Epidermal Barrier Dysfunction in Atopic Dermatitis

Michael J. Cork[1,2], Simon G. Danby[2], Yiannis Vasilopoulos[2], Jonathan Hadgraft[3], Majella E. Lane[3], Manar Moustafa[1,2], Richard H. Guy[4], Alice L. MacGowan[5], Rachid Tazi-Ahnini[2] and Simon J. Ward[2]

Atopic dermatitis (AD) is a multifactorial, heterogenous disease that arises as a result of the interaction between both environmental and genetic factors. Changes in at least three groups of genes encoding structural proteins, epidermal proteases, and protease inhibitors predispose to a defective epidermal barrier and increase the risk of developing AD. Loss-of-function mutations found within the *FLG* gene encoding the structural protein, filaggrin, represent the most significant genetic factor predisposing to AD identified to date. Enhanced protease activity and decreased synthesis of the lipid lamellae lead to exacerbated breakdown of the epidermal barrier. Environmental factors, including the use of soap and detergents, exacerbate epidermal barrier breakdown, attributed to the elevation of stratum corneum pH. A sustained increase in pH enhances the activity of degradatory proteases and decreases the activity of the lipid synthesis enzymes. The strong association between both genetic barrier defects and environmental insults to the barrier with AD suggests that epidermal barrier dysfunction is a primary event in the development of this disease. Our understanding of gene–environment interactions should lead to a better use of some topical products, avoidance of others, and the increased use and development of products that can repair the skin barrier.

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INTRODUCTION

Atopic dermatitis (AD) is a chronic, inflammatory disease of the skin, which is characterized by xerosis, pruritus (itch), and erythematous lesions with increased transepidermal water loss (TEWL). In the 1990s, Elias and Taieb were among the first to suggest that the breakdown of the skin barrier may be an initial event in the development of AD (Elias *et al.*, 1999; Taieb, 1999). At that time, the majority of research was focused on immune dysfunction in AD (Williams, 2000). However, as the hyper-reactivity of the immune response in AD is not present in all patients (Flohr *et al.*, 2004), additional explanation for the pathogenesis of this disease was needed.

To distinguish between the different immunological states, AD is often split into two sub-categories termed ''non-atopic'' dermatitis and ''true'' AD on the basis of whether the patient has elevated IgE levels, which is indicative of immune hyper-reactivity (Bieber, 2008). True AD is associated with the development of food allergy, asthma, and allergic rhinitis (Spergel and Paller, 2003). The spectrum of severity of AD is very wide; at the mild end, the dermatitis is usually non-atopic, and can normally be controlled with a complete emollient regimen (Cork, 1997), and intermittent use of calcineurin inhibitors and mild-to-moderate potency topical corticosteroids (TCS) (Wahn *et al.*, 2002; Cork *et al.*, 2003). At the other end of the spectrum, in very severe ''atopic'' dermatitis, the total IgE level may be >10,000 U. This very severe dermatitis may only be controlled using systemic agents such as cyclosporine, mycophenolate, and methotrexate (Harper *et al.*, 2000).

In 80% of patients with non-atopic AD, IgE levels subsequently increase and patients develop true AD (Illi *et al.*, 2004; Bieber, 2008). The remaining 20% of patients continue as non-atopic and never develop a raised IgE (Bieber, 2008). These findings support a non-immune causative event early in the development of AD, such as a defective epidermal barrier (Elias *et al.*, 1999; Taieb, 1999; Cork *et al.*, 2006; Callard and Harper, 2007; Bieber, 2008). A defective epidermal barrier allows the penetration of allergens through the skin, facilitating the interaction of these allergens with the local antigen-presenting cells and immune effector cells (Figure 1). This may result, in some cases, in the transition from the non-

[1]*The Paediatric Dermatology Clinic, Sheffield Children's Hospital, Sheffield, UK;* [2]*The Academic Unit of Dermatology Research, School of Medicine & Biomedical Sciences, The University of Sheffield, Sheffield, UK;* [3]*The School of Pharmacy, University of London, London, UK;* [4]*Department of Pharmacy & Pharmacology, University of Bath, Bath, England and* [5]*York Pharma (R&D) Ltd, Sheffield, UK*

Correspondence: Professor Michael J. Cork, The Academic Unit of Biomedical Genetics-Dermatology, School of Medicine & Biomedical Sciences, The University of Sheffield, E Floor, Beech Hill Road, Sheffield S10 2RX, UK.
E-mail: m.j.cork@sheffield.ac.uk

Abbreviations: AD, atopic dermatitis; CE, cornified envelope; DSG, desmoglein; LEKTI, lymphoepithelial Kazal-type 5 serine protease inhibitor; KLK, kallikrein; KC, keratinocyte; MCC, mast cell chymase; NMF, natural moisturizing factor; PAR2, protease-activated receptor 2; SC, stratum corneum; SG, stratum granulosum; SPINK5, *serine protease inhibitor Kazal-type 5; TCS, topical corticosteroids; TEWL, transepidermal water loss*

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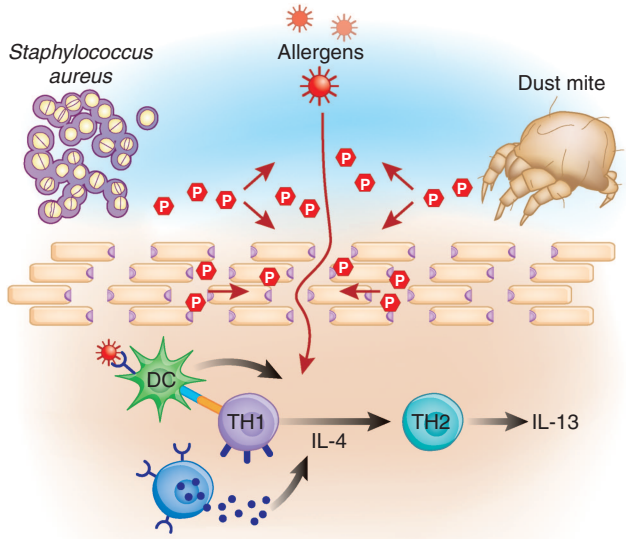

Figure 1. There is a defective epidermal barrier in individuals with atopic dermatitis. The epidermal barrier is found in the lower layers of the stratum corneum, and is composed of differentiated keratinocytes, termed corneocytes (beige rectangles), held together with corneodesmosomes (purple spheres). The hyperactivity of degradatory proteases (red hexagons) found within the epidermis, and contributed to by exogenous proteases (red hexagons), from house dust mites and *Staphylococcus aureus*, for example, facilitate the cleavage of the corneodesmosome junctions. This is just one event in the breakdown of the epidermal barrier that permits the penetration of allergens. Dendritic cells (DC) (green) found in the dermis take up and present these allergens (red stars) to helper T (TH) cells and recruit CD4+ T cells (blue). Activated DC and IL-4, expressed by CD4+ T cells, promote TH1 to TH2 switching with the subsequent release of pro-inflammatory cytokines and elevation of IgE levels (please refer to Werfel, 2009 in this series for a detailed explanation). The clinical outcome of this type of response is atopy and asthma.

atopic state to the atopic state of the disease with raised IgE (Novak *et al.*, 2003; Bieber, 2008).

The hypothesis that the xerosis (Denda *et al.*, 1998), the permeability barrier abnormality (Ghadially *et al.*, 1996; Elias *et al.*, 1999), or both can drive the activity of AD is referred to as the ''outside–inside'' hypothesis. The converse, immunological perspective, known as the ''inside–outside hypothesis'' suggests that barrier breakdown in AD is a secondary consequence of the inflammatory response to irritants and allergens (Leung, 2000). The correct hypothesis is still debated. Barrier function seems to fluctuate in relation to disease activity, suggesting that changes in barrier function may drive the disease activity (Chamlin *et al.*, 2002). In addition, barrier damage induced experimentally, for example, by surfactants (sodium lauryl sulfate) or skin stripping, causes the release and production of cytokines, such as IL-1α, IL-β, tumor necrosis factor-α, and granulocyte-macrophage colony-stimulating factor (Wood *et al.*, 1996, 1997), indicating that barrier disruption alone leads to cytokine production, inflammation, and flare of dermatitis (Elias *et al.*, 1999).

