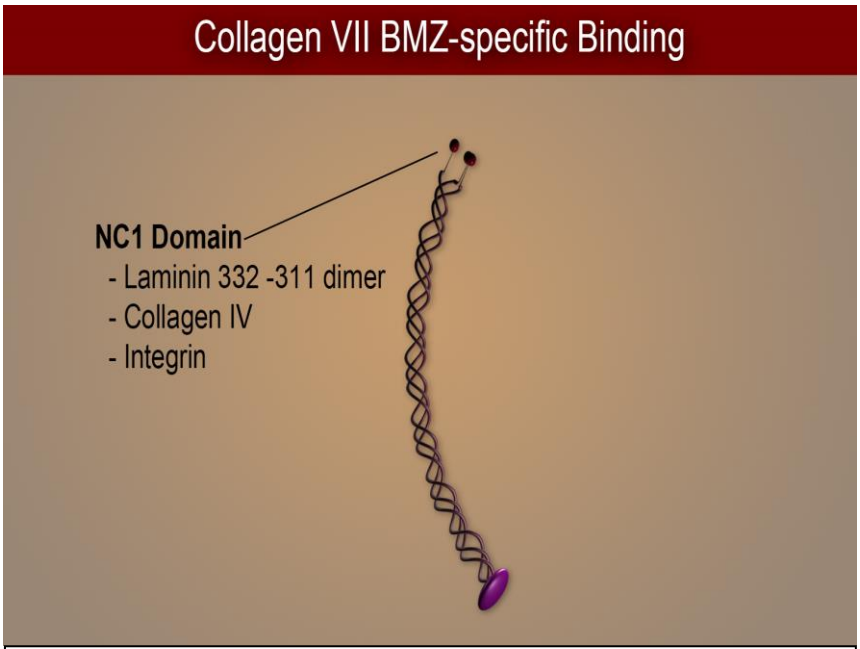
Collagen VII is secreted by both keratinocytes and dermal fibroblasts[147] as a procollagen molecule with three main domains (Figure 1.43). A large globular amino-terminal end known as NC-1, a central triple helical rod section, known as the collagenous domain, and a smaller carboxyl-terminal end, known as the NC-2 domain.[146] Intracellularly, the 424nm pro-α1 (VII) molecule forms a 290 kDa triple-helical collagen VII molecule, with disulfide-binding stabilizing the NC-1 amino-terminal portions. Extracellularly, this triple helix then self-assembles into antiparallel dimers, via the carboxyl-terminal ends (Figure 1.44A).[148] The NC-2 domain is essential for the formation of the antiparallel dimers, as this domain contains cysteine residues needed for disulfide bonding, which stabilizes the dimers.[149] Lateral association of collagen VII dimers form the semi-circular anchoring fibrils (Figure 1.44B), after cleavage of the NC-2 domain by procollagen C-proteinase/BMP-1.[150] The anchoring fibrils (~870 kDa) resemble velcro®-like loops (Figure 1.42C), with their large globular amino terminal domains embedded within the lamina densa's thick collagen IV mesh-like structure. Collagen VII's NC-1 domain interacts with the laminin 332-311 dimer, collagen IV, and integrins (Figure. 1.45).[121,151]



1.43 Collagen VII Domains. Collagen VII contains three domains: (1) a large globular amino-terminal end known as NC-1, a central triple helical rod section, known as the collagenous domain, and a smaller carboxyl-terminal end, known as the NC-2.



1.44 Anchoring fibril assembly. Intracellularly (**A**), pro-α1(VII) collagen peptides form the characteristic triple α-helical core structure (290 kDa). Extracellularly (**B**), collagen VII antiparallel dimers self-assemble, with stabilization of the dimer by disulfide bonding. The NC-2 domain, rich in cysteine residues appears essential for the formation of the antiparallel dimers. However, it is only after cleavage of the NC-2 domain by procollagen C-proteinase/BMP-1, that multiple dimers are able to align laterally to form anchoring fibrils (~870 kDa).



1.45 Collagen VII BMZ-Specific Binding.

ORIGIN

The development of the epidermal basement membrane zone is a complex process, whose origins can be traced back to the first week of life. The earliest basement membrane zone components detected are the laminins,[152] integrins (α6β4,[153] α3β1), collagen IV, and CD151[41]. Of these early components, α6β4 integrin is the most crucial, since it forms the scaffolding on which all other molecules bind.[41,78,80,82,83,154] As early as day 3, we can find laminin and collagen production. By the second week,[152,155] the developing embryo's dermis is producing collagen I, and shortly thereafter, collagen III, forming the dermal matrix upon which the basement membrane zone will form.

Origin of BMZ Components

Basal keratinocytes	Dermal fibroblasts
Intermediate filaments	Laminins 322, 311, 511
BPAG1e	Collagen IV
Plectin	Collagen VII
Collagen XVII	
Integrins	
CD151	
Nidogen	
Perlecan	
Collagen VII*	

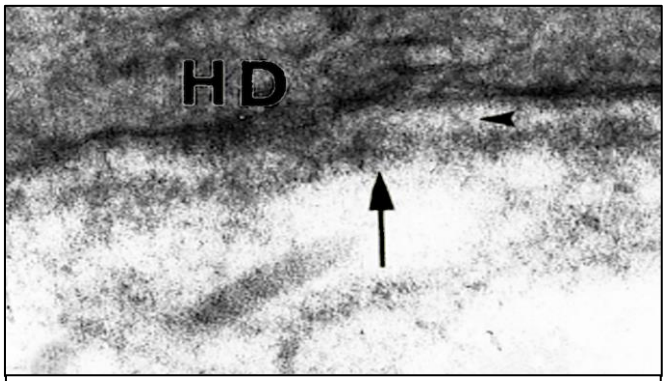
* Fetal basal keratinocytes

2.1 Origin of BMZ components.

The majority of basement membrane zone components are secreted by the basal keratinocytes, with dermal fibroblasts contributing a greater percentage of components below the level of the lamina lucida (Figure 2.1). Dermal fibroblasts secrete laminins 322, 311, and 511, found within the lamina lucida, collagen IV that localizes to the lamina densa, and collagen VII, which forms anchoring fibrils within the sublamina densa.[156] Fetal basal keratinocytes are able to produce a significant amount of collagen VII, unlike neonatal basal keratinocytes, which lose their ability to produce collagen VII.[156]

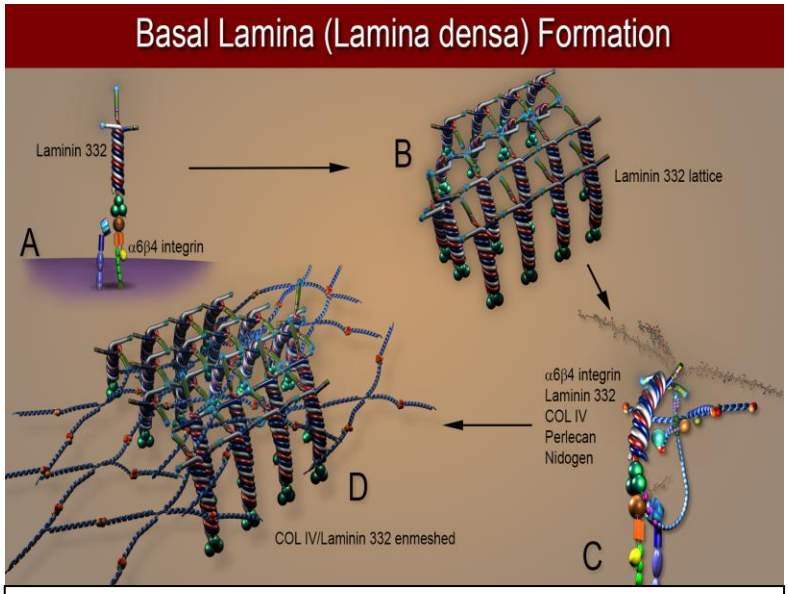
4TH WEEK OF LIFE

During the fourth week of life, external human features are not yet recognizable, and the skin is in its first stages of development. The epidermis is composed of a single layer of primordial basal cells. On electron microscopy, these early basal keratinocytes, and rudimentary forms of anchoring filaments, hemidesmosomes, lamina densa and anchoring fibrils are seen (Figure 2.2).[157] These rudimentary structures, formed around α6β4 integrin, take weeks to develop. For example, fully formed anchoring fibrils do not materialize until the 9th or 10th week.[158,159]



2.2 Electron Microscope image of BMZ at week 4 of life.

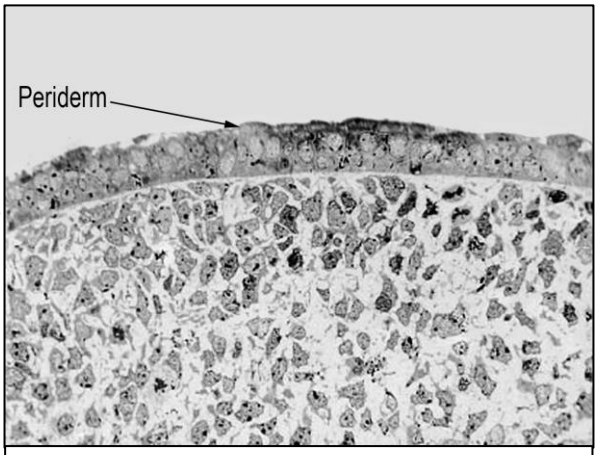
The basement membrane zone development begins when laminins bind to α6β4 integrin (Figure 2.3A). Along the inferior surface of basal keratinocyte membranes are α6β4 integrin molecules, with their extracellular domains extending into the dermis. Laminins, the most abundant non-collagenous protein in the basement membrane zone,[20] bind the β1-chains of α6β4 integrin, via the laminin LG domains. Laminins then self-assemble into a honeycomb-like network via their β- and γ-side chains. The three-arm (α-, β- γ-side chain) assembly hypothesis[56] predicts that laminins will self-assemble via the amino-terminal portions of their component chains, in a calcium dependent manner, to form a honeycomb network (Figure 2.3B).[20] Collagen IV also self-assembles into a 3-dimensional lattice structure, which combines with the laminin network to form the lamina densa. Twenty-two non-collagenous stretches within collagenous domain of collagen IV, confer significant flexibility to this molecule, and in combination with the 7S domain spider-like tetramer formation, and NC1 domain binding, ensure a stable collagen IV lattice. This structure is stabilized by Perlecan and nidogen incorporation (Figure 2.3C).



2.3 Basal lamina (lamina densa) formation. A. Laminin 332 binds the β1-chain of α6β4 integrin on the surface of basal keratinocytes, via the laminin LG domains. **B.** Laminins then self-assemble into a honeycomb-like network via their β- and γ-side chains. **C.** The collagen IV lattice enmeshes on to the laminin lattice, and is stabilized by nidogen and perlecan binding. The G3 domain of nidogen binds laminins (e.g. laminin 332-311 dimers), while the G2 domain binds collagen IV and perlecan. Perlecan's SEA domain binds collagen IV, and Lammin dimers, the NCAM domain binds nidogen and collagen IV, and the LG domain binds nidogen and β1-chains of α6β4 integrin. **D.** Completed basal lamina.

5TH WEEK OF LIFE

During the fifth week of life the nose and lips are formed, and the brain, spinal cord develops, along with the presence of flowing cerebral spinal fluid. The embryo's heart has begun to beat. A protective outer layer of cells known as the periderm will form above the primordial basal cell layer (Figure 2.4). These cells protect the developing epidermal cells beneath them, but they will not become keratinocytes. Periderm cells are covered by

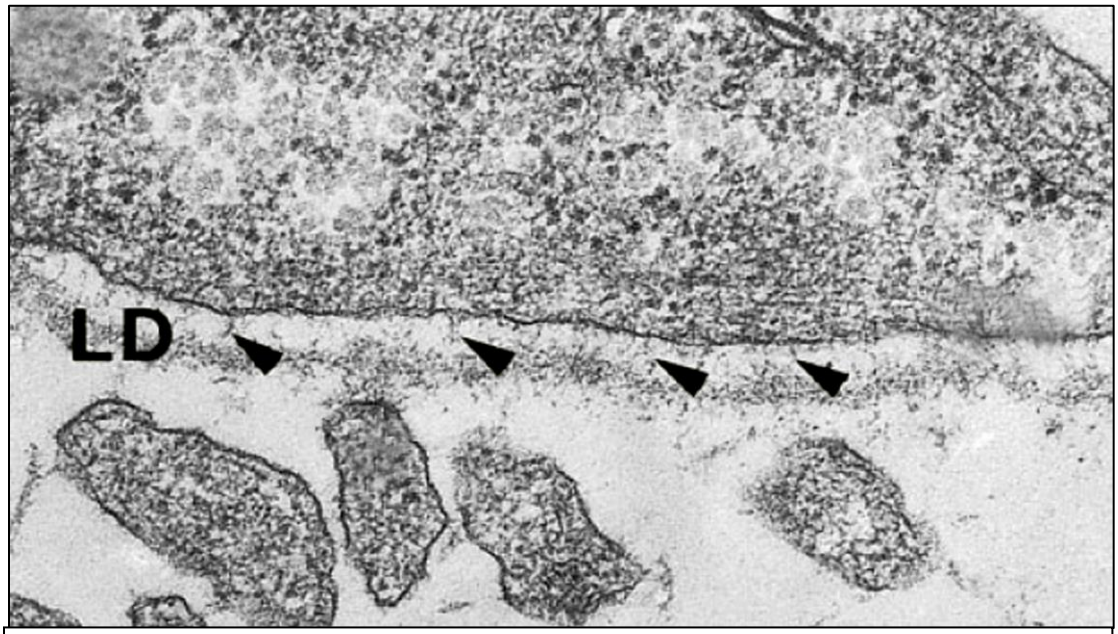


2.4 **Periderm.** Periderm cells seen on electron micrograph. McMillan JR, Eady RA. Hemidesmosome ontogeny in digit skin of the human fetus. *Arch Dermatol Res.* Feb 1996;288(2):91-97.

microvilli, and are known to demonstrate amazing and often bizarre changes in surface morphology,[160] indicating functional roles other than simply serving as a protective cell layer. Periderm cells develop independently and through different mechanisms[161] than the keratinocytes they protect. The periderm will eventually be replaced by a fully formed epidermis by the 24th week of gestation.[160]

7TH WEEK OF LIFE

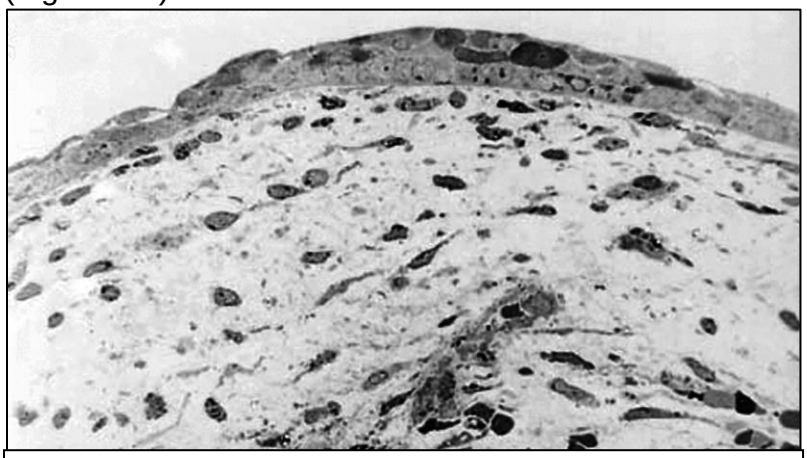
During the 7th week of life, the developing embryo's eyes and ears have begun to form. During this week its arms and legs become visible. The epidermis is still composed of two cell layers, the thin, flattened periderm, and the columnar basal keratinocytes underneath.[162] On electron microscopy,[162] the lamina densa has formed, and early anchoring filaments can be seen within the lamina lucida. Inside the basal keratinocytes, are loosely arranged networks of keratin intermediate filaments. At this time, hemidesmosomes are still not fully developed (Figure 2.5).



