ADF Perspectives

The skin: an indispensable barrier

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Abstract: The skin forms an effective barrier between the organism and the environment preventing invasion of pathogens and fending off chemical and physical assaults, as well as the unregulated loss of water and solutes. In this review we provide an overview of several components of the physical barrier, explaining how barrier function is regulated and altered in dermatoses. The physical barrier is mainly localized in the stratum corneum (SC) and consists of protein-enriched cells (corneocytes with cornified envelope and cytoskeletal elements, as well as corneodesmosomes) and lipid-enriched intercellular domains. The nucleated epidermis also contributes to the barrier through tight, gap and adherens junctions, as well as through desmosomes and cytoskeletal elements. During epidermal differentiation lipids are synthesized in the keratinocytes and extruded into the extracellular domains, where they form extracellular lipid-enriched layers. The cornified cell envelope, a tough protein/lipid polymer structure, resides below the cytoplasmic membrane on the exterior of the corneocytes. Ceramides A and B are covalently bound to cornified envelope proteins and form the backbone for the subsequent addition of free ceramides, free fatty acids and cholesterol in the SC. Filaggrin is cross-linked to the cornified envelope and aggregates keratin filaments into macrofibrils. Formation and maintenance of barrier function is influenced by cytokines, 3′,5′-cyclic adenosine monophosphate and calcium. Changes in epidermal differentiation and lipid composition lead to a disturbed skin barrier, which allows the entry of environmental allergens, immunological reaction and inflammation in atopic dermatitis. A disturbed skin barrier is important for the pathogenesis of contact dermatitis, ichthyosis, psoriasis and atopic dermatitis.

Key words: epidermal differentiation – lipids – skin barrier function – stratum corneum – tight junction

Introduction

The skin's most important function is to form an effective barrier between the 'inside' and the 'outside' of the organism (Fig. 1). The epidermis comprises the physical, the chemical/biochemical (antimicrobial, innate immunity) and the adaptive immunological barriers. The physical barrier consists mainly of the stratum corneum (SC), but the nucleated epidermis, in particular the cell–cell junctions and associated cytoskeletal proteins, provides other important components. The chemical/biochemical (antimicrobial, innate immunity) barrier consists of lipids, acids, hydrolytic enzymes, antimicrobial peptides and macrophages. The (adaptive) immunological barrier is composed of humoral and cellular constituents of the immune system.

Components of the physical epidermal barrier

Stratum corneum

The SC serves as the principal barrier against the percutaneous penetration of chemicals and microbes and is capable of withstanding mechanical forces (1). It is further involved in the regulation of water release from the organism and into the atmosphere, known as transepidermal water loss (TEWL). The SC forms a continuous sheet of protein-enriched cells (corneocytes) connected by corneodesmosomes and embedded in an intercellular matrix enriched in non-polar lipids and organized as lamellar lipid layers. The final steps in keratinocyte differentiation are associated with profound changes in their structure, resulting in their transformation into the flat and anucleated corneocytes of the SC, which are loaded with keratin filaments and surrounded by a cell envelope composed of cross-linked proteins (cornified envelope proteins) as well as a covalently bound lipid envelope. Extracellular non-polar lipids surround the corneocytes to form a hydrophobic matrix (Fig. 2).

Corneocytes

During the final stages of normal differentiation, keratins are aligned into highly ordered and condensed arrays through interactions with filaggrin, a matrix protein. The role of filaggrin in skin barrier homeostasis is only partially
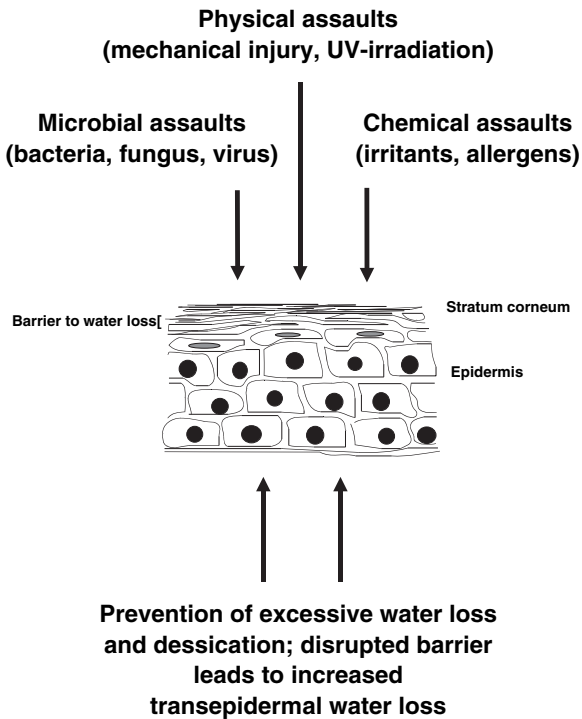
Figure 1. Functions of the epidermal 'inside-outside' and 'outside-inside' barrier.