Another area of AD research that points us to the skin barrier and the influence of the environment is the rising prevalence of AD and concomitant rise in exposure to the environmental agents. The prevalence of AD has been rising progressively in developed countries since the 1940s (Walker and Warin, 1956; Fergusson *et al.*, 1981; Taylor *et al.*, 1984; Shultz-Larsen *et al.*, 1986; Williams, 1992; Neame *et al.*, 1995; Thestrup-Pedersen, 1996; Yura and Shimizu, 2001). How can the prevalence of AD increase so dramatically if it is only determined genetically? This increase suggests that gene–environment interactions must be crucial in the expression of the disease (Williams, 1992).

Here, we aim to bring together evidence to support the hypothesis that a defective skin barrier is a critical factor in the development of AD, and to provide an overview of some key themes in the current thinking on AD. As a multifactorial disease, the number (dose) and combination of contributing factors are suggested to determine the severity and the likelihood of developing the disease. These so-called ''factors'' are discussed in the following sections, classified as either genetic or environmental. For a discussion of immunological factors contributing to AD please refer to Werfel, 2009 and De Benedetto *et al.*, 2009 in this series.

THE EPIDERMAL BARRIER

Structure of the epidermal barrier

The barrier to penetration of irritants and allergens through skin is located in the lower part of the stratum corneum (SC). The structural integrity of the SC is maintained by the presence of modified desmosomes, called corneodesmosomes. Corneodesmosomes lock the corneocytes together and provide tensile strength for the SC to resist shearing forces. Elias (1983) visualized the SC as being similar to a brick wall, with the corneocytes analogous to bricks, and the lipid lamellae acting as mortar (Figure 2). Extending this model, the corneodesmosomes may be thought of as analogous to iron rods that pass down through holes in the bricks to give the wall its tensile strength (Cork *et al.*, 2006).

Corneocytes are flattened cells that represent the final stage of differentiation of the outermost keratinocytes (KCs) of the granular layer, when these cells have lost their sub-cellular organelles and nuclei, and become densely packed with keratin fibres (Lavker and Matoltsy, 1970). In humans, the SC has an average of 20 corneocyte layers, each corneocyte being approximately 30 μm in diameter (Menon *et al.*, 1992). During the formation of corneocytes, the granular cells spill out their lamellar granule contents into the extracellular space to form the lipid lamellae matrix, which encases the

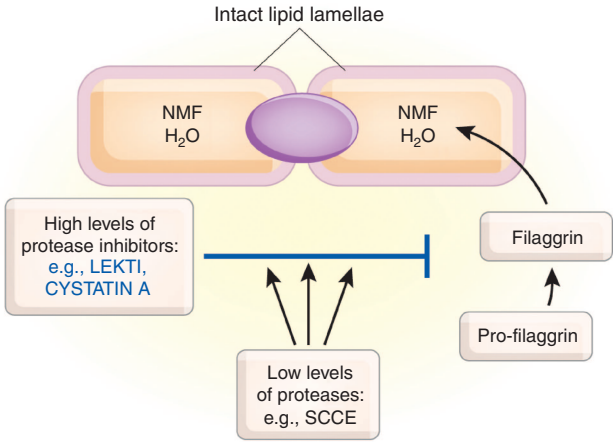

Figure 2. The structure of the epidermal barrier located in the lower part of the stratum corneum (SC). Highly differentiated flattened keratinocytes, referred to as corneocytes (beige rectangles), are the building blocks of the epidermal barrier. They contain natural moisturizing factor (NMF), derived from pro-filaggrin, a mix of hygroscopic compounds, which help maintain skin hydration. A water resistant layer of lipid lamellae (pink) encases the corneocytes preventing water loss and impeding barrier permeability. The corneocytes are held together by corneodesmosomes (purple spheres), the integrity of which is dependent on a cocktail of proteases and protease inhibitors. The balance between the expression and activity of proteases, such as KLK7 (SCCE), and protease inhibitors, such as LEKTI and cystatin A, determines the rate of desquamation (corneocytes shedding) and thereby the thickness of the barrier. Under normal conditions, the barrier is only degraded in the upper layers of the SC providing a resilient permeability barrier that prevents the penetration of allergens.

corneocytes like mortar (Lavker, 1976). The lipid lamellae help prevent internal water loss and penetration of water-soluble materials (Figure 2). They also give flexibility to the barrier and ensure that it is as tight as possible. The lipid lamellae matrix is a crystalline substance composed of ceramides, cholesterol, fatty acids, and cholesterol esters (Rawlings, 2003), and is believed to exist as a single and coherent lamellar gel (Fartasch and Diepgen, 1992).

Disturbed maturation and delivery of the lamellar granules has been shown in atopic skin (Melnik *et al.*, 1989; Fartasch and Diepgen, 1992). This results in a considerable deficiency in the acid, lipid, and enzyme constituents of the SC, leading to a defective barrier function (Mecheleidt *et al.*, 2002). An increase in sphingomyelin deacylase activity is also associated with AD and results in a decreased production of ceramide (Hara *et al.*, 2000).

During terminal differentiation, KCs replace their plasma membrane with an insoluble protein layer referred to as the ''cornified envelope'' (CE) (Candi *et al.*, 2005). This envelope confers strength to the corneocytes and acts as a scaffold for the attachment of lipids, including ceramides from the lamellae matrix, which form the ''lipid envelope'' (Elias and Menon, 1991). The CE is mainly composed of the structural proteins, loricrin, involucrin, filaggrin, and small proline-rich proteins, which are cross-linked together by the action of transglutaminases (Steven and Steinert, 1994; Steinert and Marekov, 1995). Filaggrin is particularly important, because it also aggregates the keratin fibers of the cellular cytoskeleton into bundles, thereby collapsing the corneocytes into flattened discs with a large surface area (Steinert *et al.*, 1981).

The majority of filaggrin does not persist beyond the deepest two layers of the SC (Richards *et al.*, 1988; Harding *et al.*, 2000). Filaggrin is extensively deaminated through the actions of the enzyme peptidyl deiminase. It is subsequently degraded into small peptides and then into free amino acids. The free amino acids are then catabolized into the constituents of natural moisturizing factor (NMF), such as lactic acid, sodium pyrrolidone carboxylic acid, urocanic acid, and urea (Harding *et al.*, 2000). The NMF is essential for the retention of water within corneocytes, and results in their optimal hydration and swelling. Sodium pyrrolidone carboxylic acid and lactic acid, in particular, are intensely hydroscopic; they both absorb water and dissolve in their own water, acting as very efficient humectants (Harding *et al.*, 2000), which prevents the development of gaps between the corneocytes, enhancing the integrity of the SC, and making it resistant to the penetration of irritants and allergens (Figure 2).

Corneodesmosomes are specialized desmosomes, which bind the corneocytes together in the SC (Serre *et al.*, 1991) and are incorporated into the corneocyte envelope. They consist of the cadherin family of extracellular transmembrane glycoproteins, desmoglein (DSG) and desmocollin (Rawlings, 2003). Within the corneocytes, DSG and desmocollin are linked to keratin filaments through corneodesmosomal plaque proteins, including plakoglobin, desmoplakin, and plakophilin. DSG and desmocollin pass from the corneocyte envelope into the lipid lamellae between the corneocytes and bind to the same proteins on adjacent cells (Buxton *et al.*, 1993). Corneodesmosin is a 52 kDa protein specifically expressed in keratinizing epithelia (Serre *et al.*, 1991; Lundström *et al.*, 1994; Guerrin *et al.*, 1998). After secretion into the extracellular space, corneodesmosin is translocated to the transition zone between the stratum granulosum (SG) and the SC (Haftek *et al.*, 1991), and incorporated into the desmosomes. This marks the transition from desmosome to corneodesmosome.

