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The stratum corneum: structure and function in health and disease

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ABSTRACT: Our understanding of the formation, structure, composition, and maturation of the stratum corneum (SC) has progressed enormously over the past 30 years. Today, there is a growing realization that this structure, while faithfully providing a truly magnificent barrier to water loss, is a unique, intricate biosensor that responds to environmental challenges and surface trauma by initiating a series of biologic processes which rapidly seek to repair the damage and restore barrier homeostasis. The detailed ultrastructural, biochemical, and molecular dissection of the classic "bricks and mortar" model of the SC has provided insights into the basis of dry, scaly skin disorders that range from the cosmetic problems of winter xerosis to severe conditions such as psoriasis. With this knowledge comes the promise of increasingly functional topical therapies.

KEYWORDS: ceramides, corneodesmosome, cornified cell envelope, filaggrin, intercellular lipids.

Introduction

The skin serves as a primary defense, a sensory and excretory organ, and a critical regulator of body temperature. Its barrier properties extend to protection from ultraviolet (UV) radiation, oxidants, microorganisms, and toxic agents. However, in its most widely appreciated context, the critical skin barrier function refers to the epidermal barrier to water loss. This permeability barrier resides within the stratum corneum (SC), the wafer-thin, most superficial layer of the skin that is the true interface with the environment and a prerequisite for terrestrial life itself. A highly specialized structure, the SC is essentially impermeable to water except for a small but vital flux that serves to maintain its hydration, and thereby, its flexibility. Hydration of the surface layers is also critical to facilitate desquamation, the process of skin shedding at the skin surface.

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Stratum corneum structure

Until the mid-1970s, the SC was considered to be a metabolically inactive, homogeneous tissue, analogous to a plastic film (1). In the ensuing 30 years, scientists have shown that this tissue is structurally and biochemically diverse, and can no longer be regarded as inert. As the present author shall describe, the tissue possesses a limited form of metabolic activity and, in fact, acts as a unique, sophisticated biosensor that signals the underlying epidermis to respond to external stresses.

At the simplest level, the SC has been likened to a brick wall in which the non-continuous, essentially proteinaceous, terminally differentiated keratinocytes, or corneocytes (bricks), are embedded in the continuous matrix of specialized lipids (mortar) (2). These lipids provide the essential element of the water barrier, and the corneocytes protect against the continuous abrasion by chemical and physical injury. The structure of the stratum corneum is shown schematically in Fig. 1.

The lipid matrix constitutes approximately 20% of the SC volume (about 15% of the dry weight) and is the continuous phase of the skin barrier (3,4). The lamellar bilayer organization of this lipid matrix was first observed clearly using electron microscopy to examine ruthenium tetroxide-fixed

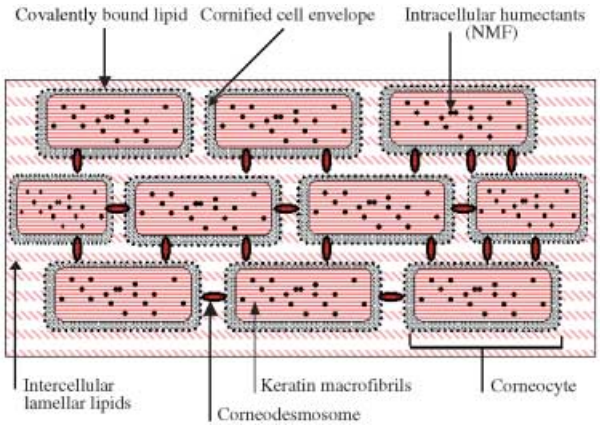

Fig. 1. Schematic "bricks and mortar" representation of the structural and functional components of the stratum corneum.

