

Netherton syndrome: skin inflammation and allergy by loss of protease inhibition

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Abstract Netherton syndrome (NS) is a rare autosomal recessive skin disease with severe skin inflammation and scaling, a specific hair shaft defect and constant allergic manifestations. NS is caused by loss-of-function mutations in *SPINK5* (serine protease inhibitor of kazal type 5) encoding LEKTI-1 (lympho-epithelial kazal type related inhibitor type 5) expressed in stratified epithelia. In vitro and in vivo studies in murine models and in NS patients have cast light on the pathogenesis of the disease and shown that LEKTI deficiency results in unopposed kallikrein-related peptidase 5 (KLK5) and KLK7 activities and to the overactivity of a new epidermal protease, elastase 2 (ELA2). Two main cascades initiated by KLK5 activity have emerged. One results in desmoglein 1 degradation and desmosome cleavage leading to stratum corneum detachment. KLK5 also activates KLK7 and ELA2, which contribute to a defective skin barrier. This facilitates allergen and microbe penetration and generates danger signals leading to caspase 1 activation and the production of active interleukin-1 β . In parallel, KLK5 activates a specific cascade of allergy and inflammation by activating protease-activated receptor-2 (PAR-2) receptors. PAR-2 activation triggers

the production of the major pro-Th2 cytokine TSLP (thymic stromal lymphopoietin) and several inflammatory cytokines, including tumour necrosis factor- α . Levels of thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) also contribute to allergy in a PAR-2-independent manner. Patient investigations have confirmed these abnormalities and revealed a wide spectrum of disease expression, sometimes associated with residual LEKTI expression. These results have demonstrated that the tight regulation of epidermal protease activity is essential for skin homeostasis and identified new targets for therapeutic intervention. They also provide a link with atopic dermatitis through deregulated protease activity, as recently supported by functional studies of the E420K LEKTI variant.

Keywords *Serine protease inhibitor of kazal type 5 (SPINK5)* · Lympho-epithelial Kazal type related inhibitor type 5 (LEKTI) · Kallikreins (KLK) · Protease-activated receptor-2 (PAR-2) · Thymic stromal lymphopoietin (TSLP)

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Introduction

Netherton syndrome (NS) is a rare genodermatosis with severe skin inflammation and allergy, and was first described by Comel in 1949 and Netherton in 1958 (Comel 1949; Netherton 1958). The defective gene, *SPINK5* (serine protease inhibitor of kazal type 5), encodes lympho-epithelial kazal type related inhibitor type 5 (LEKTI), a serine protease inhibitor of kazal type expressed in all stratified epithelia and in the Hassall corpuscles of the thymus (Magert et al. 1999; Chavanas et al. 2000; Bitoun et al. 2003). *Spink5* knock-out murine models have been essential in understanding the pathogenesis of the disease and have oriented NS patient investigations. They have identified

kallikreins as major LEKTI targets. In particular, unopposed kallikrein-related peptidase 5 (KLK5) activity plays a key role in the initiation of a dual biological cascade that has profound effects on skin barrier integrity and permeability and also induces skin inflammation and allergy through PAR-2-dependent and PAR-2-independant pathways.

Clinical presentation

NS is characterised by the clinical triad of congenital ichthyosiform erythroderma, a specific hair shaft abnormality (bamboo hair or trichorrhexis invaginata) and constant atopic manifestations with elevated immunoglobulin E (IgE) levels in the serum. The disease is often severe and can be