Published in final edited form as:

J Invest Dermatol. 2012 March ; 132(3 0 2): 949–963. doi:10.1038/jid.2011.435.

Skin Barrier Disruption - A Requirement for Allergen Sensitization?

Anna De Benedetto, M.D.[1], **Akiharu Kubo, M.D. Ph.D**[2,3], and **Lisa A. Beck, M.D.**[1]

[1]Department of Dermatology, University of Rochester Medical Center, Rochester, NY

[2]Department of Dermatology, School of Medicine, Keio University, Tokyo, Japan

[3]Center for Integrated Medical Research, School of Medicine, Keio University, Tokyo, Japan

Abstract

For at least half a century, noninvasive techniques have been available to quantify skin barrier function, and these have shown that a number of human skin conditions and disorders are associated with defects in skin permeability. In the last decade, several genes responsible for skin barrier defects observed in both monogenetic and complex, polygenic disorders have been elucidated and functionally characterized. This has led to an explosion of work in the last six years that has identified pathways connecting epidermal barrier disruption and antigen uptake as well as the quality and/or magnitude of the antigen-specific adaptive immune response. This review will introduce the notion that diseases arise from the dynamic crosstalk that occurs between the skin barrier and immune system using atopic dermatitis or eczema as the disease prototype. Nevertheless, the concepts put forth are highly relevant to a number of antigen-driven disorders for which skin barrier is at least transiently compromised such as psoriasis, allergic contact dermatitis and blistering disorders.

Keywords

Atopic dermatitis; barrier; epicutaneous sensitization; allergen

The skin provides a vital barrier structure that protects vertebrates from both routine and extreme environments including exposure to antigens, solvents, ultraviolet light, detergents, microorganisms, toxins, nanoparticles and a variety of physical insults (Elias, 2006). In terrestrial vertebrates, the epidermis, where most of the skin barrier function resides, is highly stratified and has an outermost layer that is cornified. Recent findings have shown that epidermal barrier dysfunction is pathologically involved in a variety of common, antigen-driven skin diseases, including psoriasis and atopic dermatitis (AD). In this review, we will briefly describe i) the barrier system of the human epidermis, ii) human disorders associated with skin barrier defects and allergen sensitization, iii) murine studies that have

Corresponding Author: Lisa A. Beck, M.D., University of Rochester, Dept of Dermatology, 601 Elmwood Ave, Box 697, Rochester, NY 14642, Phone: 585-275-1039, Fax: 585-276-2330, Lisa_Beck@URMC.ROCHESTER.EDU.

Conflicts of Interest: None Declared

helped to clarify pathways connecting barrier and immunity and iv) epithelial-derived immune adjuvants released in response to barrier disruption. We will focus on immune recognition of allergens as a paradigm for all incomplete and complete environmental and microbial antigens. It is important to note that the skin microbiome, which consists of both commensal and pathogenic bacteria, affects the skin barrier and epithelial innate immune responses. Consequently, skin microbes are thought to play a critical role in the development of atopic dermatitis. These topics will not be discussed further as they are reviewed in this 75th Anniversary series (Gallo, 2012; Kong and Segre, 2012).

Cutaneous allergen sensitization is a critical and early event in the pathogenesis of AD, but this may also be true for several other atopic disorders where defects in skin barrier genes associate with diseases that manifest in other organs including food allergy, asthma, allergic rhinitis and eosinophil esophagitis (Blanchard *et al.*, 2010; Bremmer *et al.*, 2008; Brown *et al.*, 2011a; Brown *et al.*, 2008; Weidinger *et al.*, 2008). In subjects with AD, the initial exposure to allergens (***sensitization phase***) induces a systemic "allergic" T Helper (Th) 2 cell response that is magnified with each subsequent exposure (***effector phase***). Critical features of a Th2 immune response include the local production of Th2 cytokines (IL4, IL5 and IL13), bone marrow production, prolonged survival and activation of eosinophils and mast cells and production of allergen-specific IgE. For a long time, allergic diseases were considered primarily immunologic disorders. As a consequence, research and drug development focused on modifying the Th2 effector phase (Hanifin *et al.*, 1985). Over the last decade, there has been a shift in our thinking, with the epithelium now recognized as a critical player in the development of allergic sensitization (Bulek *et al.*, 2010; De Benedetto *et al.*, 2009; Holgate, 2007). In particular, compelling data from human and mouse studies have implicated skin barrier impairment as an indispensable event in allergen sensitization (Cork *et al.*, 2009; Fallon *et al.*, 2009; Jin *et al.*, 2009; O'Regan and Irvine, 2010; Oyoshi *et al.*, 2009; Palmer *et al.*, 2006; Smith *et al.*, 2006; Spergel, 2010). The specific pathways connecting epidermal barrier disruption to allergen sensitization are beginning to be elucidated. The overriding hypothesis is that epidermal disruption would allow skin resident antigen presenting cells such as Langerhans cells (LC; epidermis) or dendritic cells (DC; dermis) to capture environmental antigens (Kubo *et al.*, 2009) (Figure 1 **&** 2). Additionally, barrier-disrupted keratinocytes would release immune adjuvants that activate and mature LC/DCs as well as affect their ability to direct naïve Th cell polarization and thereby affect the character of the T helper response (Figure 3). Whether the adaptive immune response that ensues can feedback and affect barrier function has not been fully explored. In this review, we will summarize the evidence for and possible mechanism(s) involved in the induction of allergen sensitization in the context of skin barrier defects.

I. Overview of the epidermal barrier

Stratum corneum – the proverbial "moat" that is the preliminary defense for the "castle"

The horny layer of the epidermis was initially considered to be a loose collection of amorphous keratin filaments separated by wide empty spaces (Rothman, 1954). The "basket-weave" appearance of this layer as it is observed in formalin-fixed specimens has long misled histopathologists. Kligman and Christophers demonstrated that this appearance

was an artifact of sample preparation. They were the first to recognize its cellular nature coining the term “corneocytes” (Christophers and Kligman, 1964b; Kligman, 2006; Kligman, 2011). Corneocytes were noted to appear as disk-shaped cells with a polygonal outline (Goldschmidt and Kligman, 1967), and the sodium hydroxide immersion technique demonstrated that the stratum corneum (SC) was about 15 cells thick in adult human skin samples from most body sites (Christophers and Kligman, 1964a). This same technique also demonstrated that corneocytes are encased by well-defined “cornified envelopes”, which have been extensively studied since then (Candi *et al.*, 2005; Eckert *et al.*, 1993; Madison, 2003; Proksch *et al.*, 2008). At first, the inter-corneocyte space seemed empty as viewed by electron microscopy. This turned out to be an artifact of fixation and sample processing. Ruthenium tetroxide fixation and staining demonstrated that the intercellular spaces were filled with stacks of lamellar structures, which are now recognized as multiple stripes of hydrophobic and hydrophilic structures formed by various intercellular lipids (Elias, 2005). These intercellular spaces also include various proteases that control desquamation and antimicrobial peptides that act as a microbial barrier and control the growth of both commensal and pathogenic bacteria (Braff *et al.*, 2005).

Studies demonstrating enhanced skin permeability after SC removal highlight the importance of the SC as a barrier structure (Blank and Gould, 1959; Malkinson, 1958; Monash, 1957). Although, it is not clear whether several or all of these approaches to disrupt the SC may also disrupt the tight junctions (TJ) found immediately below the SC. Dr. Elias and his colleagues have published a large number of studies characterizing the SC components responsible for barrier function and how they are altered in normal and pathologic conditions (reviewed in (Elias, 2005; Elias, 2006). It was initially proposed by Scheuplein in 1971 that exogenous substances transited though the SC by direct transmembrane diffusion (Scheuplein and Blank, 1971). Later, it became clear that the intercorneocyte space provided the pathway through which exogenous, more lipophilic substances pass to reach the sub-SC layers of the epidermis. The surface proteins of the “cornified envelope” were shown to be tightly bound to hydroxyl-acylsphingosine molecules, which suggests that each corneocyte envelope has its own lipid envelope providing a barrier against the passage of water and water-soluble substances (Swartzendruber *et al.*, 1987). Various physical and chemical penetration enhancers including exposure to water alone were shown to have the ability to disrupt the intercellular multi-laminar membranes, creating openings within the intercellular spaces called “lacunae” through which hydrophilic and hydrophobic substances could penetrate (Menon and Elias, 1997; Warner *et al.*, 1999). In summary, the SC is composed of two components, protein-rich corneocytes and intercellular lipid domains, and it has been likened to a “brick and mortar” structure (Elias, 1981).

Tight junction barrier - the proverbial “portcullis” that is the final defense for the “castle”

In the 1940s, Dr. Rothman first suggested that another barrier structure in the skin controlled diffusion of water and solutes through the paracellular pathway (Rothman and Flesch, 1944). In his words, “The impermeability of the skin to water and electrolytes is caused neither by the presence of a greasy-waxy cover of the skin nor by the presence of the horny layer. The seat of the absorption-barrier is to be placed in the transitional layers between

cornified and noncornified epithelium, i.e., in the stratum granulosum (SG)…" (Rothman and Flesch, 1944). This absorption-barrier is called TJ and is the only barrier structure in mono-layered or simple epithelia demarcating the body surface of urochordates but has also been recognized more recently in the stratified epithelia of vertebrate skin. Thus, TJ are an ancient barrier system of the body surface, which have been conserved from urochordates and fish that lack SC to mammals. Although Hashimoto had described the appearance of TJ structures in the granular layer of human epidermis in 1971 (Hashimoto, 1971), this barrier has long been overlooked or ignored in mammalian skin. The importance of this barrier structure in mammalian epidermis was revived by identification of claudins as integral transmembrane proteins found in all TJ. The profound epidermal permeability abnormalities observed in claudin-1 knockout mice and claudin-6 transgenic mice solidified the importance of this second skin barrier structure (Furuse *et al.*, 2002; Turksen and Troy, 2002). TJs seal the intercellular spaces between stratum granulosum keratinocytes (Furuse *et al.*, 2002; Kubo *et al.*, 2009). TJs are not just physical barriers; they exhibit ion and size selectivity and their barrier function varies significantly in 'tightness' depending on cell type and physiological requirements, enabling dynamic regulation of substances that traffic between extra-TJ and inside-TJ compartments including dendritic processes from antigen presenting cells (Kubo *et al.*, 2009; Tsukita and Furuse, 2002)(Figure 1).

II. Skin barrier defects promote allergen sensitization - evidence based on human diseases

Disorders characterized by enhanced desquamation – Netherton's Syndrome and Peeling Skin Syndrome Type B

Association of skin barrier defects with allergic manifestations has been demonstrated in two genodermatosis that are disorders of desquamation, namely Netherton's syndrome (NS; OMIM 256500) and Peeling skin syndrome, type B (PSS-B; OMIM 270300) (Table 1). Both diseases are characterized by superficial intra-epidermal detachment and atopic diathesis. In the SC, the corneocytes are tightly bound together via corneodesmosomes, the end product of epidermal desmosomes modified by the incorporation of corneodesmosin (CDSN). In the desquamation process, kallikrein (KLK)-related peptidases such as KLK5 and KLK7 degrade CDSN and induce cleavage of corneodesmosomes, resulting in detachment of corneocytes (Ovaere *et al.*, 2009). The protease activity of KLKs is inhibited by lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI), encoded by the serine protease inhibitor, Kazal type 5 (*SPINK5*) gene. Loss-of-function mutations in SPINK5 (Chr 5q32) leading to accelerated desquamation, is the defect in Netherton's syndrome, a severe autosomal recessive ichthyotic condition characterized by a chronic atopic dermatitis-like condition, allergen sensitization and other atopic disorders such as asthma and allergic rhinitis (Frenk and Mevorah, 1972). Interestingly, coding polymorphisms in the *SPINK5* gene have been found in association with AD and disease severity (Cork *et al.*, 2009; Walley *et al.*, 2001), and more recently with food allergy in children with AD (Kusunoki *et al.*, 2005). Although Hubiche *et al.* (Hubiche *et al.*, 2007) did not find an association in a French AD population, they did observe an association between the E420K polymorphism and serum IgE levels, suggesting that this barrier defect predisposes subjects to a Th2 response to environmental allergens.