known. Filaggrin aggregates the keratin filaments into tight bundles. This promotes the collapse of the cell into a flattened shape, which is characteristic of corneocytes in the cornified layer (2). Together, keratins and filaggrin constitute 80–90% of the protein mass of mammalian epidermis (3,4). The importance of filaggrin for epidermal barrier homeostasis is indicated in mouse models and human diseases with aberrant expression of this protein, ichthyosis vulgaris and atopic dermatitis (2,5,6). Overexpression of filaggrin in mice in the suprabasal epidermis resulted in a delay in barrier repair (7). Loss of normal profilaggrin and
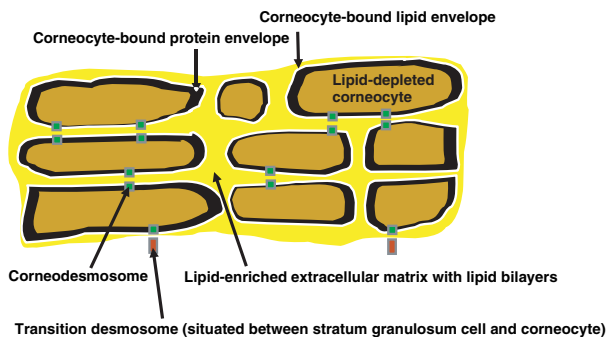

Figure 2. The lipid-depleted corneocyte is surrounded by an inner protein envelope and an outer lipid envelope. Special ceramides are covalently bound to cornified envelope proteins, in particular to involucrin. Desmosomes, terminally differentiated to corneodesmosomes, support the tightness of the stratum corneum.

filaggrin is found in flaky tail (ft/ft) mice, which characteristically display dry, flaky skin and annular tail and paw constrictions in the neonatal period (8).

The *cornified cell envelope* (CE) is a tough protein/lipid polymer structure formed just below the cytoplasmic membrane and residing on the exterior of the corneocytes (Fig. 2). It consists of two parts: a protein envelope and a lipid envelope. The protein envelope contributes to the biomechanical properties of the CE as a result of cross-linking of specialized cornified envelope structural proteins, including involucrin, loricrin, trichohyalin and to the class of small proline-rich proteins by both disulphide bonds and *N*-epsilon-(gamma-glutamyl)lysine isopeptide bonds formed by transglutaminases (4,9). The isopeptide bonds are resistant to most common proteolytic enzymes. Transglutaminase 1-deficient mice showed a defective SC and early neonatal death because of excessive water loss (10). Mutations in transglutaminase 1 in humans have been found to be the defect in lamellar ichthyosis as transglutaminase 1 affects several cornified envelope proteins and the attachment of covalently bound ceramides (11). Cathepsin D, an aspartate protease, is involved in the activation of transglutaminase 1 through cleaving the 150 kDa precursor of transglutiminase 1 and producing its active 35 kDa form. Consequently, cathepsin D-deficient mice exhibit reduced expression of involucrin, loricrin and filaggrin, and a defect in barrier function (12). Targeted ablation of the gene for the CE protein involucrin in mice resulted in no changes in skin barrier function under basal conditions but reduced barrier repair. Consequently, experimental permeability barrier disruption leads to the premature expression of involucrin (13,14). Loricrin-deficient mice did not show disturbed barrier function, but instead, a greater susceptibility to mechanical stress, which may secondarily alter skin barrier function (15,16). The variant form of Vohwinkel syndrome, mutilating keratoderma with ichthyosis, is caused by mutation in the gene for loricrin (17).

Desmosomes on corneocytes are called *(corneo)desmosomes*. Desmosomes, which connect the keratinocytes of the granular layer with the corneocytes of the SC, have been called transition desmosomes and are exclusively found at that location (18,19) (Fig. 2). Adjacently interconnected corneocytes are important for SC cohesion and are shed during the desquamation process in the SC. The corneocytes provide mechanical and chemical protection and, together with their intercellular lipid surroundings, confer water impermeability. Netherton syndrome, an autosomal recessive genodermatosis caused by mutations in SPINK5 and by encoding the serine protease inhibitor LEKTI (lympho-epithelial Kazal type inhibitor), is characterized by a congenital ichthyosiform erythroderma and bamboo hair. Often an atopic dermatitis-like skin disease with a disrupted permeability barrier is present. LEKTI deficiency causes abnormal

desmosome cleavage in the upper granular layer through degradation of desmoglein 1. This leads to defective SC adhesion and results in loss of skin barrier function (20).