2.5 7th week EM. 7th week. On electron micrograph, the lamina densa has formed, and early anchoring filaments can be seen within the lamina lucida. Inside the basal keratinocytes, are loosely arranged networks of keratin intermediate filaments. At this time, hemidesmosomes are still not fully developed. McMillan JR, Eady RA. Hemidesmosome ontogeny in digit skin of the human fetus. *Arch Dermatol Res.* Feb 1996;288(2):91-97.

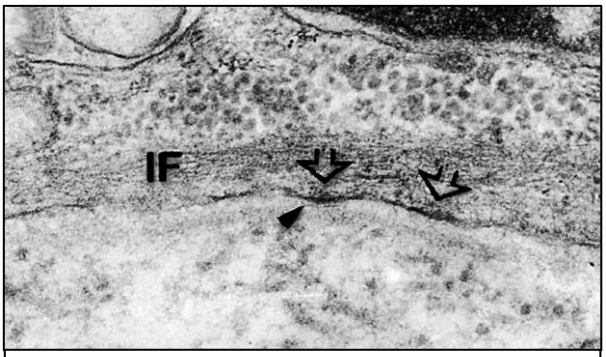
9TH WEEK OF LIFE

By the 9th week of life, the embryonic period has ended, and we are well into the fetal period. Externally, the fetus has taken on a more human appearance, with its eyes shifting forward and the ears starting to take shape. On light microscopy, a stratified layer of cells has formed between the periderm and the basal cell layer underneath (Figure 2.6).



2.6 9th week light micrograph. 9th week. Light micrograph of semithin Epon-embedded sections, demonstrating a stratified layer of cells formed between the periderm and tne basal cell layer underneath. McMillan JR, Eady RA. Hemidesmosome ontogeny in digit skin of the human fetus. *Arch Dermatol Res.* Feb 1996;288(2):91-97.

It is at this stage, that more mature-appearing hemidesmosomal-like plaques begin to appear (Figure 2.7).[163,164] There are two types of hemidemsmosomes[36]: the type I or classic hemidesmosome, and the type II hemidesmosomes. Type II hemidesmosomes are found in fetal skin and tissues such as the intestines,[43,44] and contain only α6β4 integrin and plectin.[45-47] Type II hemidesmosomes, or immature hemidesmosomes, become mature hemidesmosomes after the integration of BPAG1e and collagen XVII. The process of hemidesmosomal development begins at the fourth week and culminates in a fully formed hemidesmosome by the 15th week of life.[162,163] α6β4 integrin first binds to plectin,[80] to form the inner plaque of the hemidesmosome, and a type II or immature hemidesmosome. BPAG1e can first be detected during the 9th or 10th week of gestation.[159] Eventually, BPAG1e and collagen XVII will become incorporated into the complex,[82] transforming the immature hemidesmosome into a functioning type I, mature hemidesmosome.



2.7 9th week EM. 9th week. Electron micrograph of 9 week old fetal skin. Rudimentary hemidesmosomes are demonstrated (arrows). McMillan JR, Eady RA. Hemidesmosome ontogeny in digit skin of the human fetus. *Arch Dermatol Res.* Feb 1996;288(2):91-97.

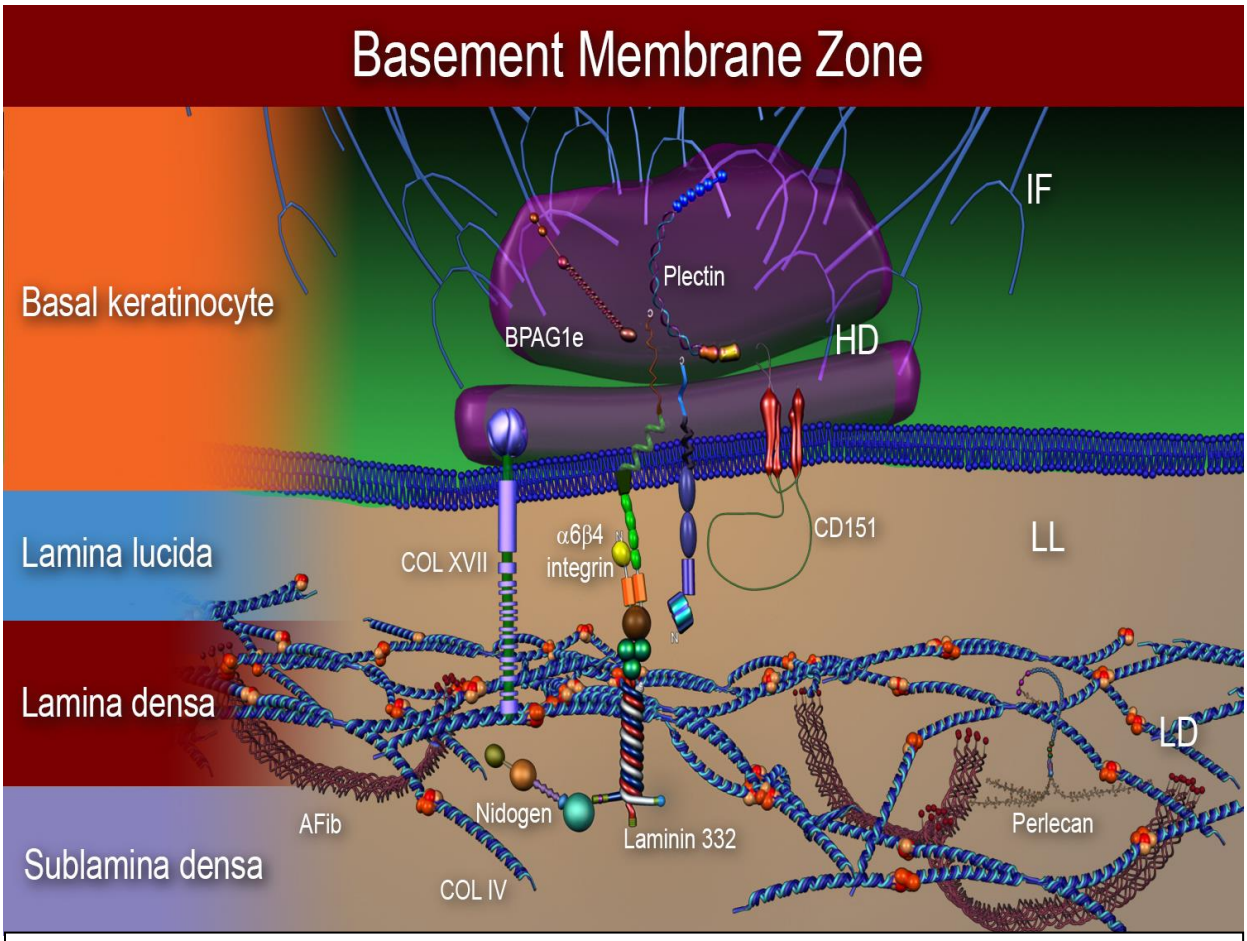
At the basal keratinocyte level, a loosely formed primitive intermediate filament network, first seen during the seventh week, forms more mature and fully formed intermediate filament networks, during this time. The basal keratinocyte expression of keratins 5 and 14[162,165,166] is known to coincide with the formation of the inner plaque, during the ninth week of life. Within the dermis, rudimentary anchoring fibrils are seen. Fetal collagen VII,[158] which is first seen in the sixth week, now forms more recognizable anchoring fibrils.[162] Collagens I and III, which have been present since the second week of gestation, continue to develop. By the 12th week, the fetal dermis will be transformed into a more adult-like structure with thickening and aggregation of collagens I and III into larger bundles.

15TH WEEK OF LIFE

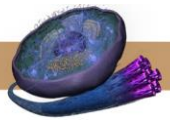
By the 15th week, the developing fetus' arms and legs are much longer, and the mother may start to notice fluttering sensations as the fetus begins to kick. The eyebrows have begun to grow, and the fetus may even begin sucking its thumb. Structures such as intermediate filaments, hemidesmosomes, and anchoring filaments can be easily identified with electron microscopy. Anchoring fibrils are still forming at this stage. On histology, there is the persistence of the embryonic periderm layer. This periderm will not be completely replaced until the 24th week.[162]

24TH WEEK OF LIFE

During the 24th week, the fetus is almost completely formed. The fingernails, taste buds, and ears will have developed. By the end of this week, the growing baby is viable and can survive outside of the womb, with intensive care unit assistance. On electron microscopy, the anchoring fibrils are now fully formed, completing the basement membrane zone development. On histology, a much more adult-like stratified epidermis is seen, and the periderm layer is no longer visible.[162] From the first week of embryonic development to the 24th week of life, the wondrous complexity of the basement membrane zone continues to amaze and inspire us all (Figure 2.8).



2.7 9th week EM. 9th week. Electron micrograph of 9 week old fetal skin. Rudimentary hemidesmosomes are demonstrated (arrows). McMillan JR, Eady RA. Hemidesmosome ontogeny in digit skin of the human fetus. *Arch Dermatol Res.* Feb 1996;288(2):91-97.



FUNCTION

The human skin is subjected to an enormous amount of stress and sheering forces. The skin owes its resiliency, in large part, to the epidermal basement membrane zone. A specialized extracellular matrix,[167] the thin sheet-like basement membrane zone primarily serves a structural function. It maintains the architectural integrity of the epidermis, provides protection against shearing forces.[156] The importance of this function has been documented in hereditary blistering disorders that disrupt the basement membrane zone, producing skin blistering with even minor trauma.[168]

Since the identification of genetic mutations in human basement membrane zone collagen IV, and their link to Alport's syndrome in 1990,[167] a host of diseases attributable to defects within the basement membrane zone have been discovered.[23,168] These findings have firmly cemented the importance of the structural function of the basement membrane. Embryonic development, for example, cannot occur without functioning basement membranes.[169]

The basement membrane is much more than just a structural support. It serves as a permeability barrier, a template or matrix for wound healing, participates in signal transduction between the epidermis and underlying connective tissues, and acts as an important regulator of cancer progression.[23,134,135,163] For example, perlecan, our prototype heparin sulfate proteoglycan within the lamina densa, contains highly negatively charged glycosaminoglycan side-chains. These side chains confer an overall charge to the basement membrane, filtering charged molecules.

The basement membrane zone functions as an important regulator of cancer progression,[23] but is the basement membrane zone simply a structural barrier for cancer, or is the basement membrane zone's regulation of cancer progression more complex? Although cancers come in a variety of different forms, one of the basic tenants of cancer is the fact that it has the ability to invade through basement membranes. Since the 1980's, theories on tumor cell invasion have incorporated the notion that basement membrane components actively participate in providing a suitable microenvironment for tumor progression.[170] The old notion that the extracellular membrane is just a physical barrier for invading tumor cells has given way to a new understanding, where the extracellular matrix molecules are active participants in the regulation of tumor growth and invasion.[171] Many of the components of the epidermal basement membrane zone have been linked to cancer, with several basement membrane zone components having a significant role in tumor invasiveness and or prognosis.[23]

The cytokeratins, which belong to the intermediate filament family of proteins, reflect tumor cell activity and are clinically useful in evaluating response to therapy for several types of cancers, as well as providing prognostic information on tumor progression and metastasis.[172-176] Integrins have also been known to have a role in

cancer.[23,177] α6β4 integrin is associated with poor prognosis and reduced survival in several cancers.[23,178] α6β4 integrin promotes tumor progression by helping cancer evade apoptosis or programmed cell death, become more invasive, and metastasize.[23] Integrins function in intracellular signaling, and even recruit signaling molecules for various functions.[71,177] At every stage of cancer progression, integrins are activated, which create signaling pathways that enable cancer cells to localize and take hold at metastatic sites.[177,179]

α6β4 integrin, with the aid of other factors, is known to control at least 538 genes, 36 of which are linked to tumor progression.[23] A tyrosine residue in the β4-subunit of its cytoplasmic domain, known as Y1494, has been proposed as a possible source of this gene regulating function.[178] α3β1-integrin, another basement membrane zone integrin, has been proposed as a possible contributor to tumor migration in malignant melanoma.[23,180]

Laminin-332, is highly expressed in epithelial tumors, and has been known to accumulate at the tumor stromal interface.[23] Laminin-332 has been found to be either up regulated or down regulated, based on tumor type. The up-regulation of laminin-332 is correlated with tumor invasiveness and poor prognosis in squamous cell carcinoma variants,[181,182] and breast cancer.[183] In contrast, laminin-332's down-regulation is correlated with tumor invasiveness in prostate cancer.[184,185] This indicates that laminin-332, when up regulated may serve as a promoter of tumor invasiveness, and when down regulated, the basement membrane zone's integrity is altered such that tumor cells are allowed to cross the basement membrane. Laminin-511, which has been associated with breast cancer,[186] has been shown to produce a proliferative signal for keratinocytes.[187] Such a proliferative signal function could explain the correlation between tumor invasiveness and up-regulation of laminins.

Collagen IV is believed to have a protective effect against tumor progression.[188] For example, collagen IV α5(IV) and α6(IV) chain assembly has been shown to be defective in advanced breast cancer.[189] Phenomenon such as methylation of genes, can enhance cancer progression, when it occurs in promoter regions of tumor suppressors. The hypermethylation can serve dual purposes for collagen IV genes. On the one hand, hypermethylation has been shown to be essential for the normal regulation of collagen IV cell-specific expression. On the other hand, methylation of collagen IV genes can lead to alterations in its α5/α6 chain expression, which can theoretically result in loss of collagen IV chains, thereby enhancing tumor progression, for cancers such as colon cancer.[188]

Collagen VII, through interaction with Laminin-332 has been shown to enhance tumorigenesis.[23,190,191] Mutations in collagen VII leading to recessive dystrophic epidermolysis bullosa, as blistering disorder, can be associated with a cumulative risk if squamous cell cancer of 70% by the age of 45.[192,193] However, the link between collagen VII and the development of squamous cell cancer in this disorder is somewhat controversial. Disease severity is inversely related to the amount of collagen VII

present within the basement membrane zone - the more severe the disease, the less collagen VII present. As disease severity increases, so does the risk of developing squamous cell cancer.[194] However, the presence of collagen VII, has been shown to be necessary for tumor formation.[191] The amino-terminal noncollagenous domain of collagen VII, known as the NC-1 domain, interacts with Laminin 332, and leads to development of signaling factors that enhance tumorigenesis.[23,190,191] Furthermore, the loss of the NC1 binding site on Laminin-332's β3-chain has been associated with decreased squamous cell tumorigenesis.[23,190] Adding to the controversy is the fact that some tumors in patients with recessive dystrophic epidermolysis bullosa do not express collagen VII.[192,195] The explanation for these seemingly contradictory findings is most likely the extracellular matrix's effect on the complex signaling pathways, which is regulated either directly or indirectly by basement membrane zone components.

