Review paper

Structure and function of the epidermal barrier in patients with atopic dermatitis – treatment options. Part one

Jagoda Pelc, Magdalena Czarnecka-Operacz, Zygmunt Adamski

Department of Dermatology and Venereology, Poznan University of Medical Sciences, Poznan, Poland

Adv Dermatol Allergol 2018; XXXV (1): 1–5
DOI: https://doi.org/10.5114/ada.2018.73159

Abstract

Atopic dermatitis is a chronic, recurrent inflammatory skin disease, which is frequently familial. The main cause of the disease seems to be a defect of the epidermal barrier resulting from a genetic predisposition concerning the epidermis, functioning of the immune system as well as environmental factors (which are not related to the immune system). Genes responsible for encoding protein S100, filaggrin, proteases and their inhibitors are the main genes related to the problem of epidermal barrier dysfunction. There is a close connection between structural and immunological processes. Increased expression of cytokine Th2 profile belongs to the latter category. The objective of the present paper is to describe the influence of aforementioned factors on epidermis structure and dysfunction which leads to clinical symptoms of atopic dermatitis.

Key words: atopic dermatitis, epidermal barrier, filaggrin, cytokines.

Introduction

Atopic dermatitis (AD) is a chronic, recurrent, inflammatory skin disease characterized by very intense itching. The condition, like other diseases of atopic origin, is familial. With both parents diagnosed, there is 60–80% chance that children will suffer as well. In the case of one ill parent, the probability of developing the condition is about 30–40% [1]. It is known that AD is heritable and multiple genes are involved in the process. Yet, it is difficult to point univocally to one certain locus or gene responsible for developing so many clinical symptoms of AD. The relation between chromosomes 1, 3, 4, 5, 11, 13, 15, 17, 18, 19 and 20 has been proved [2].

As an atopic condition AD may co-occur with symptoms of bronchial asthma and/or allergic nasal mucosa inflammation and conjunctivitis [1]. Almost half of affected patients develop the first symptoms in the first 6 months of life. More than half develop such symptoms in the first year of life and in most cases the disease appears during the first 5 years of life [3]. However, late-onset AD and very-late-onset AD are becoming more frequent.

Etiopathogenesis of atopic dermatitis

Genetic as well as environmental, immunologic and non-immunologic factors play a relevant role in the etiopathogenesis of AD. Thus, it is an unusually complex system of diverse factors which, influencing each other, condition the development of symptoms relating to an illness of remarkable phenotypic variety.

With reference to genetic factors, one should emphasize the significance of the two most important of them, namely a genetically conditioned disorder in the range of the structure and the function of the skin barrier and disorders with reference to the immunological response.

Structural and functional dysfunction of the skin barrier in patients suffering from atopic dermatitis

The skin barrier can be defined in many different ways, namely as mechanical, functional, immunologic or microbiological, etc.

From the structural point of view, the skin barrier concerns and is usually described with reference to the stratum corneum. The human stratum corneum on average

Address for correspondence: Jagoda Pelc MD, Department of Dermatology and Venereology, Poznan University of Medical Sciences, 49 Przybyszewskiego St, 60-355 Poznan, Poland, phone: +48 667 261 626, e-mail: jagmos@wp.pl
Received: 18.04.2017, **accepted:** 3.08.2017.

consists of 20 layers of corneocytes which are rich in intercellular lipids and constitute the last phase of differentiation of keratinocytes of the granular layer. They amalgamate with corneodesmosomes (which are composed of desmoglein and desmocollin). During the creation of corneocytes, the cells of the granular layer release their content into the extracellular space and, as a result, they create a lipid array, which prevents transepidermal water loss (TEWL). The lipid array is composed of ceramides, cholesterol and its esters and fatty acids [4].

Epithelium in AD which is not affected by skin lesions is characterized by bioelectric abnormalities in tight junctions, which are located on the opposite films of corneocytes of the granular layer, directly below the stratum corneum, creating a second barrier. They are made up of a complex of cell adhesion and structural molecules, which control the passage of water, ions and solutions. The abnormalities may be finally related to a consequence of lowered level of claudin-1 (crucial cell adhesive molecules of tight junctions) [2]. These disturbances in the structure of epithelium consisting in cleaving the molecules may result from the activity of cysteine and serine proteases of house dust allergens [5].