The corneocytes that are shed from the skin surface are continually replaced from underneath by KCs undergoing terminal differentiation. Thus, there is a fine balance between basal-cell proliferation and corneocyte desquamation involved in maintaining an epithelium at a constant thickness (Egelrud, 1993). Desquamation also treads a fine balance between adequate breakdown of the barrier to allow a continual renewal of epidermal cells and leaving the barrier sufficiently intact to prevent allergens and irritants

from penetrating through to the deeper layers of the skin. The current model of the processes involved in desquamation has been provided by Caubet *et al*. (2004). The model describes a network of degradatory proteases, regulated by protease inhibitors, which breaks down the extracellular corneodesmosomal adhesion proteins that bind the corneocytes together and, in doing so, allows the corneocytes to be shed from the skin surface. A cocktail of serine, cysteine, and aspartic proteases are secreted into the extracellular spaces of the SC during desquamation to facilitate the breakdown of the corneodesmosomes (Figure 2) (Horikoshi *et al*., 1999; Watkinson, 1999; Ekholm *et al*., 2000). According to the model of Caubet *et al*. (2004), inactive protease precursors are activated by tryptic cleavage and regulated by a complementary cocktail of protease inhibitors.

SC proteases and protease inhibitors

The human kallikrein (KLK)-related peptidases, including SC chymotryptic enzyme (SCCE, KLK7) and SC tryptic enzyme (SCTE, KLK5), are key proteases involved in desquamation (Egelrud and Lundström, 1991; Egelrud, 1993; Suzuki *et al*., 1994; Ekholm and Egelrud, 1998; Hansson *et al*., 2002). They are members of a family of serine proteases, with optimum activity at slightly alkaline pH, expressed in granular KCs, and present within the extracellular spaces of the SC (Sondell *et al*., 1994; Ekholm and Egelrud, 1998). KLK7, with chymotrypsin-like activity, has been shown to hydrolyze corneodesmosin and desmocollin 1, whereas KLK5, possessing trypsin-like activity, can also cleave DSG 1 (Caubet *et al*., 2004). More recently, enzymatically active KLK-related peptidase 14 (KLK14), also exhibiting trypsin-like activity, was identified in the SC and found to cleave DSG at a greater rate than KLK5 (Brattsand *et al*., 2005; Borgoño *et al*., 2007). KLK5, KLK7, and KLK14 are all produced as inactive precursors. Removal of pro-peptides by trypsin digestion leads to the formation of the proteolytically active enzymes (Egelrud and Lundström, 1991; Hansson *et al*., 2002). Studies have shown that KLK5 is capable of activating KLK7 (Caubet *et al*., 2004) and KLK14 (Emami and Diamandis, 2008), in addition to self-activation (Egelrud and Lundström, 1991; Egelrud, 1993; Ekholm and Egelrud, 1998), suggesting that KLK5 may serve as a primary regulator of the KLK cascade in the SC. KLK14 has been shown to activate KLK5, as well as KLK1 and KLK11 (Emami and Diamandis, 2008). Other enzymes capable of degrading corneodesmosomal adhesion proteins include the cysteine proteases, cathepsin L2 (SC thiol protease) and SC cathepsin-L-like enzyme (Watkinson, 1999; Bernard *et al*., 2003), and the aspartate protease, cathepsin D (Horikoshi *et al*., 1998), all with optimum activity at acidic pH.

The activity of the above-mentioned proteases, and thereby the rate of desquamation, is strictly regulated by a cocktail of protease inhibitors. KLK7 activity is inhibited by the serine leukoprotease inhibitor (Franzke *et al*., 1996), which can itself be inactivated by members of the cathepsin family (Taggart *et al*., 2001). KLK7 is also inhibited by elafin, otherwise known as skin-derived antileukoprotease, which has been shown to covalently bind to corneocytes (Molhuizen *et al*., 1993). Human epidermis also expresses the cystatin protease inhibitors, A and M/E, which are specific for cysteine proteases (Zeeuwen *et al*., 2001). Cystatin A is also secreted in sweat and forms a layer over the surface of the skin that protects the skin from exogenous proteases, such as those produced by house dust mites and *Staphylococcus aureus* (Kato *et al*., 2005).

The lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI), encoded by the serine protease inhibitor Kazal-type 5 (*SPINK5*) gene, is a particularly important, pH-dependent regulator of desquamation (Mägert *et al*., 1999; Deraison *et al*., 2007). LEKTI is composed of 15 potential serine proteinase inhibitory domains, at least four of which have confirmed activity against members of the KLK family, including KLK5, KLK7, and KLK14 (Borgoño *et al*., 2007; Deraison *et al*., 2007). LEKTI is expressed in the granular layer of the epidermis, and delivered to the SG–SC interface in lamellar bodies (Ishida-Yamamoto *et al*., 2005). Here, it is colocalized with human KLK-related peptidases in the extracellular space, where the pH is near neutral. Under these conditions, LEKTI is a potent inhibitor of both KLK5 and KLK7 (Deraison *et al*., 2007). As the pH becomes more acidic, the inhibitory potential of LEKTI is reduced. In the superficial layers of the SC, inhibition by LEKTI is sufficiently reduced to support localized desquamation.

The acid mantle

The skin has long been known to have an acidic pH (the acid mantle) that contributes to the optimal barrier function of this tissue (Schade and Marchionini, 1928). The average surface pH of the forearm of a healthy male is around 5.4–5.9 (Braun-Falco and Korting, 1986). In humans, the skin surface pH at birth is near neutral (pH 6.5) and takes several weeks after birth for the pH to reach the normal range (Taddei, 1935; Behrendt and Green, 1958; Fox *et al*., 1998; Visscher *et al*., 2000).

Although the acid mantle of the SC was initially thought to originate from exogenous sources (microbial metabolites, free fatty acids of pilo-sebaceous origin, and eccrine gland-derived products, such as amino and lactic acids) (Marchionini and Hausknecht, 1938; Puhvel *et al*., 1975; Ament *et al*., 1997), recent studies have shown that endogenous pathways, such as generation of by-products of keratinization, synthesis of free fatty acids from phospholipid hydrolysis by the secretory phospholipase A2, and the non-energy-dependent sodium–proton exchanger, are additional sources (Behne *et al*., 2002; Fluhr and Elias, 2002; Rippke *et al*., 2002). For example, NMF makes an important contribution to the acid mantle, which, in turn, has multiple effects on the skin. First, it has a strong antimicrobial effect (Rebell *et al*., 1950; Leyden and Kligman, 1978), decreases skin colonization by pathogenic bacteria (Rebell *et al*., 1950; Aly *et al*., 1975; Puhvel *et al*., 1975), and favors the adhesion of non-pathogenic bacteria to the SC (Bibel *et al*., 1987). Second, several lines of evidence indicate a role for skin

surface pH in desquamation, permeability barrier homeostasis, and SC integrity/cohesion.

Serine proteases, such as KLK5 and KLK7, involved in desquamation, exhibit a neutral pH optimum (Ekholm *et al.*, 2000). A change in pH from 7.5 to 5.5 reduces KLK7 activity by 50% (Ekholm *et al.*, 2000; Caubet *et al.*, 2004). This activity is controlled *in vivo* by the action of the pH-sensitive inhibitor, LEKTI. Conversely, cathepsin LZ and cathepsin D have an acidic pH optimum (Horikoshi *et al.*, 1999; Bernard *et al.*, 2003). The lipid-generating enzymes, β-glucocerebrosidase and sphingomyelinase, also exhibit low acid pH optimum (Holleran *et al.*, 1993; Jensen *et al.*, 1999; Schmuth *et al.*, 2000; Uchida *et al.*, 2000). Taken together, the pH gradient across the epidermis is very important in regulating desquamation and the generation of the lamellar matrix.

Epidermal barrier abnormalities are noticed when the skin pH is increased by blocking either the secretory phospholipase A2 or the non-energy-dependent sodium–proton exchanger, and these abnormalities are corrected by co-exposure of inhibitor-treated areas to an acidic buffer (Fluhr *et al.*, 2001; Behne *et al.*, 2002). Moreover, a delay in epidermal barrier recovery occurs when the skin is immersed in neutral pH buffers (Mauro *et al.*, 1998b). When hairless mice were treated with ''superbases'' that neutralize skin surface pH, a rapid activation of serine protease activity was observed with consequent degradation of corneodesmosomes (Hachem *et al.*, 2005). This was accompanied by decreased glucocerebrosidase activity, resulting in incompletely processed lipid lamellae membranes.