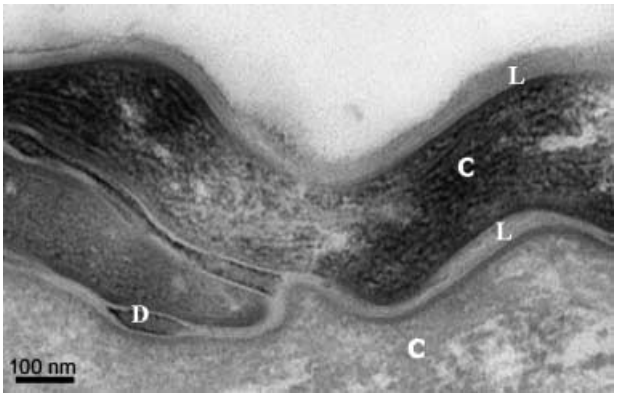

Fig. 2. Normal human stratum corneum after ruthenium tetroxide fixation showing domains of lipids (L) between differentially stained corneocytes (C). A corneodesmosome (D) is seen between adjacent corneocytes (bar = 100 nm). Adapted from Madison KC, Swartzendruber DC, Wertz PW, Downing DT. Presence of intact intercellular lipid lamellae in the upper layers of the stratum corneum. J Invest Dermatol 1987: 88: 714–718, courtesy of Blackwell Publishing, Inc.

samples (5) (Fig. 2). The SC lipid bilayers are unique among biological membranes in terms of composition, organization, and physical properties. The major lipid species of the SC are ceramides (about 50% by mass), fatty acids (10–20% by mass), and cholesterol (25% by mass) (3,4,6). Small amounts of cholesterol esters and cholesterol sulfate seem to play a critical role in normal barrier function (7). Ceramide with omega-hydroxy fatty acid (O) ester-linked [E] to linoleic acid and amide-linked to sphingosine (S) [Cer(EOS)] predominates in the SC and is highly enriched in linoleic acid, which constitutes a minimum of 20–30% of the omega-esterified fatty acid (8).

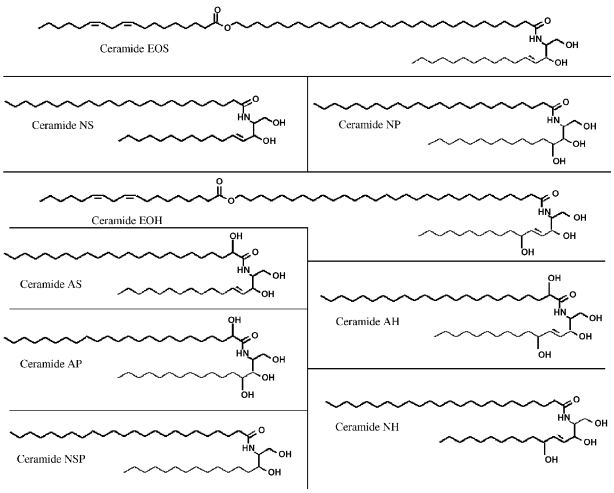

Fig. 3. Ceramide nomenclature and structures. Adapted from Robson KJ, Stewart ME, Michelson S, Lazo ND, Downing DT. 6-hydroxy-4-sphingenine in human epidermal ceramides. J Lipid Res 1994: 35: 2060–2068.

The epidermis must have linoleic acid in order to maintain barrier function. Its absence leads directly to the dramatically perturbed barrier found in animals with essential fatty acid deficiency (9,10).

In contrast to the classic cellular membranes of the epidermis, there are no phospholipids in healthy SC (4). Ceramides and fatty acids are characterized by extreme heterogeneity. Currently, nine classes of ceramides are recognized (11). Their chemical structures are shown in Fig. 3, where they are characterized essentially according to the work of Downing and colleagues (12). The main fatty acids (primarily saturated) range in chain length from C_{14} to C_{36} with the longer chain lengths (C_{20} to C_{36}) predominant (13). The very long carbon chain lengths of the SC ceramides and free fatty acids are believed to be the primary determinant of the unusual (for a biological membrane) physical properties of the SC lipid bilayers. Norlen and colleagues (13) have speculated that the existence of such a heterogeneous free fatty acid lipid subpopulation provides a broad transition temperature zone comparable to skin surface temperature, thereby maintaining skin barrier properties (e.g., transepidermal water loss). It is now recognized that triglycerides, short-chain saturated fatty acids, and unsaturated fatty acids, which are often included in a representation of SC lipid composition, in fact represent sebaceous contaminants and are hypothesized to play no significant role in barrier function (13). Rather, their presence may serve to disrupt barrier organization close to the skin surface and facilitate desquamation (11,14).