Other basement membrane zone molecules such as CD151, nidogen, and perlecan are associated with tumorigenesis. CD151 expression has been associated with Merkel cell carcinoma, ductal breast carcinoma, and adenocarcinoma of the prostate, liver, colon, and lung.[196] CD151 levels correlate with overall survival in breast and liver cancers, and inversely correlated with overall survival in Merkel cell cancer.[196] Nidogen has been suggested as a possible serum marker for ovarian cancer.[197] Growth factors and other signals from tumor cells modify the functions of proteoglycans, like perlecan, directly, and also indirectly by altering the composition of glycosaminoglycan-attached side chains.[198] Perlecan expression is increased in liver tumors, oral tumors, and in malignant melanoma, and serves as a promoter of tumor growth and angiogenesis. The large globular domain of perlecan, known as LG3, has been detected as a proteolytic fragment in pancreatic, colon and breast cancers.[199]

INTERMEDIATE FILAMENTS

First detected on x-ray diffraction analysis, during the 1930's, and then later visualized with electron microscopy, it was not until the late 1960's that researchers suggested that intermediate filaments were actually distinct cytoskeletal proteins.[24,25] It would take a few more years for this concept to take hold. In fact, the a paradigm shift from viewing these proteins as just static structural elements, to dynamic multi-functional participants in diverse cellular processes would not occur until the late 1990's.[25]

There are a number of disorders associated with keratins (Table 1.3) and other types of intermediate filaments. From studying these disease states, intermediate filament functions, other than structural roles, are becoming increasingly evident. These functions include the maintenance of structural and mechanical cellular integrity, the organization of intercellular or cytoplasmic architecture, intracellular signaling, and the regulation of transcription.[25,26,30]

Table 1.3 **Keratin intermediate filament expression patterns.**[26-28,30,31]

Keratin(s)	Expression pattern	Disease association
1	Suprabasal keratinocytes	Ichthyosis hystrix (Curth-Mackin) Palmoplantar keratoderma (striae) Palmoplantar keratoderma (mild ichthyosis) Greither's syndrome
1/10	Suprabasal keratinocytes (stratum spinosa)	Epidermolytic hyperkeratosis
1/16	Suprabasal keratinocytes	Palmoplantar keratoderma (non-epidermolytic)
2e	Stratum spinosa/granulosum	Ichthyosis bullosa of Seimens
3/12	Cornea	Meesmann corneal dystrophy
4/13	Mucosa	Oral white-sponge nevus of Cannon
5	Basal keratinocytes	Dowling-Degos Epidermolysis bullosa simplex (EBS) with migratory circinate erythema. EBS with severe palmoplantar hyperkeratosis
5/14	Basal keratinocytes	EBS-generalized other (formerly Weber-Cockayne, Dowling-Meara, Koebner types) EBS with mottled pigmentation
6a/16	Outer root sheath of hair follicle, nail bed, palmoplantar skin and orogenital mucosa	Pachyonychia congenita (type I)
6b/17	Nail bed, hair follicle, sebaceous glands	Pachyonychia congenita (type II)
6c/16	Suprabasal keratinocytes	Palmoplantar keratoderma, nonepidermolytic (focal)
6/16/17	Hyperproliferative epidermis (e.g. stress, wound healing, dermatoses)	Psoriasis Acute skin inflammation (UV exposure, infection)
8	Bowel, Liver	Chronic pancreatitis Inflammatory bowel disease
8/18	Bowel, liver	Cirrhosis and hepatitis
9	Palmoplantar	Palmoplantar keratoderma (Epidermolytic)
14	Basal keratinocytes	EBS (recessive) Dermatopathia pigmentosa reticularis Naegeli-Franceschetti-Jadassohn syndrome
17	Hair	Steatocystoma multiplex
18	liver	Hepatic artery thrombosis
74	Hair	Woolly hair (autosomal-dominant)
75	Hair	Loose-anagen syndrome Pseudofolliculitis barbae
81/86	Hair	Monilethrix
85	Hair	Ectodermal dysplasia (hair-nail type)

Keratin intermediate filaments are known to form a dense network throughout keratinocytes, with extension into hemidesmosomes and cell-to-cell adhesion sites. The staggered tetramer configuration of intermediate filaments provides them the stability needed to function as the internal supporting skeleton for mammalian cells. Keratin intermediate filaments are characterized by their high viscoelasticity and flexibility.[200] The ability to endure extensive deformation forces is a key element to the success of these filaments.[30,201,202] Even in resting cells, the keratin filament network appears to be in constant motion, with a tremendous degree of flexibilty, combined with the ability to demonstrate incredible tensile strength with applied mechanical stresses.[200] The structural role of keratins is also demonstrated in the fact that their concentration is dramatically increased at sites exposed to mechanical stressors, as opposed to the loosely dispersed cytoplasmic keratin networks seen in parenchymal tissues that experience relatively little mechanical stress.[26]

One of the first examples of the importance of intermediate filaments in response to mechanical stress came from studying trauma-induced blistering in mice,[203,204] during the 1990's.[205] There are currently greater than 90 distinct diseases attributed to mutations in intermdiate filament genes, with over 15 of these attributed to keratin, or type I and II intermediate filaments.[27]

The long list of blistering disorders attributed to mutations in keratin genes underscores the significance of keratin filaments to the mechanical stability of the epidermis.[200] Furthermore, keratin filaments have a major impact on the shape and the internal structural polarity[206] of epithelial cells on surface lining tissues, serving the vital function of maintenaning distinct cell morphology and tissue-specific polarity.[200] Intermediate filaments serve not only as the internal structural support for cells and their organelles, they also serve as molecular scaffolds or growth templates for cellular structures. Studies on genetically modified mice, demonstrate that type IV intermediate filaments are essential in providing a molecular cytoarchitectural scaffolding for developing peripheral nerve axons to achieve the minimum functional diameter, and for effective neuron dendritic aborization.[207]

Another novel structural function for keratin intermediate filaments is the protection of the placental barrier function.[26] Studies on keratin 8, keratin 18 and keratin 19 deficient mouse embryos have demonstrated trophoblast giant cell layer structural failure, with early death by development of hematomas beneath developing yolk sac, and failure of the placental barrier function.[208,209] Keratins 8 and 18 have been associated with liver disease[27], indicating a role in the protection of organs such as the liver from stress and injury.[26,210,211]

Epithelial cells are often exposured to sheer stress and other mechanical forces such as compression, and tissue injury. As with any dynamic system, the internal keratin filament skeleton of the cell must also compensate. This is demonstrated in epithelial cells undergoing cell migration after acute injury.[205] Keratinocytes proximal to wound edges demonstrate alterations in their keratin networks. Keratins 6a, 6b, 16, and 17 are upregulated, and keratins 1 and 10 are down regulated.[205,212,213] The idea is that the keratin 6 and 16 enriched keratinocytes at wound margins can provide the stability and rigidity needed at injury sites for repair. The concept that the keratin intermediate filament structural organization is dynamic and can re-organize in response to chemical signals and tissue injury, thus allowing for greater mobility or stability - as needed - has been supported by several studies.[205,214,215]

A recently appreciated function of intermediate filament proteins is the ability to act as intracellular signals during cellular functions such as responses to mechanical stress and apoptosis.[26] In response to injury, keratin 17 has been demonstrated to influence cell growth and size by regulating intracellular protein synthesis.[216] The modulation of murine tumer necrosis factor dependent and independent Fas-mediated pro-apoptotic hepatic intracellular signaling has been demonstrated in mice lacking keratins 8 and 18.[217-219]

The histopathologic typing of epithelial tumors has been greatly enhanced by keratin-specific marker proteins.[220] Also, the intermediate filament cytokeratins 8, 18 and 19, have found utility in the early detection of epithelial cell carcinoma recurrence, and the clinical assessment of therapeutic responses in patients with these carcinomas.[172,174] With an ever expanding list of non-structural functions attributed to keratin intermediate filaments, it becomes evident that the dynamic and flexible nature of this structural protein is aptly suited for its many functions.

BPAG1E

BPAG1e, is an inner plaque hemidesmosomal cytoskeletal linker[221,222] protein that connections hemidesmosomes to intermediate filaments and stabilizing cell-to-substrate adhesions. BPAG1e is thought to function as not only a structural linker, but is involved in signaling and regulation of cell polarity and migration, via binding to the β4-subunit of α6β4 integrin.[223] Despite BPAG1e's well described association with diseases such as bullous pemphigoid, and paraneoplastic pemphigus, a 1995 study involving BPAG1e-negative transgenic mice,[224] found no weakening of hemidesmosome stability nor cell substratum adhesion, indicating that BPAG1e was not absolultely essential for hemidesmosome or basement membrane zone assembly.[23] In fact, studies on BPAG1e knock-out mice demonstrate predominantly neurological defects, and not the expected epithelial defects.[224] Further strengthening this viewpoint is the fact that type II hemidesmosomes have no associated bullous pemphigoid-

associated antigens. There is no known genetic disease associated with BPAG1e.[35] Disruption of inner plaque hemidesmosomal proteins BPAG1e or plectin results in a mechanically fragile epidermal basal keratinocytes, which are vulnerable to physical stress.[225] A recent study[226] revealed that Collagen XVII, not BPAG1e demonstrated T- and B-cell reactivity in patients with bullous pemphigoid, indicating that Collagen XVII may in fact be the primary antigen in bullous pemphigoid.[53]

PLECTIN

Plectin is a large molecule that imparts a great deal of stability to hemidesmosomes.[62] Its size, numerous domains, and multiple isoforms facilitates its differse cross-linking and scaffolding-like functions.[35,55] Specifcally, it is the plectin 1a isoform, found in hemidsmosomes,[62] that binds to both intermediate fillaments, α6β4 integrin, and collagen XVII,[50] thereby stabilizing the hemidesmosome complex.[60,61] Plectin functions as a linker of cytokeratin proteins within the basal keratinocyte and serves as a molecular scaffold for diverse signaling molecules.

The stability that plectin imparts to the basement membrane zone is underscored by the multiple variants of epidermolysis bullosa simplex associated with plectin gene defects. Plectin is associated with epidermolysis bullosa simplex with muscular dystrophy, epidermolysis bullosa simplex with pyloric atresia, and the Ogna variant of epidermolysis bullosa simplex.

INTEGRINS

Like all integrins, α6β4 integrin is a two-way signaling molecule that links the keratinocyte cytoskeleton to the extracellular matrix.[42,71] Other than the obvious structural role within the hemidesmosome, integrins are necessary for maintaining epidermal homestatic functions such as adhesion, proiferation, and differentiation.[106] The extent to which α6β4 integrin participates in functions other than adhesion and structure is controversial.

β1-subunit containing integrins are vital for human hair growth, and via their adhesion function, epidermal homeostatsis.[106] Epidermal homeostasis requires the orderly renewal of the epidermal keratinocytes, which in turn, relies on a population of stem cells residing predominantly in the bulge region of hair follicles. During wound healing, β1-subunit containing integrin-laminin interactions within the bulge region stabilize stem cell populations. During steady-state operations, epidermal stem cells in sebaceous glands and interfollicular epidermal sites are responsible for epidermal homeostasis and renewal functions. In each of these sites, β1-subunit containing

integrin-laminin interactions maintain stem cell populations and help to ensure their stability. Integrin gene mutations are associated with several diseases such as junctional epidermolysis bullosa with pyloric atresia and psoriasis.

COLLAGEN XVII

Collagen XVII has a predominantly structural function within the basement membrane zone. Collagen XVII spans almost the entire length of the basement membrane zone, and contributes to multiple interactions stabilizing the basement membrane zone components. It is a major component of the hemidesmosome, and contributes to the formation of anchoring filaments. As the major antigen associated with bullous pemphigoid, its structural significance cannot be understated. Extracellular cleavage of collagen XVII results in 120 kDa soluble ectodomain, and a 97 kDa fragment, which are associated with linear IgA bullous dermatosis. Murine studies have discovered a major role in tooth enamel formation for collagen XVII.[85]

CD151

At least 33 tetraspanins have been identified in humans over the last 20 years, but many details about their function are yet unknown. The tetraspanin family of proteins mediate transmembrane signal transduction events, with potential key roles in cellular development, activation, growth, and motility, and have been linked to cancer cell invasiveness and metastatic potential.[93] It generally accepted that CD151's association with α3β1, α6β4, and α6β1, is responsible for the majority of its functions. However, the extent of CD151's dependence on this interaction is somewhat controversial.[68] CD151 forms both homodimers on the basal cell surface, and engages in many other complex interactions. These complex interactions have led to the coining of the term 'tetraspanin web'.[68] The process of sorting out the tetraspanin web, and its contributions to the varied ascribed functions of CD151, will continue for quite some time.

CD151 plays a vital role in many renal, circulatory, and integumentary functions. Point mutations resulting in a defective large extracellular loop of CD151, leads to nephropathy with pretibial epidermolysis bullosa and deafness,[42] and other mutations in CD151 are known to cause hereditary nephritis.[95] β-thalassemia minor has also been associated with CD151. Murine studies have demonstrated altered angiogenesis, abnormal wound healing, defects in platelet aggregation and function, altered T-cell proliferation, and effects on pathological angiogenesis.[68,95]

CD151 expression has been associated with Merkel cell carcinoma, ductal breast carcinoma, and adenocarcinoma of the prostate, hepatocellular cancer, and lung cancer.[68,196] CD151 levels correlate with overall poorer survival outcomes in breast,

liver, colon, non-small cell lung, and gingival squamous cell cancers, and inversely correlated with overall survival in Merkel cell cancer.[93,196] CD151 has also been linked to matrix metalloproteinase activity, with overexpression of CD151 demonstrated in human melanoma cells, resulting in increased expression of matrix metalloproteinase 9 (MMP9),[227] underscoring its role in tumor metastasis. Furthermore, antibodies to CD151 have been shown to block in vivo metastasis in tumor model systems[228,229]

CD151 mediates interactions between α3β1 and α6β4 integrins, which are important receptors for laminin-332. Recent evidence indicates that CD151 association with both other tetraspanins and α3β1-integrin are essential for initial adhesion and rapid migration on laminin-332 matrixes, as occurs during tumor metastasis.[68] In contrast, only CD151-integrin interaction is needed for α6β4-integrin attachment to laminin-332, which mediates stable adhesion complexes.[68]

LAMININS

As major components of the basement membranes, Laminins serve a myriad of functions. The mesh-like self-polymerization of Laminins in conjunction with the self-polymerization of collagen IV has been referred to as the "glue"[114] that binds structures within the basement membrane zone. So important is the structural function of laminins, that embryogenesis cannot occur in the absence of laminins. Laminins are required for organogenesis of lungs, kidneys, gastrointestinal tract, and the central nervous system. Laminins also play key roles in tissue morphogenesis. For example, Laminin-511 is required for the development of hair,[230] and the growth of hair[231] after birth.[109] Laminin-332 “supports cell migration during wound healing” and is known to enhance tumorigenesis and metastatic behavior.[109] The various domains of the laminin chains allow molecular binding and attachement to cell surfaces, helping to stabilizing the basement membrane zone. The LN domain, a calcium-dependent binding segment, that binds molecules such as heparin, and integrins, and is believed to attach the molecule to cell surfaces. Mutations in α2-chains, have been linked to congenital muscular dystrophy (CMD), and mild forms of CMD, have been mapped to mutations in the LAMA2 gene encoding the globular L4 domains.[232] Murine transgenic laminin α1- and β1-chain therapy has been shown to compensate for laminin α2-chain deficiency,[233] indicating potential future cures.

NIDOGEN

As ubiquitous members of the basement membrane zone, nidogens play a supportive role. Their primary function appears to be stabilizing interactions between laminins and collagen IV with the lamina densa. However, nidogens are not required for

epidermal basement membrane zone formation.[133,234] This is primarily due to the overlapping functions of many of the basement membrane zone components. For example, perlecan, in the absence of nidogens, can bind to collagen IV and laminins, stabilizing interactions between collagen IV and the laminins.[234] Murine studies on epidermal basement membranes indicate that the presence of nidogen-1 is dependent on laminin γ-chain binding, whereas nidogen-2 appears to be independently recruited into basement membranes by non-γ-chain binding interactions on laminins, or via binding to other basement membrane proteins. [234] Other than the supporting role within the basement membrane zone, nidogens have been suggested as serum markers for ovarian cancer,[197] adding a clinical role for these ubiquitous basement membrane zone components.

HEPARIN SULFATE PROTEOGLYCANS: PERLECAN

Perlecan confers an overall negative charge to the basement membrane, which allows this molecule to serve its primary function as a permeability barrier within the basement membrane. It has had a multitude of functions attributed to itself. These functions include: regulation of angiogenesis,[235] chondrogenesis,[236] and fibrillogenesis,[237] the filtration of solutes within renal glomeruli,[238,239] the delivery of growth factors,[240] cellular adhesion,[134,235,241] and tumorigenesis. The degradation of perlecan has also been associated with varied embryologic developmental processes.[242] For example, carboxyl terminal fragments of perlecan, present in amniotic fluid, have the potential to serve as a prognostic indicator of risk for premature rupture of fetal membranes in pregnant women.[242,243] Furthermore, perlecan is required for epidermal morphogenesis, as it controls the survival and differentiation of epidermal keratinocytes, by regulating the availability of survival and differentiation factors in human skin.[134]

Perlecan has also been implicated in tumorigenesis. Growth factors and other signals from tumor cells modify the functions of proteoglycans directly, and also indirectly by altering the composition of glycosaminoglycan-attached side chains.[198] For example, Perlecan expression is increased in liver and oral tumors, and in malignant melanoma, and serves as a promoter of tumor growth and angiogenesis. The large globular domain of perlecan, known as LG3, has been detected as a proteolytic fragment in pancreatic, colon and breast cancers.[199]

Perlecan can serve as both an enhancer of, or an inhibitor of angiogenesis.[198] Angiogenesis is promoted by the binding of perlecan to growth factor receptors. In contrast, proteolytic cleavage of perlecan into angiostatic fragments known as endostatin and endorepellin, via downstream signaling, terminate angiogenesis.