Notably, skin pH is significantly elevated in patients with AD and similar conditions compared with that in normal controls (Anderson, 1951; Locker, 1961; Eberlein-Konig *et al.*, 2000). This elevation of skin pH is evident even in the uninvolved skin of patients with AD (Seidenari and Giusti, 1995; Eberlein-Konig *et al.*, 2000). Seidenari and Giusti (1995) also showed that skin pH values are higher in patients with active lesions than in asymptomatic patients. This elevated level of skin pH can be expected to delay barrier recovery and facilitate barrier breakdown (Elias, 2004).

Maintaining epidermal barrier homeostasis

Acute barrier disruption, by tape stripping for instance, results in disruption of the calcium gradient, which is specific to the epidermis and is required to maintain the different stages of differentiation (Yuspa *et al.*, 1989; Lee *et al.*, 1992; Mauro *et al.*, 1998a; Elias *et al.*, 2002). This stimulates barrier repair by inducing the formation and secretion of lamellar bodies, and the subsequent delivery and release of their contents to the SG–SC interface (Menon *et al.*, 1994). As discussed above, the lamellar bodies deliver essential proteins and lipids for the formation and maintenance of the epidermal barrier, including epidermal proteases and protease inhibitors. Disruption of the calcium gradient results in the inhibition of differentiation, thereby promoting proliferation of the lower epidermal KC layers (Elias *et al.*, 2002). Interestingly, this pathway is counteracted by a second pathway, which responds to changes in the pH gradient across the epidermis.

Barrier disruption, caused by tape stripping, results in elevation of the pH at the uppermost layers of the epidermis, and the subsequent elevation of serine protease activity (Denda *et al.*, 1997; Hachem *et al.*, 2003; Fluhr *et al.*, 2004). The epidermal trypsin-like serine proteases, KLK5 and KLK14, then activate the protease-activated receptor 2 (PAR)2 signaling cascade by direct cleavage of PAR2 (Hachem *et al.*, 2006; Stefansson *et al.*, 2008). PAR2 is a member of the protease-activated receptor family of G-coupled receptors, involved in innate immune inflammatory responses and in pruritus (Steinhoff *et al.*, 2003; Ramachandran and Hollenberg, 2008). Activation of PAR2, in response to perturbations in SC pH, results in the inhibition of lamellar body secretion and promotion of cornification (terminal differentiation) (Demerjian *et al.*, 2008). Enhanced cornification, in response to acute barrier disruption, is accompanied by increased expression of caspase 14, a cysteine protease that regulates formation of the CE (Demerjian *et al.*, 2008). In trying to rationalize the opposing effects of these two pathways regulating lamellar body secretion in response to barrier disruption, Hachem *et al.* (2006) suggested that concomitant changes in calcium gradient and activation of PAR2 permits the rapid transition of the outermost KCs of the SG into terminally differentiated corneocytes.

Apart from its role in maintaining barrier homeostasis, PAR2 forms an important link between the SC ''proteasome'' and initiation of an inflammatory response, such as those experienced during flares of AD (Ramachandran and Hollenberg, 2008). An additional link worth consideration, not covered here, is the ability of SC proteases to process the antimicrobial peptide, cathelicidin, an effector of innate immunity important in the epidermis (see reviews by Schauber and Gallo, 2008; Niyonsaba *et al.*, 2006). In support of the outside–inside theory, dysregulation of proteases involved in desquamation, such as KLK5 and KLK14, for example, could initiate an innate immune inflammatory response and, thereby, a flare of AD. The demonstration that PAR2 mediates pruritus, associated with eczematous lesions, emphasizes the relevance of the serine protease–PAR2 signaling pathway in AD (Steinhoff *et al.*, 2003). Notably, blocking PAR2 signaling by inhibiting protease activity at the SC improves the rate of barrier recovery (Hachem *et al.*, 2006). For this reason, PAR2 has been suggested as a previously unknown target for treating AD.

Variations in epidermal barrier structure and function

Although AD can affect any area of the body, it preferentially affects the flexures and the face. In babies aged less than 6 months, the face and scalp are the most common sites affected (Kunz and Ring, 2002). In older children, the most common sites affected are the antecubital and popliteal fossae (Schudel and Wüthrich, 1985; Dotterud *et al.*, 1995). Many factors could explain the areas of predisposition to

AD, including the thickness of the SC and the variation in exposure to exogenous substances, such as irritants and allergens. The eyelids and the genitals have the thinnest epidermis, followed by the flexor forearm and posterior auricular areas (Barker, 1951; Southwood, 1955; Lee and Hwang, 2002). The number of cell layers in the SC also varies between different body sites and correlates with epidermal thickness (Ya-Xian *et al.*, 1999). A greater penetration of TCS was observed through the skin of these areas with the thinnest epidermis (Schaefer and Scheer, 1951; Cronin and Staughton, 1962; Marzulli, 1962; Feldman and Maibach, 1967).

The size of the corneocytes that make up the epidermal barrier also varies between different body sites (Plewig and Marples, 1970; Rougier *et al.*, 1988; Kashibuchi *et al.*, 2002). This variation correlates with skin permeability; for example, the postauricular and forehead SC were found to have the smallest corneocytes and the highest permeability compared with the upper arm, the forearm, and the abdomen (Rougier *et al.*, 1988). Furthermore, it was shown that corneocytes from patients with AD are significantly smaller compared with those found in normal skin (Hölzle and Plewig, 1977; Kashibuchi *et al.*, 2002). Taken together, variations in epidermal thickness, the layers of cells comprising the epidermal permeability barrier, and the size of the cells suggest region-specific variations in the susceptibility to flares of AD.

As introduced above, the level of protease activity at the epidermal barrier is an important parameter determining barrier structure and function. It is no surprise, therefore, that the level of protease activity determined at different body sites varies, and that this variation correlates with both the thickness of the SC and the size of the corneocytes (Voegeli *et al.*, 2007). The activity of the desquamatory proteases, KLK5 and KLK7, was found to be two to four times higher on the cheek compared with the forearm (Voegeli *et al.*, 2007). Similarly, the surface pH and integrity of the barrier, measured as TEWL, was higher on the cheek. The measurement of TEWL is an important method of assessing barrier functionality (its ability to retain water). Nikolovski *et al.* (2008) found that the level of TEWL is associated with the thickness of the barrier, as determined using Raman confocal microscopy.

In a further study, TEWL measured on the cheek of 10 volunteers was found to correlate positively with certain protease activities (Voegeli *et al.*, 2008). Interestingly, the amount of a range of epidermal proteases present in samples of SC, and the level of activity, was elevated in patients with AD compared with that in normal controls (Tarroux *et al.*, 2002; Komatsu *et al.*, 2007). Given the role of these proteases in desquamation, the question of whether protease activity is a marker of, or the cause of, conditions involving skin barrier breakdown is raised.

GENETIC FACTORS AFFECTING SKIN BARRIER FUNCTION

If a disturbance in epidermal barrier function represents one of the primary events in the development of AD, the genes that regulate barrier function are a logical place to look for changes/variants that predispose to the disease. Several groups (Walley *et al.*, 2001; Vasilopoulos *et al.*, 2004, 2007; Morar *et al.*, 2006; Palmer *et al.*, 2006) have identified variants in genes regulating the integrity of the epidermal barrier and have shown that they are associated or linked with AD.

Genes encoding epidermal barrier structural proteins

The most significant ''genetic factors'' associated with AD are the loss-of-function mutations found within the *FLG* gene encoding profilaggrin, the ~500-kDa precursor for the structural protein, filaggrin (reviewed in O'Regan *et al.*, 2008). In 2006, Palmer and colleagues first identified two such mutations within the *FLG* gene, which strongly predispose to AD (Palmer *et al.*, 2006). Notably, mutations of the *FLG* gene were primarily identified as the cause of ichthyosis vulgaris, which often occurs concomitantly with AD (Smith *et al.*, 2006). Since then, several additional studies have confirmed the association and found other null mutations within this gene that predispose to AD (Marenholz *et al.*, 2006; Ruether *et al.*, 2006; Sandilands *et al.*, 2006, 2007; Weidinger *et al.*, 2006, 2008; Barker *et al.*, 2007; Hubiche *et al.*, 2007; Morar *et al.*, 2007; Nomura *et al.*, 2007, 2008; Rogers *et al.*, 2007; Stemmler *et al.*, 2007; Brown *et al.*, 2008; Ekelund *et al.*, 2008). To date, there have been 20 mutations of *FLG* found within European populations and 17 in Asian populations.

