Atopic dermatitis (AD) is a multifactorial, chronic inflammatory skin disorder in which genetic mutations and cutaneous hyperreactivity to environmental stimuli play a causative role. Genetic mutations alone might not be enough to cause clinical manifestations of AD, and this review will propose a new perspective on the importance of epidermal barrier dysfunction in genetically predisposed individuals, predisposing them to the harmful effects of environmental agents. The skin barrier is known to be damaged in patients with AD, both in acute eczematous lesions and also in clinically unaffected skin. Skin barrier function can be impaired first by a genetic predisposition to produce increased levels of stratum corneum chymotryptic enzyme. This protease enzyme causes premature breakdown of cornodesmosomes, leading to impairment of the epidermal barrier. The addition of environmental interactions, such as washing with soap and detergents, or long-term application of topical corticosteroids can further increase production of stratum corneum chymotryptic enzyme and impair epidermal barrier function. The epidermal barrier can also be damaged by exogenous proteases from house dust mites and *Staphylococcus aureus*. One or more of these factors in combination might lead to a defective barrier, thereby increasing the risk of allergen penetration and succeeding inflammatory reaction, thus contributing to exacerbations of this disease. (J Allergy Clin Immunol 2006;118:3-21.)

**Key words:** Atopic dermatitis, eczema, environmental triggers, genome, proteases, protease inhibitors, skin barrier dysfunction, topical corticosteroids

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with cutaneous hyperreactivity to environmental triggers that are innocuous to healthy nonatopic individuals. Major contributors to this hyperactivity are the many changes in the cutaneous and systemic immune responses in individuals with AD. One example is the production of increased levels of total serum IgE and specific IgE to common allergens. It has been postulated that the nonallergic intrinsic dermatitis could be a pure transitional form of AD. This raises the following question: Is there a genetic and environmental basis for primary intrinsic, nonallergic dermatitis? A logical place to look is the skin barrier, given its role in protecting against environmental stimuli. Another area of AD research that points us to the skin barrier and the influence of the environment is the increasing prevalence of AD and the concomitant increase in exposure to environmental agents. The prevalence of AD has been increasing progressively in developed countries since the 1940s. How can the prevalence of AD have increased so dramatically if it is only determined genetically? This increase in prevalence suggests that environmental factors must be crucial in the expression of the disease. AD is a multifactorial, heterogeneous genetic disease arising as a result of the interaction of many genes with environmental factors. The most likely model for the
development of AD is a gene dosage and environmental dosage effect. For example, if an individual has a mutation in 5 major genes for AD, then the environmental factors required to develop the disease might be minimal. If the mutations are only present in 2 of the genes, then a much greater environmental exposure might be required for the disease to develop. Several loci have been associated with increased IgE production, linked to increased IgE production, or both, including 4q35.2, 11q13, 16q24.1, and 5q31.1. Also, see the review by Morar et al in this issue of the Journal.

Several environmental factors have been associated with AD, including washing with soap and detergents, washing with hard water, and exposure to house dust mites. However, there are few formal longitudinal studies that indicate how the home environment has changed over the past 50 years. We previously reviewed studies that indicate how the home environment has changed over the past 40 years, which includes the increase in the use of soap and detergent personal wash products between 1981 and 2001 in the United Kingdom, where the sales increased (inflation adjusted) from £76 million to £453 million while the population only increased from 56.3 million to 59.1 million (www.wales.gov.uk). 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, increasing to 51 L per person per day in 1997/1998. In the United Kingdom there have also been changes in the heating, ventilation, and insulation systems and floor coverings of houses over the past 40 years, which have created an increasingly optimal environment for the development of AD. Several loci have been associated with increased IgE production, linked to increased IgE production, or both, including 4q35.2, 11q13, 16q24.1, and 5q31.1. Also, see the review by Morar et al in this issue of the Journal.

From an immunologic perspective, it has been suggested that barrier breakdown in AD is a secondary consequence of the inflammatory response to irritants and allergens, which is known as the inside-outside hypothesis. Alternatively, it has been hypothesized that the barrier dysfunction in AD, which is known as the outside-inside hypothesis. Which is the correct hypothesis? Barrier function appears to fluctuate in relation to disease activity, suggesting that changes in barrier function might drive disease activity. In addition, barrier damage induced, for example, by surfactants (sodium lauryl sulphate) or skin stripping causes the release and production of cytokines, such as IL-1α, IL-β, TNF-α, and GM-CSF, indicating that barrier disruption alone leads to cytokine production, inflammation, and a flare of dermatitis. AD has a very wide spectrum of disease severity. At the mild end, the dermatitis is usually intrinsic, with no increase of specific or nonspecific IgE levels, and this immunologic state might be maintained for the duration of the disease. This can usually be controlled most of the time with a complete emollient regimen and intermittent use of calcineurin inhibitors and mild-to-moderate topical corticosteroids. At the other end of the disease severity spectrum, in patients with very severe AD, the total IgE level can be greater than 10,000 units, and multiple specific IgE levels are above the top of the scale. This very severe dermatitis can only be controlled with systemic agents, such as cyclosporine and mycophenolate. Are mild AD and very severe AD the same disease? Are the contributions of the inside-outside and outside-inside hypothesis mechanisms different in AD of different severities?

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. This is not a new idea. In 1999, Alain Taieb proposed that a genetic predisposition to a defective skin barrier was a primary event in the development of AD, allowing allergen penetration and enhanced TH2 responses. Two groups have identified variants in genes regulating desquamation and have shown that they are strongly associated or linked with AD. The likely functional consequence of these genetic changes is a premature breakdown of the skin barrier, resulting in a thin skin barrier. A thin, defective epidermal barrier could enhance the penetration of irritants and allergens into and through the skin barrier. This could activate the immune response by facilitating interaction between antigens and the immune effector cells present in the skin. Increased penetration of allergens through the epidermis could also promote the initiation of an inflammatory response within the stratum corneum/stratum granulosum by inducing the release of proinflammatory cytokines from keratinocytes. This review will propose a new perspective on epidermal barrier dysfunction in AD, identifying the epidermal barrier as an important site for gene–environment interactions in the development of this disease.