The majority of SC lipids are derived from the contents of the lamellar bodies formed in the keratinocytes of the stratum spinosum and stratum granulosum, the uppermost layers of the viable epidermis. At the interface between the stratum granulosum and the SC, the extruded phospholipids, sphingolipids, and plasma membrane constituents are enzymatically cleaved as they enter the SC to generate free fatty acids and ceramides (16). These components then fuse together to form the continuous lamellar bilayers characteristic of the SC. It is estimated that the skin must synthesize approximately 100–150 mg of lipid per day to replace the amount lost in normal desquamation. Therefore, the skin is one of the most active sites of lipid synthesis in the body (17,18).

Corneocytes have a mean thickness of around 1 μm and a mean surface area of approximately 1000 μm^2, but ultimately, the surface area is dependent upon age, anatomical location, and conditions that influence epidermal proliferation such as UV irradiation (19). Corneocyte size increases considerably with age, reflecting the increased transit time within the SC. On most body sites, the SC consists typically of 12–16 layers of flattened corneocytes (20).

Each individual corneocyte can be viewed simplistically as an insoluble protein complex consisting primarily of a highly organized keratin macrofibrillar matrix. Within the SC, the keratin—which can bind substantial amounts of water—is stabilized through both interkeratin and intrakeratin filament disulfide bonds, and encapsulated within a protein shell called the cornified cell envelope (CE). The CE itself is a 15–20-nm-thick structure (21) comprising a 15-nm-thick layer of defined structural proteins and a 5-nm-thick layer of specialized lipids (22,23). This lipid monolayer, characterized by long-chain ceramides covalently bound to the outer aspect of the CE, provides a hydrophobic interface between the hydrophilic surface of the CE itself and the highly hydrophobic lipid lamellae. The layer, by associating with the intercellular lipids, helps maintain water barrier function. The CE has been studied extensively and is now recognized as consisting primarily of the proteins loricrin, small proline-rich proteins, and involucrin (24,25). Compositional changes in this structure may indeed reflect an adaptation to external mechanical forces (26). The proteins are cross-linked together by N^{ε}(γ-glutamyl) lysine isopeptide bonds (27) formed by the action of the transglutaminase family of enzymes. Other protein cross-links, such as N^1,N^8-bis(γ-glutamyl) spermidine, and disulfide bonds contribute to the overall integrity of this structure. Because of its extensive protein cross-linking, the CE is the most insoluble structure of the corneocyte. Results of detailed investigations into the protein organization within the corneocyte suggest that elements of the internal keratin matrix become cross-linked to the interior aspect of the CE through both disulfide linkages and the action of transglutaminase (28). In this manner, the corneocyte structure itself can be regarded, in essence, as a single, intricately cross-linked "macro-protein" that imparts great strength to each individual corneocyte and the tissue as a whole.