The association of *FLG* mutations with AD provides the explanation for the decreased levels of both filaggrin and NMF in the skin of patients with this disorder (Seguchi *et al.*, 1996; Harding *et al.*, 2000; Kezic *et al.*, 2008). The result of this is a reduced ability of the corneocytes to retain water, owing to the reduced levels of humectants (NMF) and a defective CE, which also adversely affects the skin's elasticity and mechanical resistance (Figure 3). A barrier defect of this kind is expected to increase the risk of irritant and allergen penetration, presumably through the development of gaps between the shrunken corneocytes. In support of this, a clinical study of the effects of *FLG* loss-of-function mutations in a Scottish population found a high prevalence of fine scaling and roughness of the skin in carriers (Sergeant *et al.*, 2009). There was also a strong association between dry and sensitive skin with carriers of *FLG* loss-of-function mutations. Nemoto-Hasebe *et al.* (2009) showed a strong correlation between clinical severity and barrier impairment in AD patients carrying a *FLG* loss-of-function mutation. A separate study, however, failed to identify an effect of *FLG* mutations on skin condition assessed by clinical scoring (SCORAD) and measurement of TEWL in a French population (Hubiche *et al.*, 2007).

The *FLG* gene is located within the epidermal differentiation complex, a cluster of genes encoding a range of proteins involved in epidermal differentiation, some of which are incorporated into the CE (Mischke *et al.*, 1996). This region is strongly associated with a number of skin disorders, including AD and psoriasis (Hoffjan and Stemmler, 2007). A number of these genes encode

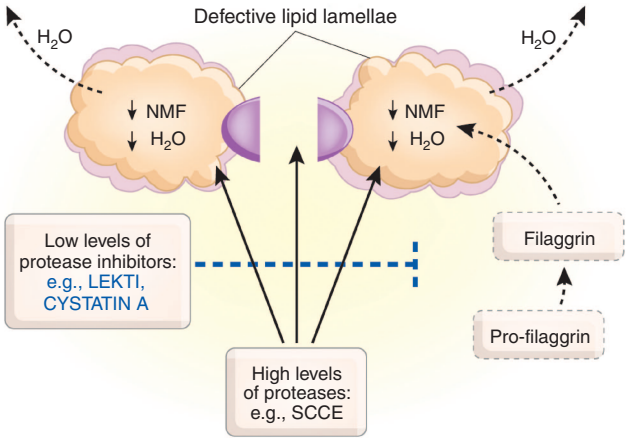

Figure 3. A defective epidermal barrier is a poor permeability barrier, which permits the entry of allergens and the loss of moisture. Changes in the *FLG* gene encoding pro-filaggrin result in reduced, or absent, expression of filaggrin thereby adversely affecting the structure of the corneocytes (beige)—the ''bricks''. The levels of natural moisturizing factor (NMF), derived from filaggrin, are also adversely affected, resulting in a decreased ability of the corneocytes to hold water and a concomitant elevation of pH. Elevated pH favors serine protease activity and inhibits enzymes involved in the synthesis of lipid lamellae (pink)—the ''mortar''. Genetic changes in the genes encoding SCCE (*KLK7*), LEKTI (*SPINK5*), and cystatin A (*CSTA*) all lead to elevated protease activity involved in desquamation—cleavage of the corneodesmosome junctions (purple spheres) between the corneocytes analogous to ''rusting'' of the ''iron rods''.

members of the family of S100 calcium-binding proteins, which includes filaggrin. In particular, the S100 calcium-binding protein, psoriasin (S100A7), was found to be a biomarker of the hyperproliferative disorder, psoriasis, and more recently also of AD (Madsen *et al.*, 1991; Gläser *et al.*, 2009).

Transgenic knockout mice have revealed the importance of several adhesion proteins for the assembly of functional desmosomes and for the maintenance of a functional skin barrier. The DSG $3^{-/-}$ mice develop traumatized skin that displays a marked separation of desmosomes under electron microscopy (Koch *et al.*, 1997). Mice lacking desmocollin 1 have been shown to have a flaky and fragile epidermis, with acanthosis in the granular layer (Chidgey *et al.*, 2001). Desmoplakin is also important in epidermal sheet formation (Vasioukhin *et al.*, 2001). Mice lacking desmoplakin have few desmosomes and a marked reduction in barrier integrity (Gallicano *et al.*, 1998). It could be hypothesized that mutations within genes encoding adhesion proteins, which alter the ability of these proteins to preserve skin barrier integrity, might also play a role in the development of AD.

The nucleated layers of the epidermis, including the SG and the upper stratum spinosum (SS), also offer protection from external insults in addition to the ''primary'' epidermal barrier located in the SC. Several groups of proteins that connect the KC cells of the SG and SS together, including tight junction, gap junction, and adheren junction proteins, are important in maintaining the integrity of this ''second'' barrier (reviewed in Proksch *et al.*, 2008). Dysregulation of these proteins is associated with various skin conditions, including AD, ichthyosis vulgaris, psoriasis vulgaris, and lichen planus. De Benedetto *et al.* (2008) recently reported a downregulation of the tight junction proteins, Claudin-1 and Claudin-23, and DSG-1, a component of corneodesmosomes, in AD.

Genes encoding SC proteases

In patients with AD, the expression of seven KLK-related peptidases is increased, with a greater abundance of these proteases found within the SC (Komatsu *et al.*, 2007) (Figure 3). Although not significant, an increase in chymotrypsin-like, and not trypsin-like, activities was found in samples of SC collected from patients with AD compared with those from normal controls (Komatsu *et al.*, 2007). Plasmin-like and furin-like activities were significantly elevated. Furthermore, it has been shown that transgenic mice overexpressing human KLK7, with chymotryptic activity, develop changes in their skin similar to those seen in chronic AD (Hansson *et al.*, 2002).

In a case–control study on 103 AD patients and 261 matched controls, a significant association was found between a 4-bp insertion in the 3′-untranslated region of the *KLK7* gene, encoding KLK7 and AD (Vasilopoulos *et al.*, 2004). It is known that the determinants of mRNA stability are frequently positioned in the 3′-untranslated region of genes and that any mutation in this region can alter the expression levels of the encoded protein (Frittitta *et al.*, 2001; Bilenoglu *et al.*, 2002; Di Paola *et al.*, 2002). Thus, the AACC insertion could potentially increase the half-life of *KLK7* mRNA, leading to an increased production of the enzyme in the skin of individuals with AD. Since the publication of this initial study, two further studies have been carried out in European populations; however, no association of the 4-bp insertion mutation with AD was found (Hubiche *et al.*, 2007; Weidinger *et al.*, 2008).

It has been suggested that the level of protease activity at the SC is an important indicator of milder forms of barrier disruption, including sensitive skin, in addition to AD (Voegeli *et al.*, 2008). This may explain the poor association between *KLK7* and AD, wherein the control populations will have comprised subjects with a wide range of skin conditions. The level of proteases quantified in samples of human SC was found to correlate with biophysical measures of skin condition, including hydration and TEWL. Trypsin-like (including KLK5 and KLK14), tryptase-like, plasmin, and urokinase activities, but not chymotrypsin-like activities (including KLK7), were positively correlated with TEWL and negatively correlated with hydration. Interestingly, the type of protease activity that correlates with barrier integrity is consistent with the types of

protease capable of activating PAR2, involved in barrier homeostasis (Stefansson *et al.*, 2008).

Cells within the inflammatory infiltrate can produce proteases that further damage the skin barrier. These proteases can be considered as a product of the inflammatory response (secondary proteases) and their levels will be proportional to the severity of a flare of AD. Mast cell chymase (MCC) is a chymotrypsin-like serine protease primarily stored in secretory mast cell granules. In one study (Badertscher *et al.*, 2005), the number of MCC-positive cells was significantly increased in the lesional skin of patients with AD in comparison with nonlesional skin. However, there was no significant difference in the number of MCC-positive cells between the nonlesional skin of patients with AD and the skin of normal controls, suggesting that increased MCC activity may be associated with active dermatitis. In another study in mice (Tomimori *et al.*, 2002), injection of MCC into the normal skin induced an inflammatory response similar to that observed in AD. There is also evidence that MCC may participate in the development of chronic dermatitis by inducing eosinophil infiltration (Mao *et al.*, 1998). Variants within the MCC gene have been associated with AD in children (Mao *et al.*, 1998). The association was strongest in individuals with low levels of total serum IgE (Mao *et al.*, 1998). Conversely, in adults with AD, a polymorphism in the promoter region of the MCC gene has been associated with high levels of total serum IgE (Iwanaga *et al.*, 2004).

