Contents

5 Skin Barrier

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KEY POINTS

- The four layers of the epidermis—stratum corneum, stratum granulosum, stratum spinosum, and stratum basale—are composed of keratinocytes, which undergo differentiation and mature as they become more superficial until they are terminally differentiated at the stratum corneum.
- The dermis provides structure, houses epidermally derived appendages, and provides vascular supply to the skin.
- There are many factors that may contribute to the development of atopic dermatitis, including defects in the components of the physical barrier (notably the lipid matrix) and structural and adhesive proteins in the stratum corneum.
- Atopic dermatitis is characterized by immune dysregulation as part of a Th2-driven disease.
- Skin in atopic dermatitis has increased permeability to microbes, allergens, and irritants causing local and systemic inflammation.

Introduction

What constitutes the skin barrier? The skin is the largest barrier between the outside environment and the internal body, which is otherwise susceptible to dehydration, loss of nutrients, infection, DNA damage from light, and ultimately loss of form and function. The skin is composed of both cellular and extracellular components that create specialized structures that make up the physical and immunologic interfaces to the outside world. This chapter focuses on physical structures and functions of this barrier and the consequences of the defective barrier in atopic dermatitis.

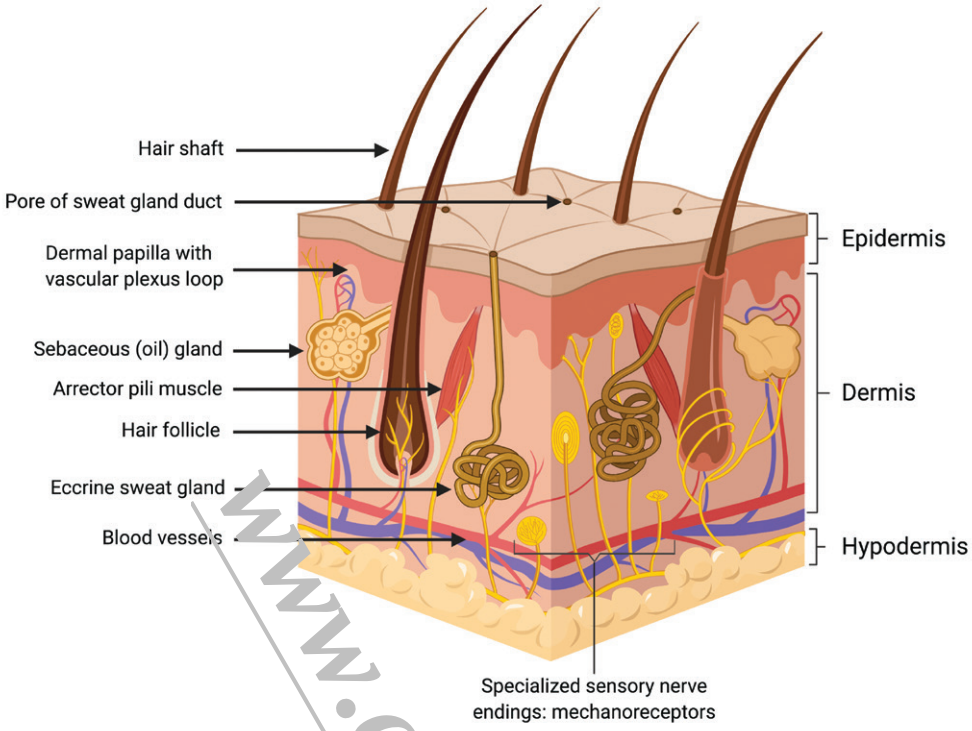
Physical composition and function of the skin

The two major layers of the skin include the epidermis and the deeper dermis. Deeper still is the subcutaneous fat and fascia, which together are also often referred to as the hypodermis, though this is not part of the integument unlike the epidermis and dermis. A drawing of a three-dimensional (3D) model depicts various major components at different levels of the skin (Fig. 5.1).