The corneocyte-bound *lipid envelope* is a plasma membrane-like structure, which replaces the plasma membrane on the external aspect of mammalian corneocytes (21). Involucrin, envoplakin and periplakin serve as substrates for the covalent attachment of ω-hydroxyceramides with very long chain *N*-acyl fatty acids by ester linkage (22). These not only provide a coating to the cell but also interdigitate with the intercellular lipid lamellae (4).

Lipids

In the upper spinous and granular layers, the epidermal lamellar bodies, lamellar vesicles which originated from within the Golgi apparatus, enriched in polar lipids, glycosphingolipids, free sterols, phospholipids and catabolic enzymes and human ß-defensin 2 as well as other (structural) proteins, appear (23,24)(Fig. 3). In response to certain signals, for example, an increase in calcium concentration in the granular layers, the lamellar bodies move to the apex of the uppermost granular cells coordinated by a vesiculo-tubular system, fuse with the plasma membrane and secrete their content into the intercellular spaces through exocytosis. The lipids derived from the lamellar bodies are subsequently modified and arranged into intercellular lamellae positioned parallel to the cell surface. The covalently bound lipid envelope acts as a scaffold for this process. The polar lipids are enzymatically converted into non-polar products. Hydrolysis of glycosphingolipids generates ceramides, while phospholipids are converted into free fatty acids. The major lipid classes in the SC are ceramides, free fatty acids and cholesterol (24).

Ceramides are amide-linked fatty acids containing a long-chain amino alcohol called sphingoid base and account for 30 to 40% of SC lipids. Ceramides are generated by serine-palmitoyl transferase as rate-limiting enzyme and by hydrolysis of both glucosylceramide (by β-glucocerebrosidase)(25) and sphingomyelin (by acid sphingomyelinase) (26). The SC contains at least nine different free ceramides (27), two of which are ceramide A and ceramide B, covalently bound to cornified envelope proteins, most importantly to involucrin (28).

The epidermis contains *free fatty acids* as well as fatty acids bound in triglycerides, phospholipids, glycosylceramides and ceramides. The chain length of free fatty acids ranges from C_{12} to C_{24}. Saturated and monounsaturated fatty acids are synthesized in the epidermis, while others must be obtained from food and blood flow. Essential fatty acid deficiency syndrome (EFAD) caused by unusual human diets or malabsorption, or experimentally induced in rats and mice, is characterized by profound changes in epithelia including the epidermis (29). The skin is red and the epidermis is rough,

scaly and displays severely disturbed permeability barrier function. In addition, severe bacterial infection, impaired wound healing and alopecia may occur. It has been proposed that the linoleic acid metabolite γ-linoleic acid is of special importance for atopic eczema (27). Disruption of the gene coding for fatty acid transport protein 4 (Fatp4) resulted in disturbed epidermal barrier function and lethality of the mice immediately after birth (30).

Cholesterol is the third major lipid class in the SC. Although basal cells are capable of resorbing cholesterol from circulation, most cholesterol in the epidermis is synthesized *in situ* from acetate. Cholesterol levels for permeability barrier function are regulated by ATP-binding cassette subgroup 1 member 12 transporter (ABCA12), a membrane transporter responsible for cholesterol efflux, liver X receptor activators and peroxisome proliferator-activated receptors via ABCA12 (31). Epidermal cholesterol synthesis is increased during permeability barrier repair (32). Inhibition of hydroxymethylglutaryl CoA reductase, the rate-limiting enzyme, by topical application of the lipid lowering drug lovastatin resulted in disturbed barrier function and epidermal hyperproliferation (29). Lamellar ichthyosis type 2 (LI2) as well as the harlequin foetus type of congenital ichthyosis are linked to mutations in ABCA12 (33,34). Because of the abnormal lamellar granule formation that was found in the harlequin foetus type, it was suggested that ABCA12 may play a critical role in the formation of lamellar granules and the release of lipids into the intercellular spaces. This might also be true for LI2 and would explain the epidermal barrier defect seen in these disorders. X-linked recessive ichthyosis (XLRI), an enzymatic lysosomal deficiency of steroid sulphatase (cholesterol backbone) or arylsulphatase C, is a relatively mild non-congenital ichthyosis characterized by the generalized desquamation of large, adherent and dark brown scales (35,36). It has been shown that cholesterol sulphate inhibits proteases that are involved in desquamation of SC (37).