Recently, two distinct mutations in the *CDSN* gene (Chr 6p21) have been shown to be associated with PSS-type B (Israeli *et al.*, 2011), an autosomal-recessive ichthyosiform erythroderma characterized by peeling of the skin and allergen sensitization (Israeli *et al.*, 2011; Oji *et al.*, 2010). PSS-B patients have been reported to have high serum IgE levels, eosinophilia and food allergies (Oji *et al.*, 2010). In summary, defects in two different proteins that result in enhanced SC desquamation are strongly associated with a Th2 adaptive immune response to environmental allergens as noted by elevated IgE levels and peripheral eosinophilia and clinical manifestations of an atopic disease(s). It is important to note that the *CDSN* gene is located in PSORS1, one of the major psoriasis (PS) susceptibility loci, and polymorphisms in this gene have been associated with PS in some populations (Capon *et al.*, 2004; Helms *et al.*, 2005; Jenisch *et al.*, 1999). Since psoriasis is a disease of delayed desquamation, it is assumed that the *CDSN* mutations observed in PS would likely inhibit their degradation by kallikreins and therefore would not likely explain PS subjects barrier defect.

Disorder of disturbed lipid metabolism - Ichthyosis Prematurity Syndrome

Ichthyosis prematurity syndrome (IPS; Chr 9q34) is a rare syndrome characterized by the clinical triad of premature birth, thick caseous desquamating epidermis, and neonatal asphyxia (Niemi *et al.*, 1993). The diagnosis is often made by characteristic ultrastructural findings demonstrating conspicuous membrane inclusions in the SC, and most cases are associated with mutations in a gene encoding for the fatty acid transport protein 4 (*FATP4*) (Klar *et al.*, 2009; Sobol *et al.*, 2011). The FATP4 protein plays a central role in the transport and activation of fatty acids in the endoplasmic reticulum of keratinocytes and is thought to be important in the maintenance of normal epidermal barrier function (Milger *et al.*, 2006). Patients with this condition can have peripheral eosinophilia, an atopic dermatitis-like condition, an extensive keratosis pilaris-like condition and a family history of atopic disorders (Bygum *et al.*, 2008). Although the clinical features of the few subjects reported in the literature to date are incomplete, there is still a suggestion that a SC lipid defect, that causes a barrier phenotype in genetically altered mice (Moulson *et al.*, 2007), is associated with markers of systemic Th2 polarity (e.g. eosinophilia) and atopy (Table 1).

Disorders characterized by reduced expression of a key SC structural protein - Ichthyosis Vulgaris and Atopic Dermatitis

Filaggrin (FLG) is expressed in SG layers as a > 400-kDa precursor protein called profilaggrin. Profilaggrin is dephosphorylated and cleaved by proteases into FLG monomers, with keratin-binding activities which are thought to contribute to the cell compaction observed in the lower SC (Dale *et al.*, 1978). FLG monomers are further degraded into “natural moisturizing factors” that maintain hydration of the upper SC (Rawlings and Matts, 2005; Sandilands *et al.*, 2009; Scott and Harding, 1986) and also have anti-staphylococcal properties *in vitro* (Miajlovic *et al.*, 2010). Mutations in the *FLG* gene were identified initially as the cause of ichthyosis vulgaris (IV; OMIM 146700; Chr 1q21) and subsequently as a major predisposing factor for AD (Table 1) (Palmer *et al.*, 2006; Smith *et al.*, 2006). To date, the strong association of *FLG* mutations with AD is one of the most robust genotype–phenotype linkages observed in human complex genetic disorders (van den Oord and Sheikh, 2009). Several case–control studies have also demonstrated

strong association between *FLG* mutations and early AD onset, disease severity, eczema herpeticum, AD-related asthma and greater allergen sensitization (Gao *et al.*, 2009; Schuttelaar *et al.*, 2009; van den Oord and Sheikh, 2009; Weidinger *et al.*, 2006). Interestingly, several studies have shown *FLG* mutations confer a substantial risk for other atopic disorders recognized as Th2 polarized diseases including allergic rhinitis, IgE-mediated peanut allergy, and eosinophilic esophagitis. These associations were observed even after controlling for co-existent AD (Blanchard *et al.*, 2010; Brown *et al.*, 2011b; Weidinger *et al.*, 2008). This is a bit difficult to understand as FLG immunostaining is restricted to skin, oral mucosa and the cornified epithelium of the nasal vestibule with no detectable staining observed in epithelium from bronchial biopsies as well as gastrointestinal epithelium (De Benedetto *et al.*, 2008; Weidinger *et al.*, 2008; Ying *et al.*, 2006). Therefore, *FLG* mutations are unlikely to affect barrier function and allergen sensitization in the organs where these diseases manifest. One interpretation of these findings is that *FLG* mutations may drive disease at distant mucosal sites by enabling allergen sensitization through a defective skin (and/or oral) barrier while subsequent allergen responsiveness occurs in these other epithelial beds (e.g. upper airway or gastrointestinal tract). This model seems feasible as we know that not all subjects with *FLG* mutations develop AD. For example, the carrier frequency is as high as 12% in healthy Northern European controls, suggesting that these mutations by themselves may not be sufficient to induce AD and/or a Th2 adaptive immune response. Additionally, the model presupposes that subjects with allergic rhinitis and food allergy likely have a barrier defect in the relevant epithelial surface that may be either genetic or acquired to explain their organ-specific disease manifestations. In the case of allergic rhinitis, the barrier defect may occur in the context of a viral upper respiratory infection which often proceeds an exacerbation of the disease, and in the case of food allergy one could argue that the infants and young children who develop this allergic disease have a delay in the normal intestinal barrier maturation that occurs shortly after birth.

Between 37 to 70% of IV patients present with atopic conditions, most commonly AD, but also allergic rhinitis and asthma (Bremmer *et al.*, 2008; Brown *et al.*, 2008; Wells, 1966). Interestingly, some IV subjects have allergic rhinitis and high IgE levels without concomitant AD providing further support for the notion that *FLG* mutations simply confer a risk for allergen sensitization through the skin (Oji *et al.*, 2009). Somewhat surprisingly, a small German IV cohort study found no difference in the prevalence of atopic disorders based on *FLG* genotype (e.g. one or double mutation) (Oji *et al.*, 2009). They also evaluated the number of epidermal dendritic cells identified by CD1a staining, as a marker of early immunologic activity, based on previous work demonstrating that subjects with more severe AD had increased epidermal DCs (Novak *et al.*, 2004). They observed increased epidermal CD1a+ cells only in the atopic IV subgroups, which was independent of *FLG* genotype suggesting that cutaneous reactivity in these subjects was not determined by the presence or absence of FLG expression. Collectively, clinical assessments of AD and IV subjects suggest that *FLG* mutations predispose subjects to allergen sensitization but that these mutations are not sufficient as other genetic as well as environmental influences are likely promoting the Th2 immune response observed in susceptible individuals. One speculation is that other epidermal barrier defects might also contribute to and/or modulate the epicutaneous sensitization and ultimately the allergic phenotype. Table 1 highlights several

human skin diseases that are thought to have a barrier defect and summarizes what we know about their local and systemic adaptive immune responses.

It is well known that the magnitude of the barrier defect in AD, as measured by transepidermal water loss (TEWL) at nonlesional sites, correlates with disease severity and serum IgE (Gupta *et al.*, 2008; Hon *et al.*, 2008; Lee *et al.*, 2006). Using this physiological measure of epidermal barrier function, Boralevi et al. demonstrated that AD infants with two or more positive allergen patch tests had higher TEWL than infants with one or no positive tests (Boralevi *et al.*, 2008). Further studies are needed to clarify the relationship between physiologic measures of barrier health and integrity (e.g. TEWL, surface pH, SC hydration, SC cohesion, lipid composition of SC, barrier recovery) and allergen sensitization in human subjects. Surprisingly, the biochemical basis for elevated TEWL is still not known. The literature strongly suggests that nonlesional TEWL abnormalities are not explained by *FLG* mutations (Hubiche *et al.*, 2007; Jungersted *et al.*, 2010; Nemoto-Hasebe *et al.*, 2009; O'Regan *et al.*, 2010). Although, Flohr *et al.*(Flohr *et al.*, 2010) showed that *FLG* mutations were associated with higher TEWL in clinically normal appearing forearms in a small cohort of 3 month-old infants, which was not dependent on AD status. Collectively, these studies strongly suggest that *FLG* mutations may play a role in the TEWL abnormalities observed in AD subjects, but they are certainly not the entire story.

FLG genotypes do seem to play a role in a number of physiologic measures of barrier function including skin hydration (Kezic *et al.*, 2008; Scott and Harding, 1986), surface pH, SC cohesion and paracellular permeability of SC with the water soluble tracer, lanthanum (Fluhr *et al.*, 2010; Gruber *et al.*, 2011; Krien and Kermici, 2000). AD subjects have a number of barrier abnormalities in addition to increased TEWL, including increased surface pH, reduced SC hydration, and enhanced barrier recovery. As we discover the biochemical basis for these abnormalities, we will be able to address how each of them interact in a bidirectional way with the immune system (Figure 3).

We have recently demonstrated that AD subjects have a defect in TJ as noted by a remarkable impairment of bioelectric properties of their *ex vivo* epidermis, and this may be due to reduced expression of the TJ components, claudin-1 and 23 (De Benedetto *et al.*, 2011). Claudin-1 levels were inversely correlated with Th2 biomarkers (IgE and eosinophilia) suggesting that reductions in this TJ protein may affect the systemic immune phenotype or vice versa (De Benedetto *et al.*, 2011). This introduces the notion that AD subjects may have defects in both barrier structures, SC and TJ, and the relative contribution of each may contribute to the heterogeneity characteristic of this disease (e.g. disease onset, natural history, magnitude of allergen sensitization, comorbid atopic conditions). Recent studies of skin biopsies from *FLG* heterozygous and homozygous IV subjects have demonstrated a *FLG* gene dose-dependent inhibition in TJ protein expression suggesting that there is a dynamic interaction between these two epidermal barrier structures, SC and TJs (Gruber *et al.*, 2011). Whether these changes affect TJ function is still not known. Developing a better understanding of how these two key epidermal barrier structures interact will likely yield information that will have broader implications than just inflammatory skin disorders. In summary, these studies suggest that damage to both epidermal barriers (SC and

TJ) might promote the elongation of LC dendrites through TJ barrier and consequently their uptake of allergens/antigens from the skin surface (Figure 2 **&** 3).