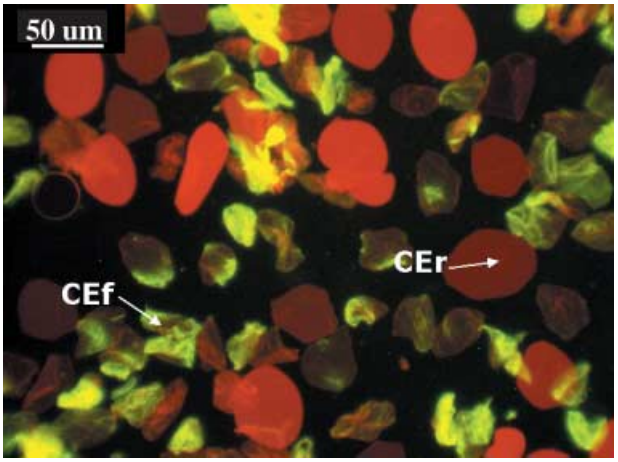

Fig. 4. Preparation of cornified envelopes from human stratum corneum. Rigid cornified envelopes (CEr) recognized by their flatter shape and strong staining with Nile Red are readily distinguishable from the fragile cornified envelopes (CEf) whose green staining with anti-involucrin reflects incomplete maturation of the structure (bar = 50 μm). Adapted from Harding CR, Long S, Richardson J, et al. The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. Int J Cosmet Sci 2003:25: 157–167, courtesy of Blackwell Publishing, Inc.

The results of recent research on the nature of the corneocyte surface points to further heterogeneity, reflecting gradual modification of the CE protein structure as mediated by transglutaminase (29). The identification of two distinct so-called "fragile" and "rigid" CE populations suggests subtle modification of the interaction of the lipid lamellae with the corneocyte structure (29,30). The heterogeneous nature of the corneocytes making up the SC can be visualized by the use of specific probes (29,30) (Fig. 4).

Differences in the nature of the intercellular lamellae and/or the corneocytes (the major components of the bricks and mortar model) provide the structural basis for the wide variations in

permeability observed on different body sites (e.g., the face versus the legs versus the palms) (31,32).

The overall integrity of the SC itself is achieved primarily through large numbers of specialized intercellular protein structures called corneodesmosomes (33), which effectively rivet neighboring corneocytes together both in the plane of the SC layer and in adjacent layers. Consistent with the "mortar" analogy, there is good evidence to indicate that lipids also contribute to the intercellular cement (34,35). Ultimately, however, it is the corneodesmosomal structures that represent the primary cohesive force and which must be degraded to facilitate desquamation. These structures are composed of certain proteins—such as desmocollin-1 and desmoglein-1 (36,37)—that are common to the desmosomal structures in the viable epidermis. Additional specialized proteins, in particular corneodesmosin (38,39), play a critical role in cohesion/dyshesion within the corneodesmosomal structure.

The exfoliative process is complex, and must be carefully controlled to maintain tissue integrity and thickness (40). Desquamation is facilitated by the action of several hydrolytic enzymes that degrade the corneodesmosomal structures in a specific pattern (41,42). Several serine (43,44), cysteine (45,46), and aspartic proteases (47,48) are involved in this process, and at least one of these enzyme classes appears to be especially adapted to function in the low-water-activity environment close to the skin surface (49). However, despite considerable progress, the precise spectrum of proteases involved and the coordinated manner in which they become activated [either through partial proteolysis (50) or through dissociation from inhibitors (51)] are still poorly understood. Although, ultimately, water and pH control the activity of these proteases because these are localized extracellularly within the lipid bilayers, it is the changing phase behavior of the intercellular lipid structure close to the skin surface that may exert fine control on their activity and on the degradative process (11).

An essential mechanism that maintains water balance within the SC, and thus, ensures flexibility and continued activity of the hydrolytic enzymes just described, is the so-called natural moisturizing factor (NMF). The term, first coined by Jacobi in 1959 (52), describes a complex "soup" of low-molecular-weight, water-soluble compounds. As will be described later (see pages 43–48 of this supplement), the NMF generated within the corneocytes is primarily derived from the complete hydrolysis of an unusual protein called filaggrin (53). In considering the importance of the NMF, it should not be forgotten that the highly structured intercellular lipid lamellae, as well as restricting water movement through the SC, also effectively prevents these highly water-soluble compounds from leaching out of the corneocytes in the surface layers of the skin.

Stratum corneum: biosensor

As described, the SC is enzymatically very active: progressively hydrolysing the corneodesmosomal linkages, cross-linking CE proteins through transglutaminase, processing lipids, and rapidly hydrolysing the protein filaggrin. In fact, the proteolysis of filaggrin, a process critical to maintaining the hydration and flexibility of this tissue, is itself initiated by changes in the water activity within the SC. The demonstration by Scott and Harding (54) that this dramatic hydrolysis is initiated within the SC in response to changes in environmental humidity was one of the earliest insights into the dynamic, responsive nature of this tissue.