Further genetic background of skin barrier dysfunction in atopic dermatitis

Currently, it seems that the genes which are part of the epidermal differentiation complex (EDC) on the 1q21 chromosome and which code the epidermis keratinization and molecule S100 [2] constitute the basic genes involved in the problem of skin barrier dysfunction in patients with AD. This molecule constitutes an important component of the cornified envelope coded in the epidermal differentiation complex on chromosome 1q21. It has an influence on the FLG and HBD-3 (human β-defensin) genes, thereby influencing the integrity of the skin barrier and the congenital immunological response [2].

The epidermal barrier dysfunction in AD is closely linked to abnormal synthesis of structural proteins, enhanced expression of serine proteases, and low activity of protease inhibitors (Figure 1). In this respect, mutation of the filaggrin genes (a basic protein in the stratum corneum linking keratin fibers) seems to be of key significance, as it leads to an evident disorder both in the structure and function of the epidermal barrier, which among other things is expressed by an increase in TEWL and as a consequence may lead to the development of dry skin [1, 3]. This is inter alia associated with the reduced synthesis of natural moisturizing factor (NMF), which is produced in the process of filaggrin deamination. NMF consists of amino acids (40%), pyrrolidone-carboxylic acid (12%), lactates (12%), urea (7%), Na, Ca, K, phosphates and chlorides (18%) as well as uric acid, glucosamine and creatinine (1,5%) (http://mcmwiktorska.pl/uploads/entries/cdec0ad5f7fdceb6479501fb2a3745d1.pdf; 13.10.2015). Natural moisturizing factor plays a key role in maintaining water within the epidermis, thus largely determining its moisturization. Pyrrolidone carboxylic acid and lactic acid are highly hygroscopic. They prevent the formation of gaps among corneocytes, strengthen the integrity of the stratum corneum, and make it resistant to the penetration of environmental irritants and allergens [4]. The FLG gene is located in the EDC on chromosome 1q21 [5]. However, mutations in the FLG gene are present in only about 30–50% of patients suffering from AD. It has been revealed that in this particular group of patients the symptoms of disease have an early onset and are characterized by elevated levels of serum IgE, while skin lesions have a relatively high intensity and can persist into adulthood as well [6]. The most prevalent FLG mutations are R501X and 2282del4. The aforementioned mutations have been described as regards the European

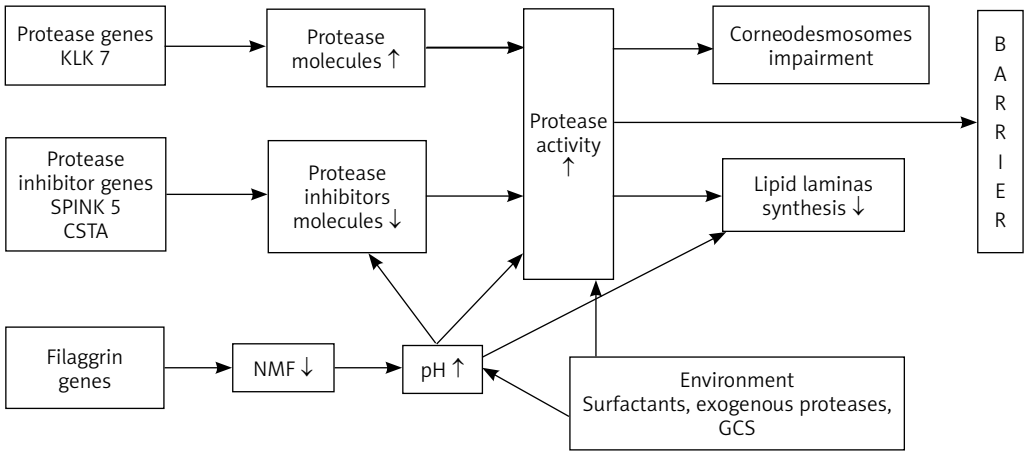

Figure 1. Genetic and environmental factors influencing skin barrier impairment in patients with atopic dermatitis [6]

population, since their presence significantly increases the likelihood of skin lesions prevalent in AD [7–10].