In a 3D cross section of skin, various components and appendages are housed with the three major layers of the skin known as the epidermis, dermis, and hypodermis. The most superficial layer of skin, the epidermis, is anchored to the deeper layer, the dermis, at the dermal-epidermal junction (DEJ) by intercalating dermal papillae and ridges. The dermis consists of extracellular matrix (ECM) that houses various functional components such as blood vessels, lymphatics, nerves, glands, and skin appendages. The deepest layer of skin that is sometimes included as a skin layer though it is not part of the integument is the hypodermis. This layer is made of subcutaneous fat and fascia.

The epidermis is largely composed of stratified squamous keratinized cells called keratinocytes that populate each of the sublayers birthed at the deepest cell layer against the basement membrane and become more differentiated as the cells become more superficial. Within the epidermis there are four sublayers (strata): From most superficial to deepest they are stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. An additional dense layer called stratum lucidum consists of enucleated flat keratinocytes between stratum granulosum and stratum corneum and exists in thick skin such as in palms and soles. The histologic features, main functions, and specialized cells of each strata are described in Table 5.1. Basal stem cells are bound to the basement membrane by hemidesmosomes, and keratinocytes are bound to adjacent cells by desmosomes and other intercellular proteins contributing to the permeability barrier created from epidermal layers. As cells travel superficially, they undergo differentiation called keratinization (also known as cornification) and enhance the amount and types of keratin filaments while they flatten, dehydrate, and eventually dispose of their nucleus and organelles at their terminal stage in the stratum corneum. At the surface, cells are surrounded by a keratinized envelope composed of transglutaminase cross-linked proteins and lipids, notably loricrin and involucrin (Agrawal & Woodfolk, 2014; Marks & Miller, 2013; Mescher, 2018) (Fig. 5.2).

The epidermis consists of four major sublayers or strata composed of keratinocytes. As cells migrate from the deepest to the most superficial strata, they undergo differentiation



• **Fig. 5.1** Structural components of skin.

in a process called keratinization. The most superficial of the strata is the stratum corneum, which possesses terminally differentiated, enucleated, and dead keratinocytes that are flattened, dehydrated, and surrounded by a dense envelop of protein and lipids that serves as a water-resistant seal. Beneath it is the stratum granulosum named for its prominent keratinohyaline granules and Golgi-derived lamellar granules containing lipids that contribute to the water barrier. Deeper yet, the stratum spinosum is the thickest of the strata and is characterized by the intercellular bridges created by desmosomes, which anchor adjacent cells to one another. The stratum basale is the deepest of the strata and is a single layer of columnar cells attached to the basement membrane by hemidesmosomes. This is where the basal stem cells produce the new keratinocytes through mitosis. An additional dense layer called stratum lucidum (not pictured in Fig. 5.2) consists of enucleated flat keratinocytes just deep to the stratum corneum and exists in thick skin such as in palms and soles.