It is interesting to note that several autoimmune disorders and genodermatosis that result in full epidermal detachment are characterized by Th2 inflammation. For example, in the autoimmune blistering disorders, bullous pemphigoid, herpes gestationis and pemphigus vulgaris there is a clear Th2 response which is observed early in the disease, but it is unclear whether it comes before the full thickness epithelial disruption or as a consequence of it (Arbesman *et al.*, 1974; Borrego *et al.*, 1999; Bushkell and Jordon, 1983; De Pita *et al.*, 1997; Fabbri *et al.*, 2003; Feliciani *et al.*, 1999; Nagel *et al.*, 2010) (Table 1). Similarly, in the genodermatosis, epidermolysis bullosa pruriginosa (OMIM 60412) (Mellerio *et al.*, 1999; Yamasaki *et al.*, 1997), a skin fragility disorder caused by mutations in the COL7A1 gene, and characterized by anchoring fibril abnormalities and sublamina densa blistering, the patients have been reported to have high serum IgE and eosinophilia (McGrath *et al.*, 1994) (Table 1). The complete repertoire of IgE antigen-specificity is unclear even in bullous pemphigoid, herpes gestationis and pemphigus vulgaris where BP180, BP230 or desmoglein 3 accounts for only part of the serum IgE measured in these patients. The assumption is that these patients have IgE reactivity to other antigens, and the specificity of these responses would be interesting to characterize further. One is left wondering if the profound barrier disruption characteristic of these disorders promotes the production of epithelial-derived Th2 adjuvants (e.g. TSLP, IL33, IL25, etc).

III. Skin barrier defects promote allergen sensitization – What have murine models taught us?

There are a remarkable number of mouse models for which a barrier defect phenotype has been observed (Table 2). We have chosen a few examples to highlight the complexity inherent to epidermal barrier integrity. We have highlighted genetically altered mice that denote the import of both SC and TJ structural proteins as well as components of other intercellular junctions such as adherens and gap junctions, transcription factors, nuclear receptors, proteases/antiproteases and proteins relevant for lipid metabolism (Table 2). Studies such as these will continue to inform us about the complex network required to form both skin barrier structures (SC and TJ) under both homeostatic and inflammatory conditions.

How epidermal barrier defects impact the adaptive immune response to an antigen has been studied by a number of groups (Herrick *et al.*, 2000; Kondo *et al.*, 1998; Oyoshi *et al.*, 2009; Spergel *et al.*, 1998; Strid *et al.*, 2006; Strid *et al.*, 2004; Wang *et al.*, 1996). These studies have demonstrated that epicutaneous sensitization with a protein allergen elicits a local and systemic Th2-predominant response, as noted by increases of IL4, IL5 and antigen-specific IgE and IgG1, but low or absent induction of IFNγ and IgG2a, in the skin and draining lymph nodes in both C57BL6 and BALB/c mouse strains which are at baseline thought to be inherently Th1 and Th2 prone, respectively (Herrick *et al.*, 2003; Kondo *et al.*, 1998; Spergel *et al.*, 1998). Importantly, in all studies, the Th2-biased immune response was achieved only after a number of barrier disrupting methods were employed including shaving, repeated tape-stripping and prolonged ($\geq$ 4 days) and often repeated patch

application(s) with an occlusive dressing that would result in maceration and thereby lead to barrier disruption. At least one group has shown that the magnitude of the Th2 response, as measured by IL-4 expression in tissue and draining lymph nodes (Kondo *et al.*, 1998), was enhanced by the degree of skin barrier disruption. Interestingly, IFNγ levels (e.g. Th1 response) did not change with variation in barrier disruption (Kondo *et al.*, 1998). No studies have evaluated IL17 levels as a marker of a Th17 response under similar experimental conditions of graded barrier disruption.

Tissue neutrophilia and the local expression of IL17, both markers of a Th17 adaptive immune response, have been observed in IL4, IL13 and dual knockout mice or in mice that are deficient in the transcription factor STAT6, which is critical for the differentiation of Th2 effector cells (He *et al.*, 2007; Herrick *et al.*, 2003). This work combined with *in vitro* studies suggesting that Th2 cytokines may reduce expression and biological functions of Th17 cytokines (Eyerich *et al.*, 2009; Nograles *et al.*, 2010) suggests that these two Th phenotypes counteract each other. This is in keeping with findings in humans where there is a remarkable paucity of tissue neutrophils in AD skin biopsies, despite modest expression of prototypic Th17 cytokines, suggesting that Th2 inflammation dampens the functional Th17 response (Guttman-Yassky *et al.*, 2011a, b; Nograles *et al.*, 2009; Suarez-Farinas *et al.*, 2011). This is in sharp contrast with psoriasis, a prototypic Th17/Th1 skin disease, characterized by tissue neutrophilia and relative paucity of Th2 cytokines, for which the antigens and conditions under which sensitization occurs remain a mystery (Guttman-Yassky *et al.*, 2008; Koga *et al.*, 2008).

These murine models of epicutaneous allergen challenge induce skin lesions that are AD-like (Spergel *et al.*, 1998). The sensitized mice have increased scratching behavior, thickened epidermis, inflammatory infiltration of CD4+ T cells and eosinophils, expression of Th2 cytokines, with modest increases in IFNγ, Th2 chemokines such as eotaxin and the Th2 adjuvant, TSLP as well as allergen-specific IgE (Herrick *et al.*, 2003; Kondo *et al.*, 1998; Spergel *et al.*, 1998). Additionally, these mice developed airway hyper-responsiveness to methacholine and eosinophilia after a single dose of inhaled OVA. This demonstrates that antigen education epicutaneously on barrier-disrupted skin is sufficient to elicit systemic Th2 allergic inflammation in a distant organ such as the lower airways and esophagus following relevant challenge (e.g. inhaled vs oral, respectively)(Akei *et al.*, 2006; Spergel *et al.*, 1998; Strid *et al.*, 2005). This may help explain the so-called “allergic march” where AD is the earliest atopic disorder to present followed, not infrequently, by the development of other atopic diseases such as allergic rhinitis, asthma, and food allergy. Additionally, it highlights the importance of cutaneous allergen sensitization even if the ultimate allergen elicitation occurs in a distant organ.

To demonstrate the importance of the route of immunization (e.g. sensitization phase), Strid et al. evaluated whether the immunologic response differed if the allergen (e.g. peanut protein) was applied on disrupted epidermis (24 hours after tape stripping) or injected subcutaneously with complete Freund’s adjuvant (CFA) (Strid *et al.*, 2004). They found that the epicutaneous route generated a Th2 immune response in draining lymph nodes and spleen (higher IL4 and antigen-specific IgE and lower IFNγ, IL10 and IgG2a) in contrast with the subcutaneous route, which induced a Th1 immune response (high IFNγ and IgG2a

and low IL4 and antigen-specific IgE). This is not too surprising since CFA is a Th1 and possibly Th17 adjuvant commonly used in many vaccines. A more interesting comparison would be to evaluate the adaptive immune response to subcutaneous administration of the antigen with a Th2 adjuvant. In subsequent work, this group demonstrated that immunization with an antigen applied on barrier-disrupted epidermis was able to switch an established Th1 response to a Th2 response (Strid *et al.*, 2006). Interestingly, the prevention of the Th1 immune response was specific to the antigen applied on the skin, as exposure to a different antigen was still able to induce a Th1 response in the same animal. Collectively, this work suggests that epidermal barrier disruption can be a "real-life" Th2 adjuvant.

A reductionist approach to barrier disruption is necessary to delineate the importance of specific barrier defects and their relevance to the immunologic abnormalities observed in AD. Fallon and colleagues have done just that by identifying the *FLG* mutation present in the spontaneous recessive mouse mutant referred to as flaky tale (ft) mouse (Fallon *et al.*, 2009). To eliminate the unknown effect of the matted mutation, Fallon et al. backcrossed the flaky tail mouse onto the C57BL/6 strain (Fallon *et al.*, 2009; Presland *et al.*, 2000). They (Fallon *et al.*, 2009) found that C57BL/6 ft/ft homozygotes generated allergen-specific IgE (as well as IgG2a and IgG1) and a mixed Th1 (IFNγ), Th2 (IL5, IL4 and IL13), and Th17 (IL17) response after epicutaneous allergen challenge as compared to heterozygotes (ft/wt) or wild type (wt/wt) mice. In this study, the epicutaneous sensitization was elicited with relatively little barrier disruption (shaving and allergen application for 5 days × 3 cycles) and with this protocol the C57BL/6 ft/wt and wt/wt had no inflammation compared to saline challenged sites. In other words, this sensitization protocol was not sufficient to induce a Th2 response in the C57BL/6 mouse strain, which Herrick et al., have shown is possible with sufficient barrier disruption (Herrick *et al.*, 2000). Oyoshi et al using the naturally occurring flaky tail mouse (with the matted mutation) and a similar epicutaneous allergen sensitization protocol found an enhanced Th17 (IL17 and IL23) with similar Th2 (IL-4 and IL-13) and Th1 (IFNγ) response compared to wild type strain controls (BALB/c and C56BL/6) (Oyoshi *et al.*, 2009). Collectively, this work highlights the permissive nature of the *FLG* knockout mouse for epicutaneous sensitization and demonstrates the promiscuous nature of the adaptive immune response, which does not favor a specific T helper profile. In the Fallon study, although baseline TEWL was not different among the three groups (ft/ft, ft/wt and wt/wt) there was a substantial increase in TEWL observed only in the ft/ft after allergen sensitization that was commensurate with the cellular infiltrate, suggesting that it was the result of an inside-out mechanism. Lastly, although human studies have shown that *FLG* mutations predispose AD subjects to the development of asthma (van den Oord and Sheikh, 2009), epicutaneous sensitization in the ft/ft mouse model did not evoke an effector response in the airway as has been seen in other barrier-disrupted, epicutaneous allergen challenge models ((Herrick *et al.*; Spergel *et al.*). It is worth mentioning that the allergen sensitization and AD-like phenotype was only observed in ft/ft and not in heterozygous (ft/wt) animals, while the majority of FLG-associated AD cases have a single *FLG* mutated allele (e.g. are heterozygotes). The authors suggested that the lack of lung sensitization in this model could reflect the use of the C57BL/6 strain, which is less susceptible to allergen-induced Th2 inflammation as compared to BALB/c. Alternatively, the lack of lung reactivity may also suggest that additional skin barrier defects need to be present at the site of allergen

sensitization, and these may prove to be more critical determinants of the Th2 response characteristically observed in AD.

One caveat to consider when comparing antigen challenge models is that the purity of the antigen preparation is a critical determinant of the immunologic outcome. For example, many allergen preparations are contaminated with ligands that can trigger innate immune receptors (e.g. LPS or peptidoglycans) and therefore would have an adjuvant effect. Even more confusing is the growing literature suggesting that many allergens themselves activate innate immune receptors (e.g. house dust mite activating dectin-1 and TLR4; ragweed activating TLR4; nickel activating TLR4; cockroach activating TLR2) (Li *et al.*, 2011; Page *et al.*, 2008; Schmidt *et al.*, 2010; Trompette *et al.*, 2009). Several other variables that may be equally important include the dose of antigen used, age and sex of mice, dressing used to apply antigen to the skin surface, anatomic site where sensitization occurs, “cleanliness” of the vivarium (e.g. determinants of microbial skin flora) and type and magnitude of barrier disruption employed (Kondo *et al.*, 1998; Oyoshi *et al.*, 2009; Spergel *et al.*, 1998; Strid *et al.*, 2004).

In summary, murine studies strongly suggest that skin barrier disruption either by physical means or by targeted gene deletion, as in the *FLG* −/− mouse, significantly enhances immunologic responses to the epicutaneous application of allergens. Similarly, the mode of barrier disruption may determine the flavor of the immune response with some favoring Th2 and others favoring a Th17 response. A better understanding of the epithelial factors that regulate effector T cell maturation and polarization at the skin surface is crucial for the development of preventive as well as therapeutic strategies in antigen-driven skin disorders such as AD, PS and allergic contact dermatitis.

