

New perspectives on epidermal barrier dysfunction in atopic dermatitis: Gene–environment interactions

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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 corneodesmosomes, 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.¹ Major contributors to this hyperactivity are the many changes in the cutaneous and systemic immune responses in individuals with AD.² One example is the production of increased levels of total serum IgE and specific IgE to common allergens.³ However, the link between AD and allergen-specific IgE remains hotly debated.⁴ A recent systematic review revealed that the association with increased IgE levels was much lower in children with mild-to-moderate AD than in children with severe disease.³ It has been postulated that the nonallergic intrinsic dermatitis could be a pure transitional form of AD.⁵ 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.⁶⁻¹²

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.⁹

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

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Abbreviations used

| | |
|-------|-------------------------------------|
| AD: | Atopic dermatitis |
| MCC: | Mast cell chymase |
| SCCE: | Stratum corneum chymotryptic enzyme |
| SCTE: | Stratum corneum tryptic enzyme |
| TEWL: | Transepidermal water loss |

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.¹³ Several loci have been associated with increased IgE production, linked to increased IgE production, or both, including 4q35.2, 11q13, 16q24.1, and 5q31.1.¹⁴⁻¹⁷ Also, see the review by Morar et al¹⁸ 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.¹⁹⁻²⁷ However, there are few formal longitudinal studies that indicate how the home environment has changed over the past 50 years. We previously reviewed data regarding exposure to soap detergents, frequency of washing, and exposure to house dust mites.²⁸ An example of these changes is the increased 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 1997/1998.²⁸ 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 house dust mite.²⁸ All these environmental agents damage the skin barrier directly, and coupled with the increasing prevalence of AD, this suggests that breakdown of the skin barrier might be a very important event in the development of the disease.

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*.²⁹ Alternatively, it has been hypothesized that the xerosis,³⁰ the permeability barrier abnormality,^{31,32} or both could drive the activity of AD, which is known as the *outside-inside hypothesis*.^{32,33} 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.³³ 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-1 α , IL- β , TNF- α , and GM-CSF,^{34,35} indicating that barrier disruption alone leads to cytokine production, inflammation, and a flare of dermatitis.³² 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.^{36,37} 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.³⁸ 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 Taieb³⁹ proposed that a genetic predisposition to a defective skin barrier was a primary event in the development of AD, allowing allergen penetration and enhanced T_H2 responses. Two groups^{40,41} have identified variants/changes 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.² 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.³² 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.⁴² In older children the most common sites affected are the antecubital and popliteal fossae.^{43,44} In addition to the classical patterns of AD, there are several site-specific variants.⁴⁵ Eyelid eczema is common in

adolescents, affecting up to 21% of these individuals,⁴³ and has been associated with hay fever and exposure to other aeroallergens, such as house dust mites.⁴⁶ The infra-auricular and retroauricular sites are particularly prone to fissuring, probably as a reaction to repeated minor trauma.^{43,47,48}

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. Hanifin⁴⁹ 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 periorbital areas because these zones are rubbed and scratched unconsciously. Only 3 studies⁵⁰⁻⁵² have evaluated epidermal thickness in multiple body sites and have shown that it is thinnest in the eyelid⁵⁰ and genitals.⁵² The next thinnest sites are the flexor forearm and posterior auricular areas (see Fig E1 in the Online Repository at www.jacionline.org).⁵² Interestingly, these are 2 of 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.^{53,54} 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 vitro* by using cadaver skin from different body sites,⁵⁵ 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 Maibach⁵⁶ 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.^{57,58}

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.⁵² 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^{52,56} 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 longer⁴² and with low epidermal barrier reserve. It is probable that these body sites are the most vulnerable to penetration of irritants and allergens⁴⁹ 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.⁵⁹ 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%.^{60,61} On the basis of the transepidermal 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.⁶⁰ 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.⁶² 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.⁶³ 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.⁶⁴ 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

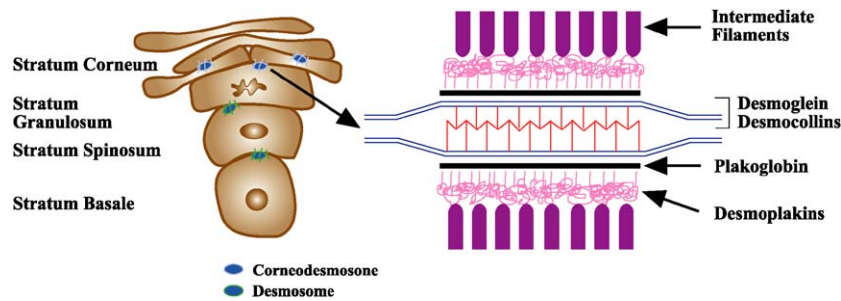


FIG 1. The barrier to the penetration of irritants, allergens, and drugs is located in the lower part of the stratum corneum. The structural integrity of the stratum corneum is maintained by modified desmosomes (corneodesmosomes), which lock together the corneocytes. As the corneocytes move up through 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.

function in nonlesional skin from patients with AD is different from that of subjects who never had this disease.⁶⁵⁻⁶⁷ In one study⁶⁵ the average thickness of the stratum corneum was 12.2 μm in nonlesional skin from individuals with AD and 19.7 μm in skin from control subjects who had not had AD. In other studies^{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.^{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,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.⁶⁹ The link between AD and allergen-specific IgE remains, however, uncertain and controversial.^{3,69-73} A recent systematic review³ 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%.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.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,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.^{74,75} Could the genetic basis of the pure, primary, intrinsic, transitional form of AD⁷⁵ 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 the presence of modified desmosomes, called corneodesmosomes (Figs 2-4 and Fig E2 in the Online Repository at www.jacionline.org). Corneodesmosomes lock the corneocytes together and provide tensile strength for the stratum corneum to resist shearing forces (Figs 1-4 and Fig E2 in the Online Repository at www.jacionline.org). Elias⁷⁶ visualized the stratum corneum as being similar to a brick wall, with the corneocytes analogous to bricks and the lipid lamellae acting as cement. Extending this model, the corneodesmosomes can be thought of as analogous to iron rods that pass down through holes in the bricks to give the wall its tensile strength (Fig 5 and Fig E3 in the Online Repository at www.jacionline.org).

Corneocytes are flattened cells that represent the final differentiation stages of the outermost keratinocytes of

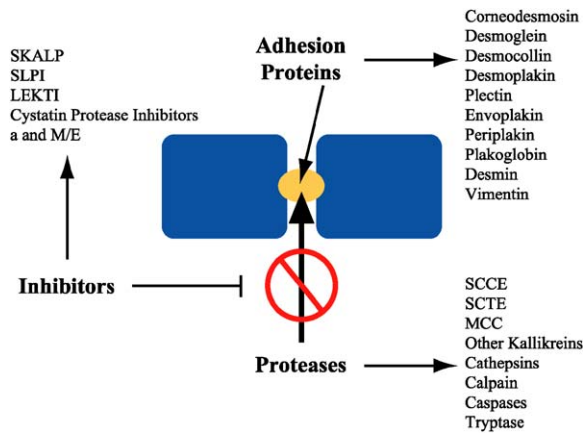


FIG 2. The corneocytes of the skin barrier are locked together by corneodesmosomes comprising several adhesion proteins. Desquamation of corneocytes can only occur once the corneodesmosome has been broken down by skin-specific proteases, such as the SCCE. The proteases are kept under control by specific protease inhibitors, such as the skin-derived antileukoprotease (*SKALP*). *SLPI*, serine leukoprotease inhibitor; *LEKTI*, lymphoepithelial Kazal-type 5 serine protease inhibitor.

the granular layer, when these cells lose their subcellular organelles and nuclei and become densely packed with keratin fibers.⁷⁷ In human subjects the stratum corneum has an average of 20 corneocyte layers, with each corneocyte being approximately 30 μm in diameter.⁷⁸ The thickness of the stratum corneum can vary in different body regions to increase the level of protection to areas that experience greater friction, such as the soles of the feet and palms of the hands.⁵² 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 corneocytes like 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.²³ 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

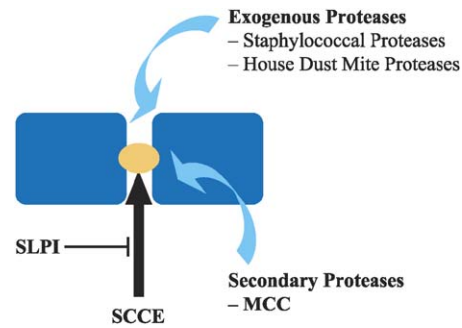


FIG 3. Corneodesmosomes are not only broken down by endogenous proteases, such as SCCE. Once a flare of AD has been triggered, cells within the inflammatory infiltrate produce secondary proteases, which can also break down the skin barrier (eg, MCC). The stratum corneum is also exposed to many exogenous proteases from the environment, such as *Staphylococcus aureus* and house dust mites. *SLPI*, serine leukoprotease inhibitor.