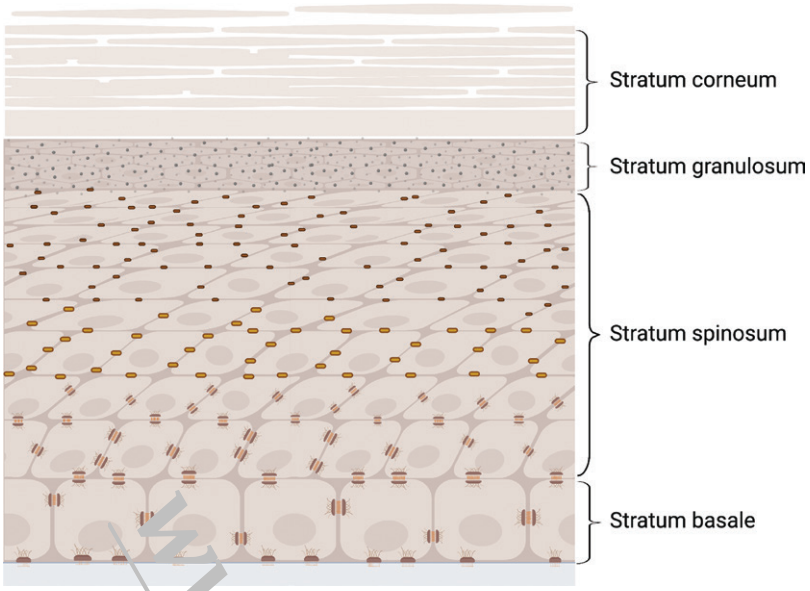
The DEJ is where the basement membrane lies and has a specialized 3D structure that anchors the epidermis to the dermis. Epidermal papillae and ridges show a wavy appearance in a superficial-to-deep cross section or a Swiss cheese model in a cross-sectional plane taken parallel to the skin surface at the DEJ.

The dermis consists of largely ECM made up of different protein fibers that provide the structural component of the skin. The dermal papillae consisting of the looser areolar connective tissue and its own vascular and lymphatic plexuses sit atop the reticular layer of the dermis, which contains more acellular irregular dense connective tissue. Fibroblasts are the main cells of the dermis producing ECM. Collagen is the predominant component of matrix responsible for the biomechanical properties associated with the strength of the dermal matrix by forming dense bundles of fibers. Other fibrillar proteins such as elastin, fibronectin, laminin, and fibrillins arrange around collagen to create a loose network that allows for the elasticity and flexibility of skin. Filling the spaces between fibers are the extrafibrillar matrix consisting of liquid binding glycosoaminoglycans (e.g., hyaluronan), proteoglycans, and glycoproteins that create a fluid medium for the movement of water, molecules, and immune cells. Additional cells in the dermal layer include macrophages, mast cells, adipocytes, Schwann cells, and stem cells.

Within the dermis there are several types of epidermally derived skin appendages, including sebaceous glands, sweat glands, and hair follicles. Blood vessels, lymphatics, and nerves also reside in the dermal and subcutaneous layers. Table 5.1 details the function of these structures (Krieg & Aumailley, 2011; Prost-Squarcioni, Fraitag, Heller, & Boehm, 2008; Ryan, Mortimer, & Jones, 1986). To sum up, the skin provides several protective barriers to the outside world, most notably the physical barrier provided by the many layers of skin, thermoinsulation from fat, water barrier created by keratinization and lipid-rich layer in epidermal stratum granulosum, and immunologic barrier created by various antigen-presenting cells and players in innate and adaptive immunity (Langerhans cells in the stratum spinosum, dendritic cells, macrophages, mast cells, and even recently discovered skin-resident T cells in the dermis and epidermis) (Clark, 2010). A properly functioning immunologic barrier is crucial in maintaining healthy skin, and these