In the case of healthy individuals, formation of the cornified envelope includes dephosphorylation and distribution of profilaggrin through active serine proteases for filaggrin. Filaggrin fills the keratin cytoskeleton in order to facilitate the process of disintegration and flattening of keratinocytes within the surface layers of the epidermis, whereas other proteins encoded by genes in the EDC (such as loricrin and involucrin) are the major components of the skin barrier. When the moisture content of the stratum corneum is decreased, filaggrin is converted to pyrrolidone carboxylic acid and trans-urocanic acid, both of which are actively involved in the process of NMF. Moreover, filaggrin breakdown products determine stratum corneum acidification, and this is why the decreased level of filaggrin metabolites may result in the increase of the pH values in the stratum corneum and secondary activation of serine proteases. These processes may lead to further damage to the epidermal barrier and may stimulate the development of Th2-dependent inflammation in patients with AD [2]. Patients suffering from AD with mutations in the filaggrin gene are characterized by the early onset of clinical signs of disease, and more severe course of disease, typically with the presence of the symptoms of acute eczema, which more often than in other cases happens to be complicated by eczema herpeticum. In addition, from the clinical point of view, the greater risk of IgE sensitization constitutes another characteristic feature, as it is dependent with regard to environmental allergens and asthma, as compared with patients without any mutations in the filaggrin gene [11].

It is evident that the expression of proteins related to the epidermal barrier and NMF is significantly limited within the inflammatory lesions, as compared to intact, seemingly healthy skin. Therefore, lower expression of epidermal barrier proteins and enzymes involved in the formation of NMF may intensify the barrier defect and increase in TEWL; however, the greater expression of proteins binding fatty acids in the epidermis, especially in patients whose skin is colonized with MRSA, may sustain the inflammatory response through signaling eicosanoids [2].

As regards the genetically determined skin barrier dysfunction in AD etiopathogenesis, it is necessary to mention mutations in the genes encoding proteases (serine and cysteine) and their inhibitors. Serine proteases are known for not only influencing the cohesion but also the proper desquamation of the stratum corneum that occurs due to degradation of corneodesmosome proteins. In addition, they regulate the synthesis of stratum corneum lipids by degradation of extracellular lipid processing enzymes (B-glucocerebrosidase and acidic sphingomyelinase) and they also reduce lamellar bodies' lipid secretion by stimulating receptor type 2 for plasminogen [6]. Cysteine proteases also affect corneodesmosome degradation. Their inhibitor – cystatin A – released during sweat secretion, in a healthy epidermis, protects the integrity of the epidermal barrier against harmful exogenous proteases produced, among other organisms, by house dust mites and *Staphylococcus aureus* [4]. The balance between proteins and their inhibitors depends largely on the pH of the skin surface. As regards AD patients, baseline skin pH is more alkaline than the average baseline skin pH of healthy skin, which is obviously associated with increased activation of proteases and subsequent impairment of skin barrier function. An increase in skin pH value enhances serine protease activity as well as kallikrein activity, resulting in corneodesmosome degradation, weakening the activity of enzymes responsible for ceramide synthesis. The decreased production of ceramides is also caused by the increased activity of sphingomyelin deacylase. Furthermore, high expression of sphingomyelin deacylase is an important determinant of ceramide deficiency. Ultimately, it dramatically increases the possibility of environmental allergens' penetration deeply through the damaged epidermal barrier [4, 12]. LEKTI (lympho-epithelial Kazal-type-related inhibitor), encoded by the SPINK5 gene, constitutes a complex of 15 serine protease inhibitors, and at least 4 of them have proven activity towards kallikrein. As the pH becomes more acidic, the inhibitory potential of LEKTI is reduced, and this leads to limited functional activity in the surface part of the stratum corneum [4].