COLLAGEN IV

Since collagen IV's initial discovery in 1966[244,245] we have learned a great deal about its role in the basement membrane zone and its function in human disease. Unlike other collagens, collagen IV is found only in basement membranes.[246] Collagen IV's primary role in the basement membrane is structural, as its three-dimensional lattice superstructure forms the basal lamina. Collagen IV also has been associated with angiogenesis, tissue remodeling, and cancer progression.[188] There are many genetic diseases attributed to collagen IV.[125,246-249] Collagen IV is associated with Goodpasture syndrome, Alport syndrome, diffuse esophageal leiomyomatosis, benign familial hematuria with thinning of the glomerular basement membrane, familial hematuria with retinal arteriolar tortuosity and contractures, and autosomal dominant hereditary cerebrovascular disease. The kidney is particularly affected by defects in collagen IV, because of the critical role of glomerular basement membranes in filtering plasma. Goodpasture syndrome is a lethal autoimmune disease characterized by glomerulonephritis and pulmonary hemorrhage, with defects in the NC1 domain of the α3(IV) chain.[125] Alport syndrome is a familial kidney disease characterized by progressive hematuria, sensorineural hearing loss, and ocular lesions with defects in glomerular basement membranes.[125,249] Diffuse esophageal leiomyomatosis is a rare disease characterized by benign smooth muscle cell proliferation of the esophagus, female genital tract, and the tracheobronchial tree, and has been associated with Alport syndrome, in 2-5% of families with juvenile Alport syndrome.[125,249] Autosomal dominant hereditary cerebrovascular disease attributed to mutations in the COL4A1 gene have a wide variety of clinical presentations, characterized by intracerebral hemorrhage, defects in the retinal vasculature, and nephropathy.[247] Collagen IV defects have also been implicated in enhancing tumor progression in breast cancer.[189]

COLLAGEN VII

Collagen VII is predominantly localized to the epidermal basement membrane zone within the lamina densa and sublamina densa, and is derived primarily from epidermal keratinocytes. It provides structural support and stability to the epidermal basement zone. Its importance to the stability of the basement membrane zone is evident in diseases related to mutations in the COL7A1 gene, such as epidermoylsis bullosa.

Chronic blistering disorders such as recessive dystrophic epidermolysis bullosa (RDEB) are often associated with the development of cutaneous squamous cell skin cancers. It is commonly thought that the most severely affected individuals are at greatest risk, because of constant skin damage. However, there is no direct evidence that having a chronic blistering disorder with skin fragility alone is sufficient to induce squamous cell skin cancer. In 1985, Susana Ortiz-Urda, and her colleges,[191] postulated

that specific collagen VII defects may be the reason that only some patients with (RDEB) go on to develop squamous cell skin cancer. They used murine models to demonstrate that RDEB cells lacking collagen VII did not form tumors, while those retaining the NC1 amino terminal domain were tumorigenic. They concluded that the fibronectin-like sequences with the NC1 domain promoted tumor cell invasion in a laminin 332-dependent manner.[191]

BASEMENT MEMBRANE DISORDERS

Bullous disorders involve every layer of the epidermis, from the stratum corneum to the superficial papillary dermis. Bullous disorders involving the basement membrane zone are associated with several key components (Table 4.1)

Table 4.1 Basement Membrane Zone Disorders

Level	Antigens	Disorders
Basal keratinocyte	Keratin 5 & 14	EB Simplex
	Plectin	EB with muscular dystrophy EB with pyloric atresia Mucocutaneous pemphigus Paraneoplasitc pemphigus
	BPAG1e	Bullous pemphigoid Mucocutaneous pemphigus
Lamina lucida	Collagen XVII	Bullous pemphigoid Pemphigoid gestationis Mucocutaneous pemphigus JEB-other Lichen planus pemphigoides Linear IgA disease
	Laminin 332	Mucocutaneous pemphigoid associated with malignancy JEB-Herlitz JEB-other
	α6β4 integrin	Junctional EB with pyloric atresia Ocular cicatricial pemphigoid (b4 integrin)
Lamina densa	Collagen IV	Alport syndrome Goodpasture syndrome Diffuse esophageal leiomyomatosis Benign familial hematuria with thinning of the glomerular basement membrane Familial hematuria with retinal arteriolar tortuosity and contractures Autosomal dominant hereditary cerebrovascular disease
Sublamina densa	Collagen VII	Epidermolysis bullosa acquisita Dystrophic EB Transient bullous dermolysis of the newborn Bullous lupus erythematosus

BASAL KERATINOCYTE LAYER

EPIDERMOLYSIS BULLOSA (KERATINS 5 & 14, LAMININ 332, COLLAGEN VII, COLLAGEN XVII, A6B4 INTEGRIN, PLECTIN)

The name epidermolysis bullosa (EB) is given to a group of inherited disorders, first described by von Hebra in 1870, and characterized by blister formation in response to mechanical trauma, and slowly or poorly healing wounds.[250] Epidermolysis bullosa subtypes encompass defects from the all areas of the basement membrane. There are four major types of epidermolysis bullosa: (1) epidermolysis bullosa simplex secondary to a defect in keratins 5 and 14, plectin; (2) junctional epidermolysis bullosa, secondary to defects in laminin 332, collagen XVII, or α6β4 integrin; (3) dystrophic epidermolysis bullosa, secondary to defects in collagen VII; and (4) Kindler syndrome arising from defects in kindling-1 encoded by the KIND1 gene (Table 4.2). There has been

considerable debate on the proper classification of the subtypes of Epidermolysis Bullosa. Molecular genetics has helped differentiate many of the subtypes[251]; however, it has become clear that there are many phenotypic variations among patient with the same inherited gene defect. Hence, clinical criteria are used to separate out the different subtypes.[252] There are currently at least 14 different genes responsible for EB, and the list continues to grow.[251,253]

Table 4.2 Epidermolysis Bullosa Classification.[251,252]

Level	Type	Subtype	Gene (Protein)
Basal keratinocyte	EBS	Suprabasal	PKP1 (Plakophilin-1)
		Basal	DSP (Desmoplakin) KRT5/14 (K5/14) PLEC1 (plectin) ITGA6, ITGB4 (α6β4 integrin) DST (Dystonin); BPAG1-e
Lamina lucida	JEB	JEB-Herlitz	LAMA3, LAMB3, LAMC2 (Laminin 332)
		JEB-Other	LAMA3, LAMB3, LAMC2 (Laminin 332) COL17A1 (collagen XVII) ITGA6, ITGB4 (α6β4 integrin)
Sublamina densa	DEB	Dominant	COL7A1 (collagen VII)
		Recessive	COL7A1 (collagen VII)
Mixed	Kindler syndrome		KIND1 (Kindlin-1)

EBS - epidermolysis bullosa simplex; JEB - junctional epidermolysis bullosa; DEB - dystrophic epidermolysis bullosa.

Epidermolysis bullosa simplex (EBS) is characterized by blistering within epidermal basal keratinocytes and contains the 'EB simplex, localized' (formerly Weber-Cockayne), 'EBS-generalized other' (formerly Koebner), and EBS-Dowling-Meara subtypes[250]. All are autosomal dominant KRT5 or KRT14 defects, except for the EBS with muscular dystrophy subtype, which is associated with a defect in PLEC, which produces the protein plectin, and is autosomal recessive. EB with pyloric atresia also stems from defects in plectin genes (PLEC1). EB with pyloric atresia presents with blisters, aplasia cutis congenita and pyloric atresia, and is considered a lethal variant, with death occurring after birth.[254] It is important to note that rare autosomal recessive subtypes have been documented such as EB with mottled pigmentation, autosomal recessive EB without muscular dystrophy, and EBS superficialis.

The diagnosis of EB is based on an initial diagnosis based on clinical findings, a careful history, family history, and a biopsy for histopathology and immunofluorescence to identify the level of the defect within the basement membrane zone, to classify the disease into one of the four major types of EB (Table 4.3).[253] There are three main techniques for laboratory diagnosis of EB: Immunofluorescence mapping, transmission EM, and mutational analysis.[251] Transmission EM studies require greater technical skill and are not widely available. Immunofluorescence is less expensive, and has greater sensitivity and specificity than EM studies. In general, the autosomal dominant forms tend to present with milder disease, and intraepidermal blistering manifests as flaccid

Table 4.3 Epidermolysis Bullosa Immunofluorescence patterns.

Type	Defect	Location	Salt Split Skin
EBS	K5/14 Plectin	Basal keratinocyte	Floor
Junctional EB	Laminin 332 Collagen XVII α6β4 integrin	Lamina lucida	Floor
Dystrophic EB	Collagen VII	Sublamina densa	Roof

EBS - epidermolysis bullosa simplex; EB - epidermolysis bullosa.

bullae. Blistering occurring below the level of the basal keratinocytes tends to be tense, and can lead to scarring, in contrast to the intraepidermal flaccid blistering, which can often heal without scarring. All forms of epidermolysis bullosa can have nail dystrophy or alopecia.

The major subtypes of EBS are contrasted in Table 4.4. The EBS-localized variant (formerly Weber-Cockayne) is the most common form, is generally milder, and tends to present in infancy or early childhood. This form rarely scars. The EBS-other generalized (formerly Koebner) and EBS-Dowling-Meara forms tent to be more severe, and occur at birth. Grouped or herpetiform blisters are seen in the EBS-Dowling-Meara subtype. The EBS-Dowling-Meara subtype also demonstrates clumped tonofilaments on electron microscopy. EB-with muscular dystrophy has two major differences between the other forms of Epidermolysis Bullosa simplex, namely, autosomal recessive inheritance, and a defect in plectin within the hemidesmosome. It is characterized by the onset of EB at birth, with the slow and gradual development of limb-girdle muscular dystrophy. The EBS-Dowling-Meara subtype is the most distinctive with several characteristic findings worth reviewing. Grouped blisters in a herpetiform or arcuate pattern can be seen. This variant can involve the oral mucosa. Plamoplantar keratoderma is more common than with the EBS-localized variant.

Table 4.4 Epidermolysis Bullosa Simplex Subtypes.

	EBS-localized	EBS-generalized other	EBS-Dowling-Meara	EB-Muscular Dystrophy
Inheritance	AD	AD	AD	AR
Defect	KRT5/14	KRT5/14	KRT5/14	Plectin
Age	Infancy/early childhood	Birth	Birth	Birth/neonatal period
Distribution	Palms/soles	generalized	generalized	generalized
Nail dystrophy	+	++	++	++
Findings			gradual PPK development, grouped herpetiform blisters, clumped tonofilaments (EM)	Gradual limb-girdle muscular dystrophy

EBS - epidermolysis bullosa simplex; AD – autosomal dominant; AR – autosomal recessive, PPK – palmoplantar keratoderma

MUCOUS MEMBRANE PEMPHIGOID (PLECTIN, LAMININ 332, COLLAGEN XVII, COLLAGEN VII, A6B4 INTEGRIN)

Mucous membrane pemphigoid (formerly cicatricial pemphigoid) is a heterogeneous groups of subepidermal blistering disorders affecting mucous membranes.[255] The phenomenon of "epitope spreading,"[256] where an autoimmune reaction exposes formerly "sequestered" antigens, and murine models demonstrating development of blistering reactions to antibodies, has been strengthening the concept that mucous membrane pemphigoid is an autoimmune disorder with no direct link to any one specific basement membrane zone component.[255]

Mucous membrane pemphigoid, which affects predominantly older women, is characterized by evanescent vesicles that rupture leaving ulcerations and erosions on oral mucosa in the majority of patients (75%)[257], with a predilection for the conjunctiva in two thirds of cases. Cases with disease limited to the oral mucosa have a better prognosis. Ocular cases are associated with defects in the β4 chain of α6β4 integrin, and tend to be chronic with possible progression to blindness. Glans penis adhesions in men, and introital obstructions in women can occur.[257] The Brunsting-Perry pemphigoid variant has no mucosal involvement, and is associated with lesions limited to the head and neck, with scaring alopecia. Mucous membrane pemphigoid associated with malignancy is associated with antibodies to laminin 332.

Diagnosis of mucous membrane pemphigoid is based on clinical findings, histology, and immunofluorescence. Anti-laminin 332 variant of mucous membrane pemphigoid is associated with malignancy in 30% of cases, so appropriate work up is necessary to exclude malignancy.[257] Patients are divided into one of two groups to determine therapeutic strategies: (1) oral mucosal lesions; (2) ocular, laryngeal, esophageal, or genital lesions.[255] For limited oral mucosal lesions, a therapeutic ladder of topical corticosteroids, low dose prednisone, dapsone, azathioprine, or mycophenolate mofetil is often satisfactory. Disease affecting mucous membranes other than the oral cavity (second group above) will require aggressive systemic immunosuppressive therapy.

BULLOUS PEMPHIGOID (BPAG1E, COLLAGEN XVII)

Bullous pemphigoid is the most common autoimmune subepidermal blistering disease of the skin.[257-260] This is a disease of the elderly, presenting after 60 years of age, with a male predominance; but can occur in children.[261] It presents as a generalized pruritic bullous eruption, and can have a myriad of triggering factors (i.e. trauma, thermal injury, neurologic disease, malignancy, drugs). Bullae are tense, developing on normal skin, or on erythematous skin. Urticarial or eczematous lesions can accompany the blisters.[260] Lesions are distributed on the trunk, and proximal extremities. Mucous membrane involvement occurs in approximately 20% of cases. Early non-bullous presentations can include excoriated, eczematous, or urticarial

lesions and present a serious diagnostic challenge.[260] Antibodies are directed against BPAg1e, and the collagen XVII NC16A domain. The NC16A domain is the first non-collagenous extracellular segment of collagen XVII, and is considered the major pathogenic antigen in bullous pemphigoid.[262] Bullous pemphigoid is also associated with autoantibodies to the α6 chain of α6β4 integrin. Despite having molecular targets in both the hemidesmosome and the lamina lucida, the split is within the lamina lucida. Clinical variants include pemphigoid gestationis, drug induced pemphigoid, dyshidrosiform pemphigoid, pemphigoid nodularis, and erythrodermic pemphigoid. These clinical variants are a testimony to the clinical polymorphic nature of this disease.

Diagnosis of bullous pemphigoid relies on clinical and histopathologic evidence. Any elderly patient with a generalized pruritic eruption that is eczematous, urticarial or presents with tense bullae should evaluated for possible bullous pemphigoid. This is important, as highly pruritic eczematous or urticarial eruptions can occur for weeks or months before any blisters appear (premonitory stage).[257] Mucosal involvement is not common. Criteria described by Vaillant, et all,[263] for the diagnosis of bullous pemphigoid include: (1) no atrophic scars (sensitivity 91%, specificity 70%), (2) no musical involvement (sensitivity 78%, specificity 66%), (3) age greater than 70 years old (sensitivity 87%, specificity 54%), and (4) no head and neck involvement (sensitivity 91%, specificity 44%). These criteria suggest the diagnosis of bullous pemphigoid if three of the above four criteria are positive, provided linear IgG or C3 is seen along the dermal-epidermal junction on immunofluorescence.

