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Type 2 Inflammation Contributes to Skin Barrier Dysfunction in Atopic Dermatitis

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Skin barrier dysfunction, a defining feature of atopic dermatitis (AD), arises from multiple interacting systems. In AD, skin inflammation is caused by host–environment interactions involving keratinocytes as well as tissue-resident immune cells such as type 2 innate lymphoid cells, basophils, mast cells, and T helper type 2 cells, which produce type 2 cytokines, including IL-4, IL-5, IL-13, and IL-31. Type 2 inflammation broadly impacts the expression of genes relevant for barrier function, such as intracellular structural proteins, extracellular lipids, and junctional proteins, and enhances *Staphylococcus aureus* skin colonization. Systemic anti-type 2 inflammation therapies may improve dysfunctional skin barrier in AD.

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INTRODUCTION

Atopic dermatitis (AD) is a chronic pruritic inflammatory skin disease, whose pathogenesis is mediated by interactions between skin barrier impairment and an abnormal immune response featuring enhanced type 2 inflammation (Figure 1). Interactions between keratinocytes (KCs), innate immune cells (e.g., type 2 innate lymphoid cells [ILC2s], dendritic cells, mast cells, basophils, and eosinophils), adaptive

immune cells (T and B cells), and an altered epidermal microbiome (with reduction of microbial diversity and predominance of *Staphylococcus aureus*) all contribute to AD pathogenesis (De Benedetto et al., 2015; Dillon et al., 2004; Gittler et al., 2012; Gschwandtner et al., 2013; Jarrett et al., 2016; Kim et al., 2014; Kong et al., 2012; Mashiko et al., 2017; Onoue et al., 2009; Oyoshi et al., 2010; Sokol et al., 2008; Sonkoly et al., 2006).

Type 2 inflammation is characterized by overexpression of the cytokines IL-4, IL-5, IL-13, and IL-31 (Giustizieri et al., 2001; Gros et al., 2009; Hamid et al., 1996, 1994; Hardman et al., 2017; Kim et al., 2014; Leyva-Castillo et al., 2013; Mashiko et al., 2017; Neill et al., 2010; Oyoshi et al., 2010; Pivarcsi et al., 2004; Schmitz et al., 2005; Sokol et al., 2008; Stott et al., 2013). These cytokines, particularly IL-4 and IL-13, act on both structural and immune cells (Figure 1). IL-4 and IL-13 signaling serves as a key initiating pathway for type 2 inflammatory diseases, whereas IL-4 amplifies the allergic inflammation observed in type 2 inflammatory diseases, including AD, asthma, allergic rhinitis, food allergy, and eosinophilic esophagitis (Davidson et al., 2019; Irvine and Mina-Osorio, 2019; Izuha et al., 2002).

The functions of IL-4 and IL-13 overlap but are not identical (Figure 2). IL-4 and, to a lesser extent, IL-13 regulate class switching and IgE production by plasma cells (Gascan et al., 1991; Punnonen et al., 1993). IL-4 but not IL-13 promotes the differentiation of T helper (Th) cells from Th0 to Th2 cells (Gandhi et al., 2016; Paul, 2015; Swain et al., 1990). Both IL-4 and IL-13 induce different transcriptional changes in mast cells (McLeod et al., 2015; Nilsson and Nilsson, 1995) and are associated with fibrotic processes (Bhagal and Bona, 2008; Elbe-Bürger et al., 2002; Fichtner-Feigl et al., 2006; Gillery et al., 1992; Jessup et al., 2008; Kaviratne et al., 2004; Kolodsick et al., 2004; Oh et al., 2011; Oriente et al., 2000; Postlethwaite et al., 1992; Rankin et al., 2010; Zheng et al., 2009). They also activate Th0 cells, recruit inflammatory effector cells, downregulate the expression of FLG and other skin barrier proteins, and, at least in murine models, favor *S. aureus* colonization in inflamed skin (Bao and Reinhart, 2015; Cho et al., 2001a; Le Floc'h et al., 2020; Leyva-Castillo et al., 2020; Liang et al., 2011; Mitamura et al., 2018a, 2018b; Swain et al., 1990). In addition, IL-4 and IL-13 directly act on sensory neurons, increasing their sensitivity to several pruritogens and contributing to the perpetuation of chronic itch in AD (Oetjen et al., 2017).

In this paper, we review the role of the components relevant to a functional skin barrier and highlight how skin barrier dysfunction promotes the development of type 2

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Abbreviations: AD, atopic dermatitis; AMP, antimicrobial peptide; CLDN, claudin; FFA, free fatty acid; hBD, human β -defensin; ILC2, type 2 innate lymphoid cell; Jaki, Jak inhibitor; K, keratin; KC, keratinocyte; MMP, matrix metalloproteinase; NMF, natural moisturizing factor; PAR, protease-activated receptor; PDE-4, phosphodiesterase-4; SC, stratum corneum; SG, stratum granulosum; TCI, topical calcineurin inhibitor; TCS, topical corticosteroid; TEWL, transepidermal water loss; Th, T helper; TJ, tight junction; TLR, toll-like receptor; TNF- α , tumor necrosis factor alpha; TYK, tyrosine kinase; ZO, zona occludens

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Figure 1. Type 2 inflammation: primary immune cells, key cytokines and alarmins, exogenous targets, and representative diseases. AD is a predominantly type 2 inflammatory disease. Other type 2 allergic diseases include allergic rhinitis, asthma, CRSwNP, eosinophilic esophagitis, and food allergy. ¹IL-25 is also known as IL-17E. AD, atopic dermatitis; CRSwNP, chronic rhinosinusitis with nasal polyps; ILC, innate lymphoid cell; TFH, T-follicular helper; Th, T helper.

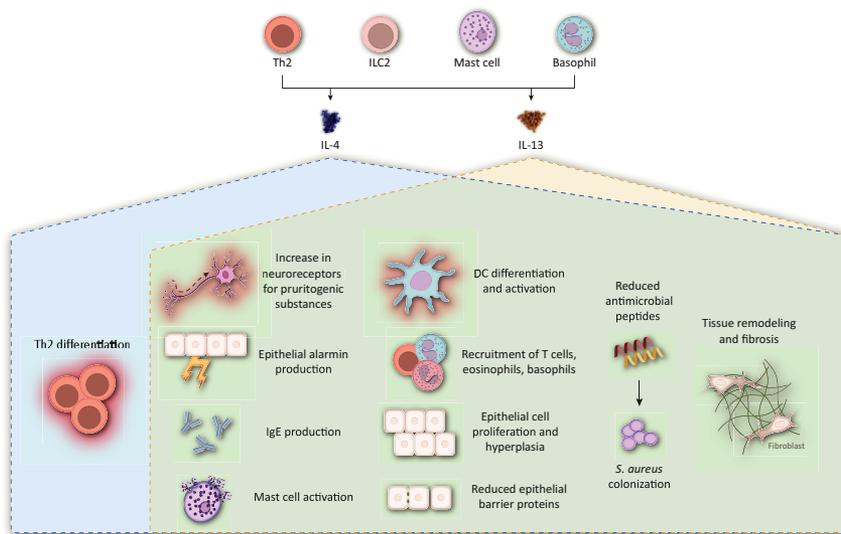
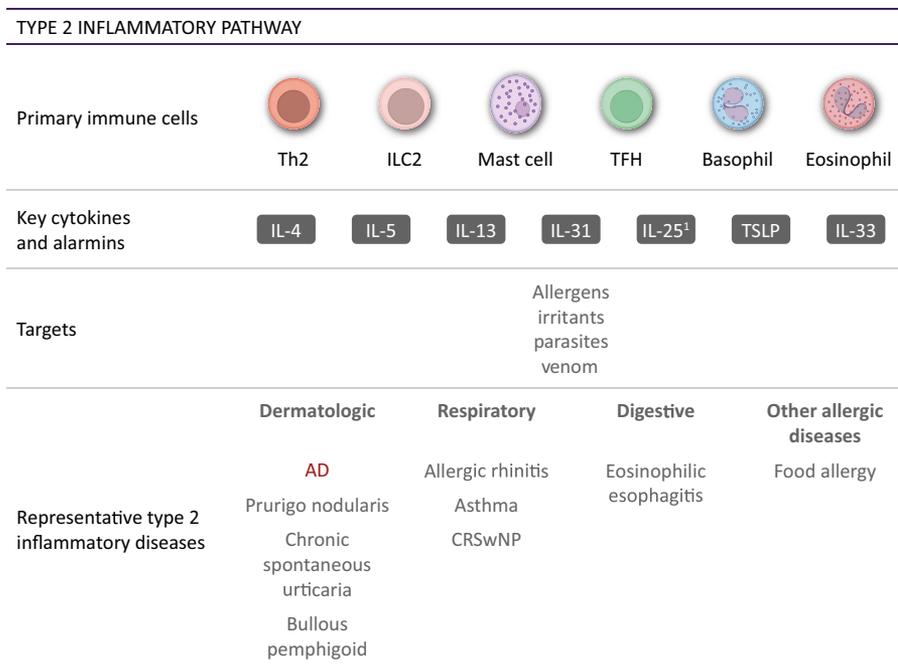


Figure 2. IL-4 and IL-13 have overlapping but not identical functions; for example, both mediate inflammation and barrier dysfunction through immunologic and structural changes, both enhance pruritogenic pathways, and both promote *Staphylococcus aureus* colonization in AD. However, IL-4 but not IL-13 promotes the differentiation of Th cells from Th0 to Th2 cells (Berdyshev et al., 2018; Campion et al., 2019; Chen et al., 2004; Danso et al., 2014; Finkelman et al., 1988; Gandhi et al., 2016; Goleva et al., 2019; Guttman-Yassky et al., 2019a; Hamilton et al., 2021, 2014; Howell et al., 2008; Jessup et al., 2008; Kim et al., 2008; Paul, 2015; Suárez-Fariñas et al., 2013; Swain et al., 1990; Zheng et al., 2009). AD, atopic dermatitis; DC, dendritic cell; ILC, innate lymphoid cell; Th, T helper.

inflammation and how type 2 inflammation, in turn, affects skin barrier dysfunction.