GENE–ENVIRONMENT INTERACTION IN AD: THE ROLE OF A DEFECTIVE EPIDERMAL BARRIER

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. In older children the most common sites affected are the antecubital and popliteal fossae. In addition to the classical patterns of AD, there are several site-specific variants. Eyelid eczema is common in
adolescents, affecting up to 21% of these individuals, and has been associated with hay fever and exposure to other aeroallergens, such as house dust mites. The infra-auricular and retroauricular sites are particularly prone to fissuring, probably as a reaction to repeated minor trauma.

Many factors could explain the areas of predisposition to AD, including the thickness of the stratum corneum and the variation in exposure to irritants and allergens at different body sites. Hanifin commented that the stratum corneum over the eyelids is extremely thin and that these areas are vulnerable to the irritants and allergens entering into contact with the peri-orbital areas because these zones are rubbed and scratched unconsciously. Only 3 studies have evaluated epidermal thickness in multiple body sites and have shown that it is thinnest in the eyelid and genitals. The next thinnest sites are the flexor forearm and posterior auricular areas (see Fig E1 in the Online Repository at www.jacionline.org). Interestingly, these 2 are the areas of predisposition to AD indicated above. The thickness of the epidermis in the antecubital fossae was not recorded.

The epidermal barrier to the penetration of exogenous substances, such as irritants, allergens, and drugs, is located in the deeper part of the stratum corneum. It is therefore expected that the percutaneous penetration of exogenous substances varies in different body areas according to differences in the thickness of the stratum corneum. The most detailed studies on the regional variation of the percutaneous penetration of an exogenous substance have been made with topical corticosteroids. In some studies, in which the percutaneous penetration of corticosteroids was measured in vivo by using cadaver skin from different body sites, the greatest percutaneous penetration was observed for scrotal and posterior auricular skin and the lowest for plantar skin. The definitive study on in vivo regional variation in percutaneous penetration of topical corticosteroids was performed in human male volunteers with normal skin. Feldman and Mabach applied carbon 14–labeled hydrocortisone to different body areas and measured the penetration of hydrocortisone by recording carbon 14 activity in the urine over the subsequent 5 days. They observed the greatest percutaneous penetration of hydrocortisone in the skin of the face, eyelid, and scrotum and the lowest penetration in plantar skin. There was a 300-fold greater penetration of hydrocortisone through the eyelid compared with that through plantar skin. These differences cannot be explained by differences in blood flow.

The percutaneous penetration of topically applied drugs in different body areas shows the same pattern of variation as the thickness of the stratum corneum, with the highest penetration through the thinnest stratum corneum. Although regional differences in the percutaneous penetration of irritants and allergens have not been investigated, it seems reasonable to speculate that the pattern might be similar to that observed for the penetration of a topically applied drug, such as hydrocortisone.

Regional variations in epidermal thickness and drug penetration indicate that the eyelids, posterior auricular areas, other parts of the face, and flexures have a thin epidermal barrier with decreased barrier function (see Fig E1 in the Online Repository at www.jacionline.org). These skin sites can be visualized as having low epidermal barrier reserve; that is, they are more vulnerable to any exogenous agent that could further decrease the thickness and functional integrity of the epidermal barrier.

Although AD can involve any body site, the eyelids, posterior auricular areas, and flexures are the earliest sites of involvement in infants, the sites where the disease persists longer and with low epidermal barrier reserve. It is probable that these body sites are the most vulnerable to penetration of irritants and allergens and therefore represent the first and most persistent sites of disease involvement.

INTERINDIVIDUAL VARIATION IN SKIN BARRIER FUNCTION

Although there are intraindividual variations in skin barrier thickness and function, which correlate with the earliest and most persistent sites of AD, not all children develop the disease. In addition to intraindividual variations in epidermal barrier function, there are also interindividual variations. The variability between different measurements at the same site and for the same individual has been estimated to be 8% by site and 21% by day to day. The variation between individuals is larger, ranging from 35% to 48%. On the basis of the transdermal water loss (TEWL) measurements, there appears to be a 20% to 40% difference in the skin barrier function at a given regional site between individuals. There is also a wide range for the percutaneous absorption of topically applied drugs, which can vary by up to 30-fold between individuals on the forearm. Between-subject differences in the absorption of drugs at different body sites are not completely explainable by variations in the thickness of the stratum corneum and corneocyte size. In certain individuals a defect in epidermal barrier function might only become apparent when the skin is stressed. An example is the skin of aged people, in which baseline TEWL measurements are similar to those seen in younger adults. However, if the barrier is damaged, recovery to normal is much slower than in a younger adult. It has been suggested that this decreased ability to repair the epidermal barrier after an environmental insult might not only explain interindividual variation in barrier function but also the increased susceptibility of some individuals to irritant contact dermatitis. The interindividual variation in skin permeability to drugs in the “normal population” suggests that there could be genetic variants associated with increased barrier permeability to drugs in some individuals. The “normal population,” from which adult healthy skin samples are obtained, might include individuals who had AD as a child or those who might have previously had irritant contact dermatitis or sensitive skin but are apparently “normal” at the time of testing. These individuals could be those who show increased skin permeability to topically applied drugs. In support of this hypothesis, there are data indicating that epidermal barrier
function in nonlesional skin from patients with AD is different from that of subjects who never had this disease.\textsuperscript{65-67} In one study\textsuperscript{65} the average thickness of the stratum corneum was 12.2 \textmu m in nonlesional skin from individuals with AD and 19.7 \textmu m in skin from control subjects who had not had AD. In other studies\textsuperscript{66,67} TEWL measurements were also much higher in nonlesional skin from individuals with AD than in skin from those with no history of AD.