IV. Potential mechanisms for a Th2 response following epidermal barrier disruption

The first step in the development of any adaptive immune response is the activation of innate immune receptors or pattern-recognition receptors (PRRs) by highly conserved pathogen-associated molecular patterns (PAMPs) common to many classes of pathogens or danger-associated molecular patterns (DAMPs) which are cellular components released in the context of cell damage/necrosis (Janeway and Medzhitov, 2002). PRR activation results in the production of specific mediators (cytokines, chemokines, and antimicrobial peptides) as well as the activation and recruitment of immune cells (immature DC, natural killer cells, and neutrophils)(De Benedetto *et al.*, 2009). Th2 responses are commonly elicited by extracellular parasites or environmental allergens (Finkelman and Urban, 1992; Paul and Zhu, 2010) and are mediated in part by the actions of a number of Th2 promoting cytokines including thymic stromal lymphopoietin [TSLP], IL33 and IL25 that are produced by tissue resident cells. By contrast, Th17 promoting cytokines include IL-1β, IL-6, IL-23 and TGFβ (Hammerich *et al.*, 2011; Wilson *et al.*, 2007). The specific innate immune pathways that mediate the effector T cell phenotype are not fully characterized (Paul and Zhu, 2010).

Immune mediators released after epidermal barrier disruption

In 1994, Nickoloff and Naidu (Nickoloff and Naidu, 1994) were the first to demonstrate the epidermal expression of several inflammatory cytokines (TNFα, CXCL8/IL8, IL10, IFNγ and TGFγ) and the Th17 promoting cytokine, TGFβ following tape-stripping of normal human skin. More recently, several groups have extended and confirmed these findings, showing enhanced epidermal mRNA expression of Th2 promoting cytokines, TSLP and IL33 as well as TNFα, heat shock protein (Hsp) 90, Hsp70, and CXCL8 after tape stripping performed on healthy subjects or in Notch deficient mice who have a chronic skin barrier defect (Angelova-Fischer *et al.*, 2010; Briot *et al.*, 2009; Demehri *et al.*, 2008; Dickel *et al.*, 2010). Importantly, this work introduced the notion that endogenous DAMPs or alarmins, such as Hsp70, Hsp90 and IL33, could activate innate receptors following epidermal injury.

TSLP is an IL7-like cytokine, that was originally characterized for its role in T and B cell development, but more recent literature strongly implicates its role in Th2 cell differentiation and in particular, in the pathogenesis of allergic inflammation (Ziegler and Artis, 2010). In 2002, Soumelis at al. (Soumelis *et al.*, 2002) demonstrated that TSLP was highly expressed in the epidermis from AD subjects and that TSLP-activated DCs produced Th2 attracting chemokines (TARC and MDC) and primed naïve T cells to differentiate into Th2 cells. Importantly, TSLP was not detected in the skin from nickel-induced allergic contact dermatitis or systemic lupus erythematosus (Soumelis *et al.*, 2002). Epithelium that is deficient in LEKT1, the protease inhibitor mutated in Netherton's Syndrome, express more TSLP which is mediated by the enhanced activity of KLK5 in a proteinase-activated receptor-2 (PAR-2)-dependent fashion (Briot *et al.*, 2009; Briot *et al.*, 2010). This finding links a genetic barrier defect (e.g. mutation in *SPINK5*) that leads to overactivity of serine proteases to TSLP expression and has important implications for the pathogenesis of AD. *In vitro* studies have demonstrated that a number of proteases can act through PAR-2 and induce TSLP expression from keratinocytes or airway epithelial cells (Kouzaki *et al.*, 2009; Zhang *et al.*, 2009). Interestingly, a number of allergens including house dust mite, cockroach, fungi, and several pollens, contain proteases that may also trigger epithelial production of TSLP through a similar mechanism (Takai and Ikeda, 2011). This may also be true for *Staphylococcus aureus* which produces extracellular proteases and chronically colonizes the skin surface of most AD patients (Takai and Ikeda, 2011). Proteases may also disrupt epithelial TJs either by direct actions on TJ proteins or by activation of PAR-2 (Runswick *et al.*, 2007; Tai *et al.*, 2006; Takai and Ikeda, 2011; Wan *et al.*, 1999). In summary, protease-mediated barrier disruption might have dual actions on the epithelium. They may induce the production of the Th2-promoting cytokine, TSLP and also facilitate allergen uptake by LC by disrupting TJs. It is intriguing to speculate that the more immunodominant allergens (e.g. house dust mite allergens) might be the ones that can both disrupt skin barriers and directly or indirectly act as Th2 adjuvants.

Epithelial TSLP is also induced through a TLR3 and 5 mediated mechanism in response to microbial products (Kinoshita *et al.*, 2009; Le *et al.*, 2010; Ma *et al.*, 2009) as well as in response to ragweed allergen which is a TLR4 mediated event (Li *et al.*, 2011). In a human skin injury model, the epidermal TSLP immunostaining was observed primarily at later time points (e.g. 48 hr) after the skin barrier perturbation (Angelova-Fischer *et al.*, 2010). This

delayed expression of TSLP suggests that TSLP may not be as critical in the sensitization phase as in the elicitation phase of an AD lesion. Therefore, Th2 adjuvants that are induced rapidly would be more likely candidates to initiate a Th2 immune response in the context of barrier disruption.

In contrast, IL33, a novel member of the IL1 family, is rapidly released in response to tissue injury and a more likely candidate to initiate Th2 polarization during the elicitation phase. IL33 was identified in 2005 using a computational database approach to search for the ligand of the orphan receptor ST2, a member of the Toll-like/IL-1R superfamily (Schmitz *et al.*, 2005). It is recognized as an alarmin or DAMP because it is released during epithelial cell death, is associated with infection or tissue injury and is induced by microbial ligands through a TLR-mediated pathway (Moussion *et al.*, 2008). Strong evidence demonstrates that IL33 plays an important role in allergic diseases (Schmitz *et al.*, 2005). Firstly, IL33 is markedly elevated in the serum of patients during anaphylactic shock (Pushparaj *et al.*, 2009), in asthmatic subjects and in the skin of subjects with atopic dermatitis (Oboki *et al.*, 2011; Pushparaj *et al.*, 2009). Secondly, IL33 plays an important role in the early phase of the Th2 immune response to intestinal helminths, acting as a bridge between innate and adaptive immunity (Humphreys *et al.*, 2008). Lastly, mast cells produce IL33 in response to IgE-dependent activation but also amplify the inflammation resulting from both IgE-dependent and independent mast cell and basophil activation (Hsu *et al.*, 2010; Silver *et al.*, 2010).

IL25 (or IL17E), a member of the IL-17 cytokine family, when overexpressed in murine models results in the production of Th2 cytokines IL-4, IL-5, and IL-13, eosinophilia, and elevated serum IgE, similar to what has been observed with TSLP and IL33 (Fort *et al.*, 2001). IL25 is expressed by mouse epithelial cells following allergen stimulation (Angkasekwinai *et al.*, 2007) and in the human skin of AD patients (Wang *et al.*, 2007). Whether barrier disruption/tissue injury induces IL25 production by human keratinocytes has not been studied. But, IL25 might contribute to barrier dysfunction in AD subjects. It was recently shown that IL25 inhibits FLG synthesis by keratinocytes (Hvid *et al.*, 2011) and therefore IL25 is capable of promoting AD both by its effects on the adaptive immune response and on epidermal barrier.

Langerhans cell activation after barrier disruption

The skin is protected by a variety of antigen presenting cells. In the epidermis, LCs form a dense network that covers the whole body. A large number of other DC subpopulations are found in the dermis and under inflammatory conditions migrate into the epidermis. LCs and dermal DCs thus form several layers of immunological defense; however, their specific roles in skin immunity are still controversial (Merad *et al.*, 2008). Skin DCs take up antigens encountered in the skin and migrate to draining lymph nodes, where they present the antigens to T cells. Mechanical or chemical stress on the epidermis has been reported to activate LCs which is thought to be mediated by the secretion of proinflammatory cytokines from keratinocytes (Lessard *et al.*, 1966, 1968; Nickoloff and Naidu, 1994; Nishijima *et al.*, 1997; Streilein *et al.*, 1982; Wood *et al.*, 1992) (Figure 3). Under steady-state conditions, LCs are on standby with their dendrites aimed outwards, positioned close to, but never

crossing, the TJ barrier (Kubo *et al.*, 2009), even though they express the key TJ component, claudin-1 (Zimmerli and Hauser, 2007) (Figure 1 **&** 2). Once they are activated, which can occur in response to mere tape-stripping of the skin surface, LCs extend their dendrites through TJ barriers, become activated and take up antigens from the extra-TJ environment (Kubo et al., 2009). Whether the tape stripping causes transient openings of TJs, the release of epidermal mediators that activate LC or direct dendrite migration to the epidermal surface or all of the above has yet to be determined. Interestingly, the epidermal TJ integrity is maintained by the *de novo* formation of TJs between keratinocytes and LCs, suggesting that LCs are able to take up foreign antigens from outside TJ barriers without penetration of the antigen through the TJ barriers (Kubo et al., 2009). As TJs are a size-limiting barrier (Tsukita *et al.*, 2001), it is possible that some smaller antigens, haptens or chemicals may penetrate the TJ barrier and through this mechanism encounter LC or DC. Thus, we need to re-evaluate each antigen and allergen individually to determine the limits of their penetration in the context of relevant “barrier disrupted” conditions.

Conclusions

In this review we have highlighted several new concepts that demonstrate the dynamic interaction between the epidermis and the immune system. Many of these concepts were the direct result of early observations made by cutaneous biologists who noted barrier defects in inflammatory skin disorders over 100 years ago. The recognition that keratinocytes are not just important to maintain tissue structure, polarity and to provide a fence-like function but also respond dynamically to environmental perturbations is still a relatively novel concept. In their role as “first-responders” they have clearly emerged as a key cell in the innate immune system. We have highlighted several human skin diseases with mutations that lead to a defective skin barrier and are often associated with markers of a Th2 response (IgE and eosinophilia) and have clinical features characteristic of an atopic disorder, most commonly, AD. It is suspected, but not known whether disorders characterized by a defect in TJ would also be associated with Th2 responses and atopic features. Interestingly, there is a monogenetic disorder called neonatal ichthyosis-sclerosing cholangitis (NISCH) syndrome, which is caused by a mutation in claudin-1, and has a skin phenotype notable for ichthyosis, as the name implies, and pruritus. Unfortunately, we do not know if these subjects have a skin barrier defect and whether they have greater allergen sensitization or atopic diseases. A number of genetically-altered mouse models have highlighted the complex network of proteins that are important for a healthy skin barrier. When this barrier is disrupted by mechanical means or genetically altered as in the case of the complete absence of FLG (ft/ft mice), the epidermis becomes permissive to allergen sensitization. The adaptive immune response that predominates when the skin barrier is physically disrupted is Th2 polarized; whereas, in the ft/ft mouse, a mixture of T helper responses (Th1, Th2 and Th17) is observed. This finding coupled with the evidence that the enhanced epidermal water loss (e.g. ↑ TEWL) characteristic of AD patients with moderate to severe disease is not fully explained by *FLG* mutations suggests that these mutations may not be the sole barrier defect in this disease. Many other abnormalities have been found in AD subjects that could affect barrier and include defects in lipid metabolism, altered expression of antimicrobial peptides, dysregulated EDC genes, altered activity of endogenous proteases, reduced activity of

endogenous antiproteases, TJ defects and simple trauma from the persistent itch-scratch cycle. Some of these defects are likely genetic (*FLG*, *SPINK5*) while others may be acquired (e.g. trauma from scratching, in response to the actions of Th2 and Th17 cytokines or on an epigenetic basis).

