Keratinocytes in allergic skin diseases

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Current Opinion in Allergy and Clinical Immunology 2010, 10:452–456

Purpose of review

The aim of the review is to provide up-to-date information on the multiple roles of epidermal keratinocytes in the immune reactions associated with allergic contact dermatitis and atopic dermatitis skin diseases.

Recent findings

In the last two decades, it has become clear that keratinocytes are highly active immunological cells, with major control over the acute and the chronic phases of skin inflammation by means of cytokine/chemokine production and surface molecule expression. Keratinocyte responses in skin allergic reactions are rather disease-specific and keratinocytes from genetically determined skin disorders, including atopic dermatitis, show intrinsic abnormalities in their capacity to respond to trigger factors.

Summary

Lymphokines and cytokines released by T lymphocytes and other immune cells represent the most important stimuli that elicit the inflammatory activation of keratinocytes. Depending on the type and extent of T-cell infiltrate present in allergic contact dermatitis and atopic dermatitis skin lesions, keratinocytes are exposed to different cytokine micromilieu and, in turn, produce flogosis mediators qualitatively and quantitatively specific for each disease. Keratinocyte-derived inflammatory molecules amplificate skin immune responses associated with allergic contact dermatitis and atopic dermatitis, and contribute to the disease process and clinical phenotype development.

Keywords

allergic contact dermatitis, atopic dermatitis, skin inflammation

Curr Opin Allergy Clin Immunol 10:452–456

1528-4050

Introduction

The skin is a frequent site of hypersensitivity reactions against apparently harmless antigens, such as the haptens causing allergic contact dermatitis (ACD), and of relapsing-remitting chronic disorders resulting from altered immune responses to environmental factors, such as atopic dermatitis. Although both ACD and atopic dermatitis diseases are driven by T cells and characterized by eczematous changes in the epidermis, the clinical features of these skin diseases as well as the pathogenetic mechanisms leading to disease expression are substantially different. In fact, although ACD affects adults primarily and occurs in the skin at sites of contact with haptens, atopic dermatitis usually develops in early childhood with a characteristic distribution pattern that changes with age, and is frequently associated with respiratory atopy and elevated serum levels of immunoglobulin E (IgE) reactive to environmental protein allergens [1,2••]. In addition, genetic factors are certainly very important in the development of atopic dermatitis whereas they have limited relevance in ACD [3•]. Concerning T-cell contribution to pathogenetic processes, although the expression of ACD is associated with the prominent presence of type I T lymphocytes and T helper (Th) 17 cells, the onset of acute atopic dermatitis is governed by Th2 cells and a percentage of Th17 lymphocytes [4,5]. Moreover, our knowledge of the immune mechanisms underlying ACD is much more detailed, thanks to the availability of valuable animal models, whose development, in the case of atopic dermatitis, is hampered by the complex and multifactorial pathogenesis. However, atopic dermatitis and ACD diseases can also share some immunological similarities. Among them, the most intriguing pathological aspect common to both allergic skin disorders is the prominent proinflammatory activity of resident keratinocytes, which serve as initiators and amplifiers of local immune responses. During ACD and atopic dermatitis development, in fact, keratinocytes sense haptens or environmental protein allergens, respectively, and, in turn, initiate a program of enhanced or de-novo expression of inflammatory molecules representing the starting point of primary skin inflammation [6••]. Furthermore, the prominent presence of T-cell infiltrate in both ACD and atopic dermatitis skin establishes an inflammatory

cytokine micromilieu responsible for the massive activation of keratinocytes. Following exposure to T cell-derived lymphokines, keratinocytes express a plethora of cytokines, chemokines and accessory receptors, which potently amplifies immune responses of innate and adaptive skin immunity [6••]. In this review, the multiple roles of keratinocytes in initiating and sustaining the inflammatory processes associated with ACD and atopic dermatitis will be discussed.