Nucleated epidermis

Although the SC is recognized as the most important physical barrier, the nucleated epidermal layers are also significant in barrier function. A low to moderate increase in TEWL occurs after removal of the SC by tape-stripping, whereas loss of the entire epidermis leads to a severe disturbance in barrier function. Loss of the SC and parts of the granular layers in staphylococcal scalded skin syndrome are not life-threatening (38). In contrast, suprabasal and subepidermal blistering diseases like pemphigus vulgaris, toxic epidermal necrolysis (Lyell syndrome) and severe burns (intra as well as subepidermal blistering) are life-threatening when large areas of the body are involved. Patients die because of extensive water loss or sepsis induced by external bacteria infection. Survival rates can be
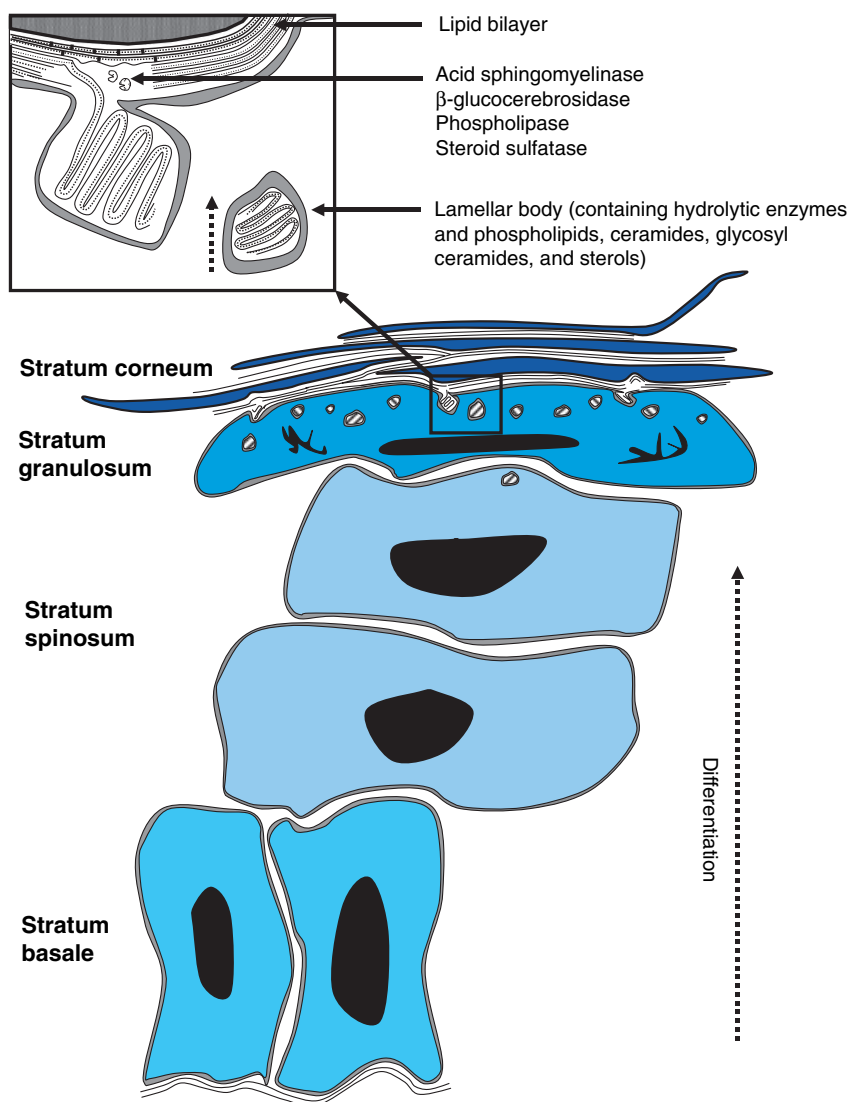
Figure 3. At the stratum granulosum/stratum corneum interface, the content of the lamellar bodies is extruded to the interface, thus forming continuous bilayers.

greatly improved with application of an artificial barrier in the form of a foil or a grease ointment. These clinical observations confirm the importance of the nucleated epidermal layers in skin barrier function by preventing both excessive water loss and the entry of harmful substances into the skin (39). After acute and chronic skin barrier disruption, an increase in DNA synthesis and increased proliferation rate of keratinocytes leading to epidermal hyperplasia occurred (40). Experimental barrier repair by occlusion partially prevented not only the increase in DNA and lipid synthesis but also in lipid synthesis (40–43).

Tight junctions
Tight junctions (TJ) are cell–cell junctions which connect neighbouring cells, control the paracellular pathway of molecules (barrier function) and separate the apical from the basolateral part of a cell membrane (fence function). In human epidermis, various TJ proteins have been identified, including occludin, claudins 1, 4, and 7, JAM-1 (junctional adhesion molecule-1), zonula occludens protein 1 and MUPP-1 (multi-PDZ protein-1) (44,45). In human skin, TJ proteins and/or discrete TJ-like structures are found in the interfollicular epidermis as well as in the skin appendages (46–50). In various diseases with perturbed SC barrier function, including psoriasis vulgaris, lichen planus and ichthyosis vulgaris, TJ proteins that were formerly restricted to the stratum granulosum and upper stratum spinosum such as occludin and claudin 4 were also found in deeper layers of the epidermis (45,47,51). Colonization of the skin by the non-pathogeneous *Staphylococcus epidermidis* also

results in an upregulation of TJ proteins (51). During epidermal (re)generation, the synthesis of TJ proteins precedes the formation of the SC (47). Therefore, TJ might be a rescue system when SC barrier is perturbed, challenged or absent. In addition, TJ proteins also appear to play a role in basic barrier function (52). This is suggested by the phenotype of several mouse models.