TABLE 5.1 Skin layers: key features and functions

Skin layer	Strata	Key histologic feature(s)	Major function(s)
Epidermis	Stratum corneum	• Most superficial layer consisting of dead enucleate flattened keratinocytes and debris	• Keratinized dead skin cells provide water barrier and some physical barrier to environmental assaults such as penetration by foreign objects, organisms, toxic substances, friction, and extreme temperature • This layer along with the other most superficial layers of epidermis where most of the skin microbiome is located
	Stratum granulosum	• A few layers of flattened keratinocytes • Where keratinization is achieved • Prominent basophilic keratinohyaline granules • Golgi-derived lamellar granules containing various lipids	• Keratinization and lipid-containing lamellar granules provide a water barrier, preventing dehydration
	Stratum spinosum	• Intercellular bridges created between cells by desmosomes • Active synthesis of keratin filaments and assembly of tonofilaments that anchor to desmosomes • Usually the thickest layer of the epidermis, especially in the epidermal ridges	• Contains Langerhans cells, the first major antigen-presenting cell in epidermis for immune response
	Stratum basale	• Single layer of columnar cells secured to dermal-epidermal junction (basement membrane) by hemidesmosomes • Mitotically active cells • Cells contain cytoskeletal keratins (intermediate filaments)	• Basal stem cells are the primary source of mitotic activity producing new keratinocytes • Contains melanocytes (pigment producing cells) involved in DNA protection from light • Contains Merkel cells (mechanoreceptors) involved in sensory response
Skin layer	**Component**	**Major function(s)**	
Dermis and skin appendages	Extracellular matrix	• Collagen is the major component of the dermis and responsible for strength • Fibrillar proteins contribute to elasticity and flexibility of skin • Extrafibrillar matrix binds liquid and allow nutrients, water, and immune cells to travel within dermis	
	Vascular plexuses	• Thermoregulation • Nutrient and waste processing • Allow circulatory immune cells to travel to and from skin	
	Lymphatic plexuses	• Clears proteins and fluid • Channel for macrophages and other immune cells to travel from the skin tissue	
	Sebaceous glands	• Produce sebum, a compound of lipids, cell debris, and keratin that nourishes and creates lubrication for skin preventing water loss	
	Apocrine glands	• Sweat glands usually associated with hair follicles that produce fatty liquid expulsed from gland tubules in response to stress • Thermoregulation by working together with eccrine glands through emulsification to create film of sweat to efficiently dissipate heat and discourage formation and loss of sweat drops • Liquid is broken to fatty acids by skin bacteria, which can cause characteristic odor	
	Eccrine glands	• Sweat glands that secrete watery sweat in response to emotional and thermal stress and sympathetic nervous system for role in thermoregulation by dissipating heat through water evaporation from skin surface	
	Hair follicles	• Extensions of the epidermal basal layer surround the base of these structures • Skin microbiome densely populates this area	
	Nerve endings	• Somatosensation. Sensory nerve endings, including free nerve endings (nociceptors) and specialized tactile mechanoreceptors (Meissner corpuscles, Ruffini endings, Pacinian corpuscles, Merkel disks, and regionally specific Kraus end bulbs) that together provide detailed information on pain, temperature, light touch, deep pressure, low and high frequency vibrations, and skin stretch. Somatosensation allows protective feature of detecting damage to the skin or threatening contacts with the skin such as sharp objects or extreme temperatures that may damage tissue if not withdrawn	
Hypodermis	Subcutaneous fascia and fat	• Adipose tissue provides thermoinsulation, energy, and storage of nutrients; provides extra padding in places that experience more pressure such as buttocks, palms, and soles	



• **Fig. 5.2** Layers of the epidermis.

immune factors are discussed in greater detail in Chapters 7, 13, and 14. In addition to the native components of skin, the healthy skin microbiome is part of a functional barrier of skin, which is elucidated in Chapter 6.