Keratinocyte contribution to allergic contact dermatitis immune responses

ACD reactions to haptens comprehend two main phases: sensitization and elicitation. In the sensitization phase, haptens are captured by resident dendritic cells that migrate to regional lymph nodes to activate and clonally expand specific T-cell precursors. Reexposure to the relevant hapten initates the elicitation phase and clinical expression of ACD, characterized by the rapid recruitment and activation of memory specific T cells at the sites of hapten challenge [7]. Although T lymphocytes are pathogenetically fundamental for ACD expression, several studies have demonstrated that also keratinocytes shape the epidermal immune responses to contact allergen. In fact, during sensitization, haptens induce a direct inflammatory activation of keratinocytes, resulting in the expression of inflammatory molecules, such as tumor necrosis factor α (TNF-α), interleukin (IL)-1, IL-6 and granulocyte-macrophage colony stimulating factor (GM-CSF), which, in turn, can activate various cell populations of the skin, including resident Langerhans cells and dermal endothelial cells [8]. TNF-α released by hapten-activated keratinocytes can act in an autocrine manner on keratinocytes themselves and induce intercellular adhesion molecule (ICAM)-1 and CXCL8 expression observed *in vivo* in these cells during ACD reactions. In the early phase of ACD, basal layer keratinocytes directly following their activation with haptens also express substantial amounts of CCL2, as assessed by in-situ hybridization performed on biopsies taken from ACD patients at different time points after hapten application [9]. Similarly, CCL27 is strongly present in epidermal keratinocytes of basal and suprabasal after nickel exposure, with this expression colocalizing with CCR10 perivascular reactivity [10]. Taken together, these data indicate that in the initial phase of ACD reactions, hapten-activated keratinocytes are important producers of inflammatory mediators and activators of other resident skin cells. At this early stage, T cells and other leukocyte subpopulations are not yet recruited to the skin, supporting the concept that keratinocytes are directly activated by contact allergens rather than by cytokines released by infiltrating cells. This hypothesis is supported by a study of transcriptomics of Gazel *et al.* [11] demonstrating that nickel alone can regulate in keratinocytes a very high number of genes, among which there are those associated with cell proliferation, inhibition of apoptosis, and remodeling of extracellular matrix. Importantly, nickel regulates a set of secreted signaling proteins potentially involved in nickel-caused allergic reactions, including IL-18, a cytokine regulating Langerhans cell migration, and T cell responses. IL-18 can be upregulated in keratinocytes also by the contact allergen trinitrochlorobenzene through the induction of inflammasome, an intracellular signaling platform for the activation of caspase I that cleaves pro-IL-18 into its active form [12,13]. The inflammasome-dependent processing and secretion of IL-18, but also of IL-1β, in keratinocytes have been suggested to trigger proinflammatory signals promoting T cell responses and expansion in the skin [12].

As mentioned above, during the elicitation phase of ACD, keratinocytes are exposed to a cytokine milieu established primarily by T cells, which accumulate in the skin in both dermal and epidermal compartments. Among hapten-specific T lymphocytes, interferon (IFN)-γ-secreting Th1 cells predominate although a substantial proportion of Th17 cells – releasing high amounts of IL-17, IL-22 and TNF-α – are present and further reinforce the proinflammatory activation of resident cells of the skin [4]. Indeed, T cell-derived cytokines, in particular IFN-γ, target primarily keratinocytes, which in turn highly express both membrane-bound and soluble ICAM-1, major histocompatibility complex (MHC) class II molecules and upregulate MHC class I and Fas [14,15]. By expressing these molecules, antigen-loaded keratinocytes can be the target of T cell-mediated cytotoxicity, with $CD8^+$ T cells being responsible for the initiation of epidermal damage during ACD [15]. In the amplification phase of ACD, cytokine-activated keratinocytes become an important source of chemotactic factors, express chemokine receptors, and hence can modulate immune responses also by selectively attracting distinct cell types into the skin. The expression of CXCL9, CXCL10 and CXCL11 begins at 12 h after hapten application on sensitive skin and reaches the maximum at 72 h, paralleling the strong infiltration of lymphocytes [9]. CXCL10, CXCL9 and CXCL11 are the chemokines which are more abundantly produced by activated keratinocytes, with the relevance of CXCR3 agonists residing in the fact that more than 70% of cells infiltrating ACD skin express CXCR3 [9,16]. Lymphokine-activated keratinocytes also upregulate CCL27, CCL5, CCL22 and CCL1, with the latter two chemokines being produced at lower levels and with delayed kinetics [9,17]. In-vitro studies performed on normal human keratinocytes activated with nickel-specific T cell-derived supernatants demonstrated that keratinocytes appear more sensitive to Th1-derived than to Th2-derived lymphokines in terms of the variety and amounts of chemokine released, and promote the