Immunological aspects of epidermal barrier dysfunction in atopic dermatitis

Chromosome 5q31-33 seems to play a potential role in the development of immune barrier dysfunction, as it contains a family gene cluster for cytokines interleukin (IL)-3, IL-4, IL-5, IL-13 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which belong to the line profile of Th2, and among other things determine the development of skin inflammation in AD [13]. Importantly, there is evident linkage between immunological and structural dysfunctions, along with the process of keratinization within the skin barrier in AD (Table 1). It appears that the increased expression of cytokines characteristic for the Th2 profile (IL-4, IL-13, IL-25, IL-33) in patients with AD leads to a rise in the level of serine proteases. In addition, TNF-α together with Th2 profile cytokine (IL-4, IL-13, IL-31) enhances the release of thymic stromal lymphopoietin (TSLP) and limits the synthesis of long-chain free fatty acids, thus leading to the destruction of the lipid skin barrier [12]. Filaggrin deficiency enhances the expression of TSLP, a chemokine which is of key importance for the development of allergic inflammation in the AD [5]. TSLP, produced by keratinocytes, activates dendritic cells which in turn stimulate the differentiation of inflammatory Th2 cells and the production of cytokines. Interferon-γ (IFN-γ) plays an important role in the release of cytokines and

Table 1. Reciprocal dependencies between immunological and structural disorders within the skin barrier in patients with atopic dermatitis

- Stimulation of epidermis hyperplasia (IL-22)
- Stimulation of spongiosis (Th2, IL-4/IL-13, TNF)
- Curbing the last path of keratinocytes (IL-4, IL-13, IL-31, IL-25/Th2, IL-22/Th2, TNF), reversible epidermis hyperplasia
- Curbing AMP synthesis (cytokines Th2, IL-4, IL-13 and IL-33)
- Curbing lipid synthesis (cytokines Th2, IL-4/IL-13, IL-31 and TNF)
- Rising the expression S100A7, S100A8 and S100A9 (IL-22, IL-17)
- Stimulation of the TSLP synthesis in keratinocytes (IL-4/IL-13, TNF)
- Intensification of pruritus (IL-31, TSLP)
- Reinforcement of the antiviral response (IFN-γ, IFN-α, IL-29)

IL – interleukin, Th2 – lymphocytes Th2, TNF – tumor necrosis factor, IFN – interferon, TSLP – thymic stromal lymphopoietin, S100A7, S100A8 and S100A9 – calcium binding proteins, AMPs – antimicrobial proteins.

chemokines by keratinocytes [14]. Interleukin-31 causes exacerbated itchiness in AD sufferers [15] and all mechanical traumas (including the impulse to scratch the skin), exposure to various environmental allergens and bacterial infections significantly increase the expression of TSLP, IL-25 and IL-33, thereby strengthening the Th2-dependent response. This creates a sequence of interdependent events, leading to the chronic and recurrent course of skin inflammation so typical of AD [11].

In terms of immunology, the inflammatory response in AD is connected with, among other factors, the activation of T lymphocytes, dendritic cells, macrophages, keratinocytes, mast cells and eosinophils [3]. Microscopy imaging of inflamed or even seemingly healthy skin in the course of AD reveals perivascular infiltrates of T lymphocytes. In the acute stage of skin inflammation, the infiltrate consists of CD41 cells, antigen-presenting cells (Langerhans cells, other dendritic cells and macrophages) with IgE molecules bound to receptors on their surface; on top of that, degranulation of mast cells is also observed. On the other hand, in the stage of chronic skin inflammation, characterized by lichenification of varying intensity, significant accumulation of collagen in the dermis is visible, coupled with reduction of the number of T cells [3] as well as a rich cellular infiltrate of eosinophils and macrophages [13, 14]. A high number of T lymphocytes results from the increased number of CD4+ cells in AD patients [14]. In AD sufferers, activated T cells present on the skin, which reflect high levels of IFN-γ, usually complete apoptosis in the circulation, redirecting the immunological response to the survival of Th2 cells as a mechanism of Th2 dominance. In the affected skin areas, those T cells stimulate effector cytokines and induce the activation and apoptosis of keratinocytes. The naturally occurring T-reg cells (CD4+ CD25+ FoxP3+) with normal immunosuppressive activity are developed in peripheral blood vessels of patients with AD. T-reg cells are capable of impeding both Th1 and Th2 responses. Mutations of the nuclear factor of T-reg cells cause immune dysregulation characterized by hyper-IgE, food allergy and eczema. Moreover, following the stimulation with a staphylococcal superantigen (enterotoxin type B) the T-reg cells lose their immunosuppressive activity [2] and thus they may exacerbate skin inflammation.