The mechanism(s) by which epidermal barrier disruption informs and directs the adaptive immune response to antigens is becoming clearer. We have presented the data that implicate several epidermal-derived alarmins (TSLP, IL25 and IL33) in directing a Th2 immune response and would likely be operative when the barrier is compromised. In addition, transient compromise in skin barrier provides the signals that lead to movement of LC dendrites through TJ and ultimately the engulfment of antigens present on the epidermal surface. We have not discussed the various subsets of skin-resident DCs or their relative effects on the adaptive immune system as this remains a highly controversial area. In conclusion, although we have summarized important evidence suggesting that skin barrier impairment appears to be an important determinant of allergen sensitization, we did not conclusively answer our initial question, is “Skin barrier disruption a requisite for allergen sensitization?” Sorting out the relative importance of specific epidermal barrier defects and the pathways by which they affect immune functions will be the charge for cutaneous biologists over the next 75 years.

Acknowledgments

Funding: Atopic Dermatitis Research Network (contract HHSN272201000020C and HHSN272201000017C) and University of Rochester Medical Center (L.A.B.); DHHS/PHS/NIH 5 T32 AR007472-21 (A.D.); Grants-in-Aid for Scientific Research and the “Promotion of Environmental Improvement for Independence of Young Researchers” program funding from the Ministry of Education, Culture, Sports, Science and Technology of Japan (A.K.)

Abbreviations

AD	atopic dermatitis
CLDN	claudin
CDSN	corneodesmosin
DAMPs	danger associated molecular patterns
DC	dendritic cells
FLG	filaggrin
Hsp	heat shock proteins
IV	ichthyosis vulgaris
KLK	kallikrein
LC	Langerhans cells
LEKTI	lymphoepithelial Kazal-type 5 serine protease inhibitor
NS	Netherton’s syndrome
OVA	ovalbumin

PAMPs	pathogen associated molecular patterns
PRR	pattern recognition receptors
PS	psoriasis
SPINK5	serine peptidase inhibitor Kazal type 5
SC	stratum corneum
SG	stratum granulosum
Th	T helper
TJ	Tight junction
TLR	toll-like receptor
TSLP	thymic stromal lymphopoietin

References

Akei HS, Brandt EB, Mishra A, et al. Epicutaneous aeroallergen exposure induces systemic TH2 immunity that predisposes to allergic nasal responses. J Allergy Clin Immunol. 2006; 118:62–9. [PubMed: 16815139]

Angelova-Fischer I, Fernandez IM, Donnadieu MH, et al. Injury to the stratum corneum induces in vivo expression of human thymic stromal lymphopoietin in the epidermis. J Invest Dermatol. 2010; 130:2505–7. [PubMed: 20555350]

Angkasekwinai P, Park H, Wang YH, et al. Interleukin 25 promotes the initiation of proallergic type 2 responses. J Exp Med. 2007; 204:1509–17. [PubMed: 17562814]

Arbesman CE, Wypych JI, Reisman RE, et al. IgE levels in sera of patients with pemphigus or bullous pemphigoid. Archives of dermatology. 1974; 110:378–81. [PubMed: 4217592]

Blanchard C, Stucke EM, Burwinkel K, et al. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. Journal of immunology. 2010; 184:4033–41.

Blank IH, Gould E. Penetration of anionic surfactants (surface active agents) into skin. I. Penetration of sodium laurate and sodium dodecyl sulfate into excised human skin. J Invest Dermatol. 1959; 33:327–36. [PubMed: 13801318]

Boralevi F, Hubiche T, Leaute-Labreze C, et al. Epicutaneous aeroallergen sensitization in atopic dermatitis infants - determining the role of epidermal barrier impairment. Allergy. 2008; 63:205–10. [PubMed: 18186810]

Borrego L, Peterson EA, Diez LI, et al. Polymorphic eruption of pregnancy and herpes gestationis: comparison of granulated cell proteins in tissue and serum. Clin Exp Dermatol. 1999; 24:213–25. [PubMed: 10354184]

Braff MH, Bardan A, Nizet V, et al. Cutaneous defense mechanisms by antimicrobial peptides. J Invest Dermatol. 2005; 125:9–13. [PubMed: 15982297]

Bremmer SF, Hanifin JM, Simpson EL. Clinical detection of ichthyosis vulgaris in an atopic dermatitis clinic: implications for allergic respiratory disease and prognosis. Journal of the American Academy of Dermatology. 2008; 59:72–8. [PubMed: 18455261]

Briot A, Deraison C, Lacroix M, et al. Kallikrein 5 induces atopic dermatitislike lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. J Exp Med. 2009; 206:1135–47. [PubMed: 19414552]

Briot A, Lacroix M, Robin A, et al. Par2 inactivation inhibits early production of TSLP, but not cutaneous inflammation, in Netherton syndrome adult mouse model. J Invest Dermatol. 2010; 130:2736–42. [PubMed: 20703245]

Brown SJ, Asai Y, Cordell HJ, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. J Allergy Clin Immunol. 2011a; 127:661–7. [PubMed: 21377035]

Brown SJ, Asai Y, Cordell HJ, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. J Allergy Clin Immunol. 2011b; 127:661–7. [PubMed: 21377035]

Brown SJ, Relton CL, Liao H, et al. Filaggrin null mutations and childhood atopic eczema: a population-based case-control study. J Allergy Clin Immunol. 2008; 121:940–46. e3. [PubMed: 18313126]

Bulek K, Swaidani S, Aronica M, et al. Epithelium: the interplay between innate and Th2 immunity. Immunol Cell Biol. 2010; 88:257–68. [PubMed: 20065993]

Bushkell LL, Jordon RE. Bullous pemphigoid: a cause of peripheral blood eosinophilia. J Am Acad Dermatol. 1983; 8:648–51. [PubMed: 6345605]

Bygum A, Westermark P, Brandrup F. Ichthyosis prematurity syndrome: a well-defined congenital ichthyosis subtype. J Am Acad Dermatol. 2008; 59:S71–4. [PubMed: 19119129]

Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol. 2005; 6:328–40. [PubMed: 15803139]

Capon F, Allen MH, Ameen M, et al. A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. Human molecular genetics. 2004; 13:2361–8. [PubMed: 15333584]

Christophers E, Kligman AM. Visualization of the cell layers of the stratum corneum. J Invest Dermatol. 1964a; 42:407–9. [PubMed: 14172195]

Christophers E, Kligman AM. Visualization of the Cell Layers of the Stratum Corneum. The Journal of investigative dermatology. 1964b; 42:407–9. [PubMed: 14172195]

Cork MJ, Danby SG, Vasilopoulos Y, et al. Epidermal barrier dysfunction in atopic dermatitis. J Invest Dermatol. 2009; 129:1892–908. [PubMed: 19494826]

Dale BA, Holbrook KA, Steinert PM. Assembly of stratum corneum basic protein and keratin filaments in macrofibrils. Nature. 1978; 276:729–31. [PubMed: 732879]

De Benedetto A, Agnihothri R, McGirt LY, et al. Atopic dermatitis: a disease caused by innate immune defects? J Invest Dermatol. 2009; 129:14–30. [PubMed: 19078985]

De Benedetto A, Qualia CM, Baroody FM, et al. Filaggrin expression in oral, nasal, and esophageal mucosa. J Invest Dermatol. 2008; 128:1594–7. [PubMed: 18172455]

De Benedetto A, Rafaels NM, McGirt LY, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol. 2011; 127:773–86. e1–7. [PubMed: 21163515]

De Pita O, Frezzolini A, Cianchini G, et al. T-helper 2 involvement in the pathogenesis of bullous pemphigoid: role of soluble CD30 (sCD30). Arch Dermatol Res. 1997; 289:667–70. [PubMed: 9452886]

Demehri S, Liu Z, Lee J, et al. Notch-deficient skin induces a lethal systemic B-lymphoproliferative disorder by secreting TSLP, a sentinel for epidermal integrity. PLoS Biol. 2008; 6:e123. [PubMed: 18507503]

Dickel H, Gambichler T, Kamphowe J, et al. Standardized tape stripping prior to patch testing induces upregulation of Hsp90, Hsp70, IL-33, TNF-alpha and IL-8/CXCL8 mRNA: new insights into the involvement of ‘alarmins’. Contact Dermatitis. 2010; 63:215–22. [PubMed: 20731692]

Eckert RL, Yaffe MB, Crish JF, et al. Involucrin--structure and role in envelope assembly. The Journal of investigative dermatology. 1993; 100:613–7. [PubMed: 8098344]

Elias PM. Epidermal lipids, membranes, and keratinization. Int J Dermatol. 1981; 20:1–19. [PubMed: 6162813]

Elias PM. Stratum corneum defensive functions: an integrated view. J Invest Dermatol. 2005; 125:183–200. [PubMed: 16098026]

Elias, PM.; Feingold, KR. Skin Barrier. Taylor and Francis; New York, USA: 2006.

Eyerich K, Pennino D, Scarponi C, et al. IL-17 in atopic eczema: linking allergen-specific adaptive and microbial-triggered innate immune response. J Allergy Clin Immunol. 2009; 123:59–66. e4. [PubMed: 19056110]

Fabbri P, Caproni M, Berti S, et al. The role of T lymphocytes and cytokines in the pathogenesis of pemphigoid gestationis. Br J Dermatol. 2003; 148:1141–8. [PubMed: 12828741]

Fallon PG, Sasaki T, Sandilands A, et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet. 2009; 41:602–8. [PubMed: 19349982]

Feliciani C, Toto P, Mohammad Pour S, et al. A Th2-like cytokine response is involved in bullous pemphigoid. the role of IL-4 and IL-5 in the pathogenesis of the disease. Int J Immunopathol Pharmacol. 1999; 12:55–61. [PubMed: 12783647]

Finkelman FD, Urban JF Jr. Cytokines: making the right choice. Parasitol Today. 1992; 8:311–4. [PubMed: 15463650]

Flohr C, England K, Radulovic S, et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. Br J Dermatol. 2010; 163:1333–6. [PubMed: 21137118]

Fluhr JW, Elias PM, Man MQ, et al. Is the filaggrin-histidine-urocanic acid pathway essential for stratum corneum acidification? J Invest Dermatol. 2010; 130:2141–4. [PubMed: 20376063]

Fort MM, Cheung J, Yen D, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity. 2001; 15:985–95. [PubMed: 11754819]

Frenk E, Mevorah B. Ichthyosis linearis circumflexa Comèl with Trichorrhexis invaginata (Netherton's Syndrom): an ultrastructural study of the skin changes. Arch Dermatol Forsch. 1972; 245:42–9. [PubMed: 4678924]

Furuse M, Hata M, Furuse K, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol. 2002; 156:1099–111. [PubMed: 11889141]

Gallo R. J Invest Dermatol. 2012

Gao PS, Rafaels NM, Hand T, et al. Filaggrin mutations that confer risk of atopic dermatitis confer greater risk for eczema herpeticum. The Journal of allergy and clinical immunology. 2009; 124:507–13. 13 e1–7. [PubMed: 19733298]

Goldschmidt H, Kligman AM. Exfoliative cytology of human horny layer. Methods of cell removal and microscopic techniques. Arch Dermatol. 1967; 96:572–6. [PubMed: 4167983]

Gruber R, Elias PM, Crumrine D, et al. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. Am J Pathol. 2011; 178:2252–63. [PubMed: 21514438]