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 plakophilin (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.^{84,86,87} 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.⁸⁸ 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

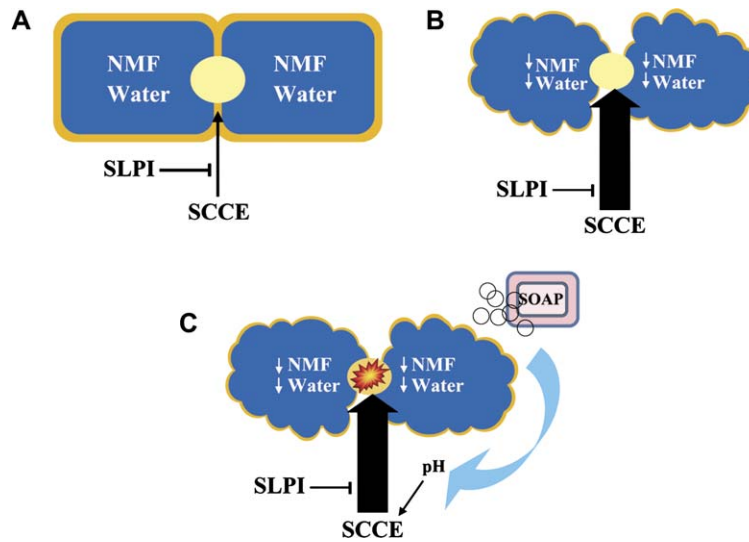


FIG 4. In the stratum corneum from healthy skin, there is a balance between the structural integrity of the corneodesmosomes and the level of proteases and protease inhibitors (**A**). In individuals genetically predisposed to AD, increased protease activity leads to premature breakdown of the corneodesmosomes 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 (**C**). *SLPI*, Serine leukoprotease inhibitor.

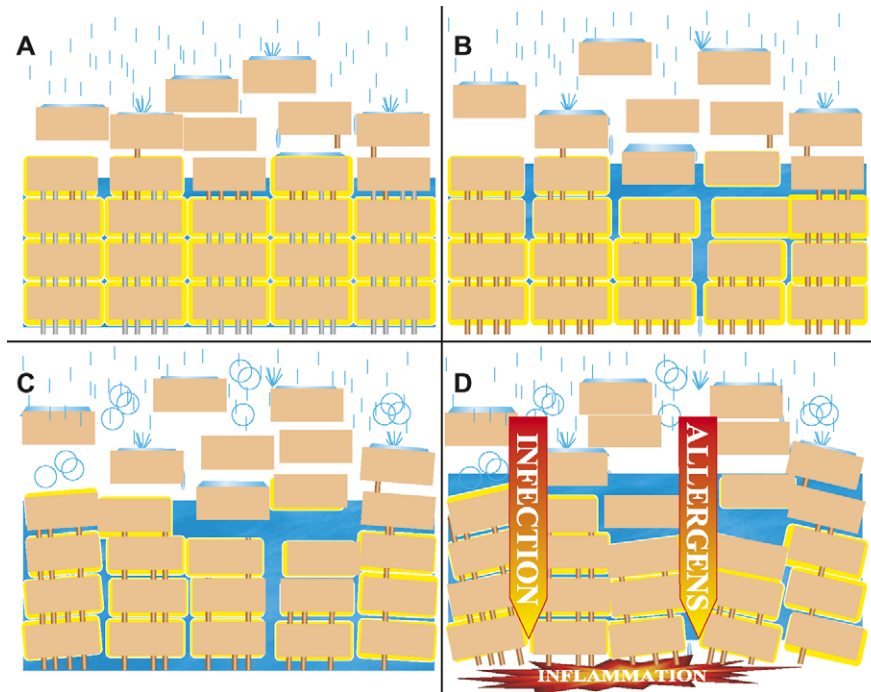


FIG 5. The brick wall analogy of the stratum corneum of the epidermal barrier. In healthy skin the corneodesmosomes (iron rods) are intact throughout the stratum corneum. At the surface, the corneodesmosomes start to break down as part of the normal desquamation process, analogous to iron rods rusting (**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**).

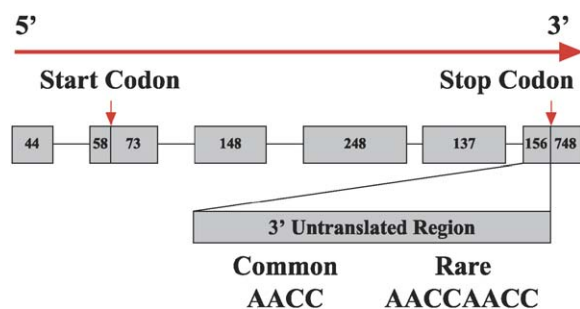


FIG 6. Genomic structure of the kallikrein 7 gene, encoding the SCCE. A 4-bp insertion (AACC) was found in the 3' untranslated region of the gene.⁴¹

provided by Caubet et al.⁹³ The model describes a network of degradatory proteases regulated by protease inhibitors, which break down the extracellular corneodesmosomal adhesion proteins that bind the corneocytes together and in doing so allow the corneocytes to shed from the skin surface (Figs 2 and 4). To refer back to the brick wall analogy, one might imagine the proteases progressively rusting the iron rods until they are completely corroded (Fig 5). A cocktail of serine, cysteine, and aspartic proteases are secreted into the extracellular spaces of the stratum corneum during desquamation to facilitate the breakdown of the corneodesmosomes.⁹⁴⁻⁹⁶ According to the model of desquamation proposed by Caubet et al,⁹³ inactive protease precursors are activated by tryptic cleavage and regulated by a complementary cocktail of protease inhibitors. Cleavage of the extracellular corneodesmosomal proteins by the proteases leads to a weakening of the bonds between the corneocytes and a reduction in corneocyte cohesion (Figs 2 and 4).

Among the proteases involved in the process of desquamation are the stratum corneum chymotryptic enzyme (SCCE) and the stratum corneum tryptic 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 corneodesmosin 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

The activities of the proteases involved in desquamation are regulated by several protease inhibitors (Fig 2 and Fig E2 in the Online Repository at www.jacionline.org) coexpressed to balance the rate of corneodesmosome cleavage. SCCE activity is inhibited by the serine

leukoprotease inhibitor,¹⁰⁴ which can itself be inactivated by members of the cathepsin family.¹⁰⁵ SCCE is also inhibited by elafin, also known as skin-derived antileukoprotease, which has been shown to covalently bind to corneocytes.¹⁰⁶ Lymphoepithelial Kazal-type 5 serine protease inhibitor, encoded by the *SPINK5* gene, might also have anti-SCCE activity. It is expressed in similar areas to SCCE and has been linked to Netherton syndrome,¹⁰⁷ a skin condition involving severe barrier dysfunction. Human epidermis also expresses the cystatin protease inhibitors α and M/E, which are specific for cysteine proteases.¹⁰⁸

GENETIC REGULATION OF SKIN BARRIER FUNCTION

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 levels^{3,74,109} 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 overexpressing 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).^{41,93,96,97,104,110-124}

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.⁴¹ 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 AACCAACC. A significant genetic association was found between the rare AACCAACC 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

TABLE I. Genetic dysfunction of epidermal integrity and effects of endogenous and exogenous proteases

| Protein | Gene | Abbreviation | Dysfunction | Reference |
|---|---------------|--------------|--|-------------|
| Stratum corneum chymotryptic enzyme | <i>KLK7</i> | SCCE | Genetic association between a mutation in the SCCE gene and AD: results suggest that altered SCCE expression might lead to an increased rate of desmosome breakdown and a weakening of the skin barrier. | 41 |
| | | | Transgenic mice overexpressing SCCE: the mice displayed epidermal hyperproliferation and decreased skin barrier function. Their phenotype was similar to that seen in chronic itchy dermatitis. | 97 |
| Stratum corneum tryptic enzyme | <i>KLK5</i> | SCTE | Shown to degrade corneodesmosomal proteins: altered function might lead to changes similar to those associated with abnormal SCCE expression. | 93 |
| Stratum corneum thiol protease | | SCTP | Responsible for the degradation of extracellular structural proteins: can function in acidic environments like the stratum corneum. Altered function might lead to changes similar to those associated with abnormal SCCE expression. | 96 |
| Desmoglein | | DSG | The distribution of different isoforms of desmoglein affects the structure and function of the stratum corneum. Transgenic mice abnormally expressing DSG3 within the stratum corneum had scaling and altered lipid lamellae. The ultrastructure of the stratum corneum showed premature loss of cohesion of comeocytes. | 117 |
| | | | Targeted disruption of the DSG3 gene in mice led to a loss of keratinocyte cell adhesion. A phenotype similar to that seen in pemphigus vulgaris developed in the mice. | 112 |
| | | | Abnormal DSG3 expression in the epidermis of transgenic mice resulted in epidermal hyperproliferation and abnormal differentiation. | 118 |
| Desmocollin | | DSC | Transgenic mice lacking desmocollin 1 develop a very weak epidermis and display barrier defects and abnormal differentiation. | 113 |
| Corneodesmosin Desmoplakin | | CDSN DP | Transgenic null mice die <i>in utero</i> . Analysis of the embryos revealed a critical role for desmoplakin in anchoring intermediate filaments to desmosomes and in desmosome assembly, stabilization, or both. Mutation in the gene encoding desmoplakin led to a null allele that was associated with the inherited skin disorder striate palmoplantar keratoderma. | 114,115 |
| Serine leukoprotease inhibitor | | SLPI | | |
| Skin derived antileukoprotease | | SKALP | | 104,119,120 |
| Lymphoepithelial Kazal-type 5 serine protease inhibitor | <i>SPINK5</i> | LEKTI | <i>SPINK5</i> -deficient mice develop the key features of Netherton syndrome. The barrier defects were attributed to increased epidermal protease activity and an increased rate of desmoglein 1 cleavage. | 110 |
| | | | <i>SPINK5</i> mutant mice have a fragile stratum corneum and die of dehydration through increased TEWL. Data suggested that the fragility of the corneodesmosomes was being caused by increased proteolysis of corneodesmosin. | 111 |
| House dust mite protease | | Der p1/p2 | Purified Der p 1 and Der p 2 were shown to elicit an allergic reaction and to have proteolytic activity. | 116 |
| Cathepsin L | | | The lysosomal protease cathepsin L is an important regulator of keratinocyte and melanocyte differentiation during hair follicle morphogenesis and cycling. | 121,122 |
| Cathepsin D | | | Cathepsin D is involved in the regulation of transglutaminase I and epidermal differentiation. | 123 |
| Cystatin M/E | | | | 124 |

expression levels of the encoded protein.¹²⁵⁻¹²⁷ Thus 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 comeocyte 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.^{40,107,128,129} Individuals with this disorder display marked barrier dysfunction, involving altered desquamation and impaired keratinization.¹³⁰ 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.¹³⁰ 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.¹¹⁰ Increased protease activity in the skin of *SPINK5*^{-/-} mice leads to increased breakdown of desmoglein 1¹¹⁰ and corneodesmosin,¹¹¹ 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.^{40,131,132}