In recent years, several research groups, most notably the group led by Elias and Feingold (55–57), have conducted many studies to show that water loss through this tissue is a critical homeostatic signaling mechanism. Perturbation in the barrier leading to altered water flux sets in motion a cascade of events within the underlying epidermis to promote barrier repair and recovery. The results of various studies have suggested that specific ions, particularly Ca^{2+}, are critically involved in this process (58–60). The SC also contains unusually high levels of certain cytokines, most notably interleukin 1-alpha and its receptor antagonist protein, and these molecules play a role in signaling to and within the epidermis (61–63).

Perturbation to barrier function

A multitude of factors, including disease, diet, race, and of course, the external environment, may render the barrier more prone to perturbation and potentially induce dryness, irritation, or itch. There is also an age-related decline in the ability to restore an impaired barrier (64), and psychological stress leading to elevated levels of circulating glucocorticoids has been shown to delay barrier recovery (65). Furthermore, there is a seasonal variation in intercellular lipid levels within the SC (66) that helps to explain the predisposition of skin to dryness in the winter months. Interestingly,

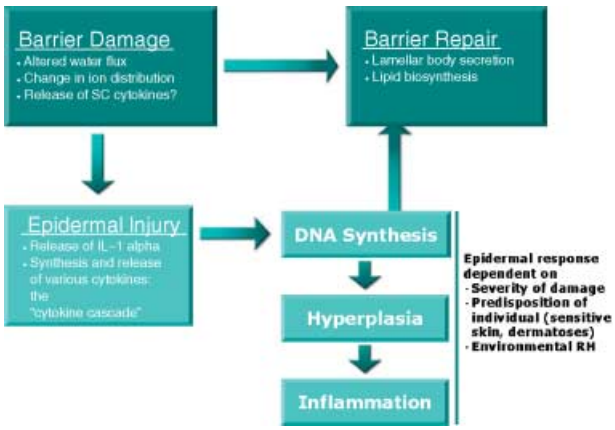

Fig. 5. Representation of the major signaling processes and events occurring following barrier perturbation of the stratum corneum.

there is also a seasonal variation in serum glucocorticoid levels (67). Recently, a circadian rhythm in the homeostatic capacity of the SC barrier was described (68).

Once perturbed, the loss of barrier function initiates a variety of signaling cascades to stimulate a metabolic response within the underlying epidermis aimed at normalizing SC function (Fig. 5). The principal response is a temporary increase in the biosynthesis of all major lipid species in the epidermis (i.e., cholesterol, fatty acids, and ceramides). Minor barrier perturbations may remain local to the epidermis. However, repeated or severe barrier disruption may stimulate signaling cascades that engage not only the desired epidermal homeostatic response, but also inflammatory events involving the deeper layers of the skin and the endothelium (69,70). Such changes have been proposed to play a role in sustaining inflammatory dermatoses. The elicitation of inflammation as a result of barrier disruption can lead to epidermal hyperplasia and abnormal keratinization. This will lead invariably to the production of an intrinsically inferior SC, in turn, thus creating a vicious cycle of events unless the environmental stress is removed.

Barrier dysfunction in skin disorders

Atopic dermatitis

Widespread regions of dry itchy skin are one of the most prominent clinical features of atopic dermatitis (71). Involuntary scratching provoked by severe itching can lead to a physical disruption of the SC, thereby exacerbating the intrinsic weakness in the barrier. Atopic dry skin displays an impaired barrier function, as indicated by increased transepidermal water loss (72) and diminished water-binding properties (73). While not the primary defect, the impaired barrier function and surface roughness associated with dryness may render the skin more susceptible to irritation. This condition is also associated with significant decreases in SC ceramide levels (74,75), particularly, Cer(EOS) (76), and the presence of unusual, possibly diagnostic ceramide species (77). Results of research over the past 5 years have emphasized that many aspects of lipid metabolism are deranged in this condition; patients with atopic dermatitis have significantly depleted covalently bound omega-hydroxyceramides (78) and reduced levels of prosaposin (79), an important regulator of sphingolipid metabolism.