THE SKIN BARRIER

The epidermis is the only epithelial surface with two barrier structures: the stratum corneum (SC), which is unique to the skin, and tight junctions (TJs), which are present in other epithelia as well (Elias, 1988; Kubo et al., 2009; Michaels et al., 1975; Yoshida et al., 2014, 2013). Both the SC and the TJs limit penetration of and reaction to microbes,

allergens/irritants, and toxins as well as prevent trans-epidermal water loss (TEWL).

SC

Corneocytes. The SC, the outer layer of the epidermis, is composed of flattened, anucleated KCs (corneocytes) surrounded by a complex lipid-enriched extracellular matrix (Figure 3). Corneocytes are analogous to bricks and lipids to mortar in the original brick and mortar model of the SC (Elias, 1988; Michaels et al., 1975). This concept has since evolved

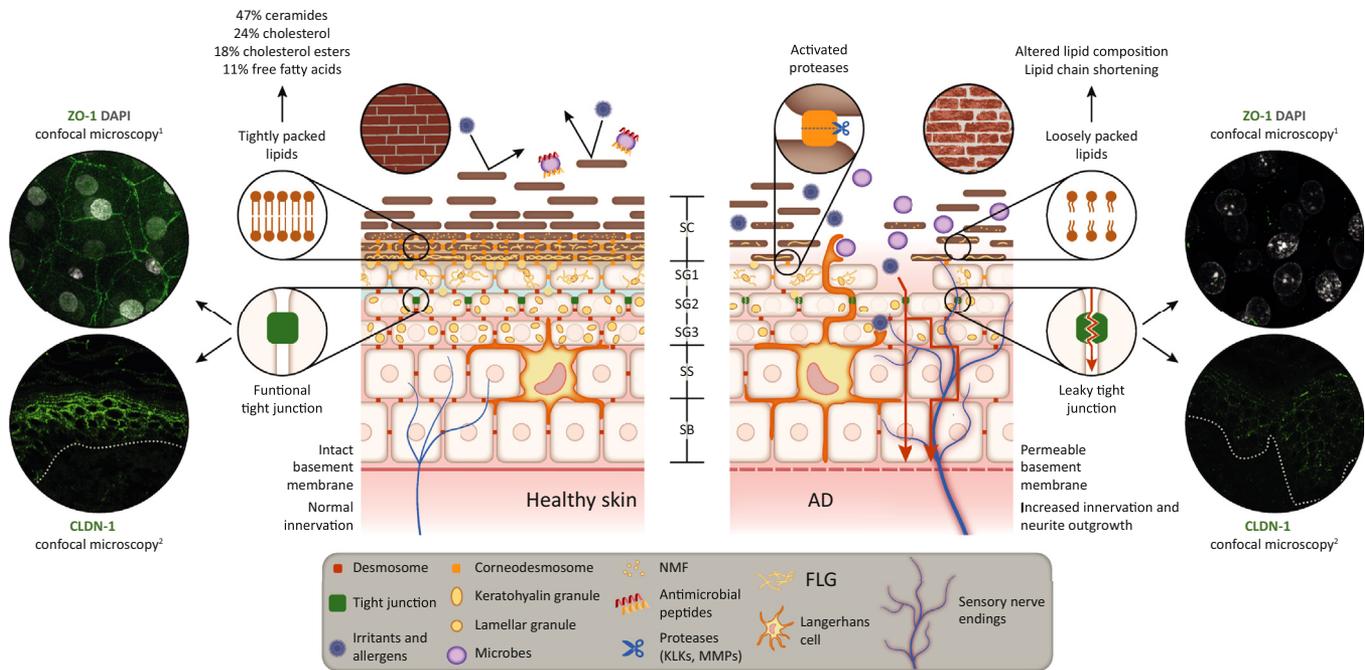


Figure 3. The key components of skin showing the differences between normal healthy skin (left side) and AD skin (right side), including the microbiome, corneocytes, antimicrobial peptides, lipids, NMFs, and tight junctions. In the brick and mortar model, the bricks represent corneocytes, and the mortar represents the extracellular lipids and other extracellular matrix components. In the confocal images, green staining represents ZO-1 and CLDN-1, and white represents cell nuclei. Confocal images from AD skin (on the right) show dramatically reduced green staining demonstrating reduced ZO-1 and CLDN-1, compared with normal skin (on the left). ¹Confocal images were adapted with permission from Takahashi et al. (2019) (<http://creativecommons.org/licenses/by/4.0>; modified), courtesy of Takaharu Okada at RIKEN IMS (Yokohama, Japan). ²Confocal images were provided with permission from De Benedetto et al. (2011a). AD, atopic dermatitis; CLDN, claudin; KLK, kallikrein; MMP, matrix metalloproteinase; NMF, natural moisturizing factor; SB, stratum basale; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum; ZO, zona occludens.

into a dynamic model in which lipid composition and alignment of the SC allow for adaptation to external factors and are altered in diseases such as AD (Pilgram et al., 2001; van Smeden and Bouwstra, 2016).

SC protein components. Keratins have both structural and regulatory functions in the epidermis. The more than 20 different epithelial keratins form specific keratin pairs composed of type I (lower molecular weight and acidic) and type II (neutral basic) components (Moll et al., 2008; Schweizer et al., 2006; Szeverenyi et al., 2008). Keratin pairs crosslink with other keratin pairs to form keratin filaments, which interact with other proteins and the cell membrane to provide structural stability and flexibility to KCs (Candi et al., 1998; Lee and Coulombe, 2009; Steinert and Marekov, 1995). In the epidermis, keratin (K) 5 and K14 predominate in the stratum basale, whereas K1 and K10 predominate in the stratum spinosum and higher layers—the change from one pair type to another reflects KC differentiation (Fuchs and Green, 1980; Moll et al., 1982; Reichelt et al., 2001). In contrast, K6, K16, and K17 are associated with the repair of an injured epidermis and are upregulated in inflammatory disorders of the skin, such as AD and psoriasis (Fu et al., 2014; Kumar et al., 2015; Lessard et al., 2013; Leung et al., 2020; Moll et al., 2008; Roth et al., 2012).

KCs undergo cornification, marking their differentiation into corneocytes; the cells become compact owing to keratin crosslinking, and keratins and other proteins form a cornified envelope that lines the cell membrane (Bowden et al., 1984;

Candi et al., 1998; Eckert et al., 2005). During cornification, keratin filaments cross-link to FLG and other proteins lining the cell membrane (e.g., involucrin and loricrin) (Candi et al., 1998; Roth et al., 2012; Steinert and Marekov, 1995). Keratin filaments also connect to cell–cell adhesion structures, such as desmosomes, stabilizing connections between KCs (Homberg and Magin, 2014; Kouklis et al., 1994; Seltsmann et al., 2013). Under normal conditions, keratin-filled corneocytes swell and expand with exposure to water, which softens the keratin and allows the SC to bend and stretch (Bouwstra et al., 2008, 2003).

Keratins have multiple regulatory functions. For example, K1 downregulates the expression and secretion of the inflammatory cytokines IL-18, IL-33, and TSLP as well as damage-associated molecular patterns such as S100A8 and S100A9 (Roth et al., 2012). K16 downregulates the expression of damage-associated molecular patterns and other inflammatory molecules involved in the innate immune response to skin barrier disruption (Lessard et al., 2013).

Keratin expression is dysregulated in AD (Guttman-Yassky et al., 2019a). K16 expression is increased in suprabasal epidermis in AD, corresponding with abnormal KC proliferation (Guttman-Yassky et al., 2019a; Suárez-Fariñas et al., 2011). In contrast, K1 and K10 expression is downregulated by IL-4 and IL-13 in AD lesional skin versus in healthy controls, which might contribute to the SC barrier defects seen in patients with AD and the release of proinflammatory and type 2–promoting alarmins (Dai et al., 2021; Imai et al., 2013; Totsuka et al., 2017).

FLG is a key structural protein in KCs. Its precursor pro-FLG is expressed in the stratum granulosum (SG) layer and is the major component of keratohyalin granules (Presland et al., 1997; Resing et al., 1995). During terminal differentiation, pro-FLG is dephosphorylated and cleaved to generate multiple FLG monomers (Presland et al., 1997; Resing et al., 1995; Sandilands et al., 2009).