Gupta J, Grube E, Ericksen MB, et al. Intrinsically defective skin barrier function in children with atopic dermatitis correlates with disease severity. J Allergy Clin Immunol. 2008; 121:725–30. e2. [PubMed: 18249438]

Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. J Immunol. 2008; 181:7420–7. [PubMed: 18981165]

Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis--part I: clinical and pathologic concepts. J Allergy Clin Immunol. 2011a; 127:1110–8. [PubMed: 21388665]

Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis--part II: immune cell subsets and therapeutic concepts. J Allergy Clin Immunol. 2011b; 127:1420–32. [PubMed: 21419481]

Hammerich L, Heymann F, Tacke F. Role of IL-17 and Th17 cells in liver diseases. Clin Dev Immunol. 2011; 2011:345803. [PubMed: 21197451]

Hanifin JM, Butler JM, Chan SC. Immunopharmacology of the atopic diseases. J Invest Dermatol. 1985; 85:161s–4s. [PubMed: 2409183]

Hashimoto K. Intercellular spaces of the human epidermis as demonstrated with lanthanum. J Invest Dermatol. 1971; 57:17–31. [PubMed: 4104141]

He R, Oyoshi MK, Jin H, et al. Epicutaneous antigen exposure induces a Th17 response that drives airway inflammation after inhalation challenge. Proc Natl Acad Sci U S A. 2007; 104:15817–22. [PubMed: 17893340]

Helms C, Saccone NL, Cao L, et al. Localization of PSORS1 to a haplotype block harboring HLA-C and distinct from corneodesmosin and HCR. Human genetics. 2005; 118:466–76. [PubMed: 16235096]

Herrick CA, MacLeod H, Glusac E, et al. Th2 responses induced by epicutaneous or inhalational protein exposure are differentially dependent on IL-4. J Clin Invest. 2000; 105:765–75. [PubMed: 10727445]

Herrick CA, Xu L, McKenzie AN, et al. IL-13 is necessary, not simply sufficient, for epicutaneously induced Th2 responses to soluble protein antigen. J Immunol. 2003; 170:2488–95. [PubMed: 12594274]

Holgate ST. The epithelium takes centre stage in asthma and atopic dermatitis. Trends Immunol. 2007; 28:248–51. [PubMed: 17466594]

Hon KL, Wong KY, Leung TF, et al. Comparison of skin hydration evaluation sites and correlations among skin hydration, transepidermal water loss, SCORAD index, Nottingham Eczema Severity Score, and quality of life in patients with atopic dermatitis. Am J Clin Dermatol. 2008; 9:45–50. [PubMed: 18092843]

Hsu CL, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. PLoS One. 2010; 5:e11944. [PubMed: 20689814]

Hubiche T, Ged C, Benard A, et al. Analysis of SPINK 5, KLK 7 and FLG genotypes in a French atopic dermatitis cohort. Acta Derm Venereol. 2007; 87:499–505. [PubMed: 17989887]

Humphreys NE, Xu D, Hepworth MR, et al. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. J Immunol. 2008; 180:2443–9. [PubMed: 18250453]

Hvid M, Vestergaard C, Kemp K, et al. IL-25 in atopic dermatitis: a possible link between inflammation and skin barrier dysfunction? J Invest Dermatol. 2011; 131:150–7. [PubMed: 20861853]

Israeli S, Zamir H, Sarig O, et al. Inflammatory peeling skin syndrome caused by a mutation in CDSN encoding corneodesmosin. J Invest Dermatol. 2011; 131:779–81. [PubMed: 21191406]

Janeway CA Jr. Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002; 20:197–216. [PubMed: 11861602]

Jenisch S, Westphal E, Nair RP, et al. Linkage disequilibrium analysis of familial psoriasis: identification of multiple disease-associated MHC haplotypes. Tissue antigens. 1999; 53:135–46. [PubMed: 10090613]

Jin H, He R, Oyoshi M, et al. Animal models of atopic dermatitis. J Invest Dermatol. 2009; 129:31–40. [PubMed: 19078986]

Jungersted JM, Scheer H, Mempel M, et al. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. Allergy. 2010; 65:911–8. [PubMed: 20132155]

Kezic S, Kemperman PM, Koster ES, et al. Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. J Invest Dermatol. 2008; 128:2117–9. [PubMed: 18305568]

Kinoshita H, Takai T, Le TA, et al. Cytokine milieu modulates release of thymic stromal lymphopoietin from human keratinocytes stimulated with double-stranded RNA. J Allergy Clin Immunol. 2009; 123:179–86. [PubMed: 19056108]

Klar J, Schweiger M, Zimmerman R, et al. Mutations in the fatty acid transport protein 4 gene cause the ichthyosis prematurity syndrome. Am J Hum Genet. 2009; 85:248–53. [PubMed: 19631310]

Kligman, AM. A brief history of how the dead stratum corneum became alive. In: Elias, PM.; Feingold, KR., editors. Skin barrier. Taylor & Francis Group; 2006.

Kligman AM. Corneobiology and corneotherapy--a final chapter. International journal of cosmetic science. 2011; 33:197–209. [PubMed: 21382057]

Koga C, Kabashima K, Shiraishi N, et al. Possible pathogenic role of Th17 cells for atopic dermatitis. J Invest Dermatol. 2008; 128:2625–30. [PubMed: 18432274]

Kondo H, Ichikawa Y, Imokawa G. Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. Eur J Immunol. 1998; 28:769–79. [PubMed: 9541570]

Kong H, Segre J. Skin Microbiome: Looking Back to Look Forward. J Invest Dermatol. 2012

Kouzaki H, O'Grady SM, Lawrence CB, et al. Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2. J Immunol. 2009; 183:1427–34. [PubMed: 19561109]

Krien PM, Kermici M. Evidence for the existence of a self-regulated enzymatic process within the human stratum corneum -an unexpected role for urocanic acid. J Invest Dermatol. 2000; 115:414–20. [PubMed: 10951277]

Kubo A, Nagao K, Yokouchi M, et al. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. J Exp Med. 2009; 206:2937–46. [PubMed: 19995951]

Kusunoki T, Okafuji I, Yoshioka T, et al. SPINK5 polymorphism is associated with disease severity and food allergy in children with atopic dermatitis. J Allergy Clin Immunol. 2005; 115:636–8. [PubMed: 15753919]

Le TA, Takai T, Vu AT, et al. Glucocorticoids inhibit double-stranded RNA-induced thymic stromal lymphopoietin release from keratinocytes in an atopic cytokine milieu more effectively than tacrolimus. Int Arch Allergy Immunol. 2010; 153:27–34. [PubMed: 20357482]

Lee CH, Chuang HY, Shih CC, et al. Transepidermal water loss, serum IgE and beta-endorphin as important and independent biological markers for development of itch intensity in atopic dermatitis. The British journal of dermatology. 2006; 154:1100–7. [PubMed: 16704640]

Lessard RJ, Wolff K, Winkelmann RK. Induced “shedding” of the epidermal Langerhans’s cells. Nature. 1966; 212:628–9. [PubMed: 5971694]

Lessard RJ, Wolff K, Winkelmann RK. The disappearance and regeneration of Langerhans cells following epidermal injury. J Invest Dermatol. 1968; 50:171–9. [PubMed: 5644195]

Li DQ, Zhang L, Pflugfelder SC, et al. Short ragweed pollen triggers allergic inflammation through Toll-like receptor 4-dependent thymic stromal lymphopoietin/OX40 ligand/OX40 signaling pathways. J Allergy Clin Immunol. 2011

Ma P, Bian F, Wang Z, et al. Human corneal epithelium-derived thymic stromal lymphopoietin links the innate and adaptive immune responses via TLRs and Th2 cytokines. Invest Ophthalmol Vis Sci. 2009; 50:2702–9. [PubMed: 19151401]

Madison KC. Barrier function of the skin: “la raison d’etre” of the epidermis. The Journal of investigative dermatology. 2003; 121:231–41. [PubMed: 12880413]

Malkinson FD. Studies on the percutaneous absorption of C14 labeled steroids by use of the gas-flow cell. J Invest Dermatol. 1958; 31:19–28. [PubMed: 13563939]

McGrath JA, Schofield OM, Eady RA. Epidermolysis bullosa pruriginosa: dystrophic epidermolysis bullosa with distinctive clinicopathological features. Br J Dermatol. 1994; 130:617–25. [PubMed: 8204470]

Mellerio JE, Ashton GH, Mohammedi R, et al. Allelic heterogeneity of dominant and recessive COL7A1 mutations underlying epidermolysis bullosa pruriginosa. J Invest Dermatol. 1999; 112:984–7. [PubMed: 10383749]

Menon GK, Elias PM. Morphologic basis for a pore-pathway in mammalian stratum corneum. Skin Pharmacol. 1997; 10:235–46. [PubMed: 9449162]

Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nat Rev Immunol. 2008; 8:935–47. [PubMed: 19029989]

Miajlovic H, Fallon PG, Irvine AD, et al. Effect of filaggrin breakdown products on growth of and protein expression by Staphylococcus aureus. The Journal of allergy and clinical immunology. 2010; 126:1184–90. e3. [PubMed: 21036388]

Milger K, Herrmann T, Becker C, et al. Cellular uptake of fatty acids driven by the ER-localized acyl-CoA synthetase FATP4. J Cell Sci. 2006; 119:4678–88. [PubMed: 17062637]

Monash S. Location of the superficial epithelial barrier to skin penetration. J Invest Dermatol. 1957; 29:367–76. [PubMed: 13502592]

Moulson CL, Lin MH, White JM, et al. Keratinocyte-specific expression of fatty acid transport protein 4 rescues the wrinkle-free phenotype in Slc27a4/Fatp4 mutant mice. J Biol Chem. 2007; 282:15912–20. [PubMed: 17401141]

Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel ‘alarmin’? PLoS One. 2008; 3:e3331. [PubMed: 18836528]

Nagel A, Lang A, Engel D, et al. Clinical activity of pemphigus vulgaris relates to IgE autoantibodies against desmoglein 3. Clin Immunol. 2010; 134:320–30. [PubMed: 20015693]

Nemoto-Hasebe I, Akiyama M, Nomura T, et al. Clinical severity correlates with impaired barrier in filaggrin-related eczema. J Invest Dermatol. 2009; 129:682–9. [PubMed: 18818676]

Nickoloff BJ, Naidu Y. Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. J Am Acad Dermatol. 1994; 30:535–46. [PubMed: 7512582]

Niemi KM, Kuokkanen K, Kanerva L, et al. Recessive ichthyosis congenita type IV. Am J Dermatopathol. 1993; 15:224–8. [PubMed: 7685984]

Nishijima T, Tokura Y, Imokawa G, et al. Altered permeability and disordered cutaneous immunoregulatory function in mice with acute barrier disruption. J Invest Dermatol. 1997; 109:175–82. [PubMed: 9242504]

Nograles KE, Suarez-Farinas M, Shemer A, et al. Atopic dermatitis keratinocytes exhibit normal T(H)17 cytokine responses. J Allergy Clin Immunol. 2010; 125:744–6. 6 e1–6 e2. [PubMed: 20226306]

Nograles KE, Zaba LC, Shemer A, et al. IL-22-producing “T22” T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. J Allergy Clin Immunol. 2009; 123:1244–52. e2. [PubMed: 19439349]

Novak N, Bieber T, Kraft S. Immunoglobulin E-bearing antigen-presenting cells in atopic dermatitis. Current allergy and asthma reports. 2004; 4:263–9. [PubMed: 15175139]