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.¹³³ Transgenic mice carrying a null mutation in the gene encoding cystatin M/E display severe barrier abnormalities and die shortly after birth.¹⁰⁸ Mice lacking cystatin M/E have abnormalities in cornification and desquamation with hyperkeratosis (Table I).¹⁰⁸

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.¹¹² Mice lacking desmocollin 1 have been shown to have a flaky and fragile epidermis, with acanthosis in the granular layer.¹¹³ Desmoplakin is also important in epidermal sheet formation (Table I).¹¹⁴ Mice lacking desmoplakin have few desmosomes and a marked reduction in barrier integrity.¹¹⁵ 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 study¹³⁴ the numbers of MCC⁺ cells were significantly increased in the lesional

skin of patients with AD in comparison with those in non-lesional 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,¹³⁵ 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.¹³⁶ Variants within the MCC gene have been associated with AD in children.¹³⁷ The association was strongest in individuals with low levels of total serum IgE.¹³⁷ 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.¹³⁸

EXOGENOUS PROTEASES

House dust mites are a source of more than 30 different proteins that can induce IgE-mediated responses.¹³⁹ Some of these proteins are cysteine and serine proteases.¹⁴⁰ Some of these proteins have been shown to cleave adhesion proteins and to increase the permeability of lung epithelium.¹⁴¹ 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.¹¹⁶

As reviewed by Storck,¹⁴² *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 14×10^6 organisms per square centimeter are present in eczematous lesions.¹⁴³ *S aureus* might play a role in the chronicity and severity of AD through its release of superantigenic exotoxins.¹⁴⁴ 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.¹⁴⁵ In addition, *S aureus* secretes sphingosine deacylase and glycerophospholipids that might interfere with the formation of the lipid lamellae.¹⁴⁶ 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.¹⁴⁷ The average surface pH of the forearm of a healthy male is around 5.4 to 5.9.¹⁴⁸ In human subjects the skin surface pH at birth is near neutral (pH 6.5)

compared with that in children and adults.¹⁴⁹⁻¹⁵¹ In newborn rats the stratum corneum reaches adult pH levels during the first few days after birth,^{152,153} whereas similar changes take a few weeks to occur in human newborns.^{151,154}

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),¹⁵⁵⁻¹⁵⁷ 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.¹⁵⁸⁻¹⁶⁰ The acid mantle has multiple effects on the skin. First, it has a strong antimicrobial effect,^{161,162} decreases skin colonization by pathogenic bacteria,^{156,161,163} and favors the adhesion of nonpathogenic bacteria to the stratum corneum.¹⁶⁴ 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.¹⁶⁵ 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.^{160,166}

Skin pH variations have been clearly documented in some skin diseases. Anderson¹⁶⁷ found a total body pH increase in patients with seborrheic dermatitis, AD, and xeroderma. Others¹⁶⁸ 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.¹⁶⁹ Similar studies^{168,170} documented that skin pH is higher in patients with AD than in healthy control subjects, even on uninvolved skin. Seidenari and Giusti,¹⁷⁰ 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.¹⁷¹ The skin protease SCCE exhibits a neutral pH optimum.⁹⁵ A change in pH from 7.5 to 5.5 reduces SCCE activity by 50%.^{95,172} 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.^{94,102,173} 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.¹⁷³ The importance of pH to the activity of skin proteases was demonstrated in hairless mice treated with "superbases" that neutralize skin surface pH.¹⁷⁴ 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.^{171,175-177} 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.¹⁷⁴ 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,⁴¹ there will be constantly high levels of SCCE protein in the stratum corneum. If 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.¹⁷⁸ White et al⁶⁵ 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 μ m) than in nonlesional eczematous skin (13.7 μ m). 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).⁴¹ 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 (Fig 5). 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).¹⁷⁹

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

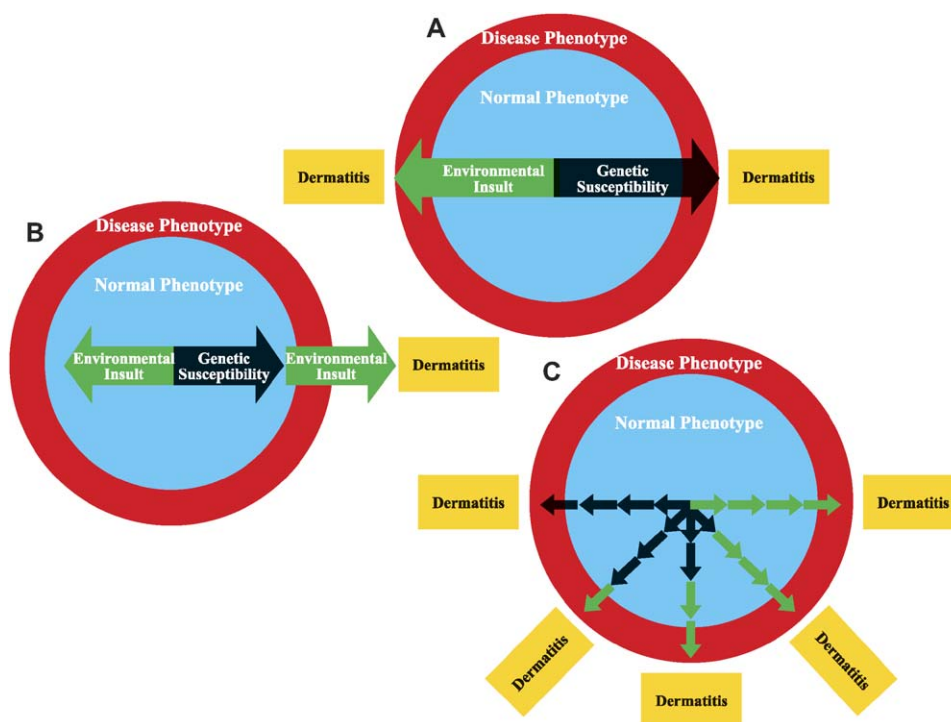


FIG 7. Different combinations of genetic and environmental factors contribute to the development of multifactorial diseases, such as AD. Focusing on the skin barrier, severe barrier breakdown could be caused by a combination of functional variants in adhesion protein, protease, and protease inhibitor genes (A) or by a single major environmental insult. Alternatively, major functional changes in the skin barrier–related genes could produce a defective skin barrier. Exposure of this defective barrier to an environmental insult, such as soap and detergents, breaks it down further, allowing penetration of irritants and allergens (B). A third possibility is the combination of several changes in skin barrier genes, resulting in small functional changes (C). Other combinations of both genetic and environmental factors can also lead to the development of AD (C). A combination of repeated environmental insults might, alone, also produce sufficient barrier breakdown to lead to the development of eczematous lesions (C).

water. Surfactants can damage the skin, provoking scaling, dryness, tightness and roughness, erythema, and swelling.¹⁸⁰⁻¹⁸³ 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.¹⁸⁴

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.¹⁸⁵ Surfactants can solubilize lipids, and it has been postulated that this could be the mechanism by which they increase TEWL.^{185,186} 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.¹⁸² The acute irritant effects of soap and detergents could be partially explained by the release of pro-inflammatory cytokines from corneocytes.^{34,35} 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,³⁶ 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 glycosaminoglycans¹⁸⁷⁻¹⁸⁹ and also against their effects on the integrity of the epidermal

barrier.¹⁹⁰⁻¹⁹² A significant increase in TEWL has been observed in patients after the long-term application of topical corticosteroids.^{193,194} Short-term application of topical corticosteroids (3 weeks) has also been associated with a significant increase in TEWL from healthy skin.¹⁹¹ 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 al¹⁹⁰ 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 al¹⁹² 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.¹⁹² 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.¹⁹² 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,¹⁹² 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 al¹⁹² also found changes in the lipid lamellae similar to those reported by Sheu et al.^{190,196}