FLG has multiple functions. It binds to keratin filaments in the KC cytoskeleton, forming an FLG–keratohyalin complex that cross-links to the cornified envelope, transforming KCs into arguably impervious corneocytes (i.e., “bricks”) (Eckhart et al., 2013; Presland et al., 1997; Sandilands et al., 2009). FLG degradation by the protease caspase-14 in outer SC layers produces natural moisturizing factors (NMFs) (see below) (Hoste et al., 2011). FLG and NMFs are controlled in a finely balanced process of production, proteolysis, and inhibition that is crucial to skin barrier structure; hydration; and function, including pH regulation, microbial ecology, and possibly even UV protection (Barker et al., 2007; Denecker et al., 2007; Kawasaki et al., 2012; Palmer et al., 2006; Sandilands et al., 2007; Smith et al., 2006).

Reduced expression and loss-of-function mutations of *FLG* are common in AD (Barker et al., 2007; Baurecht et al., 2007; Nomura et al., 2008; Palmer et al., 2006; Weidinger et al., 2008, 2007). Prevalence and types of *FLG* loss-of-function mutations vary among populations, with a very wide variation being reported for patients with AD (Barker et al., 2007; Baurecht et al., 2007; Brown and McLean, 2012; Nomura et al., 2008; Palmer et al., 2006; Weidinger et al., 2008, 2007). *FLG* loss-of-function mutations are associated with more severe AD (Brown and McLean, 2012, 2009; Brown et al., 2008a; Margolis et al., 2012; Weidinger et al., 2007), earlier onset of AD, greater risk of allergen sensitization and other atopic disorders (Brown et al., 2011; Palmer et al., 2007), and higher incidence of eczema herpeticum (Gao et al., 2009). *FLG* loss-of-function mutations are also associated with mild AD, but the association is weaker than that seen for severe disease (Brown et al., 2008b).

Notably, FLG expression may be reduced in patients with AD without *FLG* mutations. Type 2 inflammatory mediators, including IL-4, IL-13, IL-31, IL-33, and TSLP, reduce FLG expression (Howell et al., 2009, 2007; Kim et al., 2015; Sehra et al., 2010; Seltsmann et al., 2015). This has also been observed in skin inflammation mediated by Th17 (IL-17), Th22 (IL-22), and Th1 (IL-1 α , IL-1 β , and TNF- α) (Archer et al., 2019; Boniface et al., 2005; Danso et al., 2014; Gutowska-Owsiak et al., 2012, 2011; Kezic et al., 2012; Oyoshi et al., 2009; Tan et al., 2017). Repetitive scratching, detergent use, low humidity, exogenous or endogenous proteases, air pollution, and topical and oral corticosteroids can also reduce FLG expression (Danby et al., 2014; Goleva et al., 2019; Sheu et al., 1997, 1991; Thyssen and Kezic, 2014). *FLG* has multiple repeats (typically 10–12) within the locus (Brown et al., 2012). Copy number variants are associated with AD in some but not all populations. For example, in a cohort study in Ireland, reduced copy numbers were more frequent in patients with AD than in normal controls (Brown et al., 2012), whereas studies in other populations did not find any association between copy number

variation and the risk of AD (Fernandez et al., 2017; Fulton et al., 2022).

FLG deficiency is associated with reductions in SC structure, hydration, antimicrobial function, and epithelial buffering capacity in AD and increases in skin pH, percutaneous absorption, and protease activity (Brauweiler et al., 2013; Flohr et al., 2010; Kawasaki et al., 2012; Kezic et al., 2008; Thyssen and Kezic, 2014; Vávrová et al., 2014). *FLG*-knockdown KCs have reduced levels of K10, TJ proteins (zona occludens [ZO]-1, claudin [CLDN]-1, and occludin), and human β -defensin (hBD)-2; and increased cysteine proteases, which can degrade TJ proteins (Hönzke et al., 2016; Wang et al., 2017). Reduction in *FLG* expression reduces the levels of FLG metabolites such as NMFs. This results in an increase in SC pH, which activates serine proteases (Elias et al., 2008; Goleva et al., 2019; Wang et al., 2017) and induces the expression of the proinflammatory cytokines, IL-1 α , IL-1 β , and TSLP (Hönzke et al., 2016; Kezic et al., 2012; Nylander-Lundqvist and Egelrud, 1997; Wood et al., 1996). Reduced *FLG* expression is also linked to increased levels of arachidonic acid and its metabolite 12-hydroxy-eicosatetraenoic acid in KCs, leading to increased inflammation and impairing late epidermal differentiation (Blunder et al., 2017).

The manifestations of FLG-deficient skin are much more dramatic when combined with the biological actions of IL-4 and IL-13. For example, in an in vitro study, IL-4 and IL-13 stimulation induced spongiosis and increased epidermal thickening, skin pH, and permeability in both normal and FLG-deficient skin equivalents (Hönzke et al., 2016). However, in FLG-deficient equivalents, IL-4 and IL-13 decreased the levels of skin barrier proteins (e.g., involucrin and loricrin), TJ proteins (e.g., occludin), and hBD-2 and increased basal layer proliferation rates and TSLP levels to a greater extent than in normal skin equivalents. This suggests that the combination of type 2 immunity and FLG deficiency may promote AD development more than either alone.

NMFs are composed of FLG degradation products (i.e., free amino acids, urocanic acid, and pyrrolidine carboxylic acid), urea, and lactate derived from sweat. Under normal conditions, the decrease in hydration from middle to outer SC levels promotes FLG detachment from the corneocyte envelope and degradation, forming NMFs (Rawlings and Matts, 2005; Sandilands et al., 2009).

NMFs retain moisture, contributing to barrier function by promoting epidermal hydration through osmotic gradients that allow the movement of water into the corneocytes (Björklund et al., 2014; Kezic et al., 2008). NMFs maintain and buffer the acidic pH of the SC, which may reduce colonization by pathogenic bacteria (Kezic et al., 2008; Krien and Kermici, 2000; Miajlovic et al., 2010). NMFs also promote epidermal maturation and desquamation (Kezic et al., 2011). Decreased SC NMF levels are associated with dry skin and skin diseases such as ichthyosis vulgaris and AD. IL-4 and IL-13 reduce FLG levels and sweat secretion, which thereby affect NMF composition and function (Howell et al., 2009, 2007; Sehra et al., 2010).

Loricrin and involucrin are key structural proteins of the cornified envelope that anchor keratin filaments, providing mechanical strength and flexibility to the corneocytes (Candi

et al., 1998; Roth et al., 2012; Steinert and Marekov, 1995). Both loricrin and involucrin are highly insoluble in late-stage KC differentiation, resulting from disulfide and transglutaminase cross-linking within the molecules and to other proteins in the cell envelope in corneocytes (Hohl et al., 1991; Rice and Green, 1979; Steinert and Marekov, 1995). Loricrin is more prominent toward the cytoplasmic surface of the envelope, whereas involucrin is localized proximate to the lipid portion of the envelope (Jarnik et al., 2002; Steinert and Marekov, 1995).

IL-4 and IL-13 downregulate loricrin and involucrin expression in KCs (Kim et al., 2011, 2008), which may account for the reduced levels observed in AD. TNF- α reduces loricrin and involucrin expression, which also explains their reduced levels in psoriasis (Kim et al., 2011, 2008). Interestingly, silencing FLG expression in normal human KCs reduced involucrin expression but upregulated the expression of loricrin and IL-2, IL-4, IL-5, and IL-13 (Dang et al., 2015).

Proteases have multiple roles in the SC, mediated by both their direct proteolytic activity and through protease-activated receptors (PARs) (Figure 3). They influence SC cohesion, degrade corneodesmosome proteins (desmogleins and desmocollins) during homeostatic desquamation, regulate lipid synthesis by degrading enzymes that process extracellular lipids, and reduce lipid secretion into the extracellular matrix by stimulating the type 2 plasminogen receptor (Borgoño et al., 2007; Brattsand and Egelrud, 1999; Caubet et al., 2004; Hachem et al., 2006, 2005; Sales et al., 2010; Watkinson, 1999).

Serine protease activity is increased in both lesional and nonlesional AD skin (Komatsu et al., 2007; Voegeli et al., 2009). Increased serine protease activity compromises barrier function by increasing the degradation of corneodesmosomes and extracellular lipid-processing enzymes, reducing ceramide production (a characteristic abnormality of AD) (Borgoño et al., 2007; Di Nardo et al., 1998; Hachem et al., 2005, 2003; Imokawa et al., 1991). Serine proteases and cysteine proteases activate the PAR2 receptor, which regulates the secretion of lamellar bodies and cornification (Demerjian et al., 2008; Hachem et al., 2006), and is linked to increased inflammation, itch, and epidermal barrier disruption (Briot et al., 2009; Wilson et al., 2013). Both endogenous and exogenous proteases (e.g., from allergens, such as cockroach and dust mites, or from bacteria, such as *S. aureus* alpha-toxin) activate PAR2 (Ebeling et al., 2007; Hachem et al., 2006; Jeong et al., 2008; Kato et al., 2009). PAR2 activation reduces the expression of TJ proteins (occludin, CLDN-1, and ZO-1) and impairs TJ function, as assessed by reduced transepithelial electrical resistance (owing to a diminished barrier to ions) and increased permeability to small proteins (Nadeau et al., 2018). Thus both allergens and cutaneous dysbiosis may promote skin barrier disruption in AD through PAR2-mediated mechanisms. Finally, PAR2 agonists also increase the expression of IL-4 and IL-13 by mast cells, whereas PAR2 inhibition blocks IL-4 and IL-13 expression, decreases skin thickening, and suppresses itching in AD models (Barr et al., 2019). Of interest, Netherton syndrome, a monogenic AD-like syndrome characterized by the loss of serine protease inhibition due to

a mutation in *SPINK5* (which codes for the protease inhibitor LEKTI), is associated with kallikrein 5-mediated PAR2 activation resulting in the production of the pro-type 2 cytokine TSLP by KCs (Briot et al., 2010).