The lowered level of ceramides in patients with atopic dermatitis has been linked to an increased expression of the enzyme sphingomyelin deacylase (80,81). This enzyme competes with sphingomyelinase for the ceramide precursor sphingomyelin. Although sphingomyelinase remains active in those with atopic dermatitis (82), significant levels of sphingomyelin are hydrolysed by this alternative pathway to release free fatty acid and sphingosyl phosphoryl choline (81). The presence of sphingosyl phosphoryl choline may partially explain the inflammation associated with this disorder since it is a potent modulator of epidermal function, stimulating proliferation and up-regulating plasminogen activator (83). The vulnerability of the SC of atopic patients to colonization by *Staphyloccus aureus* may reflect the reduced levels of sphingosine present in the tissue (84) that, in turn, reflects the decreased levels of ceramide (substrate) and the diminished activity of its metabolic enzyme, acid ceramidase. The water content in atopic SC is low, and the free amino acid content in atopic patients is also significantly reduced, which reflects the decreased numbers of keratohyalin granules (the repository of the filaggrin precursor protein) seen in the stratum granulosum (85). Recent data from Hirao also suggests that in atopics (86), as in psoriatics (87), the CE maturation is incomplete.

Psoriasis

In this condition, transepidermal water loss levels are increased between one and 20 times, depending on the severity of the lesion (88,89). Dramatic changes in SC lipid structure are also observed

(90), which reflect both perturbation in delivery of lipids from the lamellar bodies during SC formation (91) and overall changes in lipid composition (88). These changes include increases in ceramide with non-hydroxy fatty acids (N) and sphingosine (S) [Cer(NS)], and ceramide with 6-hydroxy-4-sphingenine (H) and omega-hydroxy fatty acid (O) ester-linked [E] to linoleic acid [Cer(EOH)], and decreases in ceramide with alpha-hydroxy fatty acids (A) and sphingosine (S) [Cer(AS)]. Together with the altered cholesterol and fatty acids levels, these alterations contribute to some of the characteristic aberrations in SC function, including corneocyte cohesion and faulty desquamation. It has also been reported that the composition of the covalently bound lipids differs in psoriatic SC compared with healthy SC. In psoriatic skin, Cer(OH) decreases while other components such as ω-hydroxy acids and fatty acids, particularly the covalently bound oleate and linoleate, increase (92). As in atopic dermatitis, psoriasis is also associated with perturbed synthesis of filaggrin leading to reduced water-binding capacity in the SC (93).

Ichthyoses

The characteristic scaling of the common forms of ichthyosis, namely, ichthyosis vulgaris and recessive X-linked ichthyosis, can be explained by discrete defects in barrier formation and integrity. In ichthyosis vulgaris, defective keratohyalin granule formation (and therefore little or no filaggrin) leads to the formation of an SC essentially devoid of many components of the NMF (94). Because of the resulting defective water binding and possible alterations in skin pH (as a result of depletion of urocanic acid and pyrrolidone carboxylic acid), desquamation is severely perturbed and corneodesmosomes are poorly degraded (95).

X-linked ichthyosis is characterized by the desquamation of large, adherent scales. This condition is caused by a deficiency in the enzyme steroid sulfatase (96) that leads to an accumulation of cholesterol sulfate and a reduction of cholesterol. This specific defect leads to altered lipid organization (97), and is associated with fragmented and disrupted lamellae in the intercellular domains (98) and increased cohesion between corneocytes. Cholesterol sulfate is known to influence many biological processes. For example, as a critical factor in SC functioning, it has been shown to induce transcription of transglutaminase and significantly inhibit some of the key serine proteases involved in desquamation (99), contributing further to the abnormal retention of corneodesmosomes.