Three year mortality has been described as high as 38%, with bullous pemphigoid having a chronic relapsing course and conferring significant effect on quality of life.[260] Treatment with topical clobetasol is often effective. Oral corticosteroids are frequently prescribed for extended periods of time, but have not been found to be superior to topical regimens. The use of systemic immunosuppressive agents (i.e. dapsone, azathioprine, methotrexate, etc.) should be weighed against the potential side effects in elderly patients.

PARANEOPLASTIC PEMPHIGUS

Paraneoplastic pemphigus is a blistering disorder with a significant amount of clinical polymorphism. Lesions show a unique combination of histologic characteristics of pemphigus vulgaris, erythema multiforme, and lichen planus. The classic histologic picture demonstrates a suprabasal split, well above the basement membrane. However, subepidermal blistering does occur, as one of its many antibody targets includes plectin, which is located within the inner plaque of hemidesmosomes. It can present with severe oral ulcerations, flaccid or tense bullae, and targetoid lesions. It most often presents as hemorrhagic stomatitis with extensive mucocutaneous erosions.[257] By definition, paraneoplastic pemphigus is associated with malignancy.

Most common malignancies are lymphomas. Treatment involves treatment of the underlying malignancy.

LAMINA LUCIDA LAYER

JUNCTIONAL EPIDERMOLYSIS BULLOSA (LAMININ 332, COLLAGEN XVII, A6B4 INTEGRIN)

Junctional epidermolysis bullosa (JEB), is associated with disruptions localized primarily at the lamina lucida. Most forms of junctional epidermolysis bullosa are autosomal recessive. In 2009, the first autosomal dominant form of JEB was described.[264] JEB is divided into two subtypes: 'JEB-Herlitz; and 'JEB-others'.[250] JEB-Herlitz is associated with a complete absence of laminin 332, whereas JEB-others demonstrates reduced levels of laminin 332, collagen XVII, or α6β4 integrin and has a better prognosis.[251]

The variants of epidermolysis bullosa form a continuum. On one end we have EB simplex, with mechanically induced predominantly flaccid blisters and some degree of nail dystrophy, alopecia, and some scaring, reflecting defects within the basal keratinocyte. As we move to defects within the lamina lucida, we begin to see the tense blistering becoming more frequent. The lamina densa stays fixed to the underlying superficial papillary dermis, and forms the base of the blister. We can expect more nail dystrophy, scaring, alopecia, and even changes in dental enamel (Table 4.5).

Table 4.5 Junctional Epidermolysis Bullosa Subtypes.

	JEB-Herlitz	JEB-Other	JEB-pyloric atresia
Inheritance	AR	AR	AR
Defect	Laminin 332	Laminin 332 Collagen XVII	α6β4 Integrin
Age of presentation	Birth (early death)	Birth	Birth (early death)
Skin distribution	Generalized	Generalized	Generalized
Scaring Alopecia Milia formation	+	++	+
Enamel hypolasia (tooth pitting)	+	+ +	+
Nail dystrophy	+	+ +	+
Characteristic findings	Most severe form Excessive granulation tissue periorificial, axillae, neck, or upper back	Mild form	Pyloric atresia at birth

The JEB-Herlitz subtype is the most severe and is lethal, as infants rarely survive. They present with generalized blistering at birth, with the characteristic periorofacial erosions and excessive granulation tissue. Multisystem and mucosal involvement is seen with orotracheobronchial, conjunctival, and genitourinary disruption. A hoarse cry or cough and respiratory distress may be presenting signs. The JEB-Other subtype is non-lethal, in contrast to the JEB-Herlitz subtype. These patients also present with generalized blistering, and some may even have some periorificial erosions and hypertrophic granulation tissue. However, absent are the hoarse cry and signs of respiratory distress seen in the JEB-Herltiz subtype, and these infants survive. As these patients grow, scalp, nail and tooth abnormalities become more apparent. JEB with pyloric atresia is lethal, and although the pyloric atresia can be corrected, the infants must still survive severe generalized blistering, that can include extensive internal multisystem involvement. Some infants do survive despite these challenges.

PEMPHIGOID GESTATIONIS (COLLAGEN XVII, BPAG1E)

As a variant of bullous pemphigoid, Pemphigoid gestationis shares some similarities and some significant differences. Pemphigoid gestationis is an autoimmune inflammatory bullous disease, that occurs during pregnancy – usually in the second or third trimester - or early postpartum[265] period.[266] Pemphigoid gestationis can also be associated with hydatiform mole, trophoblastic tumors, and choriocarcinoma.[266] It presents with urticarial and pruritic papules and plaques periumbilically, that spread and become tense erythematous vesicles and bullae. Mucous membrane involvement can occur in 20% of cases.[257] Spontaneous remission occurs in approximately 3 months; however, frequent exacerbations during subsequent pregnancies, menstrual periods, or with use of oral contraceptives can occur. At least half of the cases occur during the first pregnancy. Risks to the infant include premature birth, chance of becoming small for gestational age, and skin lesions in 5-10% of cases. Neonatal pemphigoid gestationis is transient. Associations include HLA types DR3 and DR4, and Grave's disease. The antigenic site on collagen XVII is the NC16A site as in bullous pemphigoid. Several other epitopes have been documented, all within the extracellular domain of collagen XVII.

Treatment for pemphoid gestationis is generally systemic corticosteroids. Topical corticosteroids are often not effective in relieving intense pruritus. "Alternatives to corticosteroids are azathioprine, dapsone, intravenous immunoglobulin, pyridoxine, cyclosporine, plasmapheresis, minocycline/nicotinamide, methotrexate, and cyclophosphamide".[266]

LICHEN PLANUS PEMPHIGOIDES (COLLAGEN XVII)

Lichen planus pemphigoides is a relatively rare, acquired autoimmune subepidermal blistering disease, originally described in 1892.[267] Patients with this disorder have the usual lesions of lichen planus with bullous pemphigoid-like blistering in areas of normal skin. Blistering can occur in lichen planus lesions, usually on the lower extremities; however, only with lichen planus pemphigoides do they occur on normal skin as well. Oral lesions can resemble lichen planus or bullous pemphigoid. Common triggers include medications and PUVA therapy. Pruritus can be severe. Lesions are often rapidly developing tense blisters occurring either before or after lichen planus lesions. This disease most commonly affects males in their 40's or 50's, with bimodal peaking[257] between 20-40 years old, and a second peak in the 60's. Children may also be affected, with a mean age of 12 years of age, and a female predominance.[268-270] Treatment is similar to lichen planus, with no relapses of blisters, once lesions resolve.[267] Treatment for childhood lichen planus is complicated by potential side effects of systemic corticosteroids. A review of a small number of cases, indicates that children may respond to a regimen of topical steroids and dapsone, with systemic corticosteroids reserved for refractory cases.[270]

LINEAR IGA DISEASE (COLLAGEN XVII)

Linear IgA disease is an acquired autoimmune disorder characterized by subepidermal blisters, a neutrophilic infiltrate, and circulating IgA anti-basement membrane zone antibody. Both children and adults are affected. Vesicles and bullae can have a bullous pemphigoid or dermatitis herpetiformis-like appearance. Mucous membranes are involved in 50% of cases. Remission after several years occurs in 60% of patients. Linear IgA disease has been associated with drugs and malignancy.

Childhood variant of linear IgA disease is known as chronic bullous disease of childhood, and is the most common autoimmune bullous disorder of childhood, peaking at 4-5 years of age.[257] Children present with often severely pruritic blisters in circular arrangements on the face, perineal areas, and lower extremities by 2 or 3 years of age, with spontaneous remission by age 13. Polycyclic blister patterns with blistering around the edge of lesions produces the characteristic "string of pearls" sign.[271] Perioral and scalp lesions are common. In contrast to adult linear IgA disease, children with chronic bullous disease of childhood have an increased frequency of B8, DR3, and DQ2. In adults, the disease may be acute or indolent, with prominent involvement of the trunk. Extremities and face are also involved. Mucosal involvement is common with oral lesions, nasal congestion, and gritty eyes.[271] Treatment consists of dapsone, anti-inflammatory antibiotics and topical steroids, with the disease resolving within 3-6 years.[271]

LAMINA DENSA LAYER

ALPORT SYNDROME (COLLAGEN IV)

Alport syndrome is characterized by hematuria, progressive renal failure, and sensorineural hearing loss and is frequently associated with ocular abnormalities (such as lenticonus and retinal anomalies). Alport syndrome is caused by mutations in the gene COL4A5, which encodes the basement membrane specific type IV collagen alpha-5 chain. While predominantly an X-linked dominant disorder, both autosomal dominant and recessive forms are associated with COL4A3 and COL4A4 genes. Although no specific skin pathology has been identified, Alport syndrome has been associated with diffuse cutaneous leiomyomas.

GOOD PASTURE SYNDROME (COLLAGEN IV)

Goodpasture syndrome is an autoimmune disease of lungs and kidneys. This is a potentially lethal disease with pulmonary hemorrhage and glomerulonephritis. Viral and streptococcal infections and exposure to hydrocarbon fumes have been suggested as possible causes. The Goodpasture antigen is the alpha-3 chain of type IV collagen, encoded by the COL4A3 gene. Two forms exist: (1) the antineutrophil cytoplasmic autoantibody (ANCA)-negative form, present in 75% of cases, and the (2) ANCA-positive form, present in 25% of cases. Both have autoantibodies to the NC1 domain of the alpha-3 chain of type IV collagen, but the ANCA-positive form also has antibodies to myeloperoxidase.

SUBLAMINA DENSA LAYER

EPIDERMOLYSIS BULLOSA ACQUISITA (COLLAGEN VII)

Epidermolysis Bullosa Acquisita is an acquired, subepidermal blistering disorder with antibody development against Collagen VII within the sublamina densa. There is a range of clinical presentations ranging from mechanobullous lesions similar to the inherited dystrophic epidermolysis bullosa in children, to lesions resembling bullous pemphigoid or cicatricial pemphigoid. There are three clinical variants, the classic variant (mechanobullous, noninflammatory form), a generalized inflammatory form (bullous pemphigoid like), and a localized variant.[257] Unlike dystrophic epidermolysis bullosa, which affects infants and children, epidermolysis bullosa acquisita affects both children and adults, affecting persons of all ages. There is an association with HLA

types DRB1 and DR5. Clinically, non-inflammatory trauma-induced blistering occurs acrally, that heal with scaring and milia formation. There is skin fragility, and oral lesions similar to cicatricial pemphigoid have been documented. Epidermolysis bullosa acquisita has been associated with numbers systemic diseases such as multiple myeloma, Crohn's disease, colitis, diabetes, Lymphoma, leukemia, amyloidosis, carcinoma, SLE, RA, and thyroid disease. Histologically, subepidermal, classically noninflammatory blistering is seen; but there may be neutrophils or eosinophils as well. DIF and IIF demonstrate linear IgG along basement membrane. On salt split skin, EBA localizes to the floor or dermal side of the split.

DYSTROPHIC EPIDERMOLYSIS BULLOSA (COLLAGEN VII)

The final major type of epidermolysis bullosa is the dystrophic form, where the defect is located in the sublamina densa, within the anchoring fibrils. The major subtypes include dominant dystrophic EB, recessive dystrophic EB, or the Hallopeau-Siemens subtype, and the recessive dystrophic EB, non-Hallopeau-Siemens subtype. The minor subtypes are listed. All of these forms are caused by defects in Collagen VII, within the anchoring fibrils, and located in the sublamina densa. Characteristically, blistering heals with scaring and milia formation.

Dominant dystrophic EB presents at birth. Blistering may be generalized or appear only on the hands, feet, elbows or knees - usually due to mechanical trauma as in all forms of epidermolysis bullosa. Rarely does scarring cause immobility and deformity of the hands and feet. Milia are seen at sites of scarring. There may be mild involvement of the mucous membranes, nails may be thick, dystrophic or destroyed.[12,14,16]

"Recessive dystrophic EB is characterized by blistering at birth or during the early neonatal period. All skin surfaces and mucous membranes (from mouth to anus) can be covered by blisters. Large areas may be devoid of skin. There is widespread scarring and deformity. Fingers and toes may become immobile. With recurrent scarring, fingers and/or toes may fuse together – a condition referred to as pseudosyndactly. Hands and arms may become fixed in a flexed position with resulting contractures. There is usually loss of the nails of the fingers and toes. Teeth may be malformed and delayed in appearing through the gums. Because routine dental care can create blisters, a higher than normal incidence of cavities can be expected. In many cases chronic malnutrition, growth retardation and anemia may ensue. Children who survive to adulthood have an increased risk of development of squamous cell cancer (up to 53% risk by the age of 35). The non-Hallopeau-Siemens type is less severe.[12]

Transmission electron microscopy is the gold standard for diagnosis and differentiation among the subtypes of epidermolysis Bullosa. This is accomplished by determining the level of the split within the basement membrane. There are some characteristic electron micrographic findings. For example, intermediate filament clumping indicates the Dowling-Meara subtype of EB simplex. Rudimentary hemidesmosomes are often seen in Junctional EB types. Absent or altered anchoring fibrils indicate Dystrophic EB.

Immunofluorescence antigenic mapping is also an effective technique. Immunomapping antibodies from BPAG1 and collagen IV allows for differentiation between the different types of EB. In EB simplex, both antigens localize to the floor. In junctional EB, BPAg1 localizes to the roof, while col IV localizes to the floor. In dystrophic EB, both antigens localize to the roof of the blister.

BULLOUS LUPUS ERYTHEMATOSUS (COLLAGEN VII)

Intense basal cell damage at the dermal-epidermal junction in acute lupus can result in bullous lesions. However, bullous lupus erythematosus is an autoimmune disorder distinguished by its antibody-targeted attack against type VII collagen, within the sublamina densa. Clinically, there is a rapid onset widespread non-inflamed vesiculobullous eruption. Disease activity is does not correlate with systemic lupus erythematosus. Mainstay of therapy is dapsone[272] with successful treatment with mycophenolate mofetil[273] reported.

Diagnostic criteria include the following:

(1) Diagnosis of SLE based on American Rheumatism Association criteria;
(2) Vesicles and bullae arising upon but not limited to sun-exposed skin
(3) Histopathology compatible with dermatitis herpetiformis
(4) Negative indirect immunofluorescence for circulating basement membrane zone antibodies
(5) direct immunofluorescence positive for IgG and/or IgM, and often IgA at the basement membrane zone.

HISTOPATHOLOGY

BASICS

In 1953, Walter Lever[274] differentiated between the blistering disorders pemphigus vulgaris and bullous pemphigoid, based on the histopathologic level of the split - as well as clinical criteria. Despite the numerous advances in immunofluorescence and molecular biology since the 1950's, identifying the level of the histopathologic split is still the first step in the histologic diagnosis of blistering disorders. Bullous disorders are divided into subcorneal, intraepidermal (or suprabasal), and subepidermal, based on the location of the blister within the histology specimen. For epidermal basement membrane zone diseases, the level of the split is subepidermal. Once the level of the split has been identified as occurring beneath the epidermis, or subepidermal, then the presence or absence of an inflammatory cell infiltrate defines the basic differential diagnosis.[275] Correlating this information with the clinical data and the results of other studies such as immunofluorescence will usually result in a correct diagnosis.

The basic steps in correctly identifying subepidermal blistering disorders in unknown histopathology slides are: (1) Identify the level of the split; (2) exclude artifact; (3) Identify the nature of any inflammatory cell infiltrate.[99]

Step 1: Identify the level of the split.

