Skin Barrier Function and Canine Atopic Dermatitis

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The stratum corneum forms the major protective barrier between the body and the environment.

The importance of the stratum corneum is readily overlooked in histological preparations of normal skin. The normal corneal layer consists of more than 20 layers of bland, lightly-staining, polyhedral, anucleate cells, most of which are lost during biopsy sampling, cutting, and processing. The large surface area of the skin puts it in constant contact with environmental pollutants, irritants, and allergens and the stratum corneum layer of skin forms the major protective barrier between the body and the environment. In human medicine, abnormalities in the barrier function of the stratum corneum have been hypothesized to permit epicutaneous allergen exposure in atopic and asthmatic patients. Furthermore, these alterations may, in part, help to explain the dramatic increase in atopic and asthmatic disorders in humans living in industrialized nations during the past few decades.[1,2]

Key Features in the Cornification Process:

- Lipid formation
- Dissolution of the nucleus and cell organelles
- Aggregation of intermediate filaments
- Formation of the cornified envelope
- Desquamation

In the domestic animal population, the stratum corneum has had limited scientific investigation and most studies have been performed in dogs. Morphologically, many types of injury can produce various and often similar corneal changes. Specific histopathologic changes are paramount in the diagnosis of a number of hereditary and metabolic disorders including zinc responsive dermatosis, necrolytic migratory erythema, and ichthyosiform disorders. In canine atopic dermatitis, the stratum corneum has been shown to have ultrastructural features that differ from those of normal dogs.[3] The focus of research on the pathogenesis of canine atopic dermatitis has dramatically shifted over the past two decades from that of an aeroallergen approach to epicutaneous contact via patch testing.[4-6]

STRATUM CORNEUM FUNCTION

The stratum corneum is the key epidermal layer that restricts water movement into and out of the skin. In normal humans, approximately 0.5 L of water vapor is expelled

through the stratum corneum per day.[7] Even minor injuries to the corneal layer from tape stripping or applications of solvents will result in increased transepidermal water loss. The water loss in psoriasis patients can be as much as 6 L/day.[7] The lipid composition in the corneal layer is responsible for the hydrophobic function. Human atopic dermatitis patients have xerosis, which may be caused by a deficiency in ceramides that leads to increased transepidermal water loss.[8] Transepidermal water loss has not been well documented in dogs, nor have there been many investigations. One study showed no difference between the corneal hydration status of normal dogs versus dogs with atopic dermatitis.[9]

The stratum corneum prevents the access of pathogens to the body. Much of this barrier function is likely due to the physical property of continuous desquamation, which allows for the expulsion of pathogens. This layer also contains natural antimicrobial peptides such as defensins and cathelicidins. These small proteins can act on microbial cell membranes and kill through diverse mechanisms. Humans with atopic dermatitis have been shown to have a decreased ability to produce antimicrobial peptides during inflammation.[10] Cutaneous *Malassezia* and *Staphylococcus* infections are well known to exacerbate the inflammatory and immunologic changes that occur in both dogs and humans with atopic dermatitis.[1]

The stratum corneum functions to decrease natural exposure to topical drugs and these inherent properties are the targets of pharmacologic manipulation. The stratum corneum also absorbs UV light to protect the underlying tissue from free radical oxidation. This property is enhanced by the retention of melanin in corneocytes. Urocanic acid, derived from histadine in keratinocytes, also serves as a natural sunscreen.

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PROCESS OF CORNIFICATION

During normal cornification, keratinocytes undergo a dramatic change in shape, size, and function. These cells undergo a modified form of programmed cell death. In this process, keratinocytes must lose a large amount of water volume (from 70% water in nucleated layers to 15% in stratum corneum).[1] Alterations in any step can lead to hyperkeratosis, clinical scaling, and decreased barrier function.[11] There are five key features in the process of cornification:

1. Lipid formation:

New lipid-laden organelles, called lamellar granules, are synthesized in the upper stratum spinosum. At the junction of the stratum granulosum and stratum corneum, the lamellar granules fuse with the cell membrane and expel their contents into the intercellular space. As the lipid is released at the stratum corneum/stratum granulosum junction, the content of the lipid is modified by enzymes. Glycolipids are converted to ceramides by glucosidases and phospholipids to fatty acids by phospholipases. Hence, the "probarrier lipids" become "barrier lipids" (ceramides, cholesterol, free fatty acids) that create a hydrophobic seal. The lipid acts as a glue to adhere the corneocytes to one another and repel water. The lamellar granule plays a role in the synthesis and storage of cholesterol as well as corneocyte desquamation.[11] Mutations in the genes for steroid and lipid metabolism (e.g., X-linked ichthyosis) cause failure of the cornified cells to slough and retention hyperkeratosis.[11]