Genes encoding protease inhibitors

Several studies have linked mutations in the *SPINK5* gene, which encodes LEKTI, with AD, when maternally inherited (Walley *et al.*, 2001; Kato *et al.*, 2003; Nishio *et al.*, 2003; Weidinger *et al.*, 2008). It is worth noting that the association was weaker than in the case of *FLG* mutations, in part, owing to a high prevalence in the control population. In a separate study on a French population, an association between *SPINK5* and AD was not found; however, there was an association between carriers and raised IgE serum levels (Hubiche *et al.*, 2007). The association of *SPINK5* mutations with raised IgE serum levels and with other atopic conditions, such as asthma, led to the suggestion that they are risk factors for general atopy (Walley *et al.*, 2001; Nishio *et al.*, 2003). In fact, *SPINK5* mutations are the underlying cause of Netherton syndrome, a severe autosomal recessive disorder of the skin with atopic manifestations (Sprecher *et al.*, 2001; Komatsu *et al.*, 2002). Individuals with this disorder display a marked barrier dysfunction, involving altered desquamation and impaired keratinization (Comel, 1949).

Patients with Netherton syndrome carry mutations in the *SPINK5* gene on both alleles, which are expected to result in premature termination of transcription or altered translation (Sprecher *et al.*, 2001). AD, on the other hand, which shares common features with Netherton syndrome, is associated with coding polymorphisms in *SPINK5,* which are expected to effect the functionality of LEKTI domains (Walley *et al.*, 2001; Kato *et al.*, 2003). Ultrastructural analyses of skin from patients with Netherton syndrome show that there is a marked increase in corneodesmosome cleavage and a reduction in intercorneocyte cohesion (Comel, 1949). Transgenic studies using *SPINK5* (−/−) mice confirmed that LEKTI deficiency results in increased breakdown of DSG 1 (Descargues *et al.*, 2005) and corneodesmosin (Yang *et al.*, 2004), as a result of elevated KLK5 and KLK7 activity (Figure 3). The mutations associated with AD are expected to produce a similar, but milder, skin barrier defect, as a result of dysregulation of degradatory proteases, including KLK5 and KLK7. The gene−gene interactions among *SPINK5, KLK7,* and *FLG,* and association with AD were investigated in two separate studies on European populations (Hubiche *et al.*, 2007; Weidinger *et al.*, 2008). No link was determined, suggesting that combinations of the mutations identified so far, in these genes at least, do not determine subsets of AD patients.

In addition to *SPINK5,* a mutation has been identified in the *CSTA* gene encoding the cysteine protease inhibitor, cystatin A, which associates with AD. The cystatin A gene maps to chromosome 3q21, which has been identified as a major susceptibility locus for AD (Lee *et al.*, 2000). Decreased expression of cystatin A has been found in the skin of patients with AD (Seguchi *et al.*, 1996). The +344c variant results in decreased mRNA stability and, therefore, decreased levels of the cystatin A protease inhibitor, both within the skin and in sweat (Vasilopoulos *et al.*, 2007). As a consequence, inhibition of both endogenous and exogenous cysteine proteases, such as Der p1 from house dust mites, for example, is reduced, promoting deterioration of the corneodesmosomes, breaking down of the SC, and subsequently allowing the penetration of allergens (Vasilopoulos *et al.*, 2007) (Figures 3 and 4). Transgenic mice carrying a null mutation in the gene encoding cystatin M/E also display severe barrier abnormalities, affecting cornification and desquamation, and die shortly after birth (Zeeuwen *et al.*, 2001).

ENVIRONMENTAL FACTORS AFFECTING SKIN BARRIER FUNCTION

Several environmental factors have been associated with AD, including washing with soap and detergents, washing with hard water, and exposure to house dust mites and food allergens (Abe *et al.*, 1978; Al-Jaberi and Marks, 1984; White and Gohari, 1984; Hamami and Marks, 1988; Melnik *et al.*, 1989; Colloff, 1992; Tan *et al.*, 1996; McNally *et al.*, 1998, 2001; Lack *et al.*, 2003); however, there are few formal longitudinal studies that indicate how the home environment has changed over the past 50 years. A review of data regarding exposure to soap and detergents, frequency of washing, and exposure to house dust mites, indicated significant changes over the past 50 years (Cork *et al.*, 2002). An example of these changes was seen in the increased use of soap and detergent personal wash products between 1981 and 2001 in the United Kingdom, where the sales rose (infla-

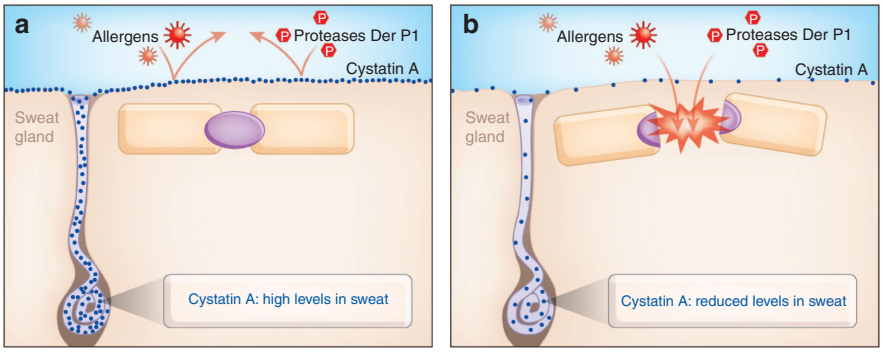

Figure 4. Protease inhibitors protect the epidermal barrier from degradation by exogenous proteases. In normal skin (panel **a**), the protease inhibitor, cystatin A (blue dots), is secreted in sweat and flows out onto the surface of the skin forming a protective layer. Exogenous proteases from, for example, house dust mites (Der P1) are inhibited by the protective layer of cystatin A and, as a result, cannot break down the corneodesmosomes (purple spheres) that lock the corneocytes (beige rectangles) of the stratum corneum together. In atopic dermatitis (panel **b**), altered expression of cystatin A leads to an incomplete protective barrier against the activity of exogenous proteases leading to breakdown of the epidermal barrier, and potential allergen, including Der P1, penetration.

tion adjusted) from £76 million to £453 million, while the population only rose from 56.3 million to 59.1 million (Cork *et al.*, 2002). The frequency of personal washing has also changed over the past 40 years. In 1961, the average use of water for personal washing was 11 l per person per day, rising to 51 l per person per day in 1997/98 (Cork *et al.*, 2002). In the United Kingdom, there have also been changes in the heating, ventilation, insulation, and floor coverings of houses over the past 40 years, which have created an optimal environment for the house dust mite (Cork *et al.*, 2002). House dust mites thrive in humid tropical conditions, which the improvement in heating, coupled with the reduction in ventilation, has gone some way to create—a warmer more humid home environment. The prevalence of AD is higher in areas where there is hard water compared with areas where the water is soft (McNally *et al.*, 1998). This may be because of irritant chemicals in hard water and/or the larger amount of soap and other detergents required to produce lather when washing with hard water.

The effects of soap and detergents on the epidermal barrier

Detergents are widely used in cleaning human skin. They work by emulsifying the skin surface lipids (both foreign and natural), which can then be washed off by water. Surfactants can damage the skin, provoking scaling, dryness, tightness and roughness, erythema, and swelling (Kligman and Wooding, 1967; Imokawa, 1980; Froebe *et al.*, 1990; Ananthapadmanabhan *et al.*, 2004). The use of soap and detergents is one of the most common causes of irritant contact dermatitis of the hands and can trigger flares of AD (Meding and Swanbeck, 1987).