As a result of the damage in the epidermal barrier, the local expression of pro-inflammatory cytokines and chemokines is increased. They bind with specific receptors within the vascular endothelium, activate the process of cell signaling and induce the expression of intercellular adhesion molecules. In line with the above, the adhesion of cells to the vessel wall is strengthened and facilitates the migration of pro-inflammatory cells, which create the infiltrate on the skin of AD sufferers [3, 14]. Those patients usually show increased expression of chemokines such as CCL22 and CCL17, which are responsible for recruitment of Th2 CCR4+ cells. The concentrations of CCL17, CCL22 and CCL27 chemokines correlates with the activity of the disease process itself, which is extremely significant and logical. The exacerbation of inflammation is closely connected with the predominance of Th2-type cytokines (e.g. IL-4, IL-13, IL-31 [3, 14]) synthesis and release. Compared to the skin of healthy individuals, seemingly healthy skin of AD sufferers is characterized by increased expression of IL-4 and IL-13, which does not concern other cytokines such as IL-5 or IFN-γ. On the other hand, the areas of inflammatory skin lesions (both acute and chronic) exhibit a significant increase in the expression of IL-4, IL-5 and IL-13 [13]. Compared to the acute skin inflammation in the course of AD, chronic skin inflammation is characterized by significantly lower expression of IL-4 and IL-13. In turn, the expression of IL-5 and IFN-γ, together with the infiltration of eosinophils and macrophages, is more prominently marked [13].

Some pro-inflammatory cytokines, mentioned above, combined with costimulatory molecules, initiate the differentiation of B lymphocytes to plasma cells, which produce antibodies of the IgE class. Receptors for those antibodies are located on the surface of myeloid dendritic cells, which include Langerhans cells and inflammatory dendritic epidermal cells (IDECs), found in the skin areas affected by lesions in AD sufferers [14]. Higher levels of IgE antibodies directed against allergens which penetrate the damaged skin barrier may facilitate capturing and presenting the allergen in atopic skin [3]. Langerhans cells play the main role in initiating the immunologic allergic response. They also control the presentation of antigens to T lymphocytes, which in turn migrate to the epidermis and release a number of cytokines inducing keratinocyte apoptosis [1]. Allergens stimulate the receptors on the Langerhans cells, which induces the release of chemotactic factors and the recruitment of IDEC progenitor cells and T lymphocytes. The stimulation of receptors on IDEC cells leads to the release of a high quantity

of inflammatory factors which contribute to the reinforcement of immunologic allergic response. The IDECs are responsible for the synthesis and release of Th1-type cytokines [14].

Plasmacytoid dendritic cells, on the other hand, are only found in AD skin lesions in small quantities. They play a major role in protection against viruses [3]. Plasmacytoid dendritic cells in peripheral blood of AD patients have receptors bound to IgE. The modified immunological function of those cells in AD patients, following the allergen stimulation of receptors, may contribute to deficiencies in the production of type I IFNs and thus cause AD patients to be more prone to viral skin infections [3].

In the case of AD, significantly increased expression of IL-31 was observed in the area of inflammation foci; the inflammation is accompanied by an exacerbated itching sensation of the skin. This phenomenon may be ascribed to a whole variety of causes, e.g. increased release of histamine as well as colonization of the skin by *Staphylococcus aureus*, i.e. the activity of *S. aureus* superantigens [14]. Chronic inflammation in the course of AD is connected with the predominance of cytokines characteristic of Th1 lymphocytes (IL-12, IL-18, IL-11, TGF-β as well as IL-31). It has been found, for instance, that increased expression of IL-12 correlates with the creation of cellular infiltrates composed of eosinophils and macrophages [13], whilst IL-5 is responsible for the maturation of eosinophils and the extension of their survival. The chronic nature of the skin inflammation process is also caused by increased production of GM-CSF and thus also by inhibited monocyte apoptosis [3].

