Skin barrier defect in the pathogenesis of canine atopic dermatitis

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The pathogenesis of canine atopic dermatitis is very complex and with many aspects still to be clarified. Thanks to studies carried out over the last 20 years, it is clear how canine atopic dermatitis is the result of a complex interaction between genetic abnormalities and the environment. In fact, the skin of atopic subjects is characterized by alterations (primary or secondary) of the skin barrier associated with excessive penetration of environmental allergens capable of excessively stimulating the local immune system. This immune system is characterized by a state of hyperactivation that results in an abnormal response to common allergens present in the environment.

Skin barrier

The skin is the organ in direct contact with the external environment and as such represents the first barrier against the penetration of external agents potentially harmful to the body. In healthy dogs, the skin is characterized by a series of defense mechanisms forming the skin barrier. In its entirety, the skin barrier includes physical, chemical, immunological and microbiological defense mechanisms. The physical barrier is characterized by the presence of the epidermis in its basic components, including keratinocytes / corneocytes, desmosomal junctions and tight junctions.

In particular, in healthy dogs, the epidermis, mostly made up of keratinocytes, represents an uninterrupted barrier in continuous remodeling. In fact, during a period of about 21 days the keratinocyte undergoes a process of keratinization and cornification that leads to the formation of corneocytes, anucleated keratinocytes. During this process of maturation immature keratinocytes undergo a dramatic but constant remodeling which leads to the formation of lamellar bodies and to the synthesis of keratohyalin granules in the spinous layer. During the transition from the granular to the horny layer, the formation of the "cornified envelope" takes place, which is the process that leads to the stiffening of the cell membrane due to the accumulation of keratin and other constituent proteins such as filaggrin, involucrin, and loricrin. During this transition the keratinocyte secretes, through the fusion of the lamellar bodies to the cell membrane, lipids such as ceramides, free fatty acids and cholesterol that will form the "lipid envelope", that is a lipid layer surrounding the corneocytes. Once this stage has been reached, the corneocyte is mature and is continuously eliminated, through the phenomenon of desquamation. With desquamation, together with the corneocytes, allergens and microorganisms adhering to the corneocytes are eliminated. Meanwhile, in the deeper layers of the epidermis, the intercellular junctions form the remaining part of the physical mechanisms that make up the skin barrier. Among these, the tight junctions represent the main block to the penetration of external agents.

As mentioned above, physical barrier mechanisms are associated with chemical, immunological and microbiological mechanisms. Among these, the microbiological barrier has acquired particular interest in the scientific community in recent years. Until more widespread use of methodologies capable of identifying microorganisms based on their molecular and non-phenotypic pattern, most of the skin microflora was considered potentially pathogenetic. But with the arrival of high-output techniques, we have begun to speak of the microbiome as an essential part of healthy skin. The microbiome represents the set of microorganisms that live on or in the body (human or canine). The microbiome of healthy subjects is characterized by a vast bacterial diversity that does not allow the overgrowth of a particular bacterial species, preventing potential diseases. Whenever there is an alteration of the microbiome, we speak of dysbiosis. Chemical mechanisms include the secretion of active antimicrobial substances such as antimicrobial peptides, enzymes (e.g. lysozyme and phospholipase A), lipids such as fatty acids and cholesterol which form the lipid barrier and lipid layers between the corneocytes. The chemical barrier includes small peptides (natural hydration factors) derived from the degradation of the filaggrin such as trans urocanic acid and carboxylic pyrrolidone, both essential for maintaining a healthy skin pH and able to limit the loss of water from the corneocytes. Finally, immunological defense mechanisms are varied and include both the innate and adaptive systems. These mechanisms include the activation of cells such as keratinocytes, Langerhans cells, dermal cells and lymphocytes.

As previously mentioned, the correct and synchronous functioning of these mechanisms are globally responsible for the skin barrier in healthy subjects. Alterations of one or more defense mechanisms lead to the formation of skin diseases including canine atopic dermatitis.

Skin changes in canine atopic dermatitis

Alterations of the skin barrier in atopic subjects are varied and include physical, chemical, immunological and microbiological alterations. Despite the various research in this area, it is not yet clear whether these skin barrier abnormalities are primary, therefore associated with genetic alterations, or whether they are secondary to immunological imbalances.

Regarding the alterations of the physical and chemical barrier, these include modifications of the stratum corneum and the intercellular junctions of the epidermis. In particular, the stratum corneum of atopic subjects lacks cellular cohesion resulting in "holes" in the first barrier to external agents. Furthermore, studies have shown that bacteria tend to adhere more tenaciously and abundantly on atopic corneocytes compared to healthy corneocytes. This phenomenon, together with other skin alterations, is potentially at the basis of the skin dysbiosis present in atopic subjects. This lack of cellular cohesion also leads to excessive and constant loss of water, decreasing skin hydration. It has recently been shown that atopic dogs, following exposure to dust mites, demonstrate a reduction in the content of natural hydration factors. This reduction may be the cause of reduced skin hydration and an increase in skin pH present in the skin. of atopic dogs. The increase in skin pH is associated with a greater activation of serum proteases responsible for the degradation of inter-corneocyte junctions, contributing to lower cell cohesion.