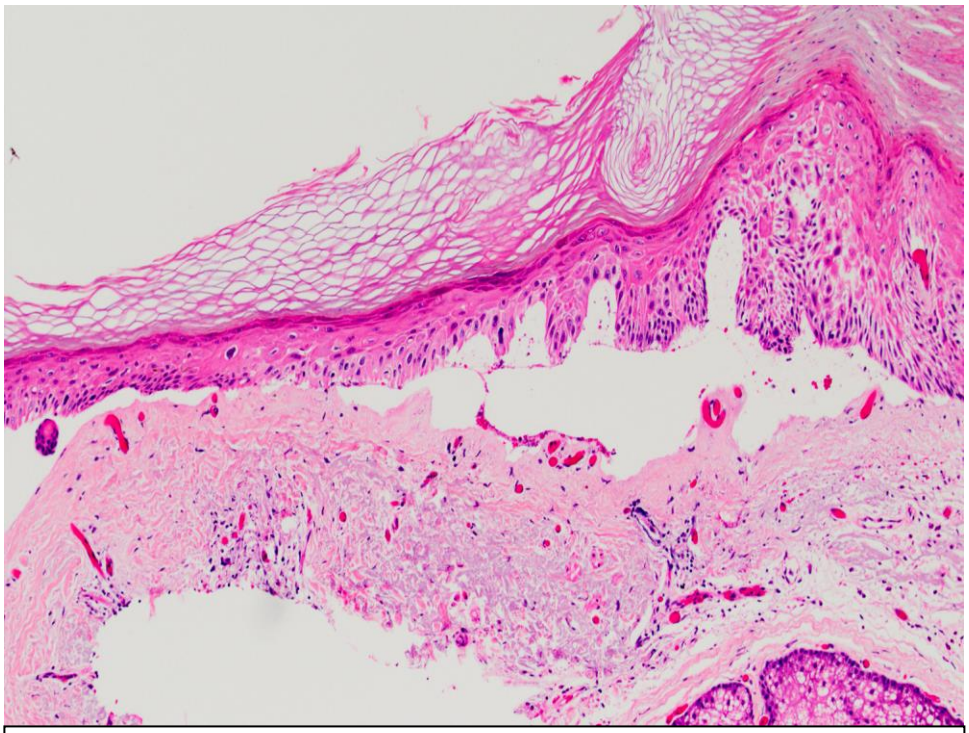
Determining the level of the split is not necessarily straightforward. The size of the biopsy specimen, the age of the lesion, the site biopsied, and laboratory processing techniques can all influence your ability to determine the level of the split. Probably the most important thing to remember is to examine the edges of each blister, in order to trace back the origin of the split. Reepitheliation artifact can lead to erroneous determination of the true level of a split.[129] The ideal biopsy specimen is a relatively nascent blister, prior to the onset of reepitheliation. A subepidermal split with reepitheliation can appear to be intraepidermal. However, fully mature epithelium, with spinous, granular, and corneal layers, is not seen on the floor of intraepidermal blisters.

Step 2: Exclude artifact.

Exclude artifactual blistering whenever possible. Trauma, radiation, burns, and external forces such as suction can lead to artifactual blistering. Frictional blisters tend to result in subcorneal splits. Generally, artifactual splits tend to have little or no associated inflammatory cell infiltrate. Dense fibrotic scars within the dermis can lead to

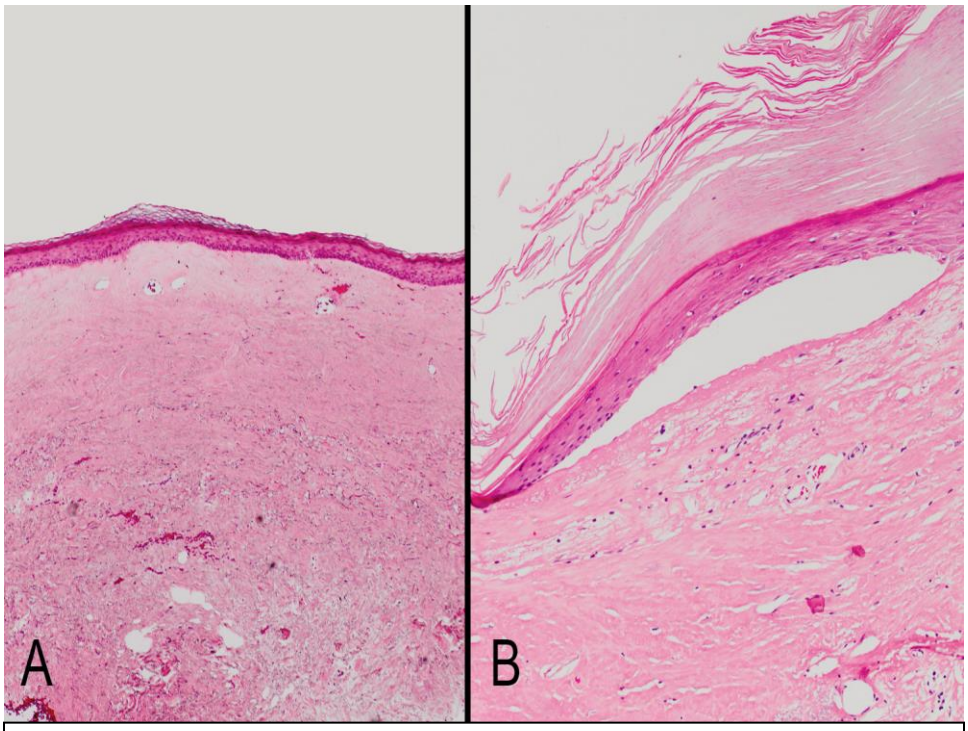
artifactual blistering directly above the scar, commonly referred to as the “bullous over scar” sign.

A recent review,[276] comparing the histology of electrical injury, thermal burns, and abrasions, found that thermal burns had the highest rate of subepidermal splitting, with electrical injuries having a higher rate intraepidermal splitting. If both intra- and subepidermal splitting is present, electrical injury was the most likely cause. Furthermore, nuclear elongation, often considered pathognomonic for electrical injuries, was present in thermal burns and abrasions as well. In fact, nuclear elongation results from a wide variety of causes, such as cauterization, drying, cryosurgery, and blunt traumatic injuries.[276] Cryosurgery can result in hemorrhagic blisters that are often cell poor and subepidermal (Figure 5.1).



5.1 **Cryosurgery.** Cryosurgery results in a hemorrhagic blister with a subepidermal cell poor split (H & E).

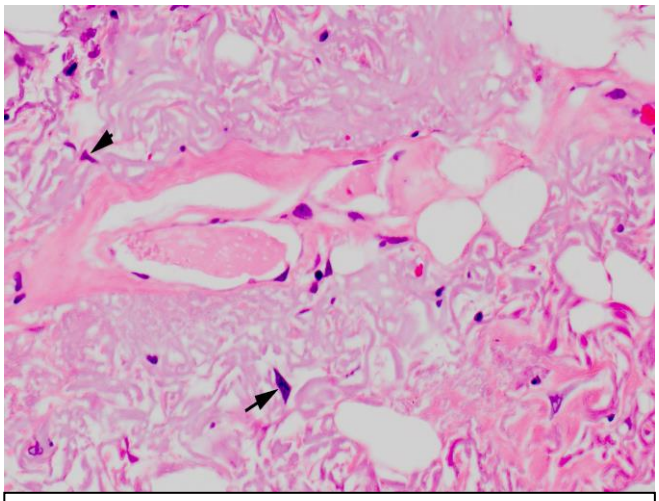
Changes associated with radiation exposure depend on many factors such as the dose of radiation. However, some features are characteristic of radiation dermatitis.[99,277] Acute radiation dermatitis can present with basal vacuolar changes, subepidermal blister formation, superficial papillary dermal edema, vascular changes such as extravasation of red blood cells, and intraluminal thrombi. Subacute or chronic radiation dermatitis can present with pronounced keratinocyte necrosis, basal vacuolar changes, hyalinization of dermal collagen, dilated telangiectatic vessels with endothelial cell swelling, and an absence of pilosebaceous structures (Figure 5.2). Another feature



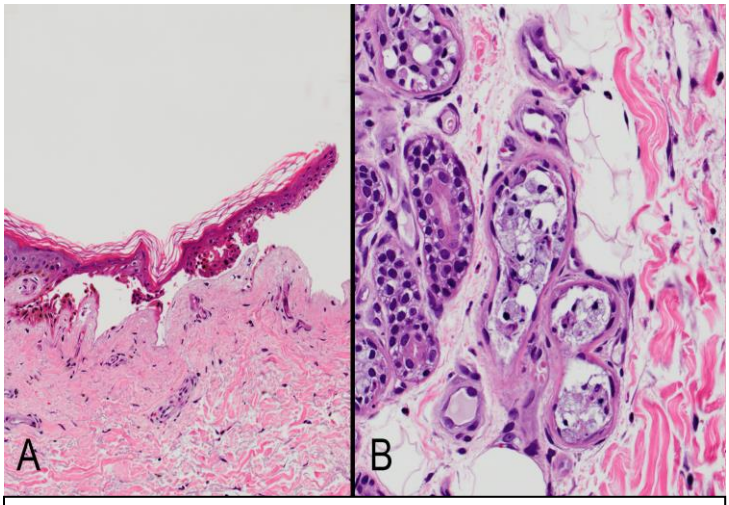
5.2 Chronic radiation changes. Chronic radiation changes. **A**. There is a characteristic absence of pilosebaceous structures, with extravasation of red blood cells, dilated vesseles, superficial papillary dermal edema, and hyalinization of dermal collagen (H & E). **B**. A subepidermal blister with hyalinization of dermal collagen, dilated vessels (H & E).

more characteristic of chronic radiation dermatitis is the appearance of radiation fibroblasts, which are bizarre and atypical stellate cells with large clumped nuclei (Figure 5.3). Suction blisters are subepidermal.[99] The absence of inflammation and the preservation of dermal papillae are characteristic. Dermal papillae will often extend into the blister cavity, a phenomenon known as festooning.[278]

Coma (pressure) blisters (Figure 5.4) occur in patients with periods of unconsciousness over a 48-72 hour period, and present with tense bullae over pressure sites.[279] These bullae are characteristically subepidermal, with varying degrees of epidermal necrosis, eccrine gland degenerative changes, and in non-drug induced coma blisters, an absence of an inflammatory cell infiltrate within the epidermis. Alteration of the outer root sheath of telogen follicles, fibrinoid thrombi, and degeneration of sebaceous glands has also been reported.[280]



5.3 **Radiation Fibroblasts**. Bizzare stellate radiation dermal fibroblasts (arrows) with hyalinized collagen (H & E).



5.4 Coma blisters. A. Subepidermal bulla with epidermal necrosis, and a paucity of inflammatory cells (H & E). **B**. Eccrine gland degenerative changes in patient with coma blister (H & E).

Step 3: Identify the nature of any inflammatory cell infiltrate.

There are characteristic inflammatory cell infiltrates seen within the blister cavity of subepidermal blisters. It is important to note that the amount of the inflammatory infiltrate can vary with each disorder and the biopsy site selected. Ideally, biopsies of blistering disorders should include the earliest lesions, and the blisters themselves should be removed entirely with a section of surrounding skin.[275] Another important feature that can assist in differentiating between blistering disorders is the presence or absence of epidermal necrosis.[275]

Subepidermal Blisters

No Inflammation (cell poor)	Bullous pemphigoid Porphyria cutanea tarda Bullous amyloidosis Kindler's syndrome Ischemic bullae Cryotherapy/Suction/Coma*	Epidermolysis bullosa Pseudoporphyria Toxic epidermal necrolysis* Bullae over scar Trauma/Burns*/Radiation Bullous solar elastosis
Lymphocytes	Erythema multiforme* Fixed drug eruption* Lichen sclerosus et atrophicus Lichen planus pemphigoides* Polymorphic light eruption	
Eosinophils	Bullous pemphigoid Herpes gestationis Bullous drug reaction Arthropod bite reaction	
Neutrophils	Dermatitis herpetiformis Bullous lupus erythematosus Linear IgA bullous dermatosis Pemphigoid Neutrophilic dermatoses (Sweet's) Pustular vasculitis Epidermolysis bullosa acquisita	

* associated with epidermal necrosis

5.5 Subepidermal Blisters.

Dermatology residents have come up with some creative mnemonics for memorizing lists of disorders associated with each of the inflammatory cell infiltrates found within blister cavities.

Mnemonics

Cell Poor Blisters	Neutrophilic Blisters
"THE P3B3 R"	"C BBLED"
• Trauma/Burn/Suction • Hereditary EB • EBA • PCT/Pseudo-PCT/Penicillamine • BP/Bullous Amyloid/Bullous over scar • Radiation	• Cicatricial Pemphgoid • BP • Bullous Lupus erythematosus • Linear IgA • EBA • DH • (Arthropod)

5.6 **Mnemonics.** Sample mneumonics used by residents at the National Capital Consortium Dermatology Residency Program, Bethesda, Maryland.

CELL POOR SUBEPIDERMAL BLISTERING DISORDERS

BULLOUS PEMPHIGOID

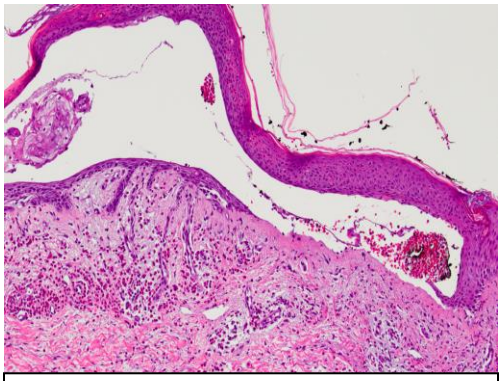
An eosinophilic infiltrate with a subepidermal blister cavity is the hallmark of bullous pemphigoid (Figure 5.7). In fact, sections of skin biopsied prior to blister formation, demonstrate a dense eosinophilic infiltrate along the dermal-epidermal junction, at the site of future bullae (Figure 5.8). The presence of eosinophils seems to mirror the level of erythema in the overlying skin. Bullous pemphigoid can either be cell poor with few eosinophils (Figure 5.9), or be rich with eosinophils. Occasionally, neutrophils or lymphocytes are seen. On direct immunofluorescence (DIF), there is a linear deposition of IgG and/or C3 along the dermal-epidermal junction (Figure 5.10).

Bullous Pemphigoid

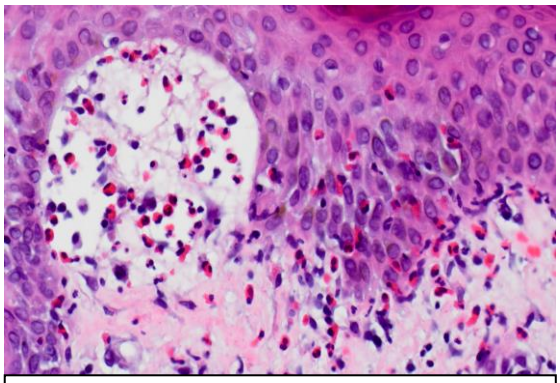
Prodromal phase
- ✓ Eosinophilic spongiosis.
- ✓ Eosinophils lined up along DEJ.

Blister phase
- ✓ Subepidermal blister.
- ✓ Cell poor to eosinophilic blister cavity infiltrate.
- ✓ Superficial papillary dermal edema.

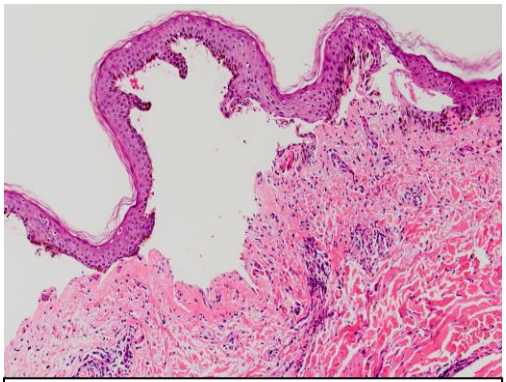
DIF
- ✓ Linear IgG and/or C3 along BMZ (lamina lucida).
- ✓ Salt-split: roof.



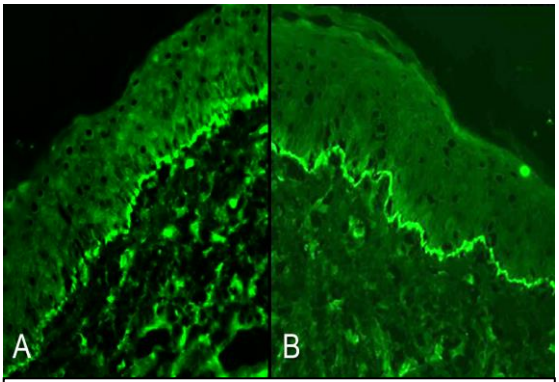
5.7 Bullous pemphigoid. Subepidermal blister with numbeous eosinophils within the blister (H & E).