**HOW ATOPIC IS AD?**

Two types of AD have been defined.\textsuperscript{68,69} The extrinsic form of the disease is associated with increased levels of total serum IgE and increased levels of specific IgE to environmental allergens,\textsuperscript{68} whereas the intrinsic form is associated with total serum IgE levels within the age-adjusted normal range and no increase of specific IgE levels to environmental allergens.\textsuperscript{69} The link between AD and allergen-specific IgE remains, however, uncertain and controversial.\textsuperscript{3,69-73} A recent systematic review\textsuperscript{3} asked the question, “How atopic is AD?” This is the first work that compared results of population-based studies with those of hospital-based studies. The comparison is important because hospital-based studies include a large proportion of patients with severe dermatitis, whereas the majority of patients included in population-based studies have mild or moderate disease. It was found that in hospital-based studies the percentage of children with extrinsic “atopic” dermatitis ranged between 47\% and 75\%. In contrast, in population-based studies the percentage of children with extrinsic “atopic” dermatitis ranged from 7.4\% to 78\%.\textsuperscript{3} Up to 66\% of patients with dermatitis did not have measurable allergen-specific IgE levels in the serum at the time of measurement. The same work also showed that high levels of specific IgE antibodies, total serum IgE levels, or both were significantly associated with the severity of dermatitis.

A major gap in the literature is the absence of longitudinal studies on IgE measurements.\textsuperscript{3} These studies could help determine whether, in some subjects, increase of total serum IgE levels and allergen-specific IgE levels occurs transiently and might remain undetected when measurements are performed only on one occasion. In a study in which children with AD were followed during the development of respiratory allergic disease,\textsuperscript{74} subjects who initially had negative responses on skin prick tests had positive responses over the next 10 years. It has been postulated that nonallergic intrinsic dermatitis might be considered as the pure, primary, transitional form of AD.\textsuperscript{74,75} Could the genetic basis of the pure, primary, intrinsic, transitional form of AD\textsuperscript{75} be represented by genetic variants that predispose to a defective epidermal barrier?

**THE SKIN BARRIER**

The epithelium serves as a first line of defense between the body and the environment. Disturbance of the epidermal barrier can favor the penetration of microbes and allergens. Enhanced penetration of agents with antigenic properties increases the risk of sensitization because it allows interaction between allergens and allergen-presenting cells in the skin and triggers the onset of inflammation once sensitization has occurred. Increased penetration of irritants through the skin facilitates the occurrence of nonallergic inflammatory reactions. Therefore the skin barrier is an important shield against environmental injury.

The barrier to the penetration of irritants and allergens is located in the lower part of the stratum corneum (Fig 1). The structural integrity of the stratum corneum is maintained by modified desmosomes (corneodesmosomes), which lock together the corneocytes. As the corneocytes move up through the stratum corneum, the corneodesmosomes are gradually broken down by the skin-specific proteases, and unattached corneocytes can then be desquamated from the surface of the skin.
cement.79 The lipid lamellae help prevent internal water loss and penetration of water-soluble materials (Fig 6). 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 esters80 and is believed to exist as a single and coherent lamellar gel.81

Disturbed maturation of the lamellar bodies has been demonstrated in atopic skin,81 consisting of a decreased release of the acid, lipid, and enzyme constituents of the stratum corneum and leading to a defective barrier function. A disturbance in the extruding mechanism of lamellar lipids, resulting in decreased lipid contents of the stratum corneum, has also been described in eczematous skin.82 Other reported alterations in AD have included a considerable deficiency in the main barrier lipid components82 and an increase in sphingomyelin deacylase activity, resulting in decreased ceramide production.83

Corneodesmosomes are specialized desmosomes that bind the corneocytes together in the stratum corneum84 and are incorporated into the corneocyte envelope (Figs 1-4 and Fig E2 in the Online Repository at www.jacionline.org). They consist of the cadherin family of extracellular transmembrane glycoproteins, desmoglein and desmocollin (reviewed by Rawlings80). Within the corneocytes, desmoglein and desmocollin are linked to keratin filaments through corneodesmosomal plaque proteins, including plakoglobin, desmoplakin, and plakophillin (Fig 1). Desmoglein and desmocollin pass from the corneocyte envelope into the lipid lamellae between the corneocytes and bind to the same proteins on adjacent cells.85 Corneodesmosin is a 52-kd protein specifically expressed in keratinizing epithelia.86,87,88 After secretion into the extracellular space, corneodesmosin is translocated to the transition zone between the stratum granulosum and the stratum corneum88 and incorporated into the desmosomes (Fig 1). This marks the transition from desmosome to corneodesmosome.

In the palmoplantar stratum corneum, corneodesmosomes are found throughout the surface of the corneocytes, whereas in the uppermost region of the stratum corneum in other body regions, they are located at the periphery of the corneocytes and particularly at the cell-cell interdigitation areas at the skin surface.89 It has been shown that cleavage of all peripheral corneodesmosomes at the skin surface must be completed for normal desquamation to occur.89,90 Persistence of peripheral corneodesmosomes has been linked to several ichthyotic, hyperkeratotic, and xerotic diseases,88,91 suggesting that abnormal corneodesmosomal processing is a key alteration in these conditions.