Other alterations of the stratum corneum include an alteration of the composition and arrangement of the lipid layers between the corneocytes. In fact, various studies have shown how the skin of atopic subjects is characterized by a drastic disorganization of the inter-

corneocyte lamellar layers associated with a significant increase in intercellular spaces. From the composition aspect, various studies have highlighted an imbalance of ceramides associated with a reduction of some ceramides, such as ceramide 1 / CER [EOS], ceramide 9 / CER [EOP], and ceramide [NP]. Furthermore, a recent study has brought to light data demonstrating not only an alteration of the composition of ceramides, cholesterol and fatty acids in the lipid layer, but also suggesting that the basis of the disorganization of the lipid lamellae is associated with an alteration of the assembly conformation of the lipids. In atopic dogs, the lipids are assembled in a hexagonal rather than an orthorhombic conformation.

Together with the alterations of lipids and natural hydration factors, in atopic subjects there are alterations of the chemical barrier, in particular alterations in the secretion of antimicrobial peptides. Various studies have demonstrated an alteration in the production of defensins and cathelicidin in atopic dogs when compared to healthy or infected subjects. More recently, two studies have shown that the antimicrobial activity, measured by skin washing, is lower in atopic subjects than in healthy subjects. This activity may be linked to the fact that these peptides tend to adhere to the external horny layer rather than being released and therefore available to attack microorganisms. Alongside the alterations of the stratum corneum are associated alterations of the lower layers of the epidermis. There is a dramatic decrease in the presence of the constituent proteins of the tight junctions, including claudin-1, occludin, and corneodesmosin for the junctions between corneocytes. Several studies in atopic dogs have shown that in these subjects there is a significant decrease in the presence and distribution of these proteins in the epidermis of atopic dogs when immunofluorescence techniques are used.

Finally, we must remember how local immune system imbalances and skin dysbiosis play a very important role in the pathogenesis of canine atopic dermatitis. Immunological alterations are more associated with an abnormal inflammatory response to harmless stimuli such as allergens. This type of response is due to several factors, including a hyperactivation of the dendritic cells capable of extending their dendrocytes to reach the most superficial layers of the epidermis, increasing contact with external agents. Not only that, but the dendritic cells of atopic subjects have been associated with an increase in the presence of IgE receptors on their surface. Among the various immune cells that play a fundamental role in atopic dermatitis, one cannot forget the keratinocyte. In fact, this cell not only has a structural function in the epidermis, but is also a very immunologically active cell. Keratinocytes produce a wide range of inflammatory cytokines and chemokines for the attraction of immune cells in the skin. As previously mentioned, the atopic keratinocyte is characterized by structural and metabolic alterations that facilitate sensitization to harmless external substances. Regarding the adaptive immune system, many studies have shown an imbalance in the lymphocytic populations present in atopic skin. Of note, there is a reduction in regulatory cells associated with an abundance of type 2 helper cells and non-specific inflammatory cytokines that characterize the immunological milieu in the different stages of the disease.

Alterations of the immune system and the physical-chemical barrier of the skin have a significant influence on the microbiological barrier. In fact, the normal microbiome is in constant interaction with the other forms of defense implemented by the epidermis. In recent years, many studies have focused on the role that the skin microbiome plays in the development of atopic dermatitis. Although knowledge on the microbiome has increased

enormously, it is not yet clear whether skin dysbiosis is a cause or a consequence of atopic dermatitis. Many microbiome studies are observational rather than causative. We certainly know that the skin microbiome of atopic subjects is characterized by a reduction in species diversity associated with a predominant increase in the Staphylococcal population. This alteration is also present in the fungal microbiome favoring the predominance of *Malassezia pachydermatis* instead of *M. restricta* predominant in healthy subjects. Furthermore, in a recent study it was shown that the presence of a dysbiotic state correlates with the presence of clinical exacerbation and with the increase in pH and loss of transdermal water.

Further studies are needed to better define the pathogenesis of canine atopic dermatitis. However, it is clear this pathology is extremely complex and characterized by a dense network of connections between immune cells, acellular components, inflammation mediators, and microbiome. Whenever there are alterations in one or more components of this network, there is the possibility of an imbalance of the skin defenses and exacerbation of the atopic pathology. Furthermore, the epigenetic effects that the environment has on the predisposition to develop atopic dermatitis should not be underestimated.

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