5.8 Bullous pemphigoid, early stage. Dense eosinophilic infiltrate along dermal-epidermal junction with early subepidermal blister formation, and focal spongiosis (H & E).



5.9 Bullous pemphigoid, cell poor. Subepidermal blister with a cell poor blister cavity. Numerous eosinophis are seen within the dermis (H & E).



5.10 Bullous pemphigoid, Direct immunofloresence. A. DIF of bullous pemphigoid demonstrating deposition of linear IgA along the dermal-epidermal junction (200x). **B.** DIF of bullous pemphigoid demonstrating deposition of linear C3 along the dermal-epidermal junction (200x).

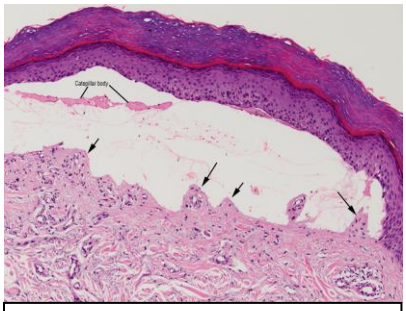
PORPHYRIA CUTANEA TARDA

Porphyria Cutanea Tarda

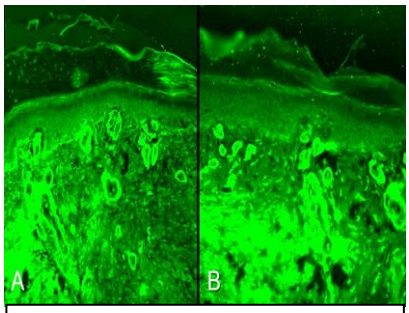
- ✓ Subepidermal blister.
- ✓ Inflammatory cell poor blister cavity.
- ✓ Festooning of dermal papillae.
- ✓ Catepillar bodies.

DIF
- ✓ IgA, IgG, IgM, C3 & Fibrin on walls/lumen of blood vessels.

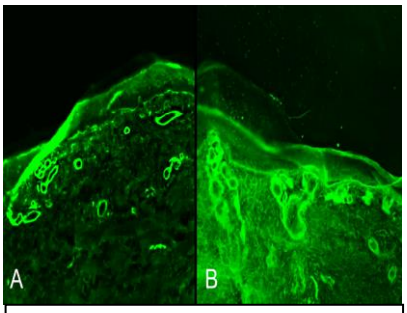
Porphyria cutanea tarda (PCT) is characterized by "festooning" of dermal papillae, in a cell poor subepidermal blister (Figure 5.11). Festooning is a phenomenon where the dermal papillae remain relatively intact within the blister cavity. Caterpillar bodies, are not unique to PCT, and are eosinophilic small buds of material studded along the roof of the subepidermal blister. On DIF, IgG, IgM, IgA, C3, and fibrin can be seen within the lumen of blood vessels and along the dermal-epidermal junction (Figures 5.12 and 5.13).[281]



5.11 Porphyria cutanea tarda. Characteristic "festooning" of dermal papillae (arrows) in cell poor blister, with "catepillar bodies" (H & E).



5.12 Porphyria cutanea tarda (PCT), Direct immunofloresence. A. DIF of PCT demonstrating deposition of IgG on blood vessel walls and along the dermal-epidermal junction (100x). **B.** DIF of PCT with C3 deposited on blood vesseal walls (100x).



5.13 **Porphyria cutanea tarda (PCT), Direct immunofloresence. A.** DIF of PCT demonstrating deposition of IgA on blood vessel walls (100x). **B.** DIF of PCT with fibrin deposited on blood vesseal walls (100x).

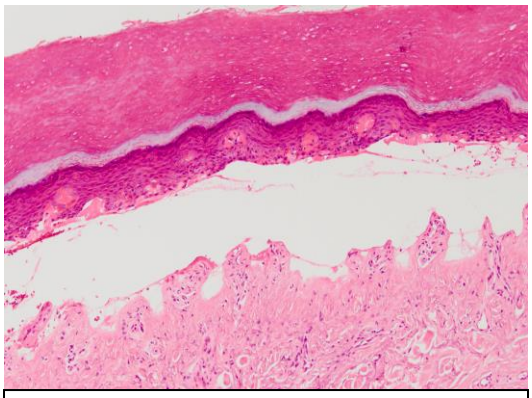
PSEUDOPORPHYRIA

Pseudoporphyria

- ✓ Identical to PCT histologically. Differentiated by laboratory work up.
- ✓ Subepidermal blister.
- ✓ Inflammatory cell poor blister cavity.
- ✓ Festooning of dermal papillae.
- ✓ Catepillar bodies.

DIF
- ✓ IgA, IgG, IgM, C3 & Fibrin on walls/lumen of blood vessels.

Like PCT, pseudoporphyria is characterized by a cell poor subepidermal blister with "festooning," and "caterpillar bodies". There is some evidence[282] that the vessel walls of PCT are thicker in comparison to pseudoporphyria; however, histologically they are essentially indistinguishable (Figure 5.14).



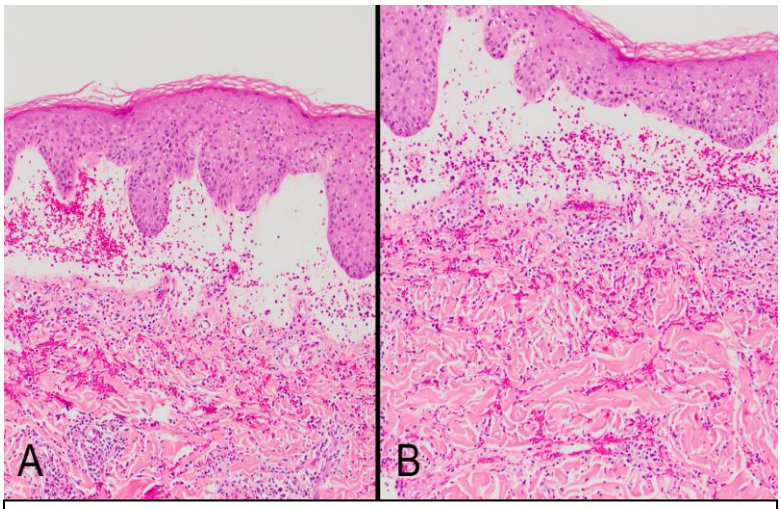
5.14 **Pseudoporphyria.** Characteristic "festooning" of dermal papillae in cell poor blister (H & E).

BULLOUS AMYLOIDOSIS

Bullous amyloidosis demonstrates hyaline deposits of amyloid within the dermis, with a hemorrhagic subepidermal blister, with a sparse inflammatory cell infiltrate (Figure 5.15).

Bullous Amyloidosis

- ✓ Subepidermal blister.
- ✓ Inflammatory cell poor blister cavity.
- ✓ Hemorrhage into blister cavity.
- ✓ Eosinophilic, amorphous material deposited along blister base and dermis.



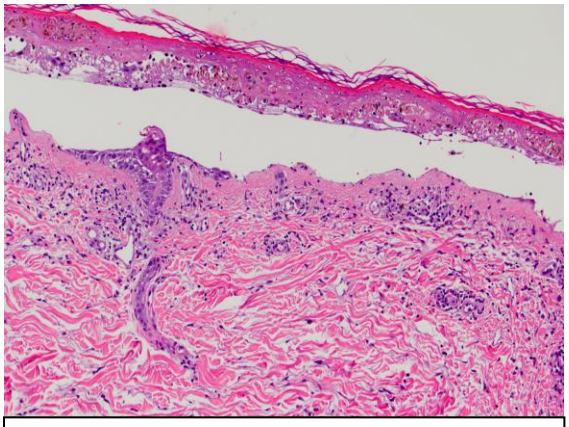
5.15 Bullous amyloidosis. A. Subepidermal blister with extravasated red blood cells, and a mixed inflammatory cell infiltrate (H & E). **B.** Deposition of hyaline amyloid along the blister base (H & E).

TOXIC EPIDERMAL NECROLYSIS

In toxic epidermal necrolysis, there is a supepidermal blister with confluent epidermal necrosis, and a sparse (or more often absent) lymphocytic infiltrate within the blister cavity (Figure 5.16).

Toxic Epidermal Necrolysis

- ✓ Subepidermal blister.
- ✓ Inflammatory cell poor blister cavity.
- ✓ Confluent epidermal necrosis.



5.16 **Toxic epidermal necrolysis. A.** Cell poor subepidermal blister with confluent epidermal necrosis (H & E).

EPIDERMOLYSIS BULLOSA

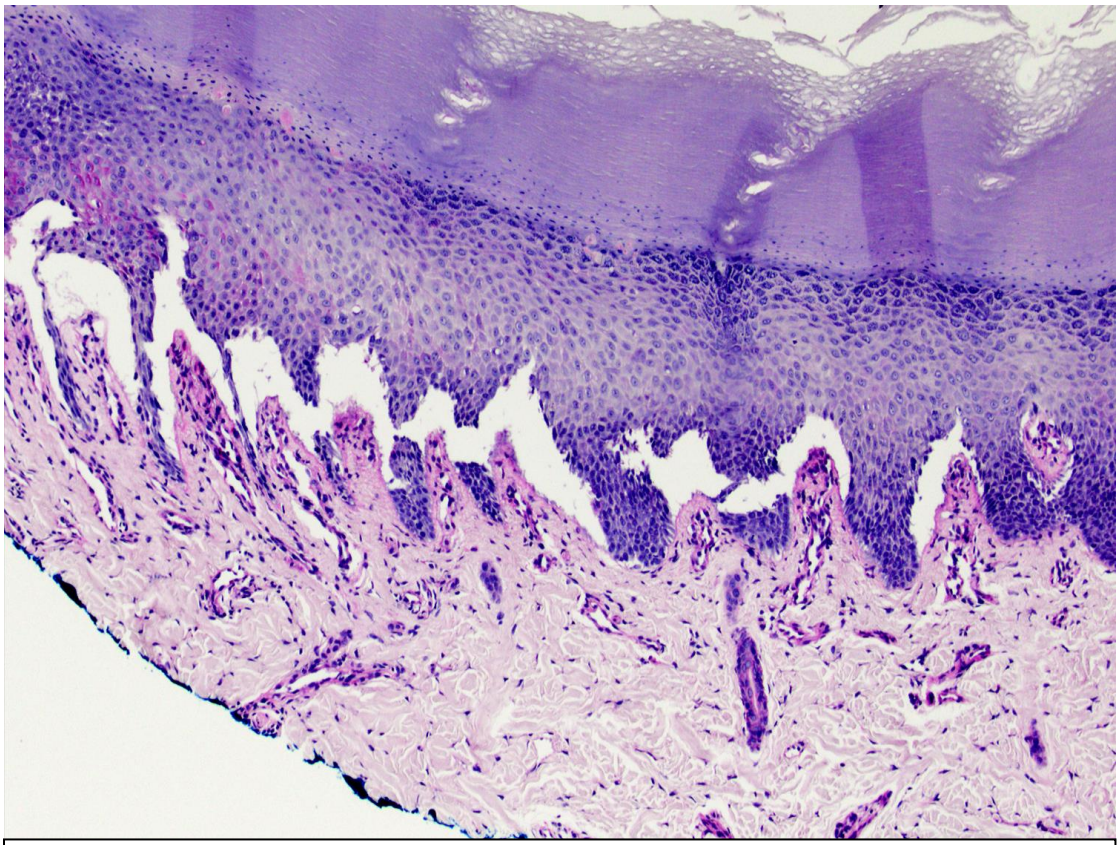
Epidermolysis Bullosa

- ✓ Subepidermal blister (esp. junctional & dystrophic subtypes).
- ✓ Inflammatory cell poor blister cavity.

EM
- ✓ Identifies level of split, differentiating subtypes.

DIF
- ✓ Negative for all types of EB, except EBA.

Epidermolysis bullosa is a general term for a group of blistering disorders that share a common characteristic of blistering upon minor skin trauma. Epidermolysis bullosa simplex is characterized by intraepidermal blistering. While junctional epidermolysis bullosa, and dystrophic epidermolysis bullosa cause blistering within the basement membrane zone. Both Junctional and dystrophic forms of epidermolysis bullosa lead to cell-poor subepidermal blisters (Figure 5.17). On PAS stains, junctional forms have a PAS-positive basement membrane on the floor of blister, whereas in dystrophic forms, the PAS-positive basement membrane is on the roof of the blister. Electron microscopy can distinguish these two entities, by demonstrating a split within the lamina lucida for junctional variants, and an absence of anchoring fibrils in dystrophic variants. Direct immunofluorescence is negative for all variants of epidermolysis bullosa (cell poor subepidermal types) except epidermolysis bullosa acquisita (neutrophilic subepidermal blister).



5.17 **Epidermolysis bullosa.** Cell poor subepidermal bullae with sparse dermal infiltrate (H&E).

LYMPHOCYTIC SUBEPIDERMAL BLISTERING DISORDERS

ERYTHEMA MULTIFORME

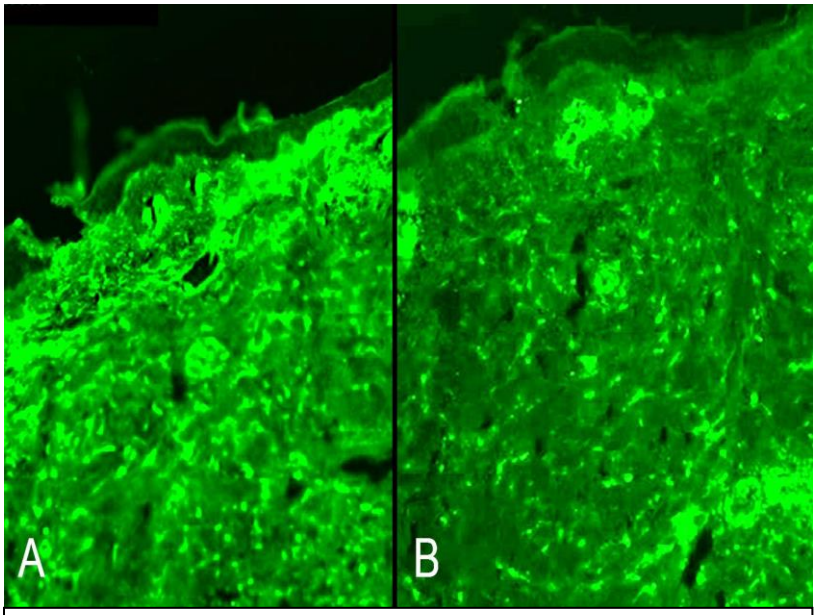
Erythema multiforme

- ✓ Subepidermal blister.
- ✓ Lymphocytic blister cavity.
- ✓ Necrotic keratinocytes.
- ✓ Papillary dermal edema.
- ✓ Spongiosis (early) prior to blister formation.
- ✓ Interface dermatitis with basal vacuolar changes.

DIF

- ✓ Nononspecific IgM, C3, and fibrin along DEJ and/or intravascularly.

With erythema multiforme, the blisters are subepidermal, with varying levels of lymphocytic infiltrate within the blister cavity. There is often a mid to upper dermal lymphocytic perivascular infiltrate. Epidermal necrosis, unlike toxic epidermal necrolysis can range from a few necrotic keratinocytes at the edge of the blister, to confluent epidermal necrosis. Papillary dermal edema is often seen. Direct immunofluorescence is often non-specific with deposition of IgM, C3, and Fibrin along dermal-epidermal junction and/or blood vessels (Figure 5.18).



5.18 Erythema multiforme (EM), Direct immunofloresence (DIF). A. DIF of EM with IgM along dermal-epidermal junction and along vessel walls (100x). **B**. DIF of EM with C3 on blood vessel walls (100x).