Desquamation is the process by which the epithelial “brick wall” is maintained at a constant thickness (Fig 5). The corneocytes that are shed from the skin surface are continually replaced from underneath by keratinocytes undergoing terminal differentiation. Thus there is a fine balance between basal cell proliferation and corneocyte desquamation involved in maintaining an epithelium of constant thickness.92 Desquamation also treads a fine balance between breaking the barrier down enough to allow a continual renewal of epidermal cells and leaving it intact enough 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
In the stratum corneum from healthy skin, there is a balance between the structural integrity of the corneodesmosomes and the level of proteases and protease inhibitors (A). In individuals genetically predisposed to AD, increased protease activity leads to premature breakdown of the corneodesmosomes and thinning of the stratum corneum (B and C). Soap use increases the skin pH from 5.5 to the optimal value for SCCE activity (>7.5), further increasing the breakdown of the corneodesmosomes and corneocyte desquamation (D). SLPI, Serine leukoprotease inhibitor.

The brick wall analogy of the stratum corneum of the epidermal barrier. In healthy skin the corneodesmosomes (iron rods) are intact throughout the stratum corneum. At the surface, the corneodesmosomes start to break down as part of the normal desquamation process, analogous to iron rods rusting (A). In an individual genetically predisposed to AD, premature breakdown of the corneodesmosomes leads to enhanced desquamation, analogous to having rusty iron rods all the way down through the brick wall (B). If the iron rods are already weakened, an environmental agent, such as soap, can corrode them much more easily. The brick wall starts falling apart (C) and allows the penetration of allergens (D).
FIG 6. Genomic structure of the kallikrein 7 gene, encoding the SCCE. A 4-bp insertion (AACC) was found in the 3′ untranslated region of the gene.93

Among the proteases involved in the process of desquamation are the stratum corneum chymotryptic enzyme (SCCE) and the stratum corneum trypsin enzyme (SCTE; Fig 2 and Fig E2 in the Online Repository at www.jacionline.org).92,97-100 These are serine proteases that are expressed in granular keratinocytes and present within the extracellular spaces of the stratum corneum.100,101 SCCE has been shown to hydrolyze corneodesmosomes and desmocollin 1, and SCTE is also capable of cleaving desmoglein 1.93 Both SCCE and SCTE are produced as inactive precursors. Removal of propeptides by means of trypsin digestion leads to the formation of the proteolytically active enzymes.97,99 Studies have shown that SCTE is capable of activating SCCE93 in addition to itself,92,99,100 suggesting that SCTE might serve as a regulator of SCCE activity. Other enzymes capable of degrading corneodesmosomal adhesion proteins include the cysteine proteases cathepsin L2/stratum corneum thiol protease and stratum corneum L-like enzyme.96,102 The aspartic protease cathepsin D,103 and several glycosidases.80

Several genes have been associated with increased IgE production, linked to increased IgE production, or both, including IL-4 and the high-affinity IgE receptor.14,15 However, observations that a large proportion of children with AD do not have increased IgE levels3,74,109 suggest that other groups of unrelated genes might play an important part in the development of AD. As discussed below, there is an increasing body of evidence that a genetically determined primary defect in the skin barrier might be central to the development of this disease.

It has been demonstrated that transgenic mice over-expressing human SCCE develop changes in their skin similar to those seen in chronic AD.97 Overexpression of SCCE in those mice might have led to a premature breakdown of the corneodesmosomes, with increasing corneocyte desquamation and thinning of the skin barrier (Fig 2 and Fig E2 in the Online Repository at www.jacionline.org). The resulting impairment of skin barrier function might have favored the penetration of irritants and allergens and the consequent development of dermatitis (Table I).41,91,96,97,104,110-112

To evaluate the possibility that genetic variations within the SCCE gene are indeed associated with dysregulation of SCCE activity in human subjects, leading to a thin skin barrier, the SCCE gene was screened for variations, and an associations study was performed in children with AD and healthy control subjects.91 A 4-bp insertion was identified in the 3′ untranslated region of the kallikrein 7 gene encoding SCCE (Fig 6). The common allele was AACC, and the rare allele was AAACCAACC. A significant genetic association was found between the rare AAACCAACC variant of the SCCE gene and AD. The patients with AD were then stratified into those who did not have high levels of serum IgE (intrinsic AD) and those who did (extrinsic AD). The highest association between the rare variant of the SCCE gene and AD was observed in the subgroup of patients who did not have increased IgE levels (odds ratio, 4.47; 95% CI, 1.49–13.38; P = .0039). The association was not significant in the subgroup of patients with high levels of serum IgE.

It is known that determinants of mRNA stability are frequently positioned in the 3′ untranslated region of the genes and that any mutation in this region can alter
The AACC insertion could increase the half-life of SCCE mRNA, leading to an increased production of the enzyme in the skin of individuals with intrinsic AD (Fig 6). The overexpression of SCCE would cause a premature lysis of the corneodesmosomal proteins (Fig 2 and Fig E2 in the Online Repository at www.jacionline.org). The consequent enhancement in corneocyte desquamation would produce a thin defective epidermal barrier that would allow penetration of irritants, thereby favoring the development of an inflammatory response (Figs 2 and 4).

Genetic mutations have also been identified in genes encoding members of the protease inhibitors involved in desquamation. Mutations in the SPINK5 gene, which
encodes lymphoepithelial Kazal-type 5 serine protease inhibitor, have been linked to Netherton syndrome. Individuals with this disorder display marked barrier dysfunction, involving altered desquamation and impaired keratinization. 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. Transgenic studies with SPINK5−/− mice have demonstrated that lymphoepithelial Kazal-type 5 serine protease inhibitor deficiency results in abnormal desmosome cleavage in the upper granular layer of the epidermis, which is caused by increased SCCE and SCTE activity. Increased protease activity in the skin of SPINK5−/− mice leads to increased breakdown of desmoglein and corneodesmosin, which is consistent with the premature cleavage of corneodesmosomes observed in the skin of patients with Netherton syndrome. Several studies have also linked mutations in the SPINK5 gene with AD.