2. Dissolution of the nucleus and cell organelles:

Dissolution of the nucleus and cell organelles occurs via newly formed proteases, which cleave DNA, RNA, and other cellular proteins. The nuclear loss occurs by apoptic mechanisms of internucleaosomal cleavage.[11] Increased turnover of the stratum corneum from minor trauma or microbial infection leads to patchy areas of

Epidermal Anatomy

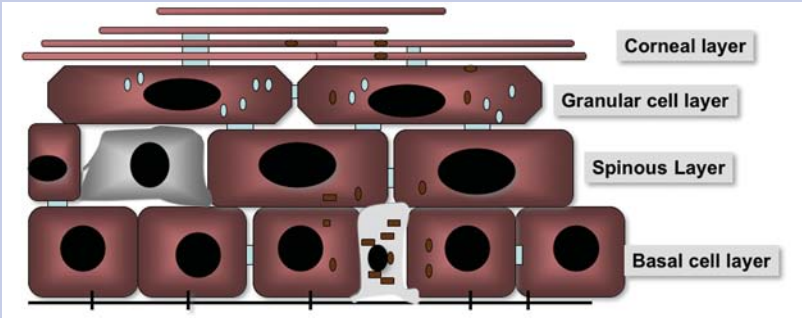

Figure 1: Schematic of normal skin. During cornification, the keratinocytes undergo a dramatic change in shape and function as the cells mature from the basal cell layer to the stratum corneum.

hyperkeratosis and nuclear retention (parakeratosis). Specific metabolic disorders (lethal acrodermatitis of Bull terrier, necrolytic migratory erythema, and zinc responsive dermatosis) produce more diffuse and severe parakeratosis.

3. *Aggregation of intermediate filaments:*

The protein core of the corneocyte provides much of the structural integrity of the stratum corneum. Keratohyalin granules contain profilaggrin, which is a precursor matrix protein that undergoes processing (proteolysis, dephosphorylation) to the active enzyme filaggrin. This protein is thought to cross-link the keratin intermediate filaments and form the protein core of the corneocyte. Absence of keratohyalin granules and abnormal filaggrin expression occur in patients with ichthyosis vulgaris.[11] A variety of congenital mutations may occur in the synthesis of different epidermal keratin proteins. The first spontaneous keratin mutation was recently identified in a family of Norfolk terrier dogs.[12]

4. *Formation of the cornified envelope:*

Transglutaminases are a group of calcium-containing enzymes located within both the epidermis and hair follicles. These proteins (in particular, transglutaminase 1 [TGM1]) catalyze the formation of the cornified envelope by cross-linking small protein molecules (e.g., involucrin, loricrin, cystatin A), which replace the cell membrane. The cornified envelope surrounds the protein core and provides the structural support for the corneocyte. The complete coding region of canine TGM1, its chromosome localization, and its map position have been documented.[13]

5. *Desquamation:*

Desquamation is now thought to be an active process. In the mature stratum corneum, multiple layers of corneocytes are sandwiched between layers of lipid, producing the so-called "mortar and bricks" analogy. Desmosomes are retained in the stratum corneum (corneodesmosomes). These are actively broken down, the corneocytes undergo desquamation, and layers of keratin squames are shed into the environment. Defects in desquamation can lead to retained squames, severe hyperkeratosis, and loss of elasticity of the skin.[7]