preferential migration of Th1 lymphocytes [17]. These findings can in part explain the Th2 to Th1 switch observed in some inflammatory skin diseases, including atopic dermatitis in the chronic phase.

Keratinocyte contribution to the pathogenesis of atopic dermatitis

An amount of evidence defines keratinocytes as enhancer cells of immune responses in atopic dermatitis. Owing to their altered genetic background, keratinocytes of atopic dermatitis skin respond peculiarly and excessively to environmental stimuli and to endogenous T cell-derived cytokines. In-vitro studies have shown that keratinocytes from patients with atopic dermatitis produce increased amounts of certain types of chemokines and cytokines compared with healthy cells or keratinocytes isolated from psoriatic skin. For instance, atopic dermatitis keratinocytes are a source of CCL5 when activated *in vitro* with IFN-γ or TNF-α, probably as a consequence of a functional mutation in CCL5 gene [18,19]. CCL5 is also strongly expressed in basal keratinocytes in atopic dermatitis skin *in vivo* and may have a role in promoting the accumulation of Th1 lymphocytes in the chronic phase of the disease. Keratinocytes cultured from patients with atopic dermatitis overproduce CCL20 [20]. Interestingly, disruption of the epidermal permeability barrier upregulates expression of CCL20, revealing an important mechanism for the initial influx of dendritic cells and T cells in the skin of patients with atopic dermatitis. Similarly to ACD lesions, both acute and chronic atopic dermatitis lesions exhibit strong expression of CCL27 in the epidermis as well as many $CCR10^+$ T cells [10]. When compared with keratinocytes from nonatopic individuals, keratinocytes of atopic dermatitis patients produce higher levels of GM-CSF and TNF-α, both basally and in response to IL-1 or IFN-γ [21]. Elevated production of cytokines by atopic dermatitis keratinocytes may be secondary to dysregulated signal transduction. For example, higher expression of GM-CSF in atopic dermatitis keratinocytes is concomitant to an upregulated activation of activator protein-1 transcription factor [22]. Activator protein-1 is prominently activated by various cytokines, including IL-4, IFN-γ and TNF-α, and activator protein-1 binding sites are located in the promoters of the genes that encode a vast array of cytokines and chemokines, including CCL5. These data support the concept that the contribution of keratinocytes to the pathogenesis of atopic dermatitis is linked to the presence of distinct alterations in their capacity to respond to proinflammatory stimuli and that these abnormalities can be important in the inflammatory hyperreactivity of atopic dermatitis lesions. In particular, interactions between epithelial cells and dendritic cells may be important in the initiation and persistence of inflammation in atopic dermatitis. The propensity of keratinocytes that are isolated from atopic dermatitis lesions to produce higher-than-normal levels of some growth factors, cytokines and chemokines may stimulate precursor cells to differentiate into dendritic cells, as well as increase the recruitment of activated dendritic cells into atopic dermatitis skin [21]. Interestingly, recent findings indicate that thymic stromal lymphopoietin (TSLP) influences dendritic cell immune responses. TSLP is highly expressed by keratinocytes from atopic dermatitis lesions and activates dendritic cells to secrete Th2-recruiting chemokines, in addition to prompting the differentiation of naïve T cells into inflammatory Th2 cells [23]. Skin-restricted overexpression of TSLP in transgenic mice results in an atopic dermatitis-like phenotype, with the development of eczematous lesions, a dramatic increase in Th2 T cells and elevated serum levels of IgE [24]. Keratinocyte-derived TSLP has also been suggested to be a prerequisite for the development of asthma in atopic dermatitis patients [25•]. Interestingly, keratinocytes can express the IL-1 and IL-33 receptor, ST2, in the acute phase of atopic dermatitis. A genetic association between atopic dermatitis and a single-nucleotide polymorphism has been found in the promoter of the ST2 gene, and this polymorphism can be responsible for the upregulation of ST2 gene expression [26]. In parallel, IL-33, induces the expression of IL-4 *in vivo*, which may explain the higher IgE serum levels found in atopic dermatitis patients with the ST2 polymorphism [27].