Matrix metalloproteinases (MMPs), which affect tissue remodeling and inflammatory cell migration into the epidermis, may also play an important role in AD pathogenesis (Groneberg et al., 2005; Harper et al., 2010; Purwar et al., 2008). MMP activity was 10–24 times greater in saline wash samples from AD lesional skin than in healthy controls, which do not normally express MMPs (Harper et al., 2010). IL-13 induces MMP-9 expression in KCs, and expression of both MMP-9 and IL-13 is increased in acute AD lesions (Purwar et al., 2008). MMP-12, which induces inflammatory cell aggregation, is also upregulated in lesional and nonlesional AD skin (Brunner et al., 2017; Pavel et al., 2020; Zhu et al., 2019).

SC lipid components. Skin barrier lipids are localized in the extracellular matrix surrounding corneocytes and are secreted from lamellar bodies before cornification (Figure 3) (Elias et al., 1998; Menon et al., 1992). By weight, these lipids include approximately 47% ceramides, 24% cholesterol, 18% cholesterol esters, and 11% free fatty acids (FFAs) (Ohno et al., 2015; Rawlings and Matts, 2005; van Smeden and Bouwstra, 2016). The SC contains several types of ceramides, many of which have very long fatty acid chains and are highly hydrophobic (Berdyshev et al., 2018; Rawlings and Matts, 2005; van Smeden and Bouwstra, 2016).

Lipids form densely packed layers in the central portion of the SC, becoming less densely packed and more gel-like closer to the surface (Brancaleon et al., 2001; Pilgram et al., 1999). Alterations of this packing pattern, resulting from altered lipid composition and lipid-chain shortening, are thought to contribute significantly to skin barrier impairment in AD (Berdyshev et al., 2018; Pilgram et al., 2001; van Smeden and Bouwstra, 2016; van Smeden et al., 2014). Fatty acid chains are lengthened by elongases (Ewald et al., 2015; van Smeden and Bouwstra, 2016). The expression of the elongases *ELOVL1*, *ELOVL3*, and *ELOVL6* is reduced in lesional AD skin, resulting in shortened fatty acid chains and increased skin barrier permeability (Berdyshev et al., 2018; Danso et al., 2017). The higher proportion of short fatty acids correlates with changes in lipid organization and skin barrier function and is associated with AD severity (Janssens et al., 2012; Li et al., 2017; van Smeden et al., 2014). IL-4 and IL-13 inhibit KCs expression of *ELOVL1*, *ELOVL3*, and *ELOVL6* (Berdyshev et al., 2018; Danso et al., 2017), and IL-4 inhibits ceramide synthesis (Hatano et al., 2005).

Sweat. Sweat is an important component of the skin barrier. Sweat forms a protective layer on the SC surface, contributing to thermoregulation, moisturizing the skin surface, and regulating water retention (Murota et al., 2015). In addition to water, electrolytes, lactate, basic nitrogenous compounds (e.g., urea, ammonia), amino acids, and proteins (Hiragun et al., 2017), sweat contains antimicrobial peptides (AMPs) (e.g., dermcidin and cathelicidin [see below]) and secretory IgA, which protect against infection (Imayama et al., 1994; Metze et al., 1991; Murakami et al., 2002). TJs

prevent sweat ducts from leaking sweat contents into the dermis. CLDN-3 is the most prevalent TJ protein that regulates sweat gland permeability (Yamaga et al., 2018).

Patients with AD may have an impaired ability to sweat (Takahashi et al., 2013; Yamaga et al., 2018), causing sweat leakage into the dermis leading to local inflammation and reducing the beneficial effects of sweating (Shiohara et al., 2011; Takahashi et al., 2013). Skin dryness and increased susceptibility to infection caused by decreased sweating at the skin surface may also worsen AD symptoms (Murota et al., 2018; Shimoda-Komatsu et al., 2018). Dermal sweat leakage is due to reduced CLDN-3 expression in AD sweat ducts (Yamaga et al., 2018). AD exacerbation may also result from sweat allergy—an IgE-mediated hypersensitivity to sweat, particularly to fungal (*Malassezia*) protein antigens commonly found in sweat (Hiragun et al., 2013; Takahagi et al., 2018). Sweat glands also express IL-13 receptors, suggesting a role for type 2 inflammation in sweat regulation (Akaiwa et al., 2001).

TJs

TJs connect epithelial cells in the SG (Figure 3) (Kubo et al., 2009; Yoshida et al., 2014, 2013). Unlike desmosomes, which are structural connectors between KCs, TJs play a more active role in skin barrier function. The continuous barrier formed by TJs limits the penetration of allergens, microbes, and irritants and regulates TEWL (De Benedetto et al., 2011b; Furuse et al., 2002; Kirschner et al., 2013; Rahn et al., 2017; Yoshida et al., 2013). In the SG, KCs flatten and form a tetra- and hexagonal shape (a 14-sided polygon with eight hexagonal and six rectangular sides). A model based on this shape showed that TJ connections can be maintained by three SG cells, ensuring that KCs never lose contact with adjacent KCs during differentiation as KCs move outward toward the SC (Yokouchi et al., 2016; Yoshida et al., 2013).

TJs contain intracellular and extracellular proteins that control the movement of water, ions, and solutions between KCs (Anderson and Van Itallie, 2009; Kubo et al., 2009; Yoshida et al., 2014, 2013). The transmembrane proteins found in TJs include occludin, CLDN family members, and junctional adhesion molecules (Brandner et al., 2002; Ebnet et al., 2004; Furuse et al., 2002, 1998, 1993; Kirschner et al., 2010; Liu et al., 2000; Morita et al., 1998; Wang et al., 2018). These transmembrane proteins connect to a cytoplasmic plaque complex, composed of ZO proteins (ZO-1, ZO-2, and ZO-3), cingulin, and other proteins (Brandner et al., 2002; Citi et al., 1988; Helfrich et al., 2007; Kirschner et al., 2013; Malminen et al., 2003; Morita et al., 1998; Pummi et al., 2001). CLDN-1 is critically important for skin barrier function, first appreciated with the CLDN-1-deficient mouse which succumbs during infancy as the consequence of extensive epidermal water loss (Furuse et al., 2002).

Reduced CLDN-1 and CLDN-3 levels in human AD skin are associated with epidermal barrier dysfunction (De Benedetto et al., 2011a; Yamaga et al., 2018). Reduced CLDN-3 levels are associated with increased sweat leakage in patients with AD (Yamaga et al., 2018). In an in vitro study, reduced CLDN-1 expression enhanced herpes simplex viral spreading, suggesting a mechanism for the susceptibility of

patients with AD to eczema herpeticum (De Benedetto et al., 2011b). A number of other CLDNs, including CLDN-4, CLDN-8, and CLDN-23, have been shown to be reduced in AD skin lesions (De Benedetto et al., 2011a; Esaki et al., 2015).

TJs also serve a fence function, separating two biologically distinct layers of the epidermis, that is, the cornifying KCs in the upper layer of the SG and the SC, and the stratum spinosum (Figure 3). Although Langerhans cell dendrites do not usually translocate through TJs in healthy skin, with minor physical trauma, they do, leading to reorganization of the TJs to preserve barrier integrity (Figure 3) (Kubo et al., 2009). This compensatory response prevents TEWL and limits antigen transit. In contrast, in AD lesional skin, greater numbers of Langerhans cell dendrites penetrate TJs, increasing their access to environmental antigens present on the epidermal surface (Figure 3) (Yoshida et al., 2014). Of interest, key innate immune receptors are only expressed on the basilar side of the epidermis (below the TJs) (Kuo et al., 2013). This loss of fence function observed in AD skin likely plays a role in allergen polysensitization, a characteristic of most patients with AD.

Type 2 inflammation may weaken TJ barrier function in AD. For example, in *FLG*-knockdown skin equivalents, IL-4 and IL-13 decreased the expression of occludin (Hönzke et al., 2016). Furthermore, it is possible that the ratio of IL-4 to IL-17 may determine the TJ barrier function because IL-4 blocks the robust barrier-enhancing effects of IL-17A on KCs (Brewer et al., 2019). In vitro studies with human epidermal equivalents have shown that IL-4, IL-13, and IL-31 reduce CLDN-1 expression (Gruber et al., 2015).