Corneal Lipid Formation

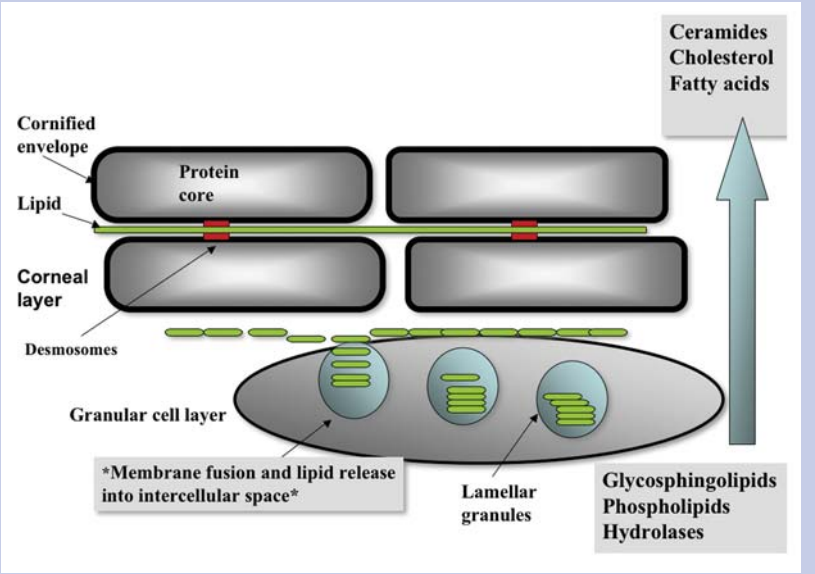

Figure 2: Lamellar granules fuse with the cell membrane of keratinocytes at the stratum granulosum/stratum corneum junction and release their contents into the intercellular space. Following release, various enzymes modify the expelled lipids, initiating a hydrophobic seal to aid in "water proofing" the epidermis.

ROUTE OF ALLERGEN EXPOSURE IN DOGS

In 1999, the American College of Veterinary Dermatology established a task force to critically review the scientific literature of canine atopic dermatitis using evidenced-based medicine. The task force addressed the route of allergen exposure and recommended that the term "allergic inhalant dermatitis" no longer be used as there was a lack of evidence to support the respiratory route as a means of pathogen exposure. The percutaneous route was favored for a number of reasons. First, the site(s) of active lesions in dogs with atopic dermatitis is either in direct contact with the environment (pedal, inguinal) or involves regions of self-trauma or friction. Second, histologic changes seen in experimental models using patch testing (e.g., aggregates of Langerhans cells in epidermis, subepidermal collections of eosinophils) are similar to those seen in dogs with atopic dermatitis. Third, there was a lack of evidence in the respiratory models that sensitization induced cutaneous lesions or pruritus. Finally, atopic dogs do not develop signs of asthma.[14]

ALLERGEN EXPOSURE AND ALTERED BARRIER FUNCTION

Studies have shown that epicutaneous allergen exposure in humans may be one route of sensitization (in addition to respiratory and oral). Altered barrier function of the stratum corneum may allow for the entry of allergens into the epidermis and induce a cytokine milieu favoring a Th2 response.[1,2] In humans, ceramides are the main polar lipids in the stratum corneum. These lipids not only play an important role in skin barrier function but they also are involved in cell adhesion and epidermal differentiation. As previously mentioned, alterations in the lipid content, particularly ceramides, occur in atopic patients as compared to healthy controls and may lead to increased transepidermal water loss.[1,2,8] It has also been shown that cholesterol levels are increased in patients with low ceramide levels. Decreased ceramide content or altered ceramide/cholesterol ratios may represent the etiology of dry skin in human atopic patients,[15] which may be due to defects in enzymatic pathways (sphingomyelin deacylase, glucosylcerebrosidase), ceramide production, or sphingolipid activated protein.[8,15,16]

It is unclear if an epidermal barrier defect exists in canine atopic patients. Normal dogs have naturally less lipid content in the epidermis than humans.[17] The lipid content also varies among breeds. The Labrador retriever and Siberian husky have more lipid content in the skin than the miniature poodle.[17] Dogs with atopic dermatitis often have corneal abnormalities, but it is not known if a functional defect precedes the cutaneous trauma caused by infection and pruritus. Recently, electron microscopic analysis of the stratum corneum of dogs with atopic dermatitis revealed abnormalities in the lipid lamellae in normal skin. These lamellae were discontinuous and thin; the stratum corneum lacked the normal "mortar and bricks" appearance of that in normal dogs.[3]

THERAPEUTIC CONSIDERATIONS

Restoring barrier function may be one means to decrease allergen exposure. In humans, damage to the skin barrier may be restored by the topical application of lipids. An applied mixture of stratum corneum lipids, ceramide, cholesterol, and free fatty acids on murine and human skin accelerated the repair of water function after damage. This was effective only when the lipid was applied as a mixture. Similar mixtures have been shown to produce significant improvements in patients with allergic contact dermatitis, irritant dermatitis, and atopic dermatitis.[18] In dogs, frequent washing or rinsing of contact areas (e.g., paws) may also help to decrease allergen exposure. Dietary supplementation may help to increase epidermal lipid content and decrease transepidermal water loss.