The compromised skin barrier in atopic dermatitis

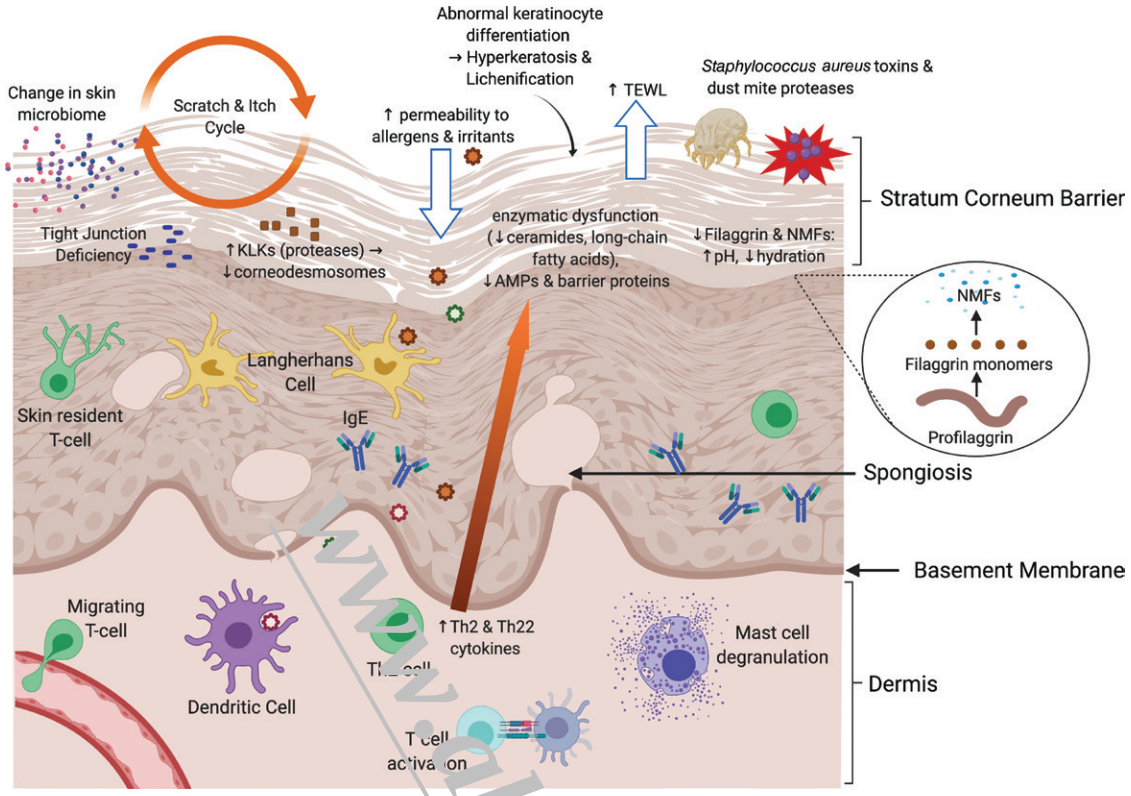
How is the skin barrier compromised in atopic dermatitis? This skin disease is caused by defective skin barrier proteins and a dysfunctional immune system. A component of atopic dermatitis can be genetic and as a result be a heritable disorder. Atopic dermatitis can also be part of other syndromes and diseases such as immunodeficiencies. In people who do not have primary immunodeficiencies, the disease is also associated with other allergic symptoms such as allergic rhinitis and asthma in the allergic triad, showing us that atopic dermatitis has a lot to do with the dysfunction of the immune system and is more than just simply a defect of the skin. There are many contributing factors in pathogenesis of atopic dermatitis and a variety of clinical presentations. In those who are diagnosed with the disease, changes to the environment or an acquired modification of the skin barrier and immune system can change its severity. In fact, some environmental factors can cause epigenetic changes that can cause atopic dermatitis. Fig. 5.3 depicts these barrier compromises in atopic dermatitis.

Loss of skin barrier function in atopic dermatitis is multifactorial. Measurable parameters of skin barrier loss include increased transepidermal water loss, increased stratum corneum pH, and decreased hydration. Histologic changes include spongiosis and hyperkeratosis of the epidermis and perivascular inflammation with prominent T-cell infiltration. Local immune dysregulation characterized by overexpression of Th2 and Th22 cytokines change the expression or functions of various enzymes. Stratum corneum lipid matrix producing water barrier is abnormal in atopic dermatitis, where ceramides are decreased in amount or structures are shorter in length. Antimicrobial peptides are also inhibited by Th2 cytokines causing increased propensity for infections such as *Staphylococcus aureus*. Increase in kallikrein (KLK) activity and impaired tight junction proteins also decrease the function of proteins responsible for intercellular adhesion.