The detergent sodium lauryl sulfate is used as the standard test of skin susceptibility to irritation. The negative effects of surfactants on skin barrier function are shown by an increased TEWL, which is more severe in patients with AD than in normal controls (Cowley and Farr, 1992). Surfactants can solubilize lipids, and it has been postulated that this could be the mechanism by which they increase TEWL (Kirk, 1966; Cowley and Farr, 1992). However, measurements of lipid solubilization by sodium lauryl sulfate suggest that, at concentrations ranging between 0.1 and 2%, it removes very small amounts of free fatty acids, cholesterol, and esters (Froebe *et al.*, 1990).

The acute irritant effects of soap and detergents could be partially explained by the release of pro-inflammatory cytokines from corneocytes (Wood *et al.*, 1996, 1997). However, it is the effect of soap and detergents on skin pH that is most compelling. Washing the skin with soap causes an increase in pH by 3 U on the palms for more than 90 minutes (Mucke *et al.*, 1993). As discussed above, elevated pH has a significant detrimental effect on the epidermal barrier. White *et al.* (1987) measured the thickness of the SC in normal skin and in non-lesional eczematous skin before and after washing with soap. Before washing, the SC was thicker in normal skin (19.7 μm) than in non-lesional eczematous skin (13.7 μm). Washing with soap caused further thinning of the SC in both the normal and the non-lesional eczematous skin, which is consistent with altered activity of epidermal proteases.

The use of detergents has also been shown to alter the expression of key markers of KC differentiation and SC degradatory enzymes, in addition to promoting the release of cytokines (Wood *et al.*, 1996, 1997; Törmä *et al.*, 2008). PAR2, as discussed above, is involved in signaling innate immune inflammatory responses and changes in gene transcription. Given that PAR2 is activated by serine proteases found within the epidermis in a pH-dependent manner, it is likely that this pathway responds to the use of soap and detergents, with consequent changes in gene expression. The involvement of PAR2 in mediating pruritus and inflammation suggests an interesting link between the use of detergents and skin irritation, which calls for further investigation.

Exogenous proteases

House dust mites are a source of over 30 different proteins that can induce IgE-mediated responses (Stewart and Thompson, 1996), including cysteine and serine proteases (Yasueda *et al.*, 1993). Some of these proteins have been shown to cleave adhesion proteins

and to increase the permeability of lung epithelium (Winton *et al.*, 1998). Patch tests have shown that two proteins with proteolytic activity derived from house dust mites, Der p1 and Der p2, can elicit irritative or immune reactions that are not linked to raised levels of IgE, suggesting that these proteins cause skin irritation or immune activation through direct proteolytic activity (Deleuran *et al.*, 1998). Jeong *et al.* (2008) showed that proteolytically active house dust mite and cockroach allergens both facilitate barrier breakdown and activate PAR2. This leads to increased penetration of allergens and pruritus. The level of IgE specific for house dust mite allergens significantly correlated with OSCORAD, a clinical scoring system for the severity of AD in patients with a defective skin barrier (presence of a *FLG* loss-of-function mutation) (Nemoto-Hasebe *et al.*, 2009). This shows the ability of such allergens to trigger/exacerbate AD; however, the lack of correlation with AD patients without a *FLG* loss-of-function mutation suggests that a predisposition to a defective barrier is required. Furthermore, Teplitsky *et al.* (2008) detected a higher percentage of samples with house dust mite from patients with AD when compared with that of controls; however, there was no difference in their prevalence in bedding or clothing between the groups. This indicates that a ''normal'' barrier resists such infestations, but that they are a ''secondary'' factor that can exacerbate or possibly even trigger AD lesions when there is an existing barrier defect.

As reviewed by Storck (1948), *S. aureus* has been implicated as an environmental factor in the pathogenesis of AD since the nineteenth century. *S. aureus* is not a member of the normal microflora colonizing the skin, apart from carriage in the nasal and perineal areas. In contrast, in the skin of patients with AD, up to 14×10^6 organisms per cm^2 are present in eczematous lesions (Leyden *et al.*, 1974). *S. aureus* may play a role in the chronicity and severity of AD through its release of superantigenic exotoxins (Leung *et al.*, 1993). In addition to their immunological effects, these toxins may also directly damage the skin barrier. *Staphylococci* produce proteinases that could break down corneodesmosomes by a mechanism similar to that described above for KLK-related peptidases (Miedzobrodzki *et al.*, 2002). In addition, *S. aureus* secretes sphingosine deacylase and glycerophospholipids that may interfere with the formation of the lipid lamellae (Otto, 2004).

The effect of topical corticosteroids on the epidermal barrier

Topical corticosteroids are successfully used to treat the immune hyperreactivity associated with AD; however, increasing evidence suggests that they do not address the epidermal barrier defect associated with this disorder. The skin of patients treated with TCS is up to 70% thinner compared with that of untreated controls (Sheu *et al.*, 1997). There is also a concomitant decrease in the amount of intercellular lipid lamellae and a reduced number of membrane-coated granules at the SC–SG interface (Sheu *et al.*, 1997; Kao *et al.*, 2003). The effect of these changes on barrier function was shown by an elevation in TEWL from the skin of patients treated with TCS (Sheu and Chang, 1991; Sheu *et al.*, 1997). The skin barrier defect has been observed for a range of treatment regimens using TCS, from the short-term use (3 days) of very potent TCS to the prolonged use (6 weeks) of very mild TCS (Kao *et al.*, 2003; Cork *et al.*, 2007a, b, 2008).

Rebound flare after discontinuation of TCS has similarities to that observed after other forms of barrier disruption, such as surfactants and tape stripping. Barrier disruption results in the initiation of cytokine cascade, followed by an inflammatory response (Nickoloff and Naidu, 1994; Wood *et al.*, 1996; Kunz and Ring, 2002). This suggests that a barrier defect triggers the inflammatory response after cessation of immune suppression by the use of TCS. Furthermore, steroids have been shown to induce the expression of the desquamatory protease, KLK7, which is associated with the barrier defect in AD (Yousef *et al.*, 2000). Taken together, although the use of TCS suppresses inflammation associated with AD, they concomitantly seem to further damage the skin barrier, thereby increasing the risk of developing further flares of the disease.

GENE–ENVIRONMENT INTERACTIONS

To date, the *FLG* loss-of-function mutations are the highest risk factor for developing AD identified so far; however, 40% of carriers never develop AD (O'Regan *et al.*, 2008) indicating that other contributing factors play a role. Recently, exposure to cat, but not dog, allergens was found to significantly increase the risk of developing AD, but only in carriers of *FLG* loss-of-function mutations (Bisgaard *et al.*, 2008). This effect was independent of allergic sensitization. The environmental factors discussed in this review represent strong candidates for additional contributing factors. Notably, no additional risk associated with house dust mite allergens was identified in the aforementioned study (Bisgaard *et al.*, 2008).

The *FLG* mutations identified so far account for between 14 and 56% of European patients with AD, depending on the study design and mutations tested (Irvine, 2007). This indicates that there is a great deal of scope for additional factors to play a part. Variants found within *KLK7*, *SPINK5,* and *CSTA* are all hypothesized to lead directly to elevated protease activity associated with desquamation, either through an increased abundance of degradatory proteases or reduction in their inhibition. In conjunction with the finding that the levels of active proteases found within the SC of AD patients is altered (discussed above), this suggests that protease activity within the SC is a very important factor affecting the development of AD. Notably, it is hypothesized that one of the effects of *FLG* loss-of-function mutations is altered SC protease activity. This is a consequence of the reduced levels of NMF acidifying agents (filaggrin breakdown products) expected to result in elevated pH (Brown and McLean, 2009). Figure 5 illustrates this model, wherein structural, protease, and protease inhibitor genes all lead to enhanced protease activity. The

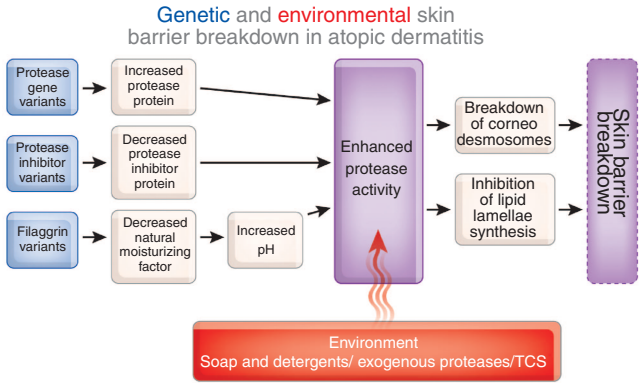

Figure 5. Three groups of genes contribute to skin barrier breakdown in atopic dermatitis (AD) coding for structural, protease, and protease inhibitor proteins. Changes in protease (such as *KLK7*) and protease inhibitor (*CSTA* and *SPINK5*) genes lead directly to enhanced protease activity within the stratum corneum (SC), resulting in exacerbated breakdown of the corneodesmosome junctions. Loss-of-function mutations in the *FLG* gene encoding filaggrin, result in decreased levels of natural moisturizing factor (NMF) within the SC. As NMF levels fall, the SC pH will rise, leading to enhanced protease activity, decreased protease inhibitor activity, and decreased lipid lamellae synthesis. Environmental insults, such as soap and other detergents; exogenous proteases from house dust mite and *Staphylococcus aureus*; and the prolonged use of topical corticosteroids (TCS) exacerbate proteolytic breakdown of the barrier resulting in increased skin barrier breakdown (detailed in the main text).