PARANEOPLASTIC PEMPHIGUS

Paraneoplastic Pemphigus

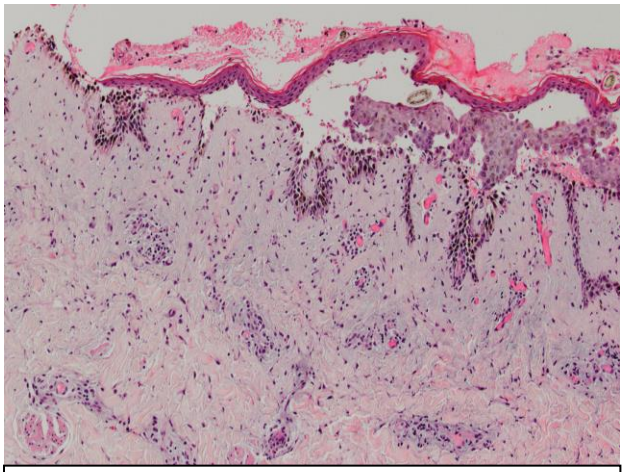
- ✓ Varied presentation.
- ✓ Subepidermal blister.
- ✓ Lymphocytic blister cavity.
- ✓ Necrotic keratinocytes.
- ✓ +/- suprabasilar acantholysis.
- ✓ Interface dermatitis with basal vacuolar changes.

DIF

- ✓ Intercellular and DEJ C3 and/or IgG.
- ✓ *Indirect:* rat bladder epithelium positive staining.

Paraneoplastic pemphigus has a varied histologic presentation, which mirrors the multiple clinical variations of this disease. There is often a lymphocytic interface dermatitis with basal vacuolar changes and varying amounts of suprabasal acantholysis and suprabasal clefting. Like erythema

multiforme, there are varying amounts of necrotic keratinocytes. Subepidermal blistering, when present, contains a lymphocytic infiltrate within the blister cavity (Figure 5.19). On direct immunofluorescence, there is intercellular and basement membrane zone C3 and/or IgG. Indirect immunofluorescence is positive for rat bladder epithelium (unlike other pemphigus variants).



5.19 Paraneoplastic pemphigus (PNP). Paraneoplastic pemphigus demonstrating suprabasal clefting.

LICHEN SCLEROSUS ET ATROPHICUS

Lichen sclerosus et atrophicus demonstrates an atrophic epidermis with diffuse papillary dermal edema, on a sclerotic base. The sclerotic base is often a broad band of hyalinized collagen in the upper to mid dermis. Lymphocytes are seen within subepidermal blister cavities, along with hemorrhage, and perivascular in the lower dermis. Dermal vessels demonstrate dilatation.

Lichen Sclerosus et Atrophicus

- ✓ Epidermal atrophy.
- ✓ Subepidermal blister.
- ✓ Lymphocytic blister cavity.
- ✓ +/- hemorrhagic blister cavity.
- ✓ Diffuse upper dermal edema.
- ✓ Broad hyalinized/sclerotic mid-dermal collagen.
- ✓ Lower dermal vascular dilatation.

LICHEN PLANUS PEMPHIGOIDES

Lichen planus pemphigoides is characterized by a mild perivascular infiltrate with a subepidermal lymphocytic blister cavity. Basal vacuolar changes and Civatte bodies (small eosinophilic necrotic keratinocytes with pyknotic nuclei) can be seen at the edges of the blister. Direct immunofluorescence demonstrates IgG and C3 along the basement membrane zone.

Lichen planus pemphigoides

- ✓ Subepidermal blister.
- ✓ Lymphocytic blister cavity.
- ✓ Basal vacuolar changes.
- ✓ Civatte bodies at blister edges.

DIF
- ✓ IgG and C3 at DEJ.

Polymorphic light eruption

Polymorphic light eruption (PMLE) is characterized by significant papillary dermal edema with subsequent subepidermal blistering. The lymphocytic perivascular infiltrate is classically "superficial and deep", and is often the first histologic feature that is noticed. Direct immunofluorescence is entirely negative.

Polymorphic Light Eruption

- ✓ Early: spongiosis leading to blisters.
- ✓ Subepidermal blister.
- ✓ Lymphocytic blister cavity.
- ✓ No basal vacuolar changes.
- ✓ "Superficial and deep" perivascular lymphocytic infiltrate.

DIF
- ✓ Negative.

EOSINOPHILIC SUBEPIDERMAL BLISTERING DISORDERS

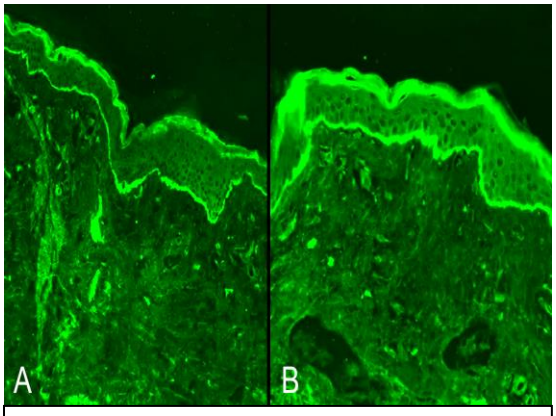
PEMPHIGOID GESTATIONIS

Pemphigoid gestationis (aka herpes gestationis) is characterized by a profusely edematous papillary superficial dermis, with a mixed dermal infiltrate of lymphocytes, eosinophils and macrophages in early lesions. Lesions with blistering demonstrate a subepidermal blister with the same mixed blister cavity infiltrate of lymphocytes, eosinophils, and macrophages seen in early lesions. A striking feature of pemphigoid gestationis is the focal spongiosis directly above dermal papillae with obliteration of dermal papillae with eosinophilic microabscesses with the dermal papillae. Direct immunofluorescence demonstrates IgG and C3 in a linear pattern along the basement membrane zone (Figure 5.20). On salt-split skin, the roof of the epidermis is highlighted (split at lamina lucida).

Pemphigoid gestationis

- ✓ Prominent papillary dermal edema.
- ✓ Subepidermal blister.
- ✓ Eosinophilic (or mixed) blister cavity.
- ✓ Mixed infiltrate of Lymphocytes, eosinophils and macrophages.
- ✓ Focal spongiosis above dermal papillae.
- ✓ Eosinophilic microabsecess in dermal papillae.

DIF
- ✓ Linear IgG and C3 along DEJ.
- ✓ Salt split: roof.



5.20 **Pemphigoid gestationis, Direct immunofloresence (DIF). A.** DIF of pemphigoid gestationis with linear IgG along dermal-epidermal junction (100x). **B.** DIF of pemphigoid gestationis with linear C3 along dermal-epidermal junction (200x).

ARTHROPOD BITES

Arthropod bites often demonstrate a wedge-shaped dense mixed (lymphocytic and eosinophilic) dermal infiltrate, with superficial dermal edema. Blister formation is variable with both intraepidermal and subepidermal blisters. Eosinophilic infiltrate within blister cavities is characteristic, but not unique to arthropod bites. Bite reaction histopathology can vary tremendously.

Arthropod Bite Reaction

- ✓ Highly variable.
- ✓ Spongiosis.
- ✓ Dermal edema.
- ✓ Intraepidermal and/or subepidermal blister.
- ✓ Eosinophilic blister cavity.
- ✓ "Superficial & deep" mixed perivascular infiltrate of eosinophils and lymphocytes.

DRUG REACTIONS

Drug reactions can present with almost any clinical or histologic pattern. Some drugs such as quinolones, can produce subepidermal eosinophilic blisters in a photo-distributed pattern. Sulfur mustard,[283] methyl bromide, and etretinate are just a few of the many drugs that can produce subepidermal blisters, often with eosinophils within the blister cavity.

NEUTROPHILIC SUBEPIDERMAL BLISTERING DISORDERS

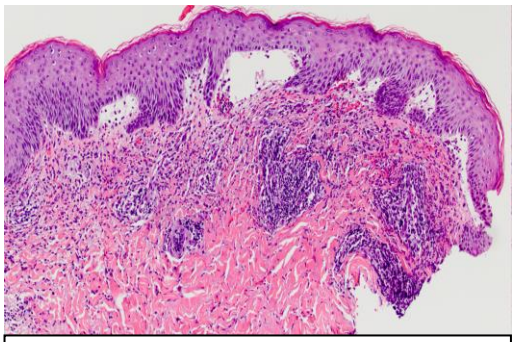
DERMATITIS HERPETIFORMIS

Dermatitis herpetiformis is characterized by "papillary (neutrophilic) microabscesses," that progress to obliteration of dermal papillae, with a subepidermal split (Figure 5.21 & 5.22). On direct immunofluorescence (DIF) dermatitis herpetiformis demonstrates IgA deposition in the dermal papillae perilesionally, with IgM (30%) and C3 (50%) deposition as well (Figure 2.23).

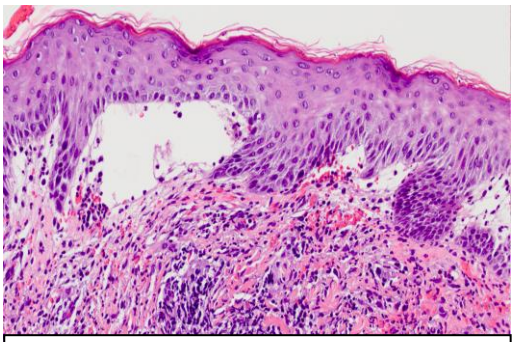
Dermatitis Herpetiformis

- ✓ Papillary microabscesses.
- ✓ Subepidermal blister.

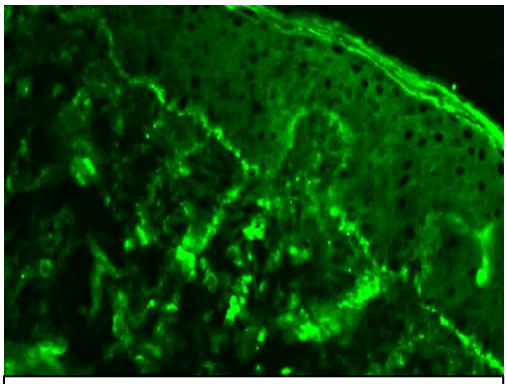
DIF
- ✓ Granular IgA, IgM, and C3 at tips of dermal papillae.



5.21 Dermatitis herpetiformis. Subepidermal bullae clustered around dermal papillae (H&E).



5.22 Dermatitis herpetiformis. Subepidermal bullae with microabscesses in dermal papillae (H&E).



5.23 **Dermatitis herpetiformis (DH), Direct immunofloresence.** DIF of DH with IgA granular deposits along dermal papilla (200x).

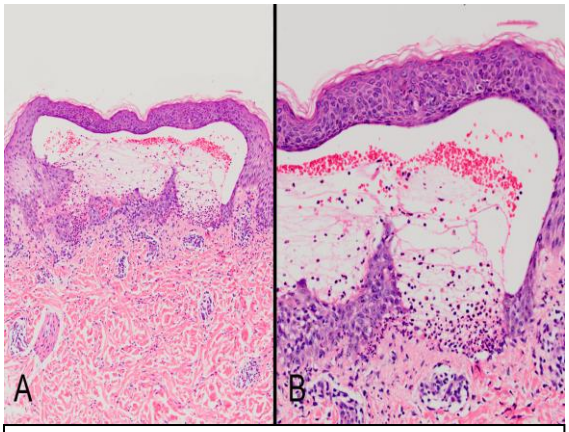
LINEAR IGA BULLOUS DERMATOSIS

Linear IgA Bullous Dermatosis

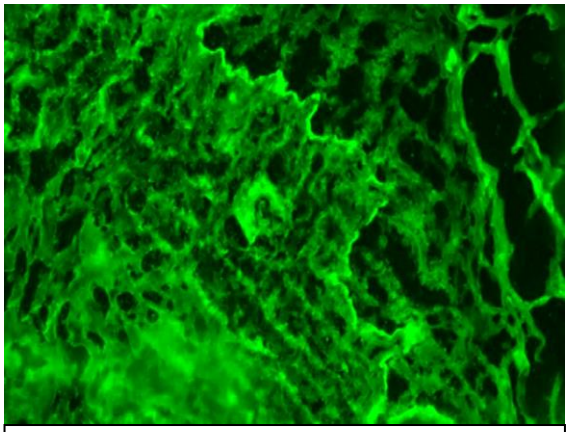
- ✓ Papillary microabscesses.
- ✓ Subepidermal blister.
- ✓ Indistinguishable from dermatitis herpetiformis histologically.

DIF
- ✓ Linear IgA along DEJ predominantly.
- ✓ ≤20% IgM and C3 along DEJ.

Linear IgA is a subepidermal blistering disease with a neutrophilic infiltrate within the blister cavity. It is usually impossible to distinguish linear IgA from dermatitis herpetiformis on histology alone. Both disorders present with neutrophilic papillary dermal microabscesses (Figure 5.30). Drug induced linear IgA can have an eosinophilic infiltrate. Direct immunofluorescence demonstrates a linear patter of IgA (predominantly) along the basement membrane zone, with IgG, IgM, and/or C3 immunoreactivity present in only approximately 20% of cases (Figure 5.31). Salt split skin studies demonstrate enhanced uptake on the roof of the blister.



5.30 Linear IgA bullous dermatosis. **A**. Subepidermal blister in childhood linear IgA bullous dermatosis. **B**. Neutrophilic microabscess in dermal papillae, within blister cavity of childhood linear IgA bullous dermatosis.



5.31 **Linear IgA bullous dermatosis, Direct immunofloresence.** DIF of linear IgA with linear homogenous staining of dermal-epidermal junction (200x).

MUCOUS MEMBRANE PEMPHIGOID

Mucous Membrane Pemphigoid

- ✓ Subepidermal blister.
- ✓ Early: papillary microabscesses.
- ✓ Variable scar formation and erosions.
- ✓ Later: eosinophilic blister cavity.
- ✓ Much later: lymphocytic blister cavity.
- ✓ Sebaceous gland(s) in blister cavity.

DIF
- ✓ Linear IgG and C3 along DEJ predominantly.
- ✓ Salt split: roof or floor.

Histologically, mucous membrane pemphigoid presents with a subepidermal blister, with varying inflammatory cell contents based on the age of the lesion. Early on (<48hrs) neutrophils are seen within the blister cavity, and in dermal papillary microabscesses, similar to those seen in dermatitis herpetiformis. Later lesions will show eosinophils, and eventually will progress to a predominantly lymphocytic infiltrate. Erosions and even scarring can be been seen. Sebaceous glands within blister cavity are considered a clue to the diagnosis of mucous membrane pemphigoid. Direct immunofluorescence demonstrates linear IgG and C3 (IgA less often) along basement membrane zone. Given the heterogeneous nature of mucous membrane pemphigoid, salt-split skin can demonstrate deposits in either the roof or the floor of the blister.

Bullous lupus erythematosus

Bullous Lupus Erythematosus

- ✓ Subepidermal blister.
- ✓ Papillary microabscesses.
- ✓ Increased dermal mucin.

In bullous lupus erythematosus, as with most subepidermal blistering dermatoses with a predominantly neutrophilic infiltrate, the histologic appearance is similar to dermatitis herpetiformis with papillary microabscesses. There is often more mucin in the dermis than is seen with dermatitis herpetiformis, and neutrophils extend deeper into the dermis than is often seen with dermatitis herpetiformis.

Epidermolysis bullosa acquisita

Epidermolysis Bullosa Acquisita

- ✓ Subepidermal blister.
- ✓ Papillary microabscesses.
- ✓ Fibrin within blister cavity.

DIF
- ✓ Linear IgG/C3 along DEJ.
- ✓ Salt split: floor.

Epidermolysis bullosa acquisita presents with neutrophilic papillary abscesses, and a subepidermal blister with fibrin within the blister cavity. The immunologic cell content of the blister cavity mirrors the extent of inflammation seen clinically in lesions. Direct immunofluorescence demonstrates linear IgG and C3 along basement membrane zone. On salt-split skin, antibodies bind to the floor of the blister.

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