THE INFLAMMATORY RESPONSE IN AD AND SKIN BARRIER DISRUPTION

A vicious circle: skin barrier disruption induces type 2 inflammation, and type 2 inflammation increases barrier disruption

Epidermal damage activates the innate immune response in a proinflammatory cascade (Figure 4). Skin barrier disruption permits access to external antigens by Langerhans cells and dermal dendritic cells, which present antigens to naive T cells and activate allergen-specific Th2 cells, leading to the release of the canonical type 2 cytokines, IL-4 and IL-13 (Dillon et al., 2004; Jarrett et al., 2016; Oyoshi et al., 2010; Sonkoly et al., 2006). In response to barrier disruption and exposure to *S. aureus* and allergens, KCs and innate immune cells release chemokines (e.g., CCL1, CCL2, CCL3, CCL4, CCL5, CCL11, CCL13, CCL17, CCL18, CCL22, CCL26, and CCL27) that attract proinflammatory cells (Gros et al., 2009; Homey et al., 2006; Pivarcsi et al., 2004). Skin barrier disruption also stimulates KCs to release alarmins (i.e., proinflammatory type 2 immunity-promoting cytokines such as TSLP, IL-18, IL-25, and IL-33). In turn, the alarmins induce ILC2s, Th2 cells, and basophils to release type 2 cytokines (Giustizieri et al., 2001; Gros et al., 2009; Hardman et al., 2017; Kim et al., 2014; Leyva-Castillo et al., 2013; Mashiko et al., 2017; Neill et al., 2010; Oyoshi et al., 2010; Pivarcsi et al., 2004; Schmitz et al., 2005; Sokol et al., 2008; Stott et al., 2013; Terada et al., 2006; Yoshimoto et al., 1999; Zedan et al., 2015). In addition, alarmins induce ILC2s

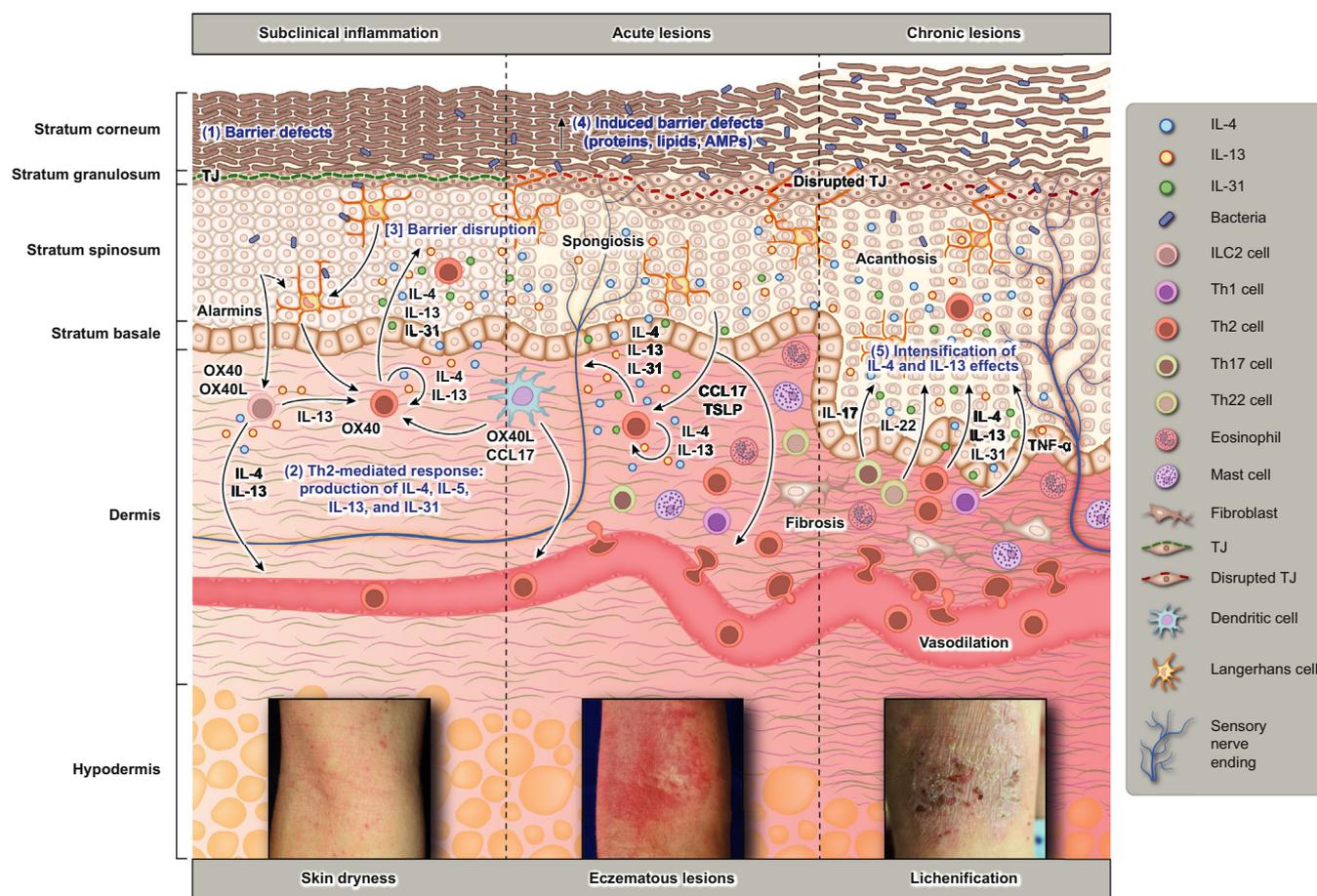


Figure 4. Skin barrier disruption and type 2 inflammation in AD. In acute AD, barrier defects in compromised skin lead to increased permeability and penetration of environmental factors (e.g., microbes, allergens) (1). Skin barrier defects and infiltration of immune cells lead to type 2–mediated production of inflammatory cytokines (e.g., IL-4, IL-13, IL-31) (2). In the epidermis, epithelial barriers predisposed to type 2–mediated disease are characterized by barrier disruption and greater numbers of Langerhans cells with dendrites penetrating through TJs (3). In chronic AD, type 2 inflammatory responses lead to epithelial remodeling and proliferation (e.g., FLG and other proteins, lipids, and AMPs) (4). The inflammatory cascade is further intensified by IL-4– and IL-13–mediated immune responses (5). AD, atopic dermatitis; AMP, antimicrobial peptide; CCL17, C–C motif chemokine ligand 17 (thymus and activation-regulated chemokine); ILC2, type 2 innate lymphoid cell; OX40L, OX40 ligand; Th, T helper; TJ, tight junction.

and dendritic cells to express the costimulatory molecule OX40 ligand, which binds to OX40 receptors on T cells, promoting Th2 differentiation (Halim et al., 2018; Ito et al., 2005).

Type 2 inflammatory cytokines contribute to skin barrier disruption through multiple pathways (Figure 5). For example, type 2 inflammatory cytokines inhibit the expression of epidermal proteins, such as FLG, loricrin, and involucrin (Amano et al., 2015; Howell et al., 2009, 2007; Hvid et al., 2011; Kim et al., 2015; Mitamura et al., 2018a; Sehra et al., 2010; Seltsmann et al., 2015) and promote the production of short-chain fatty acids (Berdyshev et al., 2018; Danso et al., 2017). In areas of active AD, type 2 inflammation leads to the recruitment of additional innate immune effector cells, including eosinophils, basophils, and mast cells (Figure 4). These effector cells release mediators such as histamine and major basic protein that not only exacerbate inflammation but also worsen skin barrier disruption by downregulating SC structural proteins and disrupting TJs (De Benedetto et al., 2015; Gschwandtner et al., 2013; Onoue et al., 2009). In addition, basophils release histamine, lipid mediators, and type 2 inflammatory cytokines, which further

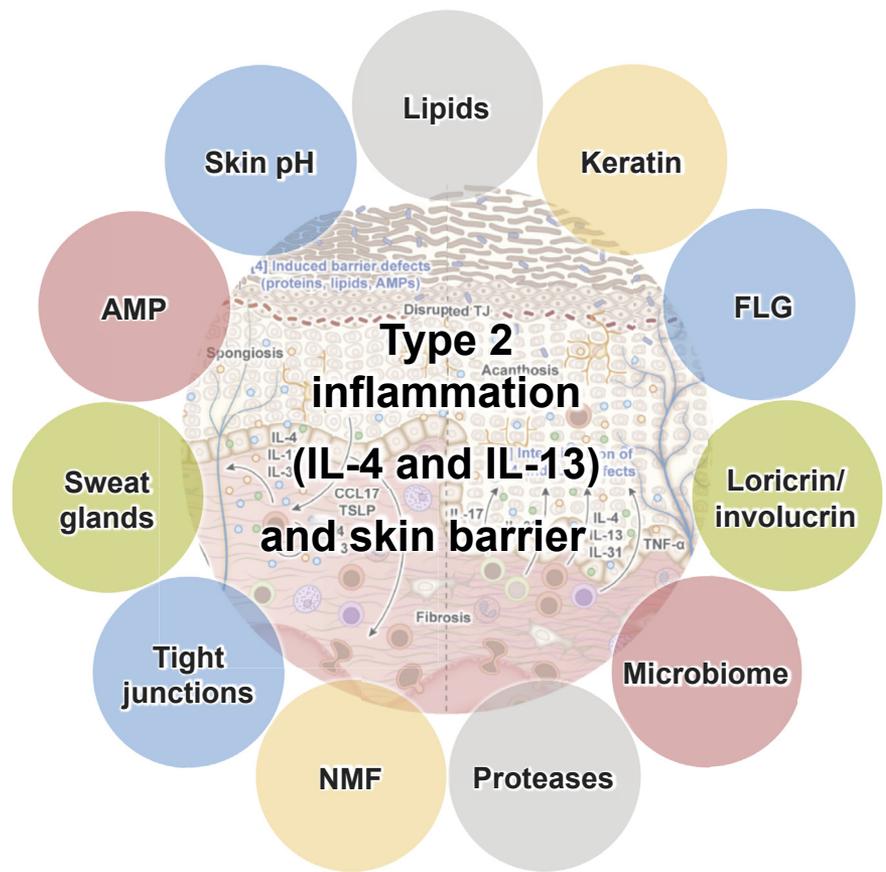
amplify AD pathogenesis (Kim et al., 2014; Mashiko et al., 2017; Sokol et al., 2008).