O’Regan GM, Irvine AD. The role of filaggrin in the atopic diathesis. Clin Exp Allergy. 2010; 40:965–72. [PubMed: 20642575]

O’Regan GM, Kemperman PM, Sandilands A, et al. Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. J Allergy Clin Immunol. 2010; 126:574–80. e1. [PubMed: 20621340]

Oboki K, Nakae S, Matsumoto K, et al. IL-33 and Airway Inflammation. Allergy Asthma Immunol Res. 2011; 3:81–8. [PubMed: 21461246]

Oji V, Eckl K-M, Aufenvenne K, et al. Loss of corneodesmosin leads to severe skin barrier defect, pruritus, and atopy: unraveling the peeling skin disease. Am J Hum Genet. 2010; 87:274–81. [PubMed: 20691404]

Oji V, Seller N, Sandilands A, et al. Ichthyosis vulgaris: novel FLG mutations in the German population and high presence of CD1a+ cells in the epidermis of the atopic subgroup. Br J Dermatol. 2009; 160:771–81. [PubMed: 19183181]

Ovaere P, Lippens S, Vandenabeele P, et al. The emerging roles of serine protease cascades in the epidermis. Trends Biochem Sci. 2009; 34:453–63. [PubMed: 19726197]

Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. J Allergy Clin Immunol. 2009; 124:485–93. 93 e1. [PubMed: 19665780]

Page K, Lierl KM, Hughes VS, et al. TLR2-mediated activation of neutrophils in response to German cockroach frass. J Immunol. 2008; 180:6317–24. [PubMed: 18424755]

Palmer CNA, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet. 2006; 38:441–6. [PubMed: 16550169]

Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat Rev Immunol. 2010; 10:225–35. [PubMed: 20336151]

Presland RB, Boggess D, Lewis SP, et al. Loss of normal profilaggrin and filaggrin in flaky tail (ft/ft) mice: an animal model for the filaggrin-deficient skin disease ichthyosis vulgaris. J Invest Dermatol. 2000; 115:1072–81. [PubMed: 11121144]

Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Experimental dermatology. 2008; 17:1063–72. [PubMed: 19043850]

Pushparaj PN, Tay HK, H’Ng SC, et al. The cytokine interleukin-33 mediates anaphylactic shock. Proc Natl Acad Sci U S A. 2009; 106:9773–8. [PubMed: 19506243]

Rawlings AV, Matts PJ. Stratum corneum moisturization at the molecular level: an update in relation to the dry skin cycle. J Invest Dermatol. 2005; 124:1099–110. [PubMed: 15955083]

Rothman, S. Physiology and Biochemistry of Skin. University of Chicago Press; Chicago, IL: 1954.

Rothman S, Flesch P. The Physiology of the Skin. Annual review of physiology. 1944; 6:195–224.

Runswick S, Mitchell T, Davies P, et al. Pollen proteolytic enzymes degrade tight junctions. Respirology. 2007; 12:834–42. [PubMed: 17986111]

Sandilands A, Sutherland C, Irvine AD, et al. Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci. 2009; 122:1285–94. [PubMed: 19386895]

Scheuplein RJ, Blank IH. Permeability of the skin. Physiol Rev. 1971; 51:702–47. [PubMed: 4940637]

Schmidt M, Raghavan B, Muller V, et al. Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel. Nat Immunol. 2010; 11:814–9. [PubMed: 20711192]

Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005; 23:479–90. [PubMed: 16286016]

Schuttelaar ML, Kerkhof M, Jonkman MF, et al. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. Allergy. 2009; 64:1758–65. [PubMed: 19839980]

Scott IR, Harding CR. Filaggrin breakdown to water binding compounds during development of the rat stratum corneum is controlled by the water activity of the environment. Dev Biol. 1986; 115:84–92. [PubMed: 3516761]

Silver MR, Margulis A, Wood N, et al. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. Inflamm Res. 2010; 59:207–18. [PubMed: 19763788]

Smith FJD, Irvine AD, Terron-Kwiatkowski A, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet. 2006; 38:337–42. [PubMed: 16444271]

Sobol M, Dahl N, Klar J. FATP4 missense and nonsense mutations cause similar features in Ichthyosis Prematurity Syndrome. BMC Res Notes. 2011; 4:90. [PubMed: 21450060]

Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol. 2002; 3:673–80. [PubMed: 12055625]

Spergel JM. From atopic dermatitis to asthma: the atopic march. Ann Allergy Asthma Immunol. 2010; 105:99–106. quiz 7-9, 17. [PubMed: 20674819]

Spergel JM, Mizoguchi E, Brewer JP, et al. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. J Clin Invest. 1998; 101:1614–22. [PubMed: 9541491]

Streilein JW, Lonsberry LW, Bergstresser PR. Depletion of epidermal langerhans cells and Ia immunogenicity from tape-stripped mouse skin. J Exp Med. 1982; 155:863–71. [PubMed: 6460830]

Strid J, Callard R, Strobel S. Epicutaneous immunization converts subsequent and established antigen-specific T helper type 1 (Th1) to Th2-type responses. Immunology. 2006; 119:27–35. [PubMed: 16764688]

Strid J, Hourihane J, Kimber I, et al. Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. Eur J Immunol. 2004; 34:2100–9. [PubMed: 15259007]

Strid J, Hourihane J, Kimber I, et al. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. Clin Exp Allergy. 2005; 35:757–66. [PubMed: 15969667]

Suarez-Farinas M, Tintle SJ, Shemer A, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. J Allergy Clin Immunol. 2011; 127:954–64. e1–4. [PubMed: 21388663]

Swartzendruber DC, Wertz PW, Madison KC, et al. Evidence that the corneocyte has a chemically bound lipid envelope. J Invest Dermatol. 1987; 88:709–13. [PubMed: 3585054]

Tai HY, Tam MF, Chou H, et al. Pen ch 13 allergen induces secretion of mediators and degradation of occludin protein of human lung epithelial cells. Allergy. 2006; 61:382–8. [PubMed: 16436150]

Takai T, Ikeda S. Barrier dysfunction caused by environmental proteases in the pathogenesis of allergic diseases. Allergol Int. 2011; 60:25–35. [PubMed: 21173566]

Trompette A, Divanovic S, Visintin A, et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. Nature. 2009; 457:585–8. [PubMed: 19060881]

Tsukita S, Furuse M. Claudin-based barrier in simple and stratified cellular sheets. Curr Opin Cell Biol. 2002; 14:531–6. [PubMed: 12231346]

Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol. 2001; 2:285–93. [PubMed: 11283726]

Turksen K, Troy T-C. Permeability barrier dysfunction in transgenic mice overexpressing claudin 6. Development. 2002; 129:1775–84. [PubMed: 11923212]

van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. BMJ. 2009; 339:b2433. [PubMed: 19589816]

Walley AJ, Chavanas S, Moffatt MF, et al. Gene polymorphism in Netherton and common atopic disease. Nat Genet. 2001; 29:175–8. [PubMed: 11544479]

Wan H, Winton HL, Soeller C, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J Clin Invest. 1999; 104:123–33. [PubMed: 10393706]

Wang LF, Lin JY, Hsieh KH, et al. Epicutaneous exposure of protein antigen induces a predominant Th2-like response with high IgE production in mice. J Immunol. 1996; 156:4077–82. [PubMed: 8666772]

Wang YH, Angkasekwinai P, Lu N, et al. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. J Exp Med. 2007; 204:1837–47. [PubMed: 17635955]

Warner RR, Boissy YL, Lilly NA, et al. Water disrupts stratum corneum lipid lamellae: damage is similar to surfactants. J Invest Dermatol. 1999; 113:960–6. [PubMed: 10594737]

Weidinger S, Illig T, Baurecht H, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol. 2006; 118:214–9. [PubMed: 16815158]

Weidinger S, O'Sullivan M, Illig T, et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol. 2008; 121:1203–9. e1. [PubMed: 18396323]

Wells RS. Ichthyosis. Br Med J. 1966; 2:1504–6. [PubMed: 5928942]

Wilson NJ, Boniface K, Chan JR, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol. 2007; 8:950–7. [PubMed: 17676044]

Wood LC, Jackson SM, Elias PM, et al. Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. J Clin Invest. 1992; 90:482–7. [PubMed: 1644919]

Yamasaki H, Tada J, Yoshioka T, et al. Epidermolysis bullosa pruriginosa (McGrath) successfully controlled by oral cyclosporin. Br J Dermatol. 1997; 137:308–10. [PubMed: 9292092]

Ying S, Meng Q, Corrigan CJ, et al. Lack of filaggrin expression in the human bronchial mucosa. J Allergy Clin Immunol. 2006; 118:1386–8. [PubMed: 17157670]

Zhang Z, Hener P, Frossard N, et al. Thymic stromal lymphopoietin overproduced by keratinocytes in mouse skin aggravates experimental asthma. Proc Natl Acad Sci U S A. 2009; 106:1536–41. [PubMed: 19188585]

Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. Nat Immunol. 2010; 11:289–93. [PubMed: 20300138]

Zimmerli S, Hauser C. Langerhans cells and lymph node dendritic cells express the tight junction component claudin-1. J Invest Dermatol. 2007; 127:2381–90. [PubMed: 17508021]

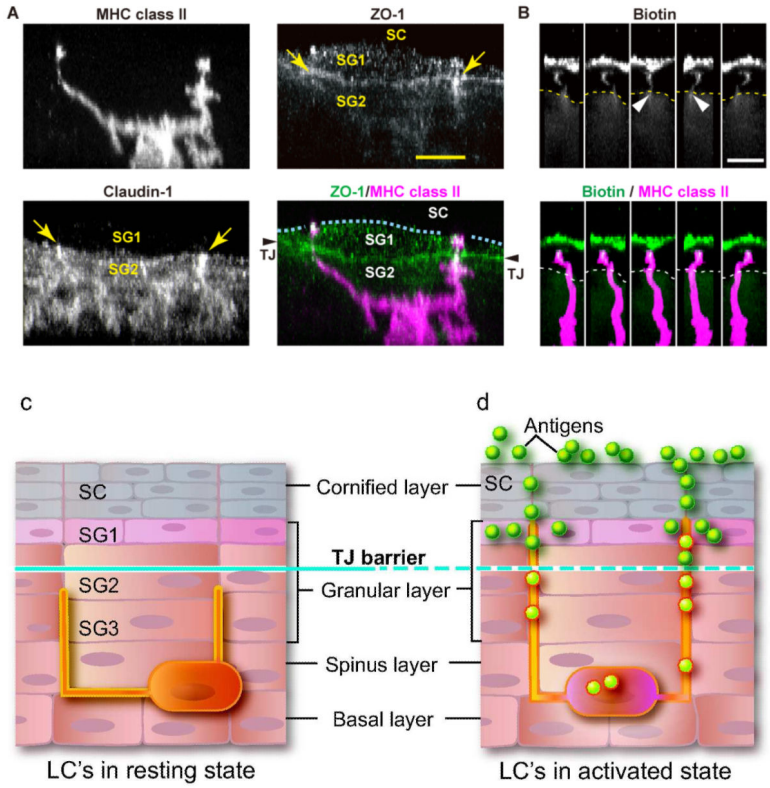

Figure 1. TJ penetration of LC dendrites

(**a** and **c**) Layers of the stratum granulosum (SG) are designated SG1, SG2, and SG3, counting from the skin surface inwards. In murine epidermis TJs are found in the intercellular spaces between SG2 cells. Blue dotted lines represent TJs. (**b** and **d**) Activated LCs (e.g. MHC class II positive) elongate their dendrites to dock with and penetrate epidermal TJs. (**a**) ZO-1 and claudin-1 accumulate at penetration points (yellow arrows), where novel tricellular TJs are formed between LC dendrites and surrounding keratinocytes to prevent significant barrier disturbance. (**b**) Rotated views of an activated LC dendrite are shown. EZ-link sulfo-NHS-LC-biotin was applied topically on mouse skin. Trace amounts of this biotin tracer are observed in LC dendrites that have crossed the TJ barrier (arrowheads; upper **b**). (The images were originally published in JOURNAL OF EXPERIMENTAL MEDICINE (Doi: 10.1084/jem.20091527 (Kubo *et al.*, 2009)).