Cystatins are cysteine protease inhibitors expressed within the epidermis. Several studies have shown that the cystatins might afford protection from proteolysis by bacterial and viral proteases. Transgenic mice carrying a null mutation in the gene encoding cystatin M/E display severe barrier abnormalities and die shortly after birth. Mice lacking cystatin M/E have abnormalities in cornification and desquamation with hyperkeratosis (Table I). Transgenic knockout mouse studies have revealed the importance of several adhesion proteins for the assembly of functional desmosomes and the maintenance of a functional skin barrier (Table I). Desmoglein 3−/− mice develop traumatized skin that displays a marked separation of desmosomes under electron microscopy. Mice lacking desmocollin 1 have been shown to have a flaky and fragile epidermis, with acanthosis in the granular layer. Desmoplakin is also important in epidermal sheet formation (Table I). Mice lacking desmoplakin have few desmosomes and a marked reduction in barrier integrity. 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.

SECONDARY PROTEASES

When endogenous proteases, such as SCCE, are produced in excessive quantities, the corneocytes desquamate prematurely, producing a thin skin barrier. This then facilitates the penetration of irritants and allergens, which can trigger a flare of the AD. 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, Fig 3), 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, the numbers of MCC− cells were significantly increased in the lesional skin of patients with AD in comparison with those in nonlesional skin. However, there was no significant difference in the number of MCC− cells between the nonlesional skin of patients with AD and the skin of healthy control subjects, suggesting that increased MCC activity might be associated with active dermatitis. In another study in mice, injection of MCC into the healthy skin induced an inflammatory response similar to that observed in AD. There is also evidence that MCC might participate in the development of chronic dermatitis by inducing eosinophil infiltration. Variants within the MCC gene have been associated with AD in children. The association was strongest in individuals with low levels of total serum IgE. Instead, in adults with AD, a polymorphism in the promoter region of the MCC gene has been associated with high levels of total serum IgE.

EXOGENOUS PROTEASES

House dust mites are a source of more than 30 different proteins that can induce IgE-mediated responses. Some of these proteins are cysteine and serine proteases. Some of these proteins have been shown to cleave adhesion proteins and to increase the permeability of lung epithelium. Patch tests have demonstrated that 2 proteins with proteolytic activity derived from house dust mites, Der p 1 and Der p 2, can elicit irritative or immune reactions that are not linked to increased levels of IgE against house dust mites, suggesting that these proteins cause skin irritation or immune activation through a direct proteolytic activity.

As reviewed by Storck, Staphylococcus aureus has been implicated as an environmental factor in the pathogenesis of AD since the 19th 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 × 10⁶ organisms per square centimeter are present in eczematous lesions. S aureus might play a role in the chronicity and severity of AD through its release of superantigenic exotoxins. In addition to their immunologic effects, these toxins might also directly damage the skin barrier. Staphylococci produce proteinases that could break down corneodesmosomes through a mechanism similar to that described above for SCCE. In addition, S aureus secretes sphingosine deacylase and glycerophospholipids that might interfere with the formation of the lipid lamellae. Thus exogenous proteases and lipases produced by house dust mites and S aureus might contribute to the breakdown of the skin barrier in AD (Fig 3).

GENE–ENVIRONMENT INTERACTIONS: pH AND DETERGENTS

The skin has long been known to have an acidic pH (the acid mantle) that contributes to the optimal barrier function of this tissue. The average surface pH of the forearm of a healthy male is around 5.4 to 5.9. In human subjects the skin surface pH at birth is near neutral (pH 6.5)
comparable with that in children and adults. In newborn rats the stratum corneum reaches adult pH levels during the first few days after birth, whereas similar changes take a few weeks to occur in human newborns. Although the acid mantle of the stratum corneum was initially thought to originate from exogenous sources (microbial metabolites, free fatty acids of pilosebaceous origin, and eccrine gland–derived products, such as amino and lactic acids), recent studies have demonstrated that endogenous pathways (generation of byproducts 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. The acid mantle has multiple effects on the skin. First, it has a strong antimicrobial effect. decreases skin colonization by pathogenic bacteria, and favors the adhesion of nonpathogenic bacteria to the stratum corneum. Second, several lines of evidence indicate a role for skin surface pH on desquamation, permeability barrier homeostasis, and stratum corneum integrity/cohesion. A delay in epidermal barrier recovery occurs when the skin is immersed in neutral pH buffers. Moreover, 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 coexposure of inhibitor-treated areas to an acidic buffer.

Skin pH variations have been clearly documented in some skin diseases. found a total body pH increase in patients with seborrheic dermatitis, AD, and xeroderma. Others demonstrated a significantly higher skin surface pH in a group of schoolchildren with AD compared with that seen in control subjects. In patients with AD, skin pH was reported to be 0.5 units higher than in control subjects. Similar studies documented that skin pH is higher in patients with AD than in healthy control subjects, even on uninvolved skin. Seidenari and Giusti also demonstrated that skin pH values are higher in patients with active lesions than in asymptomatic patients. Many enzymes involved in skin barrier homeostasis and restoration have been shown to be pH dependent. The skin protease SCCE exhibits a neutral pH optimum. A change in pH from 7.5 to 5.5 reduces SCCE activity by 50%. The thiol cysteine protein (cathepsin LZ) and the aspartate protease (cathepsin D) have an acid pH optimum and probably mediate desquamation in the upper layers of healthy skin. The SCCE/SCTE proteases could initiate the degradation of corneodesmosomes in the lower layers of the stratum corneum in healthy skin and in all layers of the stratum corneum in diseased skin, where the neutral pH (pH 7.0) predominates. The importance of pH to the activity of skin proteases was demonstrated in hairless mice treated with “superbases” that neutralize skin surface pH. This caused rapid activation of serine proteases, with consequent degradation of corneodesmosomes. The resulting decrease in skin barrier cohesion/integrity was detectable with the skin stripping/TEWL assay.