Type 2 inflammation and itch–scratch response

In the normal host-protective type 2 immune response, scratching is an adaptive response to remove ectoparasites and other irritants or toxins from the skin surface. In contrast, in AD, scratching induced by pathogenic AD itch can exacerbate skin barrier disruption, promote *S. aureus* colonization, and lead to the release of alarmins that enhance type 2 inflammation (Buhl et al., 2020; Hashimoto et al., 2011; Hu et al., 2021; Imai et al., 2014; Malhotra et al., 2016; Oetjen et al., 2017; Oyoshi et al., 2010; Wilson et al., 2013). KCs also play a role in pruritus by releasing TSLP and other itch-promoting alarmins in response to type 2 inflammatory mediators and proteases (Wilson et al., 2013). Type 2 inflammatory cytokines and alarmins promote the itch–scratch cycle by activating pruritogenic sensory neurons, which have IL-4, IL-13, IL-31, IL-33, and TSLP receptors (Cevikbas et al., 2014; Feld et al., 2016; Liu et al., 2016; Oetjen et al., 2017; Oh et al., 2013; Sonkoly et al., 2006; Wilson et al., 2013). IL-4 and IL-13 also sensitize neurons, making them more

Figure 5. Different components of the skin barrier affected by type 2 inflammation (mainly IL-4 and IL-13).

AMP, antimicrobial peptide; NMF, natural moisturizing factor.



responsive to itch-inducing substances, such as IL-31 and histamine (Oetjen et al., 2017), and act synergistically to stimulate acute and chronic itch and scratching behavior in mouse models (Campion et al., 2019). In addition, PAR2 receptors induce pruritus; activate T cells; increase the release of inflammatory mediators, including IL-13, TNF- α , and TSLP; and reduce TJ integrity (Buhl et al., 2020; Henehan and De Benedetto, 2019).

Skin barrier disruption affects sensory nerve ending positioning and density in AD (Figure 3) (Takahashi et al., 2019). Neuron density and branching are greater in AD skin than in normal skin; these changes are seen to a greater extent in chronic than in acute AD (Guseva et al., 2020; Sugiura et al., 1997; Urashima and Mihara, 1998). In normal skin, sensory nerve endings do not penetrate TJs owing to a pruning process mediated by KCs at or near TJs, but in lesional AD skin and mouse models, sensory neuron fibers penetrate TJs in areas of epidermal disruption (Takahashi et al., 2019). This is another example of the loss of the TJ fence function in patients with AD.

Type 2 inflammation and epidermal hyperplasia

Type 2 inflammatory cytokines induce epidermal hyperplasia, a characteristic histologic feature of disease activity in chronic AD. Epidermal hyperplasia is evaluated by measuring epidermal thickness, Ki-67-positive cells, and S100A8/9 and K16 expression (Esaki et al., 2016; Guttman-Yassky et al., 2019a; Hamilton et al., 2014; Ungar et al., 2017). IL-4 and IL-13 contribute to epidermal hyperplasia through disruptive

effects on the skin barrier by suppressing lipid and structural protein production and KC differentiation (Gittler et al., 2012; Guttman-Yassky et al., 2019a; Hamilton et al., 2014; Howell et al., 2008, 2007; Kim et al., 2008). In a mouse model of acute AD, basophils were the primary source of IL-4 and IL-13 in the skin (Pellefigues et al., 2021). In this model, basophils and IL-4 induced epidermal hyperplasia and skin barrier dysfunction associated with increased numbers of K10-positive KCs and Ki-67/K14-positive KCs and increased FLG expression and KC differentiation (Pellefigues et al., 2021). IL-22, which is found in AD lesional and nonlesional skin, is also a key driver of epidermal proliferation (Eyerich et al., 2009; Lou et al., 2017; Orfali et al., 2018; Suárez-Fariñas et al., 2011). IL-4 and IL-13 also promote fibrosis, leading to dermal thickening and scarring with increased collagen production, particularly in chronic AD (Figure 4) (Bhagal and Bona, 2008; Elbe-Bürger et al., 2002; Fichtner-Feigl et al., 2006; Gillery et al., 1992; Jessup et al., 2008; Kaviratne et al., 2004; Kolodsick et al., 2004; Oh et al., 2011; Oriente et al., 2000; Postlethwaite et al., 1992; Rankin et al., 2010; Zheng et al., 2009). In addition, inhibition of Notch signaling, which regulates KC differentiation, is associated with epidermal hyperplasia and enhanced the expression of TSLP, IL-4, and IL-13 (Dumortier et al., 2010; Murthy et al., 2012). Histamine binding to histamine 4 receptors stimulates KC proliferation, which may also contribute to hyperplasia (Glatzer et al., 2013). The counterbalance between epidermal hyperplasia (or proliferation) and differentiation (e.g., SC and TJ formation) suggests that

anything that leads to greater KC proliferation could lead to barrier dysfunction (Guttman-Yassky et al., 2009; Pellefigues et al., 2021; Sladek, 2012).

THE SKIN AS AN ANTIMICROBIAL BARRIER

The epidermal surface serves as an antimicrobial barrier through interactions between the skin surface microbiome and skin surface components, including AMPs, lipids, NMFs, pH, innate receptors, and resident antigen-presenting cells as well as T cells. On healthy skin, the microbiome includes diverse microorganisms that regulate local immune responses, control T-regulatory cell function, and inhibit pathologic microbes, thereby contributing to the antimicrobial barrier (Cogen et al., 2010; Grice et al., 2009; Lai et al., 2010; Naik et al., 2012; Sanford and Gallo, 2013). Its composition is affected by the integrity and function of epidermal barrier components, including long-chain unsaturated FFAs, AMPs, and NMFs (Baurecht et al., 2018; Braff et al., 2005; Feuille et al., 2018; Takigawa et al., 2005). Commensal bacteria, such as *S. hominis*, are important for skin homeostasis and host defense against *S. aureus* (Cogen et al., 2010; Nakatsuji et al., 2017). They produce proteins with antimicrobial activity and induce KCs to produce AMPs, which limit *S. aureus* growth (Cogen et al., 2010; Lai et al., 2010; Nakatsuji et al., 2017).

Dysbiosis is a feature of AD, with reduced microbial diversity compared with normal skin, and characterized by a predominance of *S. aureus* (Fyhrquist et al., 2019; Higaki et al., 1999; Kong et al., 2012; Ricci et al., 2003; Simpson et al., 2018; Totté et al., 2016). Analyses have shown that the skin surface of 55–90% of patients with AD is colonized with *S. aureus*, versus 3–20% of healthy controls (Fyhrquist et al., 2019; Higaki et al., 1999; Masenga et al., 1990; Park et al., 2013; Pascolini et al., 2011; Ricci et al., 2003). Reduced microbial diversity in patients with *S. aureus* skin colonization may result from enhanced type 2 inflammation and alterations in skin barrier function (Kong et al., 2012; Simpson et al., 2018). Interestingly, one study showed that infants' skin was colonized with *S. aureus* before their AD was diagnosed (Meylan et al., 2017). This suggests that dysbiosis may contribute to AD onset or amplification of disease activity (Kobayashi et al., 2015). Interestingly, patients with AD and *S. aureus* colonization have more severe disease, with greater type 2 deviation, allergen sensitization, and skin barrier dysfunction (Byrd et al., 2017; Fyhrquist et al., 2019; Kong et al., 2012; Simpson et al., 2018). *S. aureus* toxins can induce mast cell degranulation, leading to increased itch, inflammation, and KC death, which is made worse by coexposure with IL-4 and IL-13 (Brauweiler et al., 2014; Lin et al., 2003; Nakamura et al., 2013; Sonkoly et al., 2006). *S. aureus*-derived superantigens disrupt epithelial barrier function and enhance epithelial chemokine expression, which may promote greater leukocyte infiltration (Lin et al., 2003; Savinko et al., 2005; Schlievert et al., 2019). What remains unclear is what initiates the abnormalities commonly observed in patients with AD and the cause–effect relationships between dysbiosis, epidermal barrier defects, and type 2 inflammation.

AMPs inhibit the growth of microbial pathogens and support epidermal surface colonization by nonpathogenic

commensal microbes (Cogen et al., 2010; Lai et al., 2010; Nakatsuji et al., 2017; Ong et al., 2002). AMPs are produced by KCs and sweat gland cells in response to colonization/infection, inflammation, or barrier disruption (Braff et al., 2005; Lee et al., 2008; Murakami et al., 2002). The most commonly studied AMPs are the cathelicidin LL-37, and hBD-2 and hBD-3, which together inhibit the growth of *S. aureus*. These are typically increased with inflammation; however, IL-4 and IL-13 inhibit their production (Hönzke et al., 2016; Nomura et al., 2003; Ong et al., 2002). AMPs are also commonly produced by activation of innate immune receptors, such as toll-like receptors (TLRs), present on epidermal cells. TLR2 is important for responding to *S. aureus* but is downregulated in patients with AD (Kuo et al., 2013). AMPs have additional roles in skin barrier maintenance. For example, hBD-3 and TLR2 agonists have also been shown to increase the expression of CLDNs and other factors that regulate TJ function (Kiatsurayanon et al., 2014; Kuo et al., 2013).