The classic scaling seen in lamellar ichthyosis seems to be the result of either failed formation of the CE or defective arrangement of the intercellular lipids. Intercellular lamellae membranes are frequently truncated and fragmented in this condition, providing a basis for the barrier dysfunction (98). Individuals suffering from lamellar ichthyosis have a defective gene for transglutaminase 1 (100). The inability to link ceramides to the CE and defective cross-linking within the cornified CE have been proposed to explain the dramatic skin phenotype seen in affected individuals. However, recently obtained data suggesting normal organization of the covalently bound lipid layer cast some doubt on the role of this enzyme in covalently bound lipid attachment (101).

Although the SC lipid profiles in other ichthyotic diseases have not been fully determined, reduced levels of sphingosine have been found in a variety of patients with various ichthyoses (102). This decrease in sphingosine may, in part, explain the underlying cellular hyperproliferation observed in these conditions since, as has been proposed, sphingosine may feedback to the epidermis and down-regulate keratinocyte turnover (103).

Surfactant-induced xerosis

In our daily life, substances that include chemicals such as surfactants and solvents potentially perturb the SC barrier (104). Certain anionic surfactants such as sodium lauryl sulfate affect not only the barrier itself, but also the underlying viable cell layers (105). For example, sodium dodecyl sulfate up-regulates intercellular adhesion molecules, or vascular endothelial growth factor, suggesting that this class of surfactant has an intrinsic ability to induce epidermal hyperplasia and irritation. In general, surfactants that bind strongly to SC proteins have a higher potential to cause significant protein denaturation, leading not only to barrier damage, but also to erythema and itching.

Low-humidity-induced winter xerosis

Seasonal changes affect the condition of normal healthy skin and may trigger various cutaneous disorders. In common dermatitis, a decline in barrier function usually parallels the clinical severity of the complaint. These conditions all tend to worsen during the winter season when humidity is lower (106,107). In soap-aggregated winter xerosis, abnormalities in SC structure, lipid composition,

and corneodesmosomal degradation are readily apparent (108). At the skin surface, decreased external humidity, by reducing the water content of the peripheral SC, can decrease the activity of the enzymes involved in maturation and desquamation, leading to skin flaking (for more details, see pp. 43–48). By weakening the barrier, seasonal changes in lipid synthesis and in circulating stress hormones may further exacerbate the condition. In addition, the results of recent studies, albeit on mice, suggest that low humidity is directly or indirectly driving several additional responses within the underlying epidermis that can subsequently initiate an inflammatory response. In the first instance, exposure to a dry environment may actually enhance the epidermal permeability barrier as a response to the environmental change (57). However, decreases in humidity also drive a heightened hyperproliferative response to barrier perturbation (109), and in response to surfactant (109), initiate mast cell degranulation (110–111) and amplify the cytokine cascade initiated by barrier perturbation (112). Although extrapolating such data to the human condition must be done with caution, one or more of these processes probably contributes to the appearance of sore, chapped human skin on exposed body sites during harsh winter months, especially when there is an abrupt decline in environmental humidity.

Conclusions

Perturbations to the efficiency of the epidermal permeability barrier, whether a consequence of environmental factors or inborn metabolism error, can have profound effects on overall skin quality. There is a growing appreciation that a defective barrier is not simply a secondary consequence, but rather, a critical element driving inflammation in disorders of cornification (113). There are many instances where the severity of the disorder correlates with the degree of barrier abnormality (e.g., in psoriasis and atopic dermatitis), and similarly, where improvements in the SC barrier function can improve inflammatory disorders (e.g., in winter xerosis, atopic dermatitis, and psoriasis). Therefore, by understanding the molecular basis of the barrier perturbation in dry, flaky skin conditions—ranging from winter xerosis to psoriasis—we are able to move forward with increasing confidence to develop specific treatments that, by targeting defined deficiencies, restore barrier functionality and improve overall skin quality.

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