Mutations in filaggrin *(FLG)* gene seen in some individuals with atopic dermatitis cause abnormal filaggrin and downstream products such as natural moisturizing factors (NMF). All of these factors can compromise the paracellular barrier in the epidermis, allowing water to escape the body and increase the permeability of skin to allergens, irritants, and haptans, which reinforce local inflammation when skin resident immune cells are activated. Local inflammation causes adaptive response with T-cell recruitment and activation and leads to systemic allergic responses with imbalance in Th1 and Th2 cytokines and overexpression of immunoglobulin E (IgE) antibodies. Additional external factors such as the scratch and itch cycle, *S. aureus* toxins, and dust mite proteases further compromise the skin barrier. The increase in skin pH, various alterations in intrinsic antimicrobial components, and immune dysfunction also result in shifts in the skin microbiome (dysbiosis). The multifactorial contributors to atopic dermatitis pathology create a self-reinforcing cycle of barrier and immune dysfunction.

Physical barrier disruption

There have been several skin-associated tissue factors identified that when altered produce problems in barrier function that contribute to atopic dermatitis. These barrier dysfunctions are listed in Table 5.2.



• **Fig. 5.3** Skin barrier dysfunction in atopic dermatitis. *AMP*, Antimicrobial peptides; *KLKs*, kallikreins; *NMFs*, natural moisturizing factors; *TEWL*, transepidermal water loss.

Characteristics of skin in atopic dermatitis associated with loss of barrier function include the following:

- Biophysical measures: increased transepidermal water loss, increased stratum corneum pH, and decreased hydration
- Histological: spongiosis of epidermis, hyperkeratosis of epidermis, perivascular inflammation with prominent T-cell infiltration
- Downstream effects of barrier dysfunction: increased allergen sensitization and resulting systemic allergic responses, including characteristic increased IgE levels
- Increased permeability to microbes, allergens, haptans, irritants, and water
- Local immune dysregulation: overexpression of Th2 and Th22 cytokines altering proteins and lipids in epidermis
- Markers of keratinocyte differentiation: proteins such as desmoglein-1, desmocolin-1, loricrin, and involucrin involved in barrier function are deficient in atopic dermatitis lesional skin (Agrawal & Woodfolk, 2014; Kim & Leung, 2018)

Filaggrin

FLG is a monomer subunit of a protein packed in keratinohyaline granules that are produced in terminally differentiated keratinocytes in the granular and cornified layers of the epidermis involved in maintaining the paracellular permeability barrier. *FLG* is a structural protein that binds keratinocyte filaments to increase the density of filament bundles, flattening keratinocytes to their terminal shape, which is crucial to the strength and integrity of the skin. Profilaggrin produced from *FLG* gene is broken down into monomeric filaggrin. Filaggrin is also broken down by proteases (e.g., cysteine peptidase and caspase-14) into free amino acids that function as NMFs to retain water and maintain skin pH. NMFs also strengthen stratum corneum and provide chemical, allergen, and microbial protection (Agrawal & Woodfolk, 2014).

When *FLG* gene is mutated to be nonfunctional (*FLG*-null) or has a lower number of intraexonic repeating units, this can cause a deficiency of *FLG* enough to cause a change in the morphology of keratinocytes and enhance skin inflammation. Compromised paracellular barrier causes leaky epithelium to allergens and haptans. *FLG* mutations can contribute to atopic dermatitis but is not sufficient to cause the disease in isolation, and individuals with null alleles often do not have the disease. About 25% to 50% of atopic dermatitis patients have a *FLG* mutation as a predisposing factor (Osawa, Akiyama, & Shimizu, 2011). Individuals with these mutations often are seen in persistent or early-onset atopic dermatitis. Methylation of the gene has also been correlated with risk for atopic dermatitis and points to epigenetic contributors to this disease development (Ziyab et al., 2013). Th2 predominant imbalance in atopic dermatitis has been implicated in the downregulation of *FLG* expression and is another contributor to barrier