Extracellular matrix lipids (e.g., FFAs and sphingomyelin) contribute to the antimicrobial barrier by limiting bacterial growth, maintaining acidic pH, and activating AMPs (Baurecht et al., 2018; Bibbel et al., 1992; Jiang et al., 2013; Miller et al., 1988; Nakatsuji et al., 2010; Takigawa et al., 2005). Permeability barrier impairment due to increased surface pH and reduced levels of long-chain FFAs and sphingosine also impairs epidermal antimicrobial function (Bibbel et al., 1992; Hülpüsch et al., 2020; Miller et al., 1988; Zainal et al., 2020).

There is tremendous cross-talk between type 2 inflammation, *S. aureus* colonization, and epidermal barrier dysfunction (Figure 4). For example, patients with severe AD frequently have *S. aureus* strains on their skin that produce enterotoxins, proteases, lipases, and superantigens that enhance B-cell Ig class switching to IgE, increase epidermal expression of type 2 inflammation–promoting alarmins and cytokines, and disrupt skin barrier function (Brauweiler et al., 2019, 2017, 2013; Fyhrquist et al., 2019; Gould et al., 2007; Leung et al., 1993; Nakatsuji et al., 2017, 2016; Williams et al., 2017). Even toxin-deficient *S. aureus* strains have been shown to disrupt TJs (Ohnemus et al., 2008), supporting the notion that *S. aureus* colonization may contribute to barrier impairment and type 2 polarization in AD.

In contrast, type 2 inflammation enhances the expression of *S. aureus* adhesins, such as fibrinogen and fibronectin (Cho et al., 2001a, 2001b). In parallel, type 2 cytokines, particularly IL-4 and IL-13, inhibit the production of AMPs and FFAs from epidermal cells in patients with AD and increase skin surface pH (Berdyshev et al., 2018; Danso et al., 2017; Hönzke et al., 2016; Nomura et al., 2003; Ong et al., 2002), further evidence that IL-4 and IL-13 enhance susceptibility to *S. aureus* colonization and thereby disturb barrier integrity.

THERAPEUTIC INTERVENTIONS TARGETING THE SKIN BARRIER

Many topical and systemic therapies have been evaluated for their ability to improve skin barrier function in patients with AD. Topical medications include topical corticosteroids (TCSs), topical calcineurin inhibitors (TCIs), phosphodiesterase-4 (PDE-4) inhibitors, and Jak inhibitors

(Jakis) (Eichenfield et al., 2014; Katoh et al., 2020; Singh et al., 2020; Wollenberg et al., 2018a).

Topical medications

Topical medications, including TCS, TCI, the PDE-4 inhibitor crisaborole, and the Jakis delgocitinib and ruxolitinib, have been shown to improve AD lesional severity in clinical trials (Amano et al., 2015; Bissonnette et al., 2019a; Dähnhardt-Pfeiffer et al., 2013; Danby et al., 2014; Jensen et al., 2013, 2012, 2009; Nakagawa et al., 2020, 2019; Papp et al., 2021).

Topical medications differ in their effects on skin barrier function and type 2 inflammation. The TCIs pimecrolimus and tacrolimus have been shown to improve AD skin barrier structure and function at levels similar to or exceeding those observed with TCS (Dähnhardt-Pfeiffer et al., 2013; Danby et al., 2014; Jensen et al., 2013, 2012, 2011, 2009; Xhaufaire-Uhoda et al., 2007). In adults with mild-to-moderate AD, both TCS and pimecrolimus improved SC hydration; TEWL; epidermal differentiation; the levels of loricrin, involucrin, K5, K10, and K14; and epidermal hyperproliferation markers (e.g., K16) (Jensen et al., 2013, 2012, 2011). In contrast, TCS downregulated the expression of inflammatory biomarkers, involucrin, AMPs, hBDs, and lipid metabolism enzymes, and disrupted SC barrier structure, including lamellar body extrusion and lipid bilayer formation. In contrast, TCIs normalized these structures (Jensen et al., 2013, 2012, 2011). In similar studies, tacrolimus significantly improved multiple measures of skin barrier function and structure versus TCS in patients with quiescent (Danby et al., 2014) and/or moderate (Dähnhardt-Pfeiffer et al., 2013; Xhaufaire-Uhoda et al., 2007) AD.

In an inpatient randomized double-blinded trial in 40 adults with mild-to-moderate AD, the PDE-4 inhibitor crisaborole significantly improved lesional skin barrier function compared with vehicle, as assessed by TEWL and immunohistochemistry, and modulated inflammatory and skin barrier-related biomarkers, including CLDN-8 and K16, which correlated with improved barrier function and lesional scores (Bissonnette et al., 2019a; Paller et al., 2016).

The topical pan-Jaki delgocitinib targets Jak1, Jak2, Jak3, and tyrosine kinase (TYK) 2. In mouse models of AD, delgocitinib upregulated keratin and loricrin expression that had been downregulated by IL-4 and IL-13, improved skin barrier function (as assessed by TEWL), and increased NMF levels in KC cultures (Amano et al., 2015). Delgocitinib showed no effect on CLDN-1 and CLDN-4 staining versus vehicle, whereas TCS significantly decreased CLDN staining and caused significant skin atrophy versus delgocitinib (Anagawa-Nakamura et al., 2020).

Systemic medications

Conventional systemic immunosuppressive agents, such as corticosteroids, cyclosporine A, methotrexate, mycophenolate mofetil, and azathioprine, broadly target inflammation, but their use as treatments for AD is limited by safety concerns and the need to monitor for toxicities (Eichenfield et al., 2017; Katoh et al., 2020; Wollenberg et al., 2018b). To date, there is limited evidence that these treatments improve the skin barrier.

An increased understanding of AD pathogenesis has led to the development of systemic treatments specifically targeting

type 2 inflammatory pathways. Data on their effects on skin barrier structure and/or function are available for the oral Jakis gusacitinib and tofacitinib and the mAbs tralokinumab, fezakinumab, GBR830, and dupilumab.

Two studies evaluated the effects of Jaki on skin barrier. A randomized, placebo-controlled, double-blind phase 1b trial evaluated oral gusacitinib (Jak1/2/3, TYK2, and spleen TYK inhibitor) in patients with moderate-to-severe AD (Bissonnette et al., 2019b; Pavel et al., 2019). On the basis of transcriptomic and immunohistochemistry analyses, gusacitinib downregulated serum and skin biomarkers for type 1, type 2, Th22, and Th17 inflammatory pathways; reduced epidermal hyperplasia, K16 expression, and inflammatory cell infiltrates; increased FLG expression; and shifted the gene expression profile of lesional skin toward that of non-lesional skin (Bissonnette et al., 2019b; Pavel et al., 2019). In a three-dimensional in vitro skin model in which IL-4 and IL-13 were used to induce AD-like changes, tofacitinib prevented AD-like histologic changes, maintained FLG expression, decreased the phosphorylation of STAT 3 and 6, upregulated the expression of KC differentiation genes (including desmocollin 1, FLG, involucrin, loricrin, and K1), and downregulated the expression of genes associated with AD-related immune responses (Clarysse et al., 2019).

Four mAbs have been evaluated for their effects on the skin barrier in patients with AD (fezakinumab, GBR 830, tralokinumab, and dupilumab); of these, fezakinumab and GBR 830 are currently investigational. In a randomized, placebo-controlled, double-blinded, phase 2a clinical trial in adults with moderate-to-severe AD, fezakinumab (a mAb directed against IL-22) administered as intravenous monotherapy improved lesional skin gene expression profiles and epidermal markers for inflammation and epidermal proliferation in patients with high levels of IL-22 skin expression at baseline but not in those with low baseline IL-22 levels (Brunner et al., 2019). In a randomized, double-blind, placebo-controlled trial in adults with moderate-to-severe AD, GBR 830 (an anti-OX40 mAb) administered intravenously significantly reduced OX40-positive T-cell and OX40 ligand+ dendritic cell counts and significantly improved measures of epidermal hyperplasia, including reductions in epidermal thickness, K16 mRNA expression, and Ki-67+ cell counts, compared with placebo (Guttman-Yassky et al., 2019b).

Tralokinumab is a mAb directed against IL-13 that has been approved in Europe for the treatment of adults with moderate-to-severe AD who are candidates for systemic therapy (European Medicines Agency, 2021a). In a phase 3 trial in adults with moderate-to-severe AD, tralokinumab significantly reduced the levels of *S. aureus* colonization (on the basis of routine culture techniques) on lesional skin compared with placebo (Wollenberg et al., 2021).