It is well known that patients with atopic dermatitis frequently have bacterial and viral skin infections [28,29]. For instance, atopic dermatitis skin is heavily colonized by superantigen-releasing *Staphylococcus aureus*, which is able to induce the expansion of specific T cell subpopulations [30,31], and to stimulate an IgE-mediated hyperreactivity response [32]. In addition, herpes simplex virus infections of the skin occur frequently in atopic dermatitis. This predisposition to cutaneous infections can be related to a substantial deficiency of innate protective mechanisms in atopic dermatitis skin. In particular, a set of antimicrobial peptides, namely HBD2, HBD3 and LL-37, are significantly decreased in keratinocytes of atopic dermatitis patients. This reduced expression of antimicrobial peptides was found to be an acquired rather than intrinsic defect, as a result of the increased Th2 cytokine expression in atopic dermatitis skin [33]. The enhanced bacterial and viral dissemination in patients with atopic dermatitis has also been associated with a compromised status of the epidermal barrier. Several recent studies have demonstrated an association between atopic dermatitis and decreased keratinocyte expression of filaggrin (FLG), a protein of the epidermal differentiation complex involved in barrier function [34–36]. In particular, null mutations in the gene that encodes FLG were shown to be linked to the

phenotype of atopic dermatitis and asthma-associated atopic dermatitis whereas no associations were observed with psoriasis. However, these mutations have been observed in less than one third of general populations of atopic dermatitis patients of European descent [34–36]. Additionally, these mutations were heterozygous in most cases. Indeed, Howell *et al.* [37] demonstrated that FLG deficiency in patients with atopic dermatitis is owing to the overexpression of Th2 cytokines, which downregulate FLG during the differentiation process. Therefore, it is likely that many patients with atopic dermatitis acquire FLG deficiency and subsequent barrier disruption as a result of the local inflammatory immune responses. Similarly to FLG, other proteins of the epidermal differentiation complex, namely loricrin, involucrin, and late cornified envelope proteins, had reduced or compromised levels of expression in lesional atopic dermatitis skin [38]. The dysfunction of epidermal barrier in atopic dermatitis has also been associated with an abnormal serine protease activity in the epidermis. In particular, Hansson *et al.* [39] found that a transgenic mouse model overexpressing human stratum corneum chymotryptic enzyme (SCCE) exhibited symptoms of chronic itchy dermatitis resembling atopic dermatitis. Being SCCE active also in the proteolytic degradation of distinct lipid processing enzymes, including acidic sphingomyelinase [40], enhanced SCCE could reasonably provide a direct link between enhanced serine protease activity and reduced ceramide expression in atopic dermatitis skin. The importance of regulated proteolysis in epithelia is well demonstrated by the discovery of the lympho-epithelial kazal-type 5 serine protease inhibitor (LEKTI), encoded by the Spink5 gene. LEKTI defective inhibitory regulation results in increased protease activity in the stratum corneum and overdesquamation of corneocytes, as demonstrated in Netherton disease [41]. LEKTI is strongly expressed in differentiated keratinocytes of normal skin and contribute to the integrity and protective barrier function of the skin. Previously, Walley *et al.* [42] identified six polymorphisms in Spink5 gene and found that a particular variant (Glu420Lys) in LEKTI significantly associated with atopy, including atopic dermatitis.