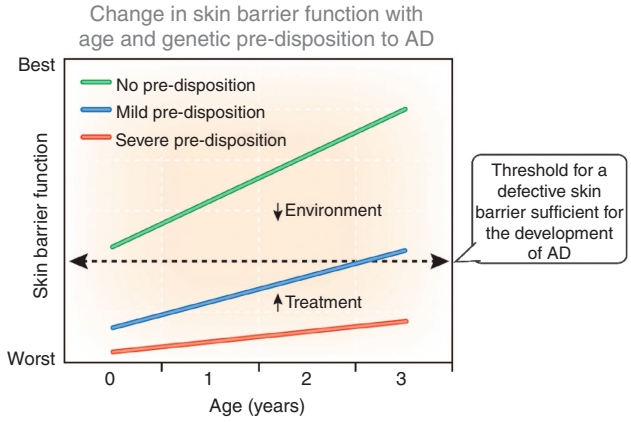

Figure 6. The ''skin barrier function'' plotted with arbitrary units against the age of a child. In children who do not have a genetic pre-disposition to a defective skin barrier (and the development of atopic dermatitis (AD)), their skin barrier is at its worst at birth then gradually improves with age (green line). At birth, the skin barrier function is just above a threshold of skin barrier function below which symptoms and signs of AD would manifest. A pre-disposition to AD can be visualized as moving the line downward (blue and red lines), wherein the distance is dependent on the severity of the combined ''genetic factors''. The natural improvement in skin barrier function may be sufficient to result in complete remission before adulthood (blue line). Environmental factors, such as the use of soap and detergents, exacerbate the skin barrier defect, visualized as moving the line downward. On the other hand, effective treatment moves the line upward. For a full description please see the main body of text.

activity of SC proteases is further affected by the environmental factors discussed above, including the use of wash products that elevate skin pH and the contribution of exogenous proteases. This shift toward elevated protease activity not only affects the rate of barrier breakdown, but also has the potential to activate PAR2, a trigger of the innate immune system.

Whether dysregulation of protease activity alone is sufficient to predispose to AD remains unknown. In the case of *FLG* mutations, several barrier functions are disrupted, including formation of the CE, modeling of corneocyte shape, moisturization through NMF, in addition to the hypothesized effects on desquamation and lipid lamellae synthesis through increased pH. A combination of both genetic and environmental factors that affect all of these barrier functions may be required for the development of AD.

Gene–environment interactions in the development of AD

The prevalence of AD is greatest in children. In 63% of children who develop AD in the first 2 years of life, it resolves by age 3, and only 18.7% of children are classified as having persistent AD by the age of 7 (Illi *et al.*, 2004; Bieber, 2008). How can AD improve with age if the primary defect is in the skin barrier? The large study of natural changes in skin barrier structure and function, from birth into adult life helps us to understand that, at its beginning, the skin barrier has relatively poor function, but this naturally improves with age (Nikolovski *et al.*, 2008). Figure 6 illustrates a hypothetical model for the early progression and development of AD, wherein the skin barrier function is plotted, using arbitrary units, against the age of a child. In children who do not have a genetic predisposition to a defective skin barrier (and the development of AD), their skin barrier is at its worst at birth then gradually improves with age (''no pre-disposition''). At birth, skin barrier function is just above a threshold of skin barrier function below which symptoms and signs of AD would manifest (Nikolovski *et al.*, 2008).

The children whose AD resolves by the age of 3 are those with mild dermatitis, without a raised IgE (non-atopic). This form of eczema has been associated with changes in the *KLK7* gene (Vasilopoulos *et al.*, 2004). In the first year of life, when skin barrier function is at its worst, the ''minor'' genetic changes bear their greatest impact, reducing skin barrier function to below the threshold for developing clinical signs of AD (see ''mild predisposition'', Figure 6). As children become older, their skin barrier function improves naturally and, therefore, it is expected to move above the

threshold for the development of AD and the condition resolves.

At the other end of the AD severity spectrum are changes in the gene encoding filaggrin (Palmer *et al*., 2006), which are associated with severe AD, wherein IgE levels are raised. In an infant carrying a loss-of-function mutation in the *FLG* gene, it can be envisaged as producing a severe skin barrier defect (see ''severe predisposition'', Figure 6), which is still severe enough at age 3 years, and into adult life, to produce continuing AD. This is because the natural improvement in skin barrier function is not sufficient to override the severe skin barrier defect caused by the filaggrin mutation.

As discussed, environmental factors, such as detergents and/or mite allergens, amplify the skin barrier defect through several mechanisms. This can be visualized as moving the line downward so that the AD persists longer (see Figure 6). It is the interplay of these environmental and genetic factors that is hypothesized to lead to flares of AD. The sum of the underlying genetic factors determines the severity of the predisposition and the overall risk of developing flares of AD in response to environmental insults. By avoiding environmental factors, such as soap and detergents, and replacing them with a regimen of emollient treatment, the skin barrier function can be improved. This is visualized by moving the line of predisposition upward (Figure 6), and may lead to earlier apparent resolution of the AD.

A regimen to protect and restore the skin barrier is essential throughout the time that the patient has any sign of AD, including even slightly dry skin, because early effective treatment to repair the defective skin barrier may prevent disease progression. Dry skin is often not recognized as the first sign of AD by parents and so the opportunity to improve skin barrier function early is often missed.

Soap and detergent wash products can be replaced with emollient wash products (Cork, 1997). For some products (such as shampoos), it is not possible to eliminate all detergents. However, it is possible to reduce the chance that they will damage the skin barrier by using the mildest surfactants in the lowest concentrations in formulations with emollient ingredients. As shampoos inevitably flow onto the face, the careful selection of these products is important. There are now emollient wash products designed for the shower, bath, and hand washing; these products should be combined with emollient creams and ointments to improve skin barrier function.

CONCLUSION

Atopic dermatitis is a multifactorial, heterogenous genetic disease arising as a result of the interaction of many genes with environmental factors. The identification variants in three groups of genes integral to epidermal barrier function in patients with AD suggests that, in the majority of cases, a primary breakdown in the skin barrier is the initial event in the development of the disease. The most likely model for the development of AD is a gene dosage and an environmental dosage effect, wherein the combination of multiple genetic and environmental factors determines the severity or the likelihood of developing the disease. The use of soap and detergents is likely to bear the greatest effect in cases in which there is an existing barrier defect, for example, as a result of a *FLG* loss-of-function mutation. Furthermore, the sites prone to the development of AD are those with an inferior epidermal barrier and enhanced protease activity. The development of AD, at least in part, seems to result from the culmination of concessions in epidermal barrier function. Our understanding of gene–environment interactions, as the initial event in the development of AD, should lead to a better use of some topical products, avoidance of others, and the increased use and development of products that can repair the skin barrier.

The opportunity for the future is to identify at birth babies who have a genetic predisposition toward developing a defective skin barrier and to modify their environment to prevent breakdown of the skin barrier. The next step is to produce formulations of wash and leave-on topical products that have the greatest positive effects on the skin barrier.

CONFLICT OF INTEREST

Alice MacGowan is employed by York Pharma R&D Ltd.

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