Thus short-term treatment (3 days) with very potent topical corticosteroid 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-responsive elements in the promoter region of multiple genes.¹⁹⁷ At concentrations as low as 10⁻¹⁰ molar, corticosteroids have been shown to upregulate SCCE gene expression *in vitro*.¹⁹⁸ 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 SCCE¹⁹⁹ 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.⁴¹ 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 corticosteroids¹⁹² 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.^{200,201} Rebound flare was observed in all of the patients studied by Sheu and colleagues.¹⁹⁰ The rebound flare after discontinuation of topical corticosteroids has similarities to that observed after other forms of barrier disruption. Barrier disruption results in the

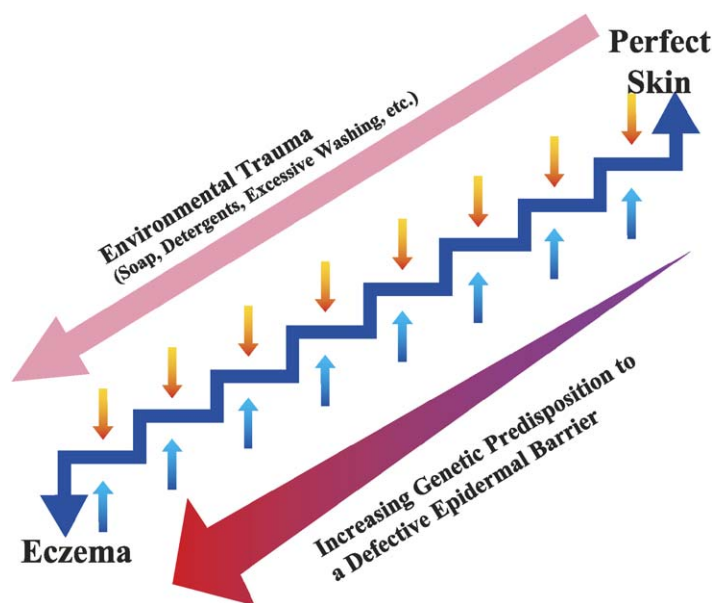


FIG 8. The skin can be considered to be on a spectrum from perfect skin to eczematous skin. Changes in skin barrier–related genes alter the epidermal barrier function, making the skin more susceptible to the development of AD. When a genetic predisposition to barrier breakdown is combined with exposure to environmental agents, such as soap, the chance of eczema development is increased substantially.

initiation of cytokine cascade, followed by an inflammatory response.^{34,35,202} Several of the cytokines released after barrier disruption can induce transcription from the protease genes and lead to further barrier breakdown.²⁰³

An extreme form of rebound flare after the discontinuation of topical corticosteroids is “the red burning skin syndrome.”²⁰⁴ 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.^{190,192} 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-outside²⁹ or outside-inside³² 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.³ It has been postulated that nonallergenic intrinsic AD can be considered a pure transitional form of the disease.⁷⁵ 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.⁷⁴

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.⁴¹

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^{34,35} 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 *S 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 disease⁷⁵ 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 T_H1 cells are most vulnerable to switching to T_H2 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 hypothesis³² might explain the entire disease process. In AD that starts as intrinsic but then switches to extrinsic, both the outside-inside hypothesis³² and the inside-outside hypothesis²⁹ 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 T_H1 and T_H2 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 T_H2 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.²⁸ *S 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 non-lesional 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 atrophy^{190,210-212} 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.

REFERENCES

1. Leung DYM, Bieber T. Atopic dermatitis. *Lancet* 2003;361:151-60.
2. Leung DYM, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. *J Clin Invest* 2004;113:651-7.

3. Flohr C, Johansson SG, Wahlgren CF, Williams H. How atopic is atopic dermatitis? *J Allergy Clin Immunol* 2004;114:150-8.
4. Pepys J. Atopy. In: Gill PGH, Coombs RRA, Lachman PJ, editors. *Clinical aspects of immunology*. 3rd ed. Oxford: Blackwell Science; 1975. p. 877-902.
5. Novak N, Allam JP, Bieber T. Allergic hyperreactivity to microbial components—a trigger factor of “intrinsic” atopic dermatitis? *J Allergy Clin Immunol* 2003;112:215-6.
6. Fergusson DM, Horwood IJ, Beatrais AI, Shannon FT, Taylor B. Eczema and infant diet. *Clin Allergy* 1981;11:325-31.
7. Taylor B, Wadsworth J, Wadsworth M, Peckham C. Changes in the reported prevalence of childhood eczema since the 1939-1945 war. *Lancet* 1984;2:1255-7.
8. Shultz-Larsen F, Holm NV, Hennigsen K. Atopic dermatitis: a genetic-epidemiological study in a population-based twin sample. *J Am Acad Dermatol* 1986;15:487-94.
9. Williams HC. Is the prevalence of atopic dermatitis increasing? *Clin Exp Dermatol* 1992;17:385-91.
10. Neame RI, Berth-Jones J, Kirinczuk JJ, Graham-Brown RAC. Prevalence of atopic dermatitis in Leicester: a study of methodology and examination of possible ethnic variation. *Br J Dermatol* 1995;132:772-7.
11. Thestrup-Pedersen K. The incidence and pathophysiology of atopic dermatitis. *J Eur Acad Dermatol Venerol* 1996;7(suppl 1):53-7.
12. Yura A, Shimizu T. Trends in the prevalence of atopic dermatitis in school children: longitudinal study in Osaka Prefecture, Japan, from 1985 to 1997. *Br J Dermatol* 2001;115:966-73.
13. Hall IP. Candidate gene approaches: gene-environmental interactions. *Clin Exp Allergy* 1998;28(suppl 1):74-6.
14. Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989;1:1292-5.
15. Marsh DG, Neeley JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
16. Deichmann KA, Heinzmann A, Forster J, Dischinger S, Mehl C, Bruegenolte E, et al. Linkage and allelic association of atopy and markers flanking the IL4-receptor gene. *Clin Exp Allergy* 1998;28:151-5.
17. Hildebrandt F, Moseler M, Kuehr J, Klüken H, Wienker T, Bieber T. Atopic eczema/dermatitis syndrome—a genetically complex disease. New advances in discovering the genetic contribution. *Allergy* 2003;58:5-12.
18. Morar N, Willis-Owen SAG, Moffatt MF, Cookson WOCM. The genetics of atopic dermatitis. *J Allergy Clin Immunol* 2006;118:24-34.
19. Abe T, Ohkido M, Yamamoto K. Studies on skin surface barrier function: skin surface lipids and transepidermal water loss in atopic skin during childhood. *J Dermatol* 1978;5:223-9.
20. Al-Jaberi H, Marks R. Studies of the clinically uninvolved skin in patients with dermatitis. *Br J Dermatol* 1984;111:437-43.
21. White FH, Gohari K. Some aspects of desmosomal morphology during differentiation of hamster cheek pouch. *J Submicrosc Cytol* 1984;16:407-22.
22. Hamami I, Marks R. Abnormalities in clinically normal skin: a possible explanation of the “angry back syndrome.” *Clin Exp Dermatol* 1988;13:328-33.
23. Melnik B, Hollman J, Erler E, Verhoeven B, Plewig G. Microanalytical thin layer chromatography of all major stratum corneum lipids. *J Invest Dermatol* 1989;92:231-4.
24. Colloff MJ. Exposure to house dust mites in houses of people with atopic dermatitis. *Br J Dermatol* 1992;127:322-7.
25. Tan BB, Weald D, Strickland I, Friedmann PS. Double-blind controlled trial of effect of housedust-mite allergen avoidance on atopic dermatitis. *Lancet* 1996;347:15-8.
26. McNally NJ, Williams HC, Phillips DR, Smallman-Raynor M, Lewis S, Venn A, et al. Atopic eczema and domestic water hardness. *Lancet* 1998;352:527-31.
27. McNally NJ, Williams HC, Phillips DR. Atopic eczema and the home environment. *Br J Dermatol* 2001;145:730-6.
28. Cork MJC, Murphy R, Carr J, Buttle D, Ward S, Båvik C, et al. The rising prevalence of atopic eczema and environmental trauma to the skin. *Dermatol Pract* 2002;10:22-6.
29. Leung DY. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000;105:860-76.
30. Denda M, Sato J, Tsuchiya T, Elias PM, Feingold KR. Low humidity stimulates epidermal DNA synthesis and amplifies the hyperproliferative response to barrier disruption: implication for seasonal exacerbations of inflammatory dermatoses. *J Invest Dermatol* 1998;111:873-8.
31. Ghadially R, Reed JT, Elias PM. Stratum corneum structure and function correlates with phenotype in psoriasis. *J Invest Dermatol* 1996;107:558-64.
32. Elias PM, Wood LC, Feingold KR. Epidermal pathogenesis of inflammatory dermatoses. *Am J Contact Dermatol* 1999;10:119-26.
33. Chamlin SL, Kao J, Freiden IJ, Sheu MY, Fowler AJ, Fluhr JW, et al. Ceramide-dominant barrier repair lipids alleviate childhood atopic dermatitis: changes in barrier function provide a sensitive indicator of disease activity. *J Am Acad Dermatol* 2002;47:198-208.
34. Wood LC, Elias PM, Calhoun C, Tsai JC, Grunfeld C, Feingold KR. Barrier disruption stimulates interleukin-1 alpha expression and release from a pre-formed pool in murine epidermis. *J Invest Dermatol* 1996;106:397-403.
35. Wood LC, Stalder AK, Liou A, Campbell IL, Grunfeld C, Elias PM, et al. Barrier disruption increases gene expression of cytokines and the 55kD TNF receptor in murine skin. *Exp Dermatol* 1997;6:98-104.
36. Cork MJ, Britton J, Butler L, Young S, Murphy R, Keohane SG. Comparison of parent knowledge, therapy utilization and severity of atopic eczema before and after explanation and demonstration of topical therapies by a specialist dermatology nurse. *Br J Dermatol* 2003;149:582-9.
37. Wahn U, Bos JD, Goodfield M, Caputo R, Papp K, Manjra A, et al. Efficacy and safety of pimecrolimus cream in the long-term management of atopic dermatitis in children. *Pediatrics* 2002;110:e2.
38. Harper JI, Ahmed I, Barclay G, Lacour M, Hoeger P, Cork MJ, et al. Cyclosporin for severe childhood atopic dermatitis: short course *versus* continuous therapy. *Br J Dermatol* 2000;142:52-8.
39. Taieb A. Hypothesis: from epidermal barrier dysfunction to atopic disorders. *Contact Dermatitis* 1999;41:177-80.
40. Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubhi B, Lawrence R, et al. Gene polymorphism in Netherton and common atopic disease. *Nat Genet* 2001;29:175-8.
41. Vasilopoulos Y, Cork MJ, Murphy R, Williams HC, Robinson DA, Duff GW, et al. Genetic association between an AACC insertion in the 3'UTR of the stratum corneum chymotryptic enzyme gene and atopic dermatitis. *J Invest Dermatol* 2004;123:62-6.
42. Kunz B, Ring J. Clinical features and diagnostic criteria of atopic dermatitis. In: Harper J, Oranje A, Prose N, editors. *Textbook of pediatric dermatology*. Oxford: Blackwell Science; 2002. p. 199-214.
43. Schudel P, Wüthrich B. Klinische Verlaufsbeobachtungen bei Neurodermitis atopica nach dem Kleinkindesalter. *Z Hautkr* 1985;60:479-86.
44. Dotterud LK, Kvammen B, Lund E, Falk ES. Prevalence and some clinical aspects of atopic dermatitis in the community of Sor-Varanger. *Acta Derm Venerol* 1995;75:50-3.
45. Przybilla B, Ring J, Ruzicka T. Clinical aspects of atopic eczema: synopsis. In: Przybilla B, Ring J, Ruzicka T, editors. *Handbook of atopic eczema*. Berlin: Springer; 1991. p. 132-8.
46. Wüthrich B. Atopic dermatitis flare provoked by inhalent allergens. *Dermatologica* 1989;178:51-3.
47. Fartasch M, Diepgen TL, Hornstein OP. Atopic dermatitis—ichthyosis vulgaris—hyperlinear palms—an ultrastructural study. *Dermatologica* 1989;178:202-5.
48. Tada J, Toi Y, Akiyama H, Arata J. Infra-auricular fissures in atopic dermatitis. *Acta Derm Venerol* 1994;74:129-31.
49. Hanifin JM. Atopic dermatitis. In: Marks R, editor. *Eczema*. London: Dunitz; 1992. p. 77-101.
50. Barker DE. Skin thickness in the human. *Plast Reconstr Surg* 1951;7:115-6.
51. Southwood WF. The thickness of the skin. *Plast Reconstr Surg* 1955;15:423-9.
52. Lee Y, Hwang K. Skin thickness of Korean adults. *Surg Radiol Anat* 2002;24:183-9.
53. Scheuplein RJ, Blank IH. Permeability of the skin. *Physiol Rev* 1971;51:702-47.
54. Schaefer H, Zesch A, Stuttgart G. *Skin permeability*. Berlin: Springer; 1982.
55. Marzulli FN. Barrier to skin penetration. *J Invest Dermatol* 1962;39:387-93.