Stratum corneum pH is also important for the generation and degradation of the lipid lamellae. The lipid-generating enzymes β-glucocerebrosidase and sphingomyelinase also exhibit low acid pH optimum. Application of superbases to hairless mouse skin has been demonstrated to decrease glucocerebrosidase activity, which has been shown to generate incompletely processed lipid lamellae membranes, as assessed by means of electron microscopy. Increasing the pH of the stratum corneum surface can therefore cause enhanced desquamation of corneocytes by increasing the activity of serine proteases, such as SCCE, and also by interfering with the normal lipid processing required for the formation of the lipid lamellae.

In an individual with a genetic predisposition to increased skin protease activity, for example, because of the rare AACCAACC variant of the SCCE gene, there will be constantly high levels of SCCE protein in the stratum corneum. In the pH of the skin is then increased from the pH of healthy skin (5.5) to 7.0 or higher, the SCCE protease activity will be further increased, with further enhancement of corneocyte desquamation and thinning of the stratum corneum (Fig 4). The most common environmental agents that can increase the pH of the skin surface are soap and other detergents. Washing the skin with soap causes an increase of the pH on the palms by 3 units for more than 90 minutes. White et al measured the thickness of the stratum corneum in healthy skin and in nonlesional eczematous skin before and after washing with soap. Before washing, the stratum corneum was thicker in healthy skin (19.7 μm) than in nonlesional eczematous skin (13.7 μm). Washing with soap caused further thinning of the stratum corneum in both the healthy and the nonlesional eczematous skin, which is consistent with an increased activity of skin proteases, such as SCCE, resulting in premature breakdown of the corneodesmosomes. The observed differences between healthy skin and nonlesional eczematous skin could be explained by differences in the level of SCCE expression in the skin determined by genetic variants in the SCCE gene (Figs 4 and 6). Considering again the brick wall model of the stratum corneum, the genetic predisposition to skin barrier breakdown in individuals with AD who have the rare allele of the SCCE gene variant is analogous to having rusty iron rods all the way down through the brick wall. In this case an environmental agent that per se induces some iron rod rusting, such as soap, can corrode the iron rods completely. Once the iron rods have been completely corroded and broken, the brick wall can no longer resist shearing forces and falls apart. The stratum corneum can no longer resist the penetration of allergens, and increased allergen penetration through the skin leads to a flare of AD. This is an excellent example of a gene–environment interaction producing the AD clinical phenotype (Fig 7).

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 with
Surfactants can damage the skin, provoking scaling, dryness, tightness and roughness, erythema, and swelling. 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. The detergent sodium lauryl sulphate is used as the standard test of skin susceptibility to irritation. The negative effects of surfactants on skin barrier function are demonstrated by an increased TEWL, which is more severe in subjects with AD than in healthy control subjects. Surfactants can solubilize lipids, and it has been postulated that this could be the mechanism by which they increase TEWL. However, measurements of lipid solubilization by sodium lauryl sulphate suggest that at concentrations ranging from 0.1% and 2%, it removes very small amounts of free fatty acids, cholesterol, and esters. The acute irritant effects of soap and detergents could be partially explained by the release of pro-inflammatory cytokines from corneocytes. However, enhanced desquamation and thinning of the stratum corneum associated with changes in skin pH probably explain the negative effects of many detergents on skin barrier function. The potential negative effects of surfactants on the skin barrier of persons with AD should be taken into account when choosing topical products. For example, aqueous cream is a generic emollient soap substitute designed to be used instead of soap in persons with AD and related disease and contains sodium lauryl sulphate at 1% concentration. The use of aqueous cream as a leave-on emollient rather than as a wash-off soap substitute has been associated with irritant reactions and exacerbations of AD, probably occurring as a result of the irritative effects of sodium lauryl sulphate described above. This illustrates the importance of understanding that topical pharmaceutical and cosmetic products can have both positive and negative effects on the skin. If used incorrectly, these products can exacerbate rather than improve the control of AD.

**GENE–ENVIRONMENT INTERACTIONS: TOPICAL CORTICOSTEROIDS**

The positive anti-inflammatory effects of topical corticosteroids have to be balanced with their potential to induce cutaneous atrophy as a result of the inhibition of the synthesis of collagen and glycosaminoglycans and also against their effects on the integrity of the epidermal
barrier. A significant increase in TEWL has been observed in patients after the long-term application of topical corticosteroids. Short-term application of topical corticosteroids (3 weeks) has also been associated with a significant increase in TEWL from healthy skin. Therefore it appears that within 3 weeks, topical corticosteroids can cause significant disruption of the epidermal barrier. These findings should not be surprising, considering that even a single supraphysiologic dose of endogenous glucocorticoids induced by stress has been shown to impair epidermal barrier homeostasis.30,195

Sheu et al performed skin biopsies on the facial skin of patients previously treated with topical corticosteroids on the face for 4 months to 4 years. The skin of patients treated with topical corticosteroids differed from that of control subjects in that it showed up to a 70% reduction in the thickness of the stratum corneum by means of light microscopy, a marked decrease in the number of intercellular lipid lamellae, and a marked reduction in the number of membrane-coated granules at the stratum granulosum/stratum corneum interface by means of electron microscopy. The reduction in the number of cell layers in the stratum corneum and reduced lipid lamellae was reflected in an increased TEWL in the topical corticosteroid-treated patients (21.3 ± 11.8 g/m² per hour) compared with that seen in healthy control subjects (6.7 ± 1.29 g/m² per hour).190,194