REFERENCES

1. Leung D, Eichenfield F, Boguniewicz M. Atopic dermatitis (atopic eczema). In: Freedberg IM, Eisen AZ, Wolff K, et al, eds. *Fitzpatrick's dermatology in general medicine*. ed 6. New York: McGraw-Hill, 2003;1181–1194.
2. Leung D, Jain N, Leo HL. New concepts in the pathogenesis of atopic dermatitis. *Curr Opin Immunol* 2003;15:634–638.
3. Inman AO, Olivry T, Dunston SM, et al. Electron microscopic observations of stratum corneum intercellular lipids in normal and atopic dogs. *Vet Pathol* 2001;38:720–723.
4. Marsella R, Olivry T, Nicklin C, et al. Pilot investigation of a model for canine atopic dermatitis: environmental house dust mite challenge of high-IgE-producing beagles, mite hypersensitive dogs with atopic dermatitis and normal dogs. *Vet Dermatol* 2006;17:24.
5. Olivry T, Buckler KE, Dunston SM, et al. Positive "atopy patch tests" reactions in IgE-hyperresponsive beagle dogs are dependent upon elevated allergen-specific IgE serum levels and are associated with IgE-expressing dendritic cells. *Vet Dermatol* 2002;13:211–229.
6. Marsella R, Nicklin C, Lopez J. Atopy patch test reactions in high-IgE beagles to different sources and concentrations of house dust mites. *Vet Dermatol* 2005;16:308–314.
7. Marks R. The stratum corneum barrier: the final frontier. *J Nutr* 2004;134:2017S–2021S.
8. Choi MJ, Maibach HI. Role of ceramides in barrier function of healthy and diseased skin. *Am J Clin Dermatol* 2005;6:215–223.
9. Chesney CJ. Measurement of skin hydration in normal dogs and in dogs with atopy or a scaling dermatosis. *J Small Anim Pract* 1995;36:305–309.
10. Izadpanah A, Gallo RL. Antimicrobial peptides. *J Am Acad Dermatol* 2005;53(3Pt1):381–390.
11. Chu DH, Haake AR, Holbrook K, et al. The structure and development of the skin. In: Freedberg IM, Eisen AZ, Wolff K, et al, eds. *Fitzpatrick's dermatology in general medicine*. ed 6. New York: McGraw-Hill, 2003;58–88.
12. Credille KM, Barnhart KF, Minor JS, et al. Mild recessive epidermolytic hyperkeratosis with a novel keratin 10 donor splice-site mutation in a family of Norfolk terrier dogs. *Br J Dermatol* 2005;153:51–58.
13. Credille KM, Ventab PJ, Breen M, et al. DNA sequence and physical mapping of the canine transglutaminase 1 gene. *Cytogenet Cell Genet* 2001;93:73–76.
14. Olivry T, DeBoer DJ, Griffin CE, et al. The ACVD task force on canine atopic dermatitis. *Vet Immunol Immunopath* 2001;81:143–384.
15. Di Nardo A, Wertz P, Giannetti A, et al. Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. *Acta Dermatol Venereol* 1998;78:27–30.
16. Hara J, Higuchi K, Okamoto R, et al. High expression of sphingomyelin deacylase is an important determinant of ceramide deficiency leading to barrier disruption in atopic dermatitis. *J Invest Dermatol* 2000;115:406–413.
17. Dunstan RW, Herdt TH, Olivier T, et al. Age- and breed-related differences in canine skin surface lipids and pH. In: Thoday KL, Foil CS, Bond R, eds. *Advances in veterinary dermatology, Vol. 4*. Oxford, UK: Blackwell, 2002;37–42.
18. Man MQM, Feingold KR, Thornfeldt CR, et al. Optimization of physiological lipid mixtures for barrier repair. *J Invest Dermatol* 1996;106:1096–1101.

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