56. Feldman RJ, Maibach HI. Regional variation in percutaneous penetration of ¹⁴C cortisol in man. *J Invest Dermatol* 1967;48:181-3.
57. Schaefer KE, Scheer K. Regional differences in CO₂ elimination through the skin. *Exp Med Surg* 1951;9:449-57.
58. Cronin E, Staughton RB. Percutaneous absorption: regional variations and the effect of hydration and epidermal stripping. *Br J Dermatol* 1962;74:265-72.
59. Schaefer H, Stutgen G, Zesch A, Schalla W, Gazith J. Quantitative determination of percutaneous absorption of radiolabeled drugs in vitro and in vivo by human skin. *Curr Probl Dermatol* 1978;7:80-94.
60. Blichmann CW, Serup J. Reproducibility and variability of transdermal water loss measurements. *Acta Derm Venereol* 1989;67:206-10.
61. Oestmann E, Lavrijsen AP, Hermans J, Ponc M. Skin barrier function in healthy volunteers as assessed by transepidermal water loss and vascular response to hexyl nicotinate: intra- and inter-individual variability. *Br J Dermatol* 1993;128:130-6.
62. Maibach HI. In vivo percutaneous penetration of corticoids in man and unresolved problems in their efficacy. *Dermatologica* 1976;152 (suppl 1):11-25.
63. Ghadially R, Brown B, Sequeiara-Martin SM, Feingold KR, Elias PM. The aged permeability barrier: structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Invest* 1995;95:2281-90.
64. Halkier-Sorenson L, Thestrup-Pedersen K. The efficacy of a moisturizer (locobase) among cleaners and kitchen assistants during everyday exposure to water and detergents. *Contact Dermatitis* 1993;29:1-6.
65. White MI, McEwan Jenkinson D, Lloyd DH. The effect of washing on the thickness of the stratum corneum in normal and atopic individuals. *Br J Dermatol* 1987;116:525-30.
66. Werner Y, Linberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. *Acta Derm Venereol* 1985;65:102-5.
67. Ogawa H, Yoshiike T. Atopic dermatitis: studies of skin permeability and effectiveness of topical PUVA treatment. *Pediatr Dermatol* 1992; 9:383-5.
68. Wollenberg A, Bieber T. Allergy review series V: the skin as target for IgE-mediated allergic reactions. Atopic dermatitis: from the genes to skin lesions. *Allergy* 2000;55:205-13.
69. Schmid-Grendelmeier P, Simon D, Simon HU, Akdis CA, Wuthrich B. Epidemiology, clinical features, and immunology of the "intrinsic" (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 2001;56:841-9.
70. Johansson SGO, Hourihane JOB, Bousquet J, Brujnzeel-Koomen C, Dreborg S, Haachtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;56:813-24.
71. Johansson C, Tengvall Linder M, Aalberse RC, Scheynius A. Elevated levels of IgG and IgG4 to *Malassezia* allergens in atopic eczema patients with IgE reactivity to *Malassezia*. *Int Arch Allergy Immunol* 2004;135:93-100.
72. Bos JD. Atopiform dermatitis. *Br J Dermatol* 2002;147:426-9.
73. Hanifin JM. Atopiform dermatitis: do we need another confusing name for atopic dermatitis? *Br J Dermatol* 2002;147:430-2.
74. Novembre E, Cianferoni A, Lombardi E, Bernardini R, Pucci N, Vierucci A. Natural history of "intrinsic" atopic dermatitis. *Allergy* 2001;56:452-3.
75. Novak N, Bieber T. Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 2003;112:252-62.
76. Elias PM. Epidermal lipids, barrier function and desquamation. *J Invest Dermatol* 1983;80:44-9.
77. Lavker RM, Matoltsy AG. Formation of horny cells: the fate of organelles and differentiation products in ruminal epithelium. *J Cell Biol* 1970;44:501-12.
78. Menon GK, Feinfeld KR, Elias PM. The lamellar secretory response to barrier disruption. *J Invest Dermatol* 1992;98:279-89.
79. Lavker RL. Membrane coating granules: the fate of the discharged lamellae. *J Ultrastruct Res* 1976;55:79-86.
80. Rawlings AV. Trends in stratum corneum research and the management of dry skin conditions. *Int J Cosmetic Sci* 2003;25:63-95.
81. Fartasch M, Diepgen TL. The barrier function in atopic dry skin: disturbance of membrane-coating granule exocytosis and formation of epidermal lipids? *Acta Derm Venereol* 1992;176:26-31.
82. Mecheleidt O, Kaiser HW, Sanhoff K. Deficiency of epidermal protein-bound omega-hydroxyceramides in atopic dermatitis. *J Invest Dermatol* 2002;119:166-73.
83. Hara J, Higuchi K, Okamoto R, Kawashima Y, Imokawa G. High-expression of sphingomyelin deacylase is an important determinant of ceramide deficiency leading to barrier disruption in atopic dermatitis. *J Invest Dermatol* 2000;115:406-13.
84. Serre G, Mils V, Haftek M, Vincent C, Croute F, Réano A, et al. Identification of late differentiation antigens of human cornified epithelia, expressed in re-organized desmosomes and bound to cross-linked envelope. *J Invest Dermatol* 1991;97:1061-72.
85. Buxton RS, Cowin P, Franke WW, Garrod DR, Green KJ, King IA, et al. Nomenclature of the desmosomal cadherins. *J Cell Biol* 1993;121: 481-3.
86. Guerrin M, Simon M, Montezin M, Haftek M, Vincent C, Serre G. Expression cloning of human corneodesmosin proves its identity with the product of the S gene and allows improved characterization of its processing during keratinocyte differentiation. *J Biol Chem* 1998;273: 22640-7.
87. Lundström A, Serre G, Haftek M, Egelrud T. Evidence for a role of corneodesmosin, a protein which may serve to modify desmosomes during cornification, in stratum corneum cell cohesion and desquamation. *Arch Dermatol Res* 1994;286:369-75.
88. Haftek M, Serre G, Thivolet J. Immunohistochemical evidence for a possible role of cross-linked keratinocyte envelopes in stratum corneum cohesion. *J Histochem Cytochem* 1991;39:1531-8.
89. Lundström A, Egelrud T. Cell shedding from human plantar skin in vitro: evidence of its dependence on endogenous proteolysis. *J Invest Dermatol* 1988;91:340-3.
90. Egelrud T, Lundström A. The dependence of detergent-induced cell dissociation in non-palmo-plantar stratum corneum on endogenous proteolysis. *J Invest Dermatol* 1990;95:456-9.
91. Simon M, Bernard D, Minondo AM, Camus C, Fiat F, Corcuff P, et al. Persistence of both peripheral and non-peripheral corneodesmosomes in the upper stratum corneum of winter xerosis skin versus only peripheral in normal skin. *J Invest Dermatol* 2001;117:710-7.
92. Egelrud T. Purification and preliminary characterization of stratum corneum chymotryptic enzyme: a proteinase that may be involved in desquamation. *J Invest Dermatol* 1993;101:200-4.
93. Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, et al. Degradation of corneodesmosome protein by two serine proteases of the kallikrein family, SCTE/ KLK5/hK5 and SCCE/ KLK7/hK7. *J Invest Dermatol* 2004;122:1235-44.
94. Horikoshi T, Igarashi S, Uchiwa H, Brysk H, Brysk MM. Role of endogenous cathepsin D-like and chymotrypsin-like proteolysis in human epidermal desquamation. *Br J Dermatol* 1999;141:453-9.
95. Ekholm IE, Brattsand M, Egelrud T. Stratum corneum tryptic enzyme in normal epidermis: a missing link in the desquamation process? *J Invest Dermatol* 2000;114:56-63.
96. Watkinson A. Stratum corneum thiol protease (SCTP): a novel cysteine protease of late epidermal differentiation. *Arch Dermatol Res* 1999;291: 260-8.
97. Hansson L, Backman A, Ny A, Edlund M, Ekholm E, Ekstrand Hammarstrom B, et al. Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. *J Invest Dermatol* 2002;118:444-9.
98. Suzuki Y, Nomura J, Koyama J, Horii I. The role of proteases in stratum corneum: involvement in stratum corneum desquamation. *Arch Dermatol Res* 1994;286:369-75.
99. Egelrud T, Lundström A. A chymotrypsin-like proteinase that may be involved in desquamation in plantar stratum corneum. *Arch Dermatol* 1991;283:108-12.
100. Ekholm IE, Egelrud T. The expression of stratum corneum chymotryptic enzyme in human anagen hair follicles: further evidence for its involvement in desquamation-like process. *Br J Dermatol* 1998;139:585-90.
101. Sondell B, Thornell LE, Stigbrand T, Egelrud T. Immunolocalisation of stratum corneum chymotryptic enzyme in human skin and oral epithelium with monoclonal antibodies: evidence of a proteinase specifically expressed in keratinizing squamous epithelia. *J Histochem Cytochem* 1994;42:459-65.
102. Bernard D, Mehul B, Thomas-Collignon A, Simonetti L, Remy V, Bernard MA, et al. Analysis of proteins with caseinolytic activity in a

- human stratum corneum extract revealed a yet unidentified cysteine protease and identified the so-called "stratum corneum thiol protease" as cathepsin 12. *J Invest Dermatol* 2003;120:592-600.
103. Horikoshi T, Chen S-H, Rajaraman S, Brysk H, Brysk MM. Involvement of cathepsin D in the desquamation of human stratum corneum [abstract]. *J Invest Dermatol* 1998;110:547.
 104. Franke CW, Baici A, Bartel J, Christophers E, Wiedow O. Antileukoprotease inhibits stratum corneum chymotryptic enzyme. *J Biol Chem* 1996;271:21886-90.
 105. Taggart CC, Lowe GJ, Greene CM, Mulgrew AT, O'Neill SJ, Levine RL, et al. Cleave and inactivate secretory leukoprotease inhibitor. *J Biol Chem* 2001;276:33345-52.
 106. Molhuizen HO, Alkemade HA, Zeeuwen PL, de Jongh GJ, Wieringa B, Schalkwijk J. SKALP/elafin: an elastase inhibitor from cultured human keratinocytes. Purification, cDNA sequence, and evidence for transglutaminase cross-linking. *J Biol Chem* 1993;268:12028-32.
 107. Chavanas S, Bodemer C, Rochat A. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 2000;25:141-2.
 108. Zeeuwen PL, Van Vlijmen-Willems IM, Jensen BJ, Sotiropoulou G, Curfs JH, Meis JF, et al. Cystatin M/E expression is restricted to differentiated epidermal keratinocytes and sweat glands: a new skin-specific proteinase inhibitor that is a target for cross-linking by transglutaminase. *J Invest Dermatol* 2001;116:693-701.
 109. Murphy R, Williams HC, Duff GW, Cork MJ. Total and specific IgE and definitions of atopy. *Br J Dermatol* 1999;141(suppl):25.
 110. Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A, et al. SPINK5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat Genet* 2005;37:56-65.
 111. Yang T, Liang D, Koch PJ, Hohl D, Kheradman F, Overbeek PA. Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in SPINK5^{-/-} mice. *Genes Dev* 2004;18:2354-8.
 112. Koch PJ, Mahoney MG, Ishikawa H, Pulkkinen L, Uitto J, Shultz L, et al. Target disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. *J Cell Biol* 1997;137:1091-102.
 113. Chidgey M, Brakebusch C, Gustafsson E, Cruchley A, Hail C, Kirk S, et al. Mice lacking desmocollin 1 show epidermal fragility accompanied by barrier defects and abnormal differentiation. *J Cell Biol* 2001;155:821-32.
 114. Vasioukhin V, Bowers E, Bauer C, Degenstein L, Fuchs E. Desmoplakin is essential in epidermal sheet formation. *Nat Cell Biol* 2001;3:1076-85.
 115. Gallicano GI, Kouklis P, Bauer C, Yin M, Vasioukhin V, Degenstein L, et al. Desmoplakin is required early in development for assembly of desmosomes and cytoskeletal linkage. *J Cell Biol* 1998;143:2009-22.
 116. Deleuran M, Ellingsen AR, Paludan K, Schou C, Thestrup-Pedersen K. Purified Der p1 and p2 patch tests in patients with atopic dermatitis: evidence for both allergenicity and proteolytic irritancy. *Acta Derm Venereol* 1998;78:241-3.
 117. Elias PM, Matsuyoshi N, Wu H, Lin C, Wang ZH, Brown BE, et al. Desmoglein isoform distribution affects stratum corneum structure and function. *J Cell Biol* 2001;153:243-9.
 118. Merritt AJ, Berika MY, Zhai W, Kirk SE, Ji B, Hardman MJ, et al. Suprabasal desmoglein 3 expression in the epidermis of transgenic mice results in hyperproliferation and abnormal differentiation. *Mol Cell Biol* 2002;22:5846-58.
 119. Alkemade JA, Molhuizen HO, Ponc M, Kempenaar JA, Zeeuwen PL, de Jongh GJ, et al. SKALP/elafin is an inducible proteinase inhibitor in human epidermal keratinocytes. *J Cell Sci* 1994;107:2335-42.
 120. Wiedow O, Young JA, Davison MD, Christophers E. Antileukoprotease in psoriatic scales. *J Invest Dermatol* 1993;101:305-9.
 121. Tobin D, Foitzik K, Reinheckel T, Mecklenburg L, Botchkarev VA, Peters C, et al. The lysosomal protease cathepsin L is an important regulator of keratinocyte and melanocyte differentiation during hair follicle morphogenesis and cycling. *Am J Pathol* 2002;160:1807-21.
 122. Roth W, Deussing J, Botchkarev VA, Pauly-Evers M, Saftiq P, Hafner A, et al. Cathepsin L deficiency as molecular defect of furless: hyperproliferation of keratinocytes and perturbation of hair follicle cycling. *FASEB J* 2000;14:2075-86.
 123. Egberts F, Heinrich M, Jensen JM, Winoto-Morbach S, Pfeiffer S, Wickel M, et al. Cathepsin D is involved in the regulation of transglutaminase 1 and epidermal differentiation. *J Cell Sci* 2004;117:2295-307.
 124. Zeeuwen PL, van Vlijmen-Willems IM, Olthuis D, Johansen HT, Hitomi K, Hara-Nishimura I, et al. Evidence that unrestricted legumain activity is involved in disturbed epidermal cornification in cystatin M/E deficient mice. *Hum Mol Genet* 2004;13:1069-79.
 125. Bilenoglu O, Basak AN, Russell JE. A 3'UTR mutation affects beta-globin expression without altering the stability of its fully processed mRNA. *Br J Haematol* 2002;119:1106-14.
 126. Frittitta L, Ercolino T, Bozzali M, Argiolas A, Graci S, Santagati MG, et al. A cluster of three single nucleotide polymorphisms in the 3'-untranslated region of human glycoprotein PC-1 gene stabilizes PC-1 mRNA and is associated with increased PC-1 protein content and insulin resistance-related abnormalities. *Diabetes* 2001;50:1952-5.
 127. Di Paola R, Frittitta L, Miscio G, Bozzali M, Baratta R, Centra M, et al. A variation in 3'UTR of hPTP1B increases specific gene expression and associates with insulin resistance. *Am J Hum Genet* 2002;70:806-12.
 128. Sprecher E, Chavanas S, DiGiovanna JJ, Amin S, Nielsen K, Prendiville JS, et al. The spectrum of pathogenic mutations in SPINK5 in 19 families with Netherton syndrome: implications for mutation detection and first case of prenatal diagnosis. *J Invest Dermatol* 2001;117:179-87.
 129. Komatsu N, Takata M, Otsuki N, Ohka R, Amano O, Takehara K, et al. Elevated stratum corneum hydrolytic activity in Netherton syndrome suggests an inhibitory regulation of desquamation by SPINK 5-derived peptides. *J Invest Dermatol* 2002;118:436-43.
 130. Comel M. Ichthyosis linearis circumflexa. *Dermatologica* 1949;98:133-6.
 131. Kato A, Fukai K, Oiso N, Hosomi N, Murakami T, Ishii M. Association of SPINK5 gene polymorphisms with atopic dermatitis in the Japanese population. *Br J Dermatol* 2003;148:665-9.
 132. Nishio Y, Noguchi E, Shibasaki M, Kamioka M, Ichikawa E, Ichikawa K, et al. Association between polymorphisms in the SPINK5 gene and atopic dermatitis in the Japanese. *Genes Immun* 2003;4:515-7.
 133. Dubin G. Proteinaceous cysteine protease inhibitors. *Cell Mol Life Sci* 2005;62:653-69.
 134. Badertscher K, Bronnimann M, Karlen S, Braathen LR, Yawalkar N. Mast cell chymase is increased in atopic dermatitis but not in psoriasis. *Arch Dermatol Res* 2005;296:503-6.
 135. Tomimori Y, Tsuruoka N, Fukami H, Saito K, Horikawa C, Saito M, et al. Role of mast cell chymase in allergen-induced biphasic skin reaction. *Biochem Pharmacol* 2002;64:1187-93.
 136. Tomimori Y, Muto T, Fukami H, Saito K, Horikawa C, Tsuruoka N, et al. Chymase participates in chronic dermatitis by inducing eosinophil infiltration. *Lab Invest* 2002;82:789-94.
 137. Mao XQ, Shirakawa T, Enomoto T, Shimazu S, Dake Y, Kitano H, et al. Association between variants of mast cell chymase gene and serum IgE levels in eczema. *Hum Hered* 1998;48:38-41.
 138. Iwanaga T, McEuen A, Walls AF, Clough JB, Keith TP, Rorke S, et al. Polymorphism of the mast cell chymase gene (CMA1) promoter region: lack of association with asthma but association with serum total immunoglobulin E levels in adult atopic dermatitis. *Clin Exp Allergy* 2004;34:1037-42.
 139. Stewart GA, Thompson PJ. The biochemistry of common aeroallergens. *Clin Exp Allergy* 1996;26:1020-44.
 140. Yasueda H, Mita H, Akiyama K, Shida T, Ando T, Sugiyama S, et al. Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin Exp Allergy* 1993;23:384-90.
 141. Winton HL, Wan H, Cannell MB, Thompson PJ, Garrod DR, Stewart GA, et al. Class specific inhibition of house dust mite proteinases which cleave cell adhesion, induce cell death and which increase the permeability of lung epithelium. *Br J Pharmacol* 1998;124:1048-59.
 142. Storck H. Experimentelle Untersuchung zur Frage der Bedeutung von Mikroben in der Ekzemgenese. *Dermatologica Helvetica* 1948;96:177-262.
 143. Leyden J, Marples R, Klingman A. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* 1974;90:523-30.
 144. Leung DY, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* 1993;92:1374-80.

145. Miedzobrodzki J, Kaszycki P, Bialecka A, Kasprowicz A. Proteolytic activity of *Staphylococcus aureus* strains isolated from the colonized skin of patients with acute-phase atopic dermatitis. *Eur J Clin Microbiol Infect Dis* 2002;21:269-76.
146. Otto M. Virulence factors of the coagulase-negative staphylococci. *Front Biosci* 2004;9:841-63.
147. Schade H, Marchionini A. Der Säuremantel der Haut (nach Gaskettenmessung). *Klin Wochenschr* 1928;7:12-4.
148. Braun-Falco O, Korting HC. Der Normale pH—Wert der Haut. *Hautarzt* 1986;3:126-9.
149. Taddei A. Ricerche, mediante indicatori, sulla relazione attuale della cute nel neonato. *Riv Ital Ginecol* 1935;18:496-501.
150. Behrendt H, Green M. Skin pH pattern in the newborn infant. *AMA J Dis Child* 1958;95:35-41.
151. Visscher MO, Chatterjee R, Munson KA, Pickens WL, Hoath SB. Changes in diapered and non diapered infant skin over the first month of life. *Pediatr Dermatol* 2000;17:45-51.
152. Behne MJ, Barry NP, Hanson KM, Aronchik I, Clegg RW, Gratton E, et al. Neonatal development of the stratum corneum pH gradient: localisation and mechanisms leading to emergence of optimal barrier function. *J Invest Dermatol* 2003;120:998-1006.
153. Fluhr JW, Mao-Qiang M, Brown BE, Hachem JP, Moskowicz DG, Demerjian M, et al. Functional consequences of a neutral pH in neonatal rat stratum corneum. *J Invest Dermatol* 2004;123:140-51.
154. Fox C, Nelson D, Wareham J. The timing of skin acidification in very low birth weight infants. *J Perinatol* 1998;18:272-5.
155. Marchionini A, Hausknecht W. Säuremantel der haut und bakterienabwehr. *Säuremantel Haut Bakterienabwehr* 1938;17:663-6.
156. Puhvel SM, Reisner RM, Sakamoto M. Analysis of lipid composition of isolated human sebaceous gland homogenates after incubation with cutaneous bacteria: thin-layer chromatography. *J Invest Dermatol* 1975;64:406-11.
157. Ament W, Huizenga JR, Mook GA, Gips CH, Verkee GJ. Lactate and ammonia concentration in blood and sweat during incremental cycle ergometer exercise. *Int J Sport Med* 1997;18:35-9.
158. Fluhr JW, Elias PM. Stratum corneum pH: formation and function of the "acid mantle." *Exog Dermatol* 2002;1:163-75.
159. Rippke F, Schreiner V, Schwanz HJ. The acidic milieu of the horny layer: new findings on the physiology and pathophysiology of the skin pH. *Am J Clin Dermatol* 2002;3:261-72.
160. Behne MJ, Meyer JW, Hanson KM, Barry NP, Murata S, Crumrine D, et al. NHE1 regulates the stratum corneum permeability barrier homeostasis. Microenvironment acidification assessed with fluorescence lifetime imaging. *J Biol Chem* 2002;277:47399-406.
161. Rebell G, Pillsbury DM, de Saint Phalle M, Ginsburg D. Factors affecting the rapid disappearance of bacteria placed on the normal skin. *J Invest Dermatol* 1950;14:247-63.
162. Leyden JJ, Kligman AM. The role of microorganisms in diaper dermatitis. *Arch Dermatol* 1978;114:56-9.
163. Aly R, Maibach HI, Rahman R, Shinefield HR, Mandel AD. Correlation of human *in vivo* and *in vitro* cutaneous antimicrobial factors. *J Infect Dis* 1975;131:579-83.
164. Bibel DJ, Aly R, Lahti L, Shinefield HR, Maibach HI. Microbial adherence to vulvar epithelial cells. *J Med Microbiol* 1987;23:75-82.
165. Mauro T, Holleran WM, Grayson S, Gao WN, Man MQ, Kriehuber E, et al. Barrier recovery is impeded at neutral pH, independent of ionic effects: implications for extracellular lipid processing. *Arch Dermatol* 1998;290:215-22.
166. Fluhr JW, Kao J, Jain M, Ahn SK, Feingold KR, Elias PM. Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. *J Invest Dermatol* 2001;117:44-51.
167. Anderson DS. The acid-base balance of the skin. *Br J Dermatol* 1951;63:283-96.
168. Eberlein-Konig B, Schafer T, Huss-Marp J, Darsow U, Mohrenschlager M, Herbert O, et al. Skin surface pH, stratum corneum hydration, transepidermal water loss and skin roughness related to atopic eczema and skin dryness in a population of primary school children. *Acta Derm Venereol* 2000;80:188-91.
169. Locker G. Permeabilitätsprüfung der Haut Ekzemkranker und Hautgesunder für den neun Indikator Nitrazingelb "Geigy," Modifizierung der alkaliresistenzprobe, pH-verlauf in der Tiefe des stratum corneum. *Dermatologica* 1961;124:159-82.
170. Seidenari S, Giusti G. Objective assessment of the skin of children affected by atopic dermatitis: a study on pH, capacitance and TEWL in eczematous and clinically uninvolved skin. *Acta Derm Venereol* 1995;75:429-33.
171. Schmuth M, Man MQ, Weber F, Gao W, Feingold KR, Fritsch P, et al. Permeability barrier disorder in Nieman-Pick disease: sphingomyelin-ceramide processing required for normal barrier homeostasis. *J Invest Dermatol* 2000;115:459-66.
172. Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, et al. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. *J Invest Dermatol* 2004;83:761-73.
173. Elias PM. The epidermal permeability barrier: from the early days at Harvard to emerging concepts. *J Invest Dermatol* 2004;122:xxvix-ix.
174. Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM. pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J Invest Dermatol* 2003;121:345-53.
175. Uchida Y, Hara M, Nishio H, Sidransky E, Inoue S, Otsuka F, et al. Epidermal sphingomyelins are precursors for selected stratum corneum ceramides. *J Lipid Res* 2000;41:2071-82.
176. Holleran WM, Takagi Y, Menon GK, Legler G, Feingold KR, Elias PM. Processing of epidermal glycosylceramides is required for optimal mammalian cutaneous permeability barrier function. *J Clin Invest* 1993;91:1656-64.
177. Jensen JM, Schutze S, Forl M, Kronke M, Proksch E. Roles for tumour necrosis factor receptor p55 and sphingomyelinase in repairing the cutaneous permeability barrier. *J Clin Invest* 1999;104:1761-70.
178. Mucke H, Mohr K-T, Rummeler A, Wutzler P. Untersuchungen über den haut-pH-wert der hand nach anwendung von seife. *Reinigungs- und Händedesinfektionsmitteln. Pharmazie* 1993;48:468-9.
179. Hornby SJ, Ward SJ, Gilbert CE, Dandona L, Foster A, Jones RB. Environmental risk factors in congenital malformations of the eye. *Ann Trop Paediatr* 2002;22:67-77.
180. Kligman AM, Wooding WM. A method for the measurement and evaluation of irritants on human skin. *J Invest Dermatol* 1967;49:78-94.
181. Imokawa G. Comparative study on the mechanism of irritation by sulphate and phosphate type of anionic surfactants. *J Soc Cosmet Chem* 1980;31:45-66.
182. Froebe CL, Simion FA, Rhein LD, Cagan RH, Kligman A. Stratum corneum lipid removal by surfactants: relation to *in vivo* irritation. *Dermatologica* 1990;181:277-83.
183. Ananthapadmanabhan KP, Moore DJ, Subramanyan L, Misra M, Meyer F. Cleansing without compromise; the impact of cleansers on the skin barrier and the technology of mild cleansing. *Dermatol Ther* 2004;17(suppl 1):16-25.
184. Meding B, Swanbeck G. Prevalence of hand eczema in an industrial city. *Br J Dermatol* 1987;116:627-34.
185. Cowley NC, Farr PM. A dose-response study of irritant reactions to sodium lauryl sulphate in patients with seborrhoeic dermatitis and atopic eczema. *Acta Derm Venereol* 1992;72:432-5.
186. Kirk JF. Effect of handwashing on skin lipid removal. *Acta Derm Venereol* 1966;57:24-71.
187. Haapasaari KM, Risteli J, Koivukangas V, Oikarinen A. Comparison of the effect of hydrocortisone, hydrocortisone-17-butyrate and betamethasone on collagen synthesis in human skin *in vivo*. *Acta Derm Venereol* 1995;75:269-71.
188. Haapasaari KM, Risteli J, Karvonen J, Oikarinen A. Effect of hydrocortisone, methylprednisolone and mometasone furoate on collagen synthesis in human skin *in vivo*. *Skin Pharmacol* 1997;10:261-4.
189. Oikarinen A, Haapasaari KM, Sutinen M, Tasanen K. The molecular basis of glucocorticoid-induced skin atrophy: topical glucocorticoid apparently decreases both collagen synthesis and the corresponding collagen mRNA level in human skin *in vivo*. *Br J Dermatol* 1998;139:1106-10.
190. Sheu HM, Lee JYY, Chai CY, Kuo K. Depletion of stratum corneum intercellular lipid lamellae and barrier function abnormalities after long-term topical corticosteroids. *Br J Dermatol* 1997;136:884-90.
191. Kolbe L, Kligman AM, Schreiner V, Stoudemayer T. Corticosteroid-induced atrophy and barrier impairment measured by non-invasive methods in human skin. *Skin Res Technol* 2001;7:73-7.
192. Kao JS, Fluhr JW, Man MQ, Fowler AJ, Hachem JP, Crumrine D, et al. Short-term glucocorticoid treatment compromises both permeability barrier homeostasis and stratum corneum integrity: inhibition of

- epidermal lipid synthesis accounts for functional abnormalities. *J Invest Dermatol* 2003;120:456-64.
193. Frosch PJ, Wendt H. Human models for quantification of corticosteroid adverse effects. In: Maibach HI, Lowe NJ, editors. *Models in dermatology*. Volume 2. Basel: Karger; 1985. p. 5-15.
194. Sheu HM, Chang CH. Alterations in water content of the stratum corneum following long-term topical corticosteroids. *J Formos Med Assoc* 1991;90:664-9.
195. Garg A, Chren MM, Sands LP, Matsui MS, Marenus KD, Feingold KR, et al. Psychological stress perturbs epidermal permeability barrier homeostasis: implications for the pathogenesis of stress-associated skin disorders. *Arch Dermatol* 2001;137:53-9.
196. Sheu HM, Lee JY, Kuo KW, Tsai JC. Permeability barrier abnormality of hairless mouse epidermis after topical corticosteroid: characterization of stratum corneum lipids by ruthenium tetroxide staining and high-performance thin-layer chromatography. *J Dermatol* 1998;25:281-9.
197. Goulding NJ. The molecular complexity of glucocorticoid actions in inflammation—a four-ring circus. *Curr Opin Pharmacol* 2004;4:629-36.
198. Yousef GM, Scorilas A, Magklara A, Soosaipillai A, Diamandis EP. The KLK7 (PRSS6) gene, encoding for the stratum corneum chymotryptic enzyme is a new member of the human kallikrein gene family—genomic characterization, mapping, tissue expression and hormonal regulation. *Gene* 2000;254:119-28.
199. Cork MJ, Robinson D, Vasipoulos Y, Ferguson A, Moustafa M, Tazi-Ahmini R, et al. Interaction of topical corticosteroids and pimecrolimus with the skin barrier: implications for efficacy and safety of treatment for atopic dermatitis. *J Am Acad Dermatol* 2006;54(suppl S):AB3.
200. Zheng PS, Lavker RM, Lehmann P, Kligman AM. Morphologic investigations on the rebound phenomenon after corticosteroid-induced atrophy in human skin. *J Invest Dermatol* 1984;82:345-52.
201. Björnberg A. Erythema craquele provoked by corticosteroids on normal skin. *Acta Derm Venereol* 1982;62:147-51.
202. Nickoloff BJ, Naidu Y. Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. *J Am Acad Dermatol* 1994;30:535-46.
203. Esche C, de Benedetto A, Beck LA. Keratinocytes in atopic dermatitis: inflammatory signals. *Curr Allergy Asthma Rep* 2004;4:276-84.
204. Rapaport MJ, Lebwohl M. Corticosteroid addiction and withdrawal in the atopic: the red burning skin syndrome. *Clin Dermatol* 2003;21:201-14.
205. Holt PG. The role of genetic and environmental factors in the development of T-cell mediated allergic disease in early life. *Paediatr Respir Rev* 2004;5(suppl A):S27-30.
206. Cork MJ. The importance of skin barrier function. *J Dermatol Treat* 1997;8(suppl):S7-13.
207. Cork MJ, Timmins J, Holden C, Carr J, Berry V, Tazi-Ahmini, et al. An audit of adverse drug reactions to aqueous cream in children with atopic eczema. *Pharm J* 2003;271:747-8.
208. Gough L, Schultz O, Sewell HF, Shakib F. The cysteine protease activities of the major dust mite allergen Der p 1 selectively enhance the immunoglobulin E antibody response. *J Exp Med* 1999;190:1897-902.
209. Comoy EE, Pestel J, Duez C, Stewart GA, Vendeville C, Fournier C, et al. The house dust mite allergen, *Dermatophagoides pteronyssinus*, promotes type 2 responses by modulating the balance between IL-4 and IFN-gamma. *J Immunol* 1998;160:2456-62.
210. Furue M, Terao H, Rikihisa W, Urabe K, Kinukawa N, Nose Y, et al. Clinical dose and adverse effects of topical corticosteroids in daily management of atopic dermatitis. *Br J Dermatol* 2003;148:128-33.
211. Cork MJ. Treatment of atopic dermatitis from a skin barrier perspective. *J Invest Dermatol* 2005;125:611.
212. Cork MJ. Skin barrier damage: Cause or consequence of atopic dermatitis? *J Am Acad Dermatol* 2005;52(suppl 3):9.
213. Eichenfield LF, Lucky AW, Boguniewicz M, Langley RGB, Cherill R, Marshall K, et al. Safety and efficacy of pimecrolimus (ASM 981) cream 1% in the treatment of mild and moderate atopic dermatitis in children and adolescents. *J Am Acad Dermatol* 2002;46:495-503.
214. Kapp A, Papp K, Bingham A, Folster Holst R, Ortonne JP, Potter P, et al. Long-term management of atopic dermatitis in infants with topical pimecrolimus, a non-steroid, anti-inflammatory drug. *J Allergy Clin Immunol* 2002;110:277-84.
215. Reitamo S, Ortonne JP, Sand C, Cambazard F, Bieber T, Folster-Holst R, et al. A multicentre, randomized, double-blind, controlled study of long-term treatment with 0.1% tacrolimus ointment in adults with moderate to severe atopic dermatitis. *Br J Dermatol* 2005;152:1282-9.