Kao et al investigated the effects of short-term (3 days) application of very potent topical corticosteroids (0.05% clobetasol propionate) in healthy human volunteers. The baseline TEWL was not changed after this treatment compared with that seen in control subjects. However, when the skin was tape stripped, the TEWL was much higher from the clobetasol-treated skin than from that treated with vehicle. Similar results were obtained in murine skin treated with 0.05% clobetasol propionate. Measurements of the amount of proteins on the tape strips removed from the mouse skin revealed larger quantities from the clobetasol-treated site than from sites treated with vehicle. This indicates that tape stripping removed more corneocytes from the skin treated with clobetasol than from the skin treated with vehicle. The ability of the stratum corneum to resist tape stripping is imparted by the corneodesmosomes, which lock the corneocytes together. As increasing numbers of corneodesmosomes are cleaved, more corneocytes will be removed with successive tape strips. The more corneocytes that are removed per tape strip, the greater the disruption to the skin barrier and the higher the TEWL. In the study by Kao et al, the number of corneocytes lost by tape stripping and the TEWL increased in a dose-dependent manner. Electron micrographs of the skin of these mice revealed a 35% reduction in the number of corneodesmosomes in the lower part of the stratum corneum in mice treated with clobetasol compared with those treated with vehicle, which explains why tape stripping removed significantly more corneocytes from clobetasol-treated skin than from vehicle-treated skin. Kao et al also found changes in the lipid lamellae similar to those reported by Sheu et al. Thus short-term treatment (3 days) with very potent topical corticosteroids appears to cause disruption of both the corneodesmosomes and lipid lamellae, resulting in a decrease in the functional integrity of the epidermal barrier. Corticosteroids bind to glucocorticoid nuclear receptors, which in turn bind to corticosteroid-responsible elements in the promoter region of multiple genes. At concentrations as low as 10^-10 molar, corticosteroids have been shown to upregulate SCCE gene expression. An increased production of SCCE protein after topical application of corticosteroids would help explain the degradation of corneodesmosomes observed after 3 days’ application of clobetasol propionate to the skin of mice.192

We have shown that application of clobetasol propionate, one finger-tip unit twice daily for 4 days, to healthy human skin induces the expression of the mRNA for SCCE and might therefore have a detrimental effect on epidermal barrier function by promoting corneodesmosome breakdown. However, topical corticosteroids are an extremely effective treatment for severe flares of AD. How is this compatible with the negative effects of topical corticosteroids on the skin barrier as a result of increased SCCE protease production? The most likely explanation is that during a severe flare of AD, there are several other sources of proteases, including inflammatory cells (secondary proteases) and S aureus (exogenous proteases; see Fig E4 in the Online Repository at www.jacionline.org). The anti-inflammatory actions of topical corticosteroids can decrease production of all these sources of proteases, and the overall effects of topical corticosteroids in the middle of a flare on the skin barrier will therefore be positive, improving barrier function. Before development of a severe flare of AD or after resolution of the flare, the main sources of proteases in the stratum corneum are endogenous proteases, such as SCCE (see Fig E4 in the Online Repository). The levels of SCCE will be increased in nonlesional eczematous skin as a result of the variation in the SCCE gene associated with AD. A further increase in the levels of SCCE induced by topical corticosteroids will worsen the epidermal barrier dysfunction (see Fig E4 in the Online Repository). The disruption of the stratum corneum barrier observed after even short-term exposure to topical corticosteroids supports this hypothesis. Outside a flare of AD, the overall effects of topical corticosteroids on the skin barrier might therefore be negative because these drugs can enhance its breakdown. This helps explain why short-term treatment of a flare of AD with topical corticosteroids is very effective, whereas long-term use can lead to problems, such as flare rebound and steroid addiction.

Rebound flare after the discontinuation of topical corticosteroids is not uncommon. It occurs both in the context of an underlying skin disease, such as AD, and also in healthy skin after prolonged application of topical corticosteroids. Rebound flare was observed in all of the patients studied by Sheu and colleagues. The rebound flare after discontinuation of topical corticosteroids has similarities to that observed after other forms of barrier disruption. Barrier disruption results in the
initiation of cytokine cascade, followed by an inflammatory response. Several of the cytokines released after barrier disruption can induce transcription from the protease genes and lead to further barrier breakdown. An extreme form of rebound flare after the discontinuation of topical corticosteroids is “the red burning skin syndrome.” In all the reported cases, patients had used topical corticosteroids for prolonged periods on delicate skin sites, such as the face and genitals. Patients initially had pruritus, followed by burning and erythema. Further application of topical corticosteroids led to an exacerbation of the condition, described as corticosteroid addiction. A possible mechanism is that because the potent topical corticosteroid causes a thinning of the naturally thin stratum corneum on the face, it allows more allergens to penetrate, inducing persistent flares of the AD. As a result, the patient uses more topical corticosteroid to treat the flare, but this causes further thinning of the stratum corneum and, consequently, greater allergen penetration, causing more flares. A vicious circle is therefore established.

Thus an understanding of the kinetics of protease production around a flare of AD helps us understand how to use treatments such as topical corticosteroids more safely.

CONCLUSIONS

Inside-outside or outside-inside hypothesis: which is correct? We suggest that both might be important at different times in the evolution of AD, in intrinsic and extrinsic AD, and in AD of different severities. Intrinsic AD, without an increased level of nonspecific or specific IgE, is common (up to 66% of cases) in children with mild or moderate AD recruited from the community. It has been postulated that nonallergenic intrinsic AD can be considered a pure transitional form of the disease. In a proportion of children with intrinsic AD, the disease will remain intrinsic, whereas in others the allergic nature of the disease will manifest with time.