doi: 10.1084/jem.20091527

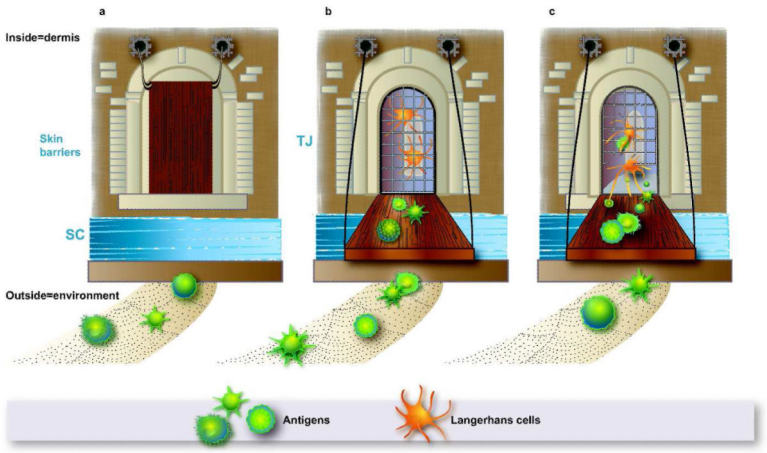

Figure 2.

The epidermis has two formidable barrier structures that are analogous to a castle's moat (SC; blue water) and portcullis (TJ; white gate). Epicutaneous sensitization requires that the antigen be engulfed by an antigen presenting cell such as an epidermal dendritic cell or Langerhans cell (LC). (**a**) Under resting conditions the immune system does not respond to environmental factors such as allergens. (**b**) When the SC is breached (e.g. drawbridge is down) allergens may cross the moat, but will still be blocked by an intact TJ barrier (portcullis). LC dendrites are found below TJ (e.g. behind the portcullis). (**c**) When the portcullis is opened (TJ loosened), LC dendrites extend through these weakened TJ and take up allergens and initiate an adaptive immune response. Additionally, it is hypothesized that small allergens may penetrate leaky TJ and be taken up by LC/DC whose dendrites are below the TJ. It is not clear whether a transient break in both epidermal barriers (SC and TJ) are required for LC dendrites to penetrate TJs. This dual barrier system uniquely found in the skin may explain why we do not respond to a myriad of antigens that reach our skin surface daily and therefore why our skin is usually uninflammed.

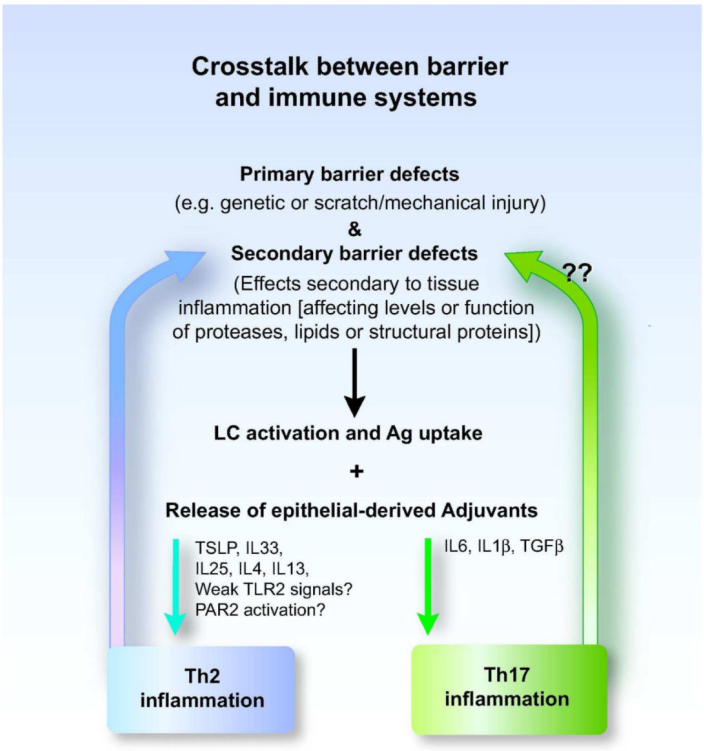

Figure 3. Epidermal barrier function and immune responses are tightly linked

Primary barrier defects lead to the release of a number of epidermal-derived mediators including ones that are considered pro-Th2 and pro-Th17. Some of these cytokines are released in an autocrine fashion through proteinase-activated receptors (PAR)-2 activation or by epithelial–derived “danger signals” that act on innate immune receptors expressed on keratinocytes. The presumption is that barrier disruption comes in different flavors each resulting in a specific adjuvant profile that would either favor a Th2, Th17 or another adaptive immune response to an antigen. Several Th2 and Th17 products establish an autocrine feedback loop and further aggravate barrier disruption (e.g. secondary barrier defects). To date, this has been best characterized for Th2 cytokines (Huppert *et al.*, 2010; Sehra *et al.*, 2010).

Table I

Epidermal barrier defects and allergen sensitization in human diseases

Human disease	*Barrier-related defects*	*Local Immune phenotype*	*ATOPY/ Th2 immune response*	*Reference (PMID)*
Desquamation abnormalities				
Netherton's syndrome	↓ SPINK5; ↑ activity of proteases (ELA2, KLK5, 7 and 14); abnormal FLG processing & lipid lamellae	↑ TSLP	Association with atopic disorders and ↑ IgE	10835624 19414552 20179351
Peeling Skin Type B	↓ CDSN	ND	Association with atopic disorders, ↑ IgE and ↑ EOS	21191406 20691404
Disturbed Lipid Metabolism				
Ichthyosis Prematurity Syndrome	↓ FATP4; membrane inclusions in SC	ND	Association with AD-like condition, ↑ EOS	19119129
Abnormalities in SC structural proteins				
Ichthyosis Vulgaris	↓ or absent FLG	ND	Association with atopic disorders. ↑IgE	16550169 18159904
Atopic Dermatitis	Dysregulated EDC (including ↓ FLG); ↓ proteases inhibitors and ↑ proteases; lipid abnormalities; TJ abnormalities, ↓ CLDN1 and 23, ↑ Cx26	↑ pro-Th2 cytokines (e.g. TSLP, IL25, IL33); Th2, Th22 and Th17 (only in acute lesions); Th2 and Th1 in chronic lesions.	Association with atopic disorders, ↑ IgE and ↑ EOS	16550169 18396323 19494826 21163515 21388665 21419481
Psoriasis	Dysregulated EDC	Th1 and Th17	Rarely observe modest ↑ IgE	18432274 21388665 21419481
Full thickness epidermal disruption				
Bullous Pemphigoid	Subepidermal blisters	Th2 cytokines, tissue eosinophils (early lesions)	↑ IgE and ↑ EOS	6345605 9452886 12783647
Pemphigus Vulgaris	Suprabasilar acantholysis	Th2 cytokines, tissue eosinophils (early lesions)	↑ IgE	4217592 20015693 11161984
Epidermolysis bullosa pruriginosa	Anchoring fibril abnormalities, mutation in COL7A1	ND	↑ IgE	8204470

Abbreviations: Abs, antibodies; CDSN, corneodesmosin;CLDN, claudin; COL7A1, collagen, type VII, alpha-1; Cx26, connexin 26; EDC, epidermal differentiation complex; ELA2, elastase 2; EOS, eosinophilia; FATP4, fatty acid transport protein 4; FLG, filaggrin; KLK, kallikrein; ND, not determined; SPINK5, serine peptidase inhibitor Kazal type 5; SC, stratum corneum; TJ, Tight junction; TSLP, thymic stromal lymphopoietin.

Table 2

Overview of transgenic mouse models characterized by epidermal barrier defects and atopy

Mouse model	*Barrier-related defects*	*BARRIER effects*	*ATOPY/ Th2 immune response*	*Reference (PMID)*
Cornified envelope/SC				
SPINK5$^{R820X/R820X}$	Absence of LEKTI; ↑ FLG monomers	Detachment of SC; dehydration	ND; ↑ TSLP; Early death	15590704
LOR−/−	Absence of L-granule	≈ percutaneous dye penetration (outside-in barrier) after E17.5; ≈ TEWL.	ND	11038185
Intercellular junctions				
CLDN1−/−	↓ CLDN1	↑ TEWL; ↑ biotin permeability (inside-out barrier)	ND	11889141
(k14-cre)Ecadherin^{Fl}	Abnormal TJ; ↓ CLDN1	↑ TEWL; ≈ percutaneous dye penetration (outside-in barrier); ↑ biotin permeability (inside-out barrier)	ND	15775979
Inv-CLDN6-tg	↑ CLDN6; ↓ CLDN1 and abnormal differentiation	ND, Lethal	ND	17914196
Inv-DSG3-tg	Thin SC with a compact lamellar pattern; Loss of corneocyte adhesion	↑ TEWL;	ND	11309406
Inv-Cx26-tg	↑ gap junctions in SB	↑ TEWL; ↑ percutaneous dye penetration (outside-in barrier)	ND	16628254
Transcription Factors				
(k14-cre)IKK1^{EKO}	↓ CLDN23; ↓ OCLD; ↑ DSG3; Lipids abnormalities in SC	↑ TEWL; ↑ percutaneous dye penetration (outside-in barrier)	ND	17351639
p63 null	↓ CLDN1; SC abnormality	↑ TEWL	ND	18648642
RARα mutant	Absence of multilamellar structure	↑ TEWL	ND	7867929
Spontaneous dermatitis				
NC/Nga (ARC)	Unknown; spontaneous dermatitis at week 8	↑ TEWL	After EC	11167677

Mouse model	*Barrier-related defects*	*BARRIER effects*	*ATOPY/ Th2 immune response*	*Reference (PMID)*
Flaky tail (ft/ma)	Lack FLG; unknown matted phenotype	≈ basal TEWL	After EC	11121144
Flaky tail (ft/ft)	Lack FLG	≈ basal TEWL	After EC	19349982
Miscellaneous				
Epidermal IL4-tg	Spontaneous dermatitis	ND	↑ IgE; asthma	11676841
APOC1+/+	Lipid abnormalities	↑ TEWL	↑ IgE	18049452
ADAM10−/−	Reduction of SS	↑ TEWL	↑ TSLP	21205794

ARC, air-regulated conditions; ADAM, A Disintegrin And Metalloprotease; APOC1, apolipoprotein C1; CLDN, claudin; Cx26, connexin26; DSG, desmoglin; EC: epicutaneous allergen sensitization; FLG, filaggrin; IKK1, I-kappa-B kinase 1; Inv, involucrin; ND: not determined; LEKTI, lymphoepithelial Kazal-type-related inhibitor; LOR, loricrin; OCLD, occludin; RAR, retinoic acid receptor; SB, stratum basale; SC, stratum corneum; SS, spinous layers; TEWL, trans epidermal water loss; tg, transgenic; TJ, Tight junction; TSLP, thymic stromal lymphopoietin; ≈, no difference.