Conclusion

Keratinocytes are immunological cells deeply involved in allergic skin responses, as delineated in this review. Although they play a pathogenetic role in both ACD and atopic dermatitis, the keratinocyte immune function in ACD and atopic dermatitis skin is quite different. In fact, keratinocytes of ACD and atopic dermatitis lesions, being exposed to different cytokines micromilieu, produce flogosis mediators qualitatively and quantitatively specific for each skin disorder. Moreover, atopic dermatitis keratinocytes have genetic defects rendering them more susceptible to allergen insults. Further advancements in the understanding of the keratinocyte-driven pathogenetic events may afford the identification of novel targets for therapeutic intervention of ACD and atopic dermatitis.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 512).

1 Nosbaum A, Vocanson M, Rozieres A, *et al.* Allergic and irritant contact dermatitis. Eur J Dermatol 2009; 19:325–332.

2 •• Werfel T. The role of leukocytes, keratinocytes, and allergenspecific IgE in the development of atopic dermatitis. J Invest Dermatol 2009; 129:1878–1891.
This is an extensive review on the inflammatory mechanisms and immunological aspects of atopic dermatitis.

3 • Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. J Invest Dermatol 2009; 129:543–552.
This provides an update on current understanding of the genetic basis of atopic eczema, and suggests possible future research goals in this fast-moving field.

4 Pennino D, Eyerich K, Scarponi C, *et al.* IL-17 amplifies human contact hypersensitivity by licensing hapten nonspecific Th1 cells to kill autologous keratinocytes. J Immunol 2010; 184:4880–4888.

5 Eyerich K, Pennino D, Scarponi C, *et al.* IL-17 in atopic eczema: linking allergen-specific adaptive and microbial-triggered innate immune response. J Allergy Clin Immunol 2009; 123:59–66.

6 •• Albanesi C, Pastore S. Pathobiology of chronic inflammatory skin diseases: interplay between keratinocytes and immune cells as a target for anti-inflammatory drugs eczema genetics: current state of knowledge and future goals. Curr Drug Metab 2010; 11:210–227.
This is fully exhaustive up-to-date information on the immunological basis of ACD, atopic dermatitis and psoriasis, with special emphasis on potentially effective targets for novel anti-inflammatory drugs.

7 Cavani A, De Pità O, Girolomoni G. New aspects of the molecular basis of contact allergy. Curr Opin Allergy Clin Immunol 2007; 7:404–408.

8 Bonneville M, Chavagnac C, Vocanson M, *et al.* Skin contact irritation conditions the development and severity of allergic contact dermatitis. J Invest Dermatol 2007; 127:1430–1435.

9 Goebeler M, Trautmann A, Voss A, *et al.* Differential and sequential expression of multiple chemokines during elicitation of allergic contact hypersensitivity. Am J Pathol 2001; 158:431–440.

10 Homey B, Alenius H, Müller A, *et al.* CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat Med 2002; 8:157–165.

11 Gazel A, Rosdy M, Tornier C, *et al.* Transcriptional profiling defines the effects of nickel in human epidermal keratinocytes. J Cell Physiol 2008; 217:686–692.

12 Watanabe H, Gaide O, Pétrilli V, *et al.* Activation of the IL-1beta-processing inflammasome is involved in contact hypersensitivity. J Invest Dermatol 2007; 127:1956–1963.

13 Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. Annu Rev Immunol 2009; 27:229–265.

14 Albanesi C, Cavani A, Girolomoni G. Interferon-gamma-stimulated human keratinocytes express the genes necessary for the production of peptide-loaded MHC class II molecules. J Invest Dermatol 1998; 110:138–142.

15 Traidl C, Sebastiani S, Albanesi C, *et al.* Disparate cytotoxic activity of nickel-specific CD8+ and CD4+ T cell subsets against keratinocytes. J Immunol 2000; 165:3058–3064.

16 Albanesi C, Scarponi C, Sebastiani S, *et al.* IL-4 enhances keratinocyte expression of CXCR3 agonistic chemokines. J Immunol 2000; 165:1395–1402.

17 Albanesi C, Scarponi C, Sebastiani S, *et al.* A cytokine-to-chemokine axis between T lymphocytes and keratinocytes can favor Th1 cell accumulation in chronic inflammatory skin diseases. J Leukoc Biol 2001; 70:617–623.