In children with intrinsic AD, there is a strong association with an insertion in the 3′ untranslated region of the gene encoding the protease SCCE. In mild intrinsic AD the use of an irritant, such as soap, in patients with the genetic predisposition to a skin barrier breakdown related to the variant of the SCCE gene might be sufficient on its own to produce barrier disruption. This stimulates the production of inflammatory cytokines and leads to the development and persistence of eczematous lesions. These would be eczematous lesions produced according to the outside-inside hypothesis. AD is an example of a gene dosage and environmental dosage effect disease (Fig 8). At one end of the spectrum, a single change in one skin barrier gene might predispose to AD but require exposure to an environmental agent, such as soap and detergents, for the disease to be expressed. At the other end of the spectrum, a combination of changes in several skin barrier genes could, on their own, lead to severe skin barrier breakdown and the development of more severe AD. Environmental factors, such as soap, detergents, and exogenous proteases derived from house dust mites and Staphylococcus aureus, would further exacerbate the barrier breakdown and AD. At the severe end of the spectrum, other genetic changes might also be important, such as changes in the genes that regulate the production of IgE.

The hypothesis that nonallergenic intrinsic AD might be considered a pure transitional form of the disease is
compatible with the skin barrier genetic data. The SCCE variant is strongly associated with intrinsic, but not extrinsic, AD.41 A proportion of the patients with intrinsic AD will never have increased levels of serum IgE. In some infants the disease might start as the intrinsic nonallergenic form of AD, with a defective epidermal barrier. The alterations in epidermal barrier integrity and function allow the penetration of allergens through the skin, facilitating the interaction of these allergens with the local antigen-presenting cells and immune effector cells. During the first 6 months of a baby’s life, the TH1 cells are most vulnerable to switching to TH2 cells, resulting in increased production of IL-4 and IL-5 and increased production of IgE.205 By this chain of events, the intrinsic AD of some young children can become extrinsic AD. In very mild, permanently intrinsic AD the outside-inside hypothesis2 might explain the entire disease process. In AD that starts as intrinsic but then switches to extrinsic, both the outside-inside hypothesis2 and the inside-inside hypothesis20 probably explain different aspects of the disease process at different points in time of the disease development. The genetic predisposition to a defective skin barrier could be considered a starting point in the atopic march. The number and functional significance of changes in skin barrier genes could help determine the severity of barrier breakdown and allergen penetration. In addition, an understanding of the kinetics of protease production in the skin of patients with AD could help to use treatments such as topical corticosteroids more safely and effectively. The environmental exposure to irritants and allergens would also be very important in unmasking/exacerbating defective skin barrier function. This, in turn, could influence TH1 and TH2 switching and the change from intrinsic nonallergic AD to extrinsic allergic AD.

CLINICAL IMPLICATIONS OF SKIN BARRIER DYSFUNCTION IN AD

Our increasing awareness that epidermal barrier dysfunction is an extremely important component of the pathophysiology of AD should focus our attention on everything that comes into contact with the skin. This includes environmental agents, such as soap, detergents, bacterial infection, and inhalant allergens, such as house dust mites, and the topical formulations used to treat AD. Exposure to soap and detergents has been recognized as an exacerbating environmental factor in AD for more than 40 years. The detrimental effects were thought to arise through damage to the lipid lamellae. It now appears that the increase in skin pH produced by soap and detergents is also very important in enhancing the activity of skin proteases. Ensuring that the washing regimen of persons with AD is completely free from any type of soap or detergent wash product is therefore very important. Soap and detergent wash products can be replaced with emollient wash products.206 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. Because shampoos inevitably flow onto the face, the careful selection of these products is important. There are now emollient wash products designed for the shower and bath and for hand washing, such as Aveeno cream and wash; Balneum Plus cream and wash; E45 cream, bath, and wash; Hydromol cream and bath; Lipobase cream; and Oilatum cream and bath. Emollient bath, shower, and wash products should be combined with emollient creams and ointments to improve skin barrier function. In view of the damaging effect of detergents, it is important to select appropriately formulated products. Emollient creams containing high concentrations of surfactants have been shown to induce irritant reactions in the majority of children attending a pediatric AD clinic.207 The ideal approach is to let the patient select which product or products they find most suitable for their skin.

Environmental agents, such as house dust mites, produce cysteine proteases that enhance TH2 responses and the production of specific IgE.208,209 However, the same proteases can also break down corneodesmosomes and lead to an increased barrier dysfunction. Measures to reduce exposure to house dust mites might therefore be important in all patients with AD.25 Staphylococcus aureus is also a source of exogenous proteases, which could break down the skin barrier. These proteases are probably very important in secondarily infected lesions of AD, but their negative effects on the skin barrier might also be important in nonlesional eczematous skin.

Topical corticosteroids are an important short-term treatment for severe flares of AD. However, if topical corticosteroids are used for prolonged periods and particularly on delicate skin sites, they can cause cutaneous atrophy100,210-212 and damage the stratum corneum. Prolonged use of topical corticosteroids might damage the skin barrier on delicate skin sites enough to enhance the penetration of irritants and allergens. This could provide the explanation for the phenomenon of posttopical steroid rebound and steroid addiction.204 One way to reduce the chronic use of topical corticosteroids is to introduce calcineurin inhibitors, such as pimecrolimus and tacrolimus, into treatment regimens. Mild-to-moderate flares of AD can be treated with pimecrolimus, which does not damage the skin barrier.37,211-214 In patients with recurrent flares of severe AD who require large quantities of potent topical corticosteroids, tacrolimus can be used as an alternative or it can be rotated with the potent topical corticosteroid.215 The key message is to control everything that comes into contact with the skin to reduce the damage to the skin barrier and the number of flares of AD. It is important to convey this message to your patients in a way they can understand,28 such as using cartoons or other patient education materials that are easy for the child (and their parents) to understand and are fun.

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