Conclusions

Maintaining the integrity of the epidermal barrier is a key factor in preventing the symptoms of AD and in alleviating their exacerbation. The pathogenesis of AD features genetic factors, including immunological ones, leading to a number of processes which have a significant effect on the dysfunction of the epidermal barrier. This pathology may be further exacerbated by epigenetic factors, described in detail in part 2 of this paper, which outlines the role of skin care as an exceptionally significant element of AD prevention and treatment.

Conflict of interest

Professor Magdalena Czarnecka-Operacz MD, PhD: training courses, lecturing (post-graduate training), (Almiral, Astellas, Meda, Berlin Chemie, Novartis), member of the Skin Academy and Advisory Board (Almiral), clinical trals (phase 2,3) and Scientific projects (Almiral, Astellas, Meda, Berlin Chemie, Novartis). Others authors declare no conflict of interest.

References

1. Ring J, Darsow U. (eds.) Atopowe zapalenie skóry. In: Braun-Falco Dermatologia. Burgdorf WHC, Plewig G, Wolff HH, et al. (eds.) Czelej, Lublin 2010; 425-41.
2. Boguniewicz M, Leung DYM. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunol Rev 2011; 242: 233-46.
3. Akdis CA, Akdis M, Bieber T, et al. Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. Allergy 2006; 61: 969-87.
4. Cork MJ, Danby SG, Vasilopoulos Y, et al. Epidermal barrier dysfunction in atopic dermatitis. J Invest Dermatol 2009; 129: 1892-908.
5. McAleer MA, Irvine AD. The multifunctional role of fillagrin in allergic skin disease. J Allergy Clin Immunol 2013; 131: 280-91.
6. Samochocki Z. Wpływ zaburzeń bariery naskórkowej na rozwój i przebieg atopowego zapalenia skóry. Alergia 2010; 4: 19-24.
7. Kurowski M, Kowalski ML. Filagryna i jej rola w patomechanizmie chorób alergicznych. Alergia Astma Immunologia 2009; 15: 95-100.
8. Trzeciak M, Gleń J, Rębała K, et al. Coexistence of 2282del4 FLG gene mutation and IL-18 –137G/C gene polymorphism enhances the risk of atopic dermatitis. Adv Dermatol Allergol 2016; 33: 57-62.
9. Filipowska-Grońska A, Weryńska-Kalemba M, Bożek A, et al. The frequency of polymorphic variants of filaggrin gene and clinical atopic dermatitis. Adv Dermatol Allergol 2016; 33: 37-41.
10. Woźniak M, Kaczmarek-Skamira E, Romańska-Gocka K, et al. The prevalence of mutations in the gene encoding filaggrin in the population of Polish patients with atopic dermatitis. Adv Dermatol Allergol 2016; 33: 128-33.
11. Leung DYM. New insights into atopic dermatitis: role of skin barrier and immune dysregulation. Allergol Int 2013; 62: 151-61.
12. Peng W, Novak N. Pathogenesis of atopic dermatitis. Clin Exp Allergy 2015; 45: 566-74.
13. Leung DYM. Pathogenesis of atopic dermatitis. J Allergy Clin Immunol 1999; 104: 99-108.
14. Silny W, Czarnecka-Operacz M, Gliński W, et al. Atopowe zapalenie skóry – współczesne poglądy na patomechanizm oraz metody postępowania diagnostyczno-leczniczego. Stanowisko grupy specjalistów Polskiego Towarzystwa Dermatologicznego. Postep Dermatol Alergol 2010; 27: 365-83.
15. Furue M, Yamamura K, Kido-Nakahara M, et al. Emerging role of interleukin-31 and interleukin-31 receptor in pruritus in atopic dermatitis. Allergy 2018; 73: 29-36.