18 Giustizieri ML, Mascia F, Frezzolini A, *et al.* Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. J Allergy Clin Immunol 2001; 107:871–877.

19 Nickel RG, Casolaro V, Wahn U, *et al.* Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. J Immunol 2000; 164:1612–1616.

20 Schmuth M, Neyer S, Rainer C, *et al.* Expression of the C-C chemokine MIP-3 alpha/CCL20 in human epidermis with impaired permeability barrier function. Exp Dermatol 2002; 11:135–142.

21 Pastore S, Fanales-Belasio E, Albanesi C, *et al.* Granulocyte macrophage colony stimulating factor is overproduced by keratinocytes in atopic dermatitis: implications for sustained dendritic cell activation in the skin. J Clin Invest 1997; 99:3009–3017.

22 Pastore S, Giustizieri ML, Mascia F, *et al.* Dysregulated activation of activator protein 1 in keratinocytes of atopic dermatitis patients with enhanced expression of granulocyte/macrophage-colony stimulating factor. J Invest Dermatol 2000; 115:1134–1143.

23 Ito T, Wang YH, Duramad O, Hori T, *et al.* TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40. J Exp Med 2005; 202:1213–1223.

24 Yoo J, Omori M, Gyarmati D, *et al.* Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. J Exp Med 2005; 202:541–549.

25 Demehri S, Morimoto M, Holtzman MJ, *et al.* Skin-derived TSLP triggers • progression from epidermal-barrier defects to asthma. PLoS Biol 2009; 7:e1000067.

A paper providing the experimental proof that keratinocyte-derived TSLP initiates a cascade of allergic inflammation in atopic dermatitis skin, and it is responsible for the progression from atopic dermatitis to asthma.

26 Shimizu M, Matsuda A, Yanagisawa K, *et al.* Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. Hum Mol Genet 2005; 14:2919–2927.

27 Schmitz J, Owyang A, Oldham E, *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005; 23:479–490.

28 Nishijima S, Namura S, Kawai S, *et al.* Staphylococcus aureus on hand surface and nasal carriage in patients with atopic dermatitis. J Am Acad Dermatol 1995; 32:677–679.

29 Cho SH, Strickland I, Boguniewicz M, *et al.* Fibronectin and fibrinogen contribute to the enhanced binding of Staphylococcus aureus to atopic skin. J Allergy Clin Immunol 2001; 108:269–274.

30 Blauvelt A, Hwang ST, Udey MC. Allergic and immunologic diseases of the skin. J Allergy Clin Immunol 2003; 111:S560–S570.

31 Zasloff M. Antimicrobial peptides in health and disease. N Engl J Med 2002; 347:1199–1200.

32 Leung DY, Boguniewicz M, Howell MD, *et al.* New insights into atopic dermatitis. J Clin Invest 2004; 113:651–657.

33 Nomura I, Goleva E, Howell MD, *et al.* Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. J Immunol 2003; 171:3262–3269.

34 Palmer CN, Irvine AD, Terron-Kwiatkowski A, *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006; 38:441–446.

35 Weidinger S, Illig T, Baurecht H, *et al.* Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 2006; 118:214–219.

36 Marenholz I, Nickel R, Ruschendorf F, *et al.* Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006; 118:866–871.

37 Howell MD, Kim BE, Gao P, *et al.* Cytokine modulation of AD filaggrin skin expression. J Allergy Clin Immunol 2007; 120:150–155.

38 Sugiura H, Ebise H, Tazawa T, *et al.* Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope. Br J Dermatol 2005; 152:146–149.

39 Hansson L, Backman A, Ny A, *et al.* Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. J Invest Dermatol 2002; 118:444–449.

40 Hachem JP, Man MQ, Crumrine D, *et al.* Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. J Invest Dermatol 2005; 125:510–520.

41 Descargues P, Deraison C, Bonnart C, *et al.* Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet 2005; 37:56–65.

42 Walley AJ, Chavanas S, Moffatt MF, *et al.* Gene polymorphism in Netherton and common atopic disease. Nat Gen 2001; 29:175–178.