Dupilumab, a fully human mAb that inhibits the signaling of both IL-4 and IL-13 by blocking their shared receptor component (IL-4R α), is approved for AD, asthma, and chronic rhinosinusitis with nasal polyps, which are diseases driven by type 2 inflammation (European Medicines Agency; 2021b; Pharmaceuticals and Medical Devices Agency, 2021; Regeneron, 2021). Dupilumab significantly improved abnormalities in skin barrier gene expression and epidermal

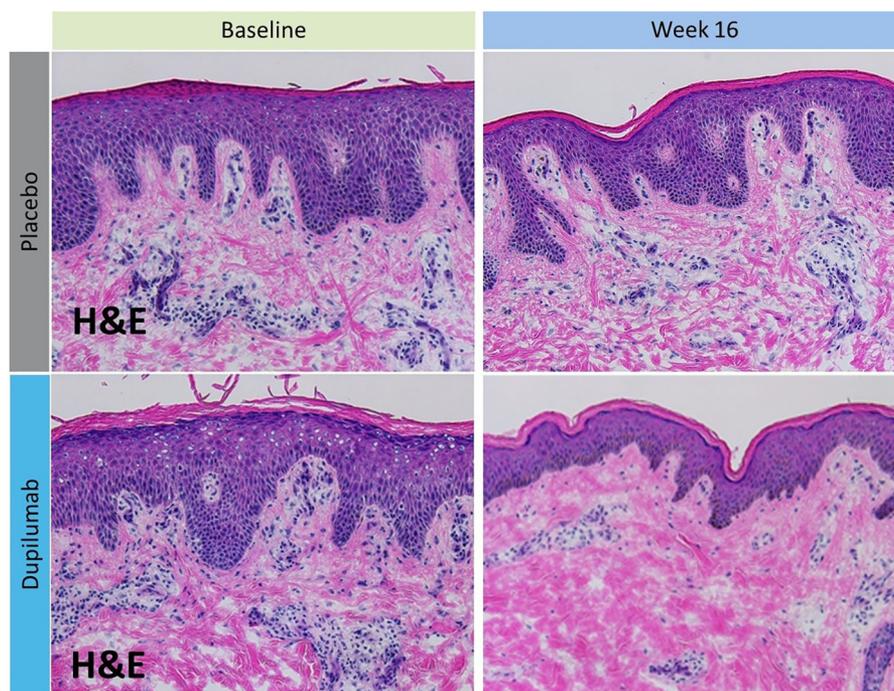


Figure 6. Histologic changes observed with dupilumab treatment. Lesional skin samples taken from AD subjects before and 16 weeks after treatment with placebo or dupilumab (300 mg subcutaneously every 2 weeks) were stained with H&E. These two representative cases are notable for epidermal hyperplasia, spongiosis with elongated rete ridges, and a disordered basket-weave pattern to the SC layers in the placebo (baseline and 16 weeks) and dupilumab (baseline) images. After dupilumab treatment (16 weeks), the epidermis is no longer hyperplastic or spongiotic, rete ridges have normalized, and the SC is compact. In addition, these changes are seen in the context of reduced inflammatory perivascular infiltrates. Images were provided with permission from Guttman-Yassky et al. (2019a), courtesy of Emma Guttman-Yassky, Center for Excellence in Eczema, Department of Dermatology, Icahn School of Medicine at Mount Sinai (New York, NY). AD, atopic dermatitis; SC, stratum corneum.

proliferation in patients with AD (Beck et al., 2014; Guttman-Yassky et al., 2019a; Hamilton et al., 2014; Rohner et al., 2021). In biopsy data from adult patients with moderate-to-severe AD in two randomized, placebo-controlled, double-blinded phase 1 studies, dupilumab significantly reduced the expression of inflammatory biomarkers and epidermal proliferation biomarkers (e.g., K6b, K16, Ki-67); upregulated the expression of epidermal barrier structure and function markers (e.g., MATN4, CLDN8, ELN, CLDN11); and shifted AD-related gene expression in lesional skin toward that resembling nonlesional skin (Beck et al., 2014; Hamilton et al., 2014). Similarly, in a randomized, placebo-controlled, double-blinded phase 2 study in adult patients with moderate-to-severe AD, dupilumab significantly reduced the expression of genes important in type 2 inflammation (e.g., IL-13, IL-31), epidermal hyperplasia (e.g., Ki-67, K16), T cells, and dendritic cells; reduced Th17 and Th22 activity; and shifted the expression profile of the AD-related transcriptome of lesional skin toward that of nonlesional skin (Guttman-Yassky et al., 2019a). Dupilumab also increased the expression of epidermal differentiation factors, barrier components, inflammatory proteases (e.g., MMP12), FLG, loricrin, CLDN-8 and CLDN-23, and ELOVL3; significantly reduced epidermal hyperplasia in lesional skin (Figure 6); significantly decreased epidermal *S. aureus* abundance (qPCR); and normalized microbial diversity in patients with AD (Bieber, 2020; Callewaert et al., 2020; Guttman-Yassky et al., 2019a). A retrospective study in a tertiary treatment center comparing skin biopsies between

before and after 6–8 weeks of dupilumab treatment in patients with severe AD reported substantial reductions in the expression of AD-related inflammatory cytokines and alarmins and increased expression of FLG, LEKT11, hBD-3, and LL-37 (Rohner et al., 2021). In an open-label transcriptomic analysis of tape strips and biopsies in adult patients with moderate-to-severe AD, biomarkers associated with healthy skin barrier that were decreased at baseline (e.g., keratins, FLG, periplakin, and lipid metabolism markers) showed increased expression after 16 weeks of treatment with dupilumab. Improvement in several of these skin barrier markers was significantly correlated with improvement in clinical signs (Mikhaylov et al., 2021). Finally, transcriptomic data from a European registry study (TREATgermany [German National Clinical Registry for Patients With Moderate-to-severe Atopic Dermatitis]) of adults with moderate-to-severe AD showed that dupilumab treatment for 12 weeks led to a stronger normalization of skin barrier-related genes than cyclosporin (Möbus et al., 2021). In this observational analysis of a nonrandomized cohort of patients, dupilumab treatment decreased the expression of type 2-related chemokines (e.g., CCL13, CCL17, CCL18, and CCL22) and normalized the expression of barrier function-related markers (e.g., CLDN8, ELOVL3, FLG, K1, K10, and loricrin gene LOR) (Möbus et al., 2021). In contrast, cyclosporine resulted in stronger improvement in the expression of certain KC differentiation markers (e.g., K16, K6A, P13, and LCE3A) and some genes related to immune pathways (e.g., IL2RA, IL9R, CXCL3, CTLA4, OX40-R/OX40, S100A8, and S100A9)

and the Th17/IL-23 axis (e.g., *IL-22*, *IL-23A/p19*, *IL-12B/p40*, and *P13/elafin*).

CONCLUSIONS

Skin barrier function depends on multiple interacting systems that affect structural and functional components of the skin, including the SC and TJs, type 2 inflammatory pathways, cellular and extracellular components of the epithelium, and interactions with the microbiome and other environmental factors. Skin barrier dysfunction in AD is driven by genetic predisposition, environmental factors, and inflammation. It has been postulated that the rise in allergic diseases (including AD) in industrialized countries may be due to substances present in modern urban environments that can damage epithelial barriers, such as detergents and their additives, emulsifiers in food, and various pollutants (Akdis, 2021). The type 2 inflammatory cytokines IL-4 and IL-13 play an important role in the disruption of skin barrier function, affecting multiple components of the skin barrier and at the same time being induced by barrier disruption (Figure 6). Targeting type 2 cytokines in AD has a broad range of beneficial effects on the components of the skin barrier, including lipids, proteins, skin pH, corneocyte structure, TJs, sweat glands, and the microbiome, reinforcing the notion that AD is a systemic type 2 inflammatory disease. Further research is needed to confirm to what extent type 2-targeted therapies can improve skin barrier function and what role this barrier repair plays in the clinical improvement seen in AD.

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CONFLICT OF INTEREST

LAB is a consultant for AbbVie, Allakos, AstraZeneca, BenevolentAI Bio, DermTech, Galderma, Incyte, Janssen, LEO Pharma, Lilly, Novartis, Pfizer, Principia Biopharma, Rapt Therapeutics, Regeneron Pharmaceuticals, Inc., Ribon Therapeutics, Sanofi Genzyme, Sanofi-Aventis, and Stealth Biotherapeutics; an investigator for AbbVie, AstraZeneca, Kiniksa, LEO Pharma, Pfizer, Regeneron Pharmaceuticals, Inc., and Sanofi; and a stock owner of Medtronic, 3M, Moderna, and Gilead. MJC is an investigator and/or consultant for AbbVie, Astellas, Atopic, Boots, Dermavant, Eli Lilly, Galapagos, Galderma, Harvey Water Softeners, Hyphens Pharma, Johnson &

Johnson, Kymab, LEO Pharma, L'Oréal, Menlo Therapeutics, Novartis, Oxagen, Perrigo (ACO Nordic), Pfizer, Procter & Gamble, Reckitt Benckiser, Regeneron Pharmaceuticals, Inc., Sanofi Genzyme, and UCB Pharma. MA has received consulting fees, honoraria, grant support, and/or lecturing fees from Maruho, Mitsubishi Tanabe, Ono Pharmaceutical, Sanofi, Torii Pharmaceutical, Pola Pharma, and Kyowa Kirin. ADB is an investigator and/or consultant for Allakos, Dermira, Kiniksa, Regeneron Pharmaceuticals, Inc., Pfizer, and Sanofi Genzyme. KK has received consulting fees, honoraria, grant support, and/or lecturing fees from Japan Tobacco, LEO Pharma, Maruho, Mitsubishi Tanabe, Ono Pharmaceutical, Procter & Gamble, Sanofi, Taiho, and Torii Pharmaceutical. JDH is an employee and shareholder of Regeneron Pharmaceuticals, Inc. and is an inventor in Regeneron patents. ABR is an employee and may hold stock and/or stock options in Sanofi Genzyme.

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