Claudin-1-deficient mice die within 1 day of birth because of tremendous water loss (53). The group of Tsukita could show very elegantly that these mice are characterized by a loss of TJ barrier function in the *stratum granulosum*. In humans, mutation in claudin-1 results in neonatal sclerosing cholangitis associated with ichthyosis (54). Epidermis specific E-cadherin knock-out mice also died perinatally because of excessive water loss. Absence of E-cadherin leads again to the absence of claudin-1 in the granular cell layer and the loss of TJ barrier function. In addition, an improper localization of other TJal proteins was observed (55). A loss of TJ barrier function was also observed in mice deficient for channel activating protease I, a serine protease. In these mice occludin is missing in the stratum granulosum. Again, these mice died soon after birth because of extremely high water loss (56). Altered barrier function of the skin has also been demonstrated in mice overexpressing claudin-6 in the epidermis (57). Therefore, as has already been shown for other cell types and tissues [for review see (58)], the composition of TJ, especially concerning claudins, seems to be very important for their barrier function in the epidermis. Downregulation as well as overexpression of certain proteins perturbs this barrier.

Adherens junctions and desmosomes

A perturbation in barrier function has also been found after the alteration of adherens junctions and desmosomes. Desmogleins are desmosomal cadherins that play major roles in stabilizing cell–cell adhesion in the living layers of the epidermis. Autoantibodies against these transmembrane glycoproteins cause blistering in pemphigus vulgaris because of the loss of keratinocyte adhesion. In transgenic mice, where the distribution of desmoglein 3 in epidermis was similar to that in mucous membrane, a highly increased TEWL resulted in lethality during the first week of life because of dehydration (59). E-cadherin is an important component of adherens junctions. In acute eczema, which shows disturbed skin barrier function, a reduction in keratinocyte membrane E-cadherin in areas of spongiosis has been found (60,61). Epidermal E-cadherin knock-out mice exhibit leaky TJ and impaired barrier functions [see above (56)].

Gap junctions

Connexins are transmembrane proteins that homo- or heteromerize on the plasma membrane to form connexons. Connexons on adjoining cells associate to form gap junctional channels and allow the passage of ions and small molecules between cells. Therefore, gap junctions are important for cell–cell communication. The involvement of connexins in barrier function of the epidermis is indicated by the phenotypes of several mouse models and by observations in patients with genetic disorders. Mice lacking the C-terminal region of connexin 43 – which is the most abundant connexin in the human epidermis – show a defective epidermal barrier and die within the first week of birth (62). These mice are characterized by an alteration of filaggrin processing among other features. Connexin 26 is almost absent in healthy epidermis but is one of the most highly upregulated genes in psoriatic plaques and is also found in other hyperproliferative conditions such as wound healing (63–65). Missense mutations in connexin 26 result in several distinct skin disorders, including the classic Vohwinkel syndrome (66). Homozygous and heterozygous patients with R143W mutation, which is associated with deafness but not ichthyosis-like phenotype, exhibit thicker epidermis and might have an advantage in malaria-contaminated areas (67). The theory of an advantage of this mutation – and therefore of malfunction of connexin 26 for barrier function – was recently supported by the finding that keratinocytes bearing this mutation are less susceptible to cellular invasion by the enteric pathogen *Shigella flexneri* (68). Consequently, mice that overexpress connexin 26 in the epidermis exhibit delayed epidermal barrier recovery (69).

Keratins

Keratins are major structural proteins synthesized in keratinocytes. They assemble into a web-like pattern of intermediate filaments that emanate from a perinuclear ring extend throughout the cytoplasm and terminate at desmosomes and hemidesmosomes. In keratin disorders, the filament networks collapse around the nucleus and interaction between neighbouring cells is therefore altered. Acute (tape-stripping) or chronic (EFAD diet) experimental barrier disruption leads to increased expression of the basal keratins K5 and K14, reduction of the differentiation-related keratins K1 and K10 and induction of proliferation-associated keratins K6 and K16, as well as inflammation-associated K17 (13). Homozygous neonatal K10-deficient mice exhibited an extremely delicate epidermis and died a few hours after birth. Heterozygous littermates showed normal skin at birth but developed increasing hyperkeratosis as they matured (70). Barrier repair in heterozygous K10-deficient mice was delayed and skin hydration was impaired (71). In humans, genetic defects in the suprabasal keratins result in hyperkeratosis and a mild barrier defect, epidermolytic hyperkeratosis has spinous layer K1 or K10 defects, while epidermolytic palmoplantar keratoderma has granular layer K9 defects (in palmar and plantar skin)

and ichthyosis bullosa of Siemens has granular layer K2 (formerly K2e) defects (reviewed in 3).

Intracellular and extracellular signalling and barrier function

Cytokines

Interleukin (IL-1), tumor necrosis factor (TNF) and IL-6 are potent mitogens and stimulators of lipid synthesis. After acute permeability barrier disruption, an increase in the epidermal expression of TNF, IL-1 and IL-6 occurs (26,43,76). In TNF receptor 1-, IL-1 receptor 1-, and in IL-6-deficient mice, a delay in permeability barrier formation occurs (26,76). In TNF receptor 1-deficient mice, the generation of lipids for skin barrier repair was delayed and the activity of acid sphingomyelinase which generates ceramides for skin barrier repair was reduced (26). The acute increase in TNF, IL-1 and IL-6 after barrier disruption seems to be crucial for skin barrier repair. However, if barrier disruption is prolonged and a chronic increase in cytokine production occurs, it could have a harmful effect leading to inflammation and epidermal proliferation. The occurrence of disrupted permeability barrier, epidermal hyperproliferation and inflammation is well known in several diseases like irritant and allergic contact dermatitis, atopic dermatitis and psoriasis and could aggravate the disease.

Calcium

A perturbed barrier recovers normally when exposed to an isotonic, hyper- or hypotonic external solution instead of air. If the solution contains both calcium and potassium barrier recovery is inhibited. Subsequent studies have shown that there is a calcium gradient in the epidermis (77,78). The highest calcium concentrations are found in the granular layers, while calcium concentration in the SC is very low because the relatively dry SC, with its extracellular lipid content, is not able to dissolve the ions. After disruption of the permeability barrier, an influx of water into the SC occurs and the calcium ion gradient is lost (Fig. 4). This depletion of calcium regulates lamellar body exocytosis (77,79,80). Calcium is a very important regulator of protein synthesis in the epidermis, including regulation of transglutaminase 1 activity (78). Furthermore, extracellular calcium ions are important for cell–cell adhesion and epidermal differentiation.

Disturbed regulation of calcium metabolism and increased TEWL (81) is observed in Darier's and Hailey–Hailey diseases, which are both characterized by loss of adhesion between suprabasal epidermal cells. The gene for Darier's disease (ATP2A2) encodes a calcium transport ATPase of the sarco-endoplasmic reticulum (82,83), while the gene for Hailey–Hailey (ATP2C1) codes for a secretory
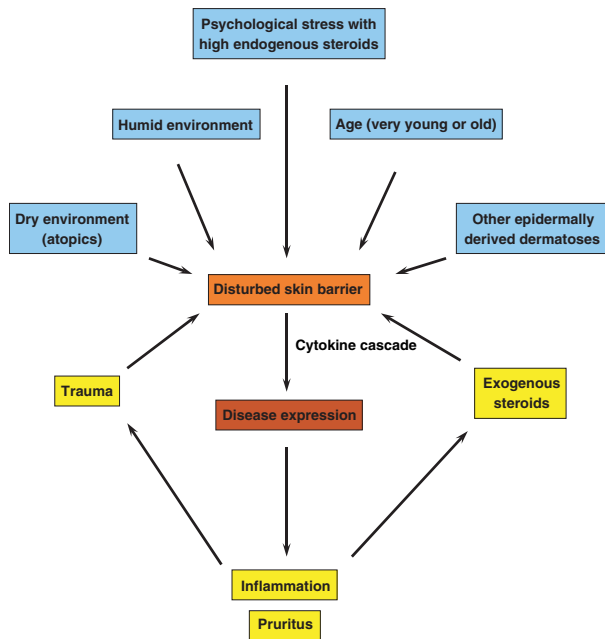

Figure 4. Changes in calcium gradient after barrier disruption regulates lamellar body secretion and epidermal differentiation.

pathway for calcium and manganese transport ATPase of the Golgi apparatus (SPCA1) (84,85).

Several publications have focused on the influence of pH on the permeability barrier (86). We think that the ion concentration in specific cell compartments and the amount of free fatty acids on the cell surface, which is influenced by bacterial colonization, are most important.

3′,5′-cyclic adenosine monophosphate

An increase of intracellular 3′,5′-cyclic adenosine monophosphate (cAMP) in epidermal keratinocytes delays barrier recovery, while cAMP antagonists accelerate barrier recovery. Activation of dopamine 2-like receptors, melatonin receptors or serotonin receptors (type 5-hydroxytryptamine 1) decreases intracellular cAMP and consequently accelerates barrier recovery, while activation of adrenergic ß2 receptors increases intracellular cAMP and delays barrier repair. Many agonists or antagonists of the mentioned neurotransmitter receptors are used clinically to treat nervous system disorders. Some of them may also be effective for treating skin diseases (87).

Determination of skin barrier function

The skin barrier protects against extensive water loss in one direction (inside-outside barrier) and against the invasion of harmful substances from the environment (outside-inside barrier). Many dermatologists and researchers believe that TEWL is the barrier. However, the TEWL

as determined by the Tewameter® (Courage + Khazaka, Cologne, Germany), Evaporimeter® (EPI Servomed, Stockholm, Sweden), the Meeco® Water Analyzer (Meeco, Warrington, PA, USA) or similar devices is a marker for the inside-outside barrier only. The inside-outside barrier often correlates with the outside-inside barrier, but in several cases this correlation does not exist. A severely disturbed inside-outside barrier (highly significant increased TEWL) resulting in perinatal death because of excessive fluid loss has been shown for example in E-cadherin-deficient mice. However, the outside-inside barrier was normal (55). The TEWL may not even be a reliable marker for the inside-outside barrier under certain circumstances. Topical treatment with corticosteroids reduces the enhanced TEWL in atopic dermatitis without a repair in the skin barrier as shown by electron microscopy studies (88). The decrease in TEWL may be explained in part by the decrease of blood flow caused by blood vessel constriction after corticosteroid treatment (89). Endo et al. (90) very recently showed that the TEWL depends on the barrier component plus a driving force component. Part of the driving force component is the blood flow as determined by laser-Doppler flowmetry.

Electron microscopy studies using ruthenium- and osmium tetroxid fixation which represent skin barrier morphology are a marker for the inside-outside as well as the outside-inside barrier. Electron microscopy studies are still the 'gold standard' for the determination of skin barrier. With this method, the lipid bilayers of the SC as well as the epidermal lamellar bodies and lipid extrusion during the transition from the stratum granulosum to the SC can be visualized (91).

For determination of outside-inside skin barrier function penetration studies must be performed. For example, it has been shown that the penetration of polyethylene glycol or sodium laurylsulfate is increased in atopic dermatitis (92). Measurements of skin penetration are often difficult to perform because the compound which penetrated into the skin must be detected by chemical analysis in tape-stripped material, biopsies, through radioactive labelling, which can no longer be performed in humans, or by the penetration of dyes which can be traced with devices like the Chromameter® (Minolta, Osaka, Japan) (93).

Skin barrier function and SC hydration

An inverse relationship between TEWL and SC hydration is well known. High TEWL values, as a marker of disturbed skin barrier function, are frequently correlated with low hydration of the SC. This was shown in experimental settings after skin cleansing with soaps and detergents (72) or in diseased skin, e.g. lesional atopic dermatitis (73). In atopic dermatitis the skin barrier defect is also shown by elec-

tron microscopy and penetration studies (92,94). Also, it is well known that moisturizers often improve skin barrier function (74). The mechanisms of this interplay have not been studied systematically. We and others think that disturbed skin barrier function leads to changes in epidermal differentiation, in particular filaggrin expression, which in turn, influences water-binding filaggrin break-down products (14,73,75).

Skin barrier function in dermatoses

The potential role of the cutaneous barrier in the pathophysiology of skin disorders can be divided into three subgroups (I). Barrier abnormality represents a primary or intrinsic process. This is found in irritant contact dermatitis, allergic contact dermatitis, burns, ulcers (ischaemic, vascular, diabetic), bullous disorders by friction or keratin abnormalities, premature infant's skin and ichthyosis (II). In a second subgroup, immunological abnormality triggers barrier abnormality. This is found in T-cell lymphoma (Mycosis fungoides) and autoimmune bullous diseases (III). In the third group of diseases, a primary barrier abnormality triggers immunological reactions, but in other, yet unknown subgroups, primary immunological reactions may trigger barrier abnormalities. This third compound subgroup integrates elements of the two others. Barrier disruption and immunological mechanisms may reciprocally play enhancing roles in initiating and sustaining skin lesions. If the causative mechanisms were known, the diseases from this subgroup could be assigned to one of the remaining groups. This might be the case in atopic dermatitis and psoriasis.

A mild impairment of the skin barrier is found in monogenetic diseases expressing an impaired epidermal differentiation or lipid composition without inflammation, for example, ichthyosis vulgaris (filaggrin mutations) (2,5) and XLRI (steroid sulphatase deficiency) (36). The diseases with more pronounced barrier disruption are inflammatory diseases, such as irritant and allergic contact dermatitis, atopic dermatitis and psoriasis. Also, blistering diseases, most of them inflammatory-related, show an increase in TEWL especially after the loosening of the blister roof and the development of erosions.

Most inflammatory skin lesions are covered with dry scales or scale-like crusts because of disturbed epidermal differentiation and a SC with poor water-holding capacity. Inflammatory skin diseases can be produced by either exogenous or endogenous causes. In contact dermatitis, disruption of the barrier by irritants and allergens is the primary event, followed by sensitization, inflammation, increased epidermal proliferation and changes in differentiation. In T-cell lymphoma (mycosis fungoides), an endogenous cause for barrier disruption, changes in epidermal

proliferation and differentiation by expansion of clonal malignant CD4+ T-cells is obvious (95).

In atopic dermatitis and in psoriasis, it is debatable whether permeability barrier disruption is followed by inflammation or whether inflammation leads to epidermal changes including barrier dysfunction. The vast majority of reports on the pathogenesis of atopic dermatitis and even more so regarding psoriasis, focused on the primary role of abnormalities in the immune system [reviewed in (96) and (97)]. However, others have proposed an 'outside-inside' pathogenesis for atopic dermatitis and other inflammatory dermatoses with barrier abnormalities (98,99). The theory of outside-inside pathogenesis is also supported by mouse models which exhibit a psoriasis-like phenotype because of alterations in keratinocytes. Inducible downregulation of JunB and c-*Jun* in keratinocytes of adult mice results in a psoriasis-like skin phenotye and arthritic lesions. The skin phenotype was also found but to a weaker extent in mice without functional T cells (100). Also mice with a knock-down of IKK2 in epidermal keratinocytes showed a psoria-sis-like skin phenotype which was independent of T cells (101).

The existence of defective permeability barrier function in *atopic dermatitis* is now widely accepted. Genetically impaired skin barrier function is already present in non-lesional skin and is more pronounced in lesional skin of atopic dermatitis. Increased epidermal proliferation and disturbed differentiation including changes in keratins and cornified envelope proteins involucrin, loricrin and filaggrin and in lipid content and composition cause impaired barrier function (73,102). Genome-wide linkage scans have identified a locus linked to the epidermal differentiation complex on chromosomes 1q21 in atopic dermatitis (103). Recently, loss-of-function genetic variants in the gene encoding filaggrin with very high significance have been shown to be strong predisposing factors for atopic dermatitis and have been found in about 20% of all atopic dermatitis patients and in about 50% of patients with severe disease (2,6,102). Defective permeability barrier function enables penetration of environmental allergens into the skin and initiates immunological reactions and inflammation. A polymorphism in the SPINK 5 gene, another barrier-related gene, has been found in atopic dermatitis (104). However, this could not be confirmed in studies made by our department (105).

In *psoriasis*, the level of TEWL is directly related to the clinical severity of the lesion; high TEWL is observed in acute exanthematous psoriasis, while a moderate increase in TEWL is seen with chronic plaques (thickening of the SC). In non-lesional skin in psoriasis TEWL is normal. Abnormalities in the SC intercellular lipids, especially a significant reduction in ceramide 1, have been found in lesional skin (106). Electron microscopy studies disclosed severe structural alteration of the intercellular lipid lamellae
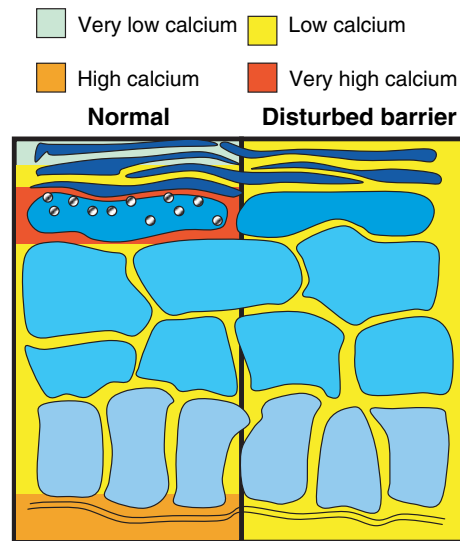

Figure 5. Endogenous and exogenous insults lead to a disturbance in skin barrier function, thus inducing inflammatory skin diseases.

(107). As in atopic dermatitis, a genetic linkage of psoriasis to the epidermal differentiation complex 1q21 has been found alongside other markers (103). However, mutations with a very high significance comparable with the filaggrin mutations in atopic dermatitis have not been found yet (103).

Conclusion

The skin barrier is a very complex system formed by a tremendous number of inter-related components. The modification of any of these components results in altered barrier function, which culminates in mild to lethal phenotype. Therefore, altered barrier function is a central event in various skin alterations and diseases (Fig. 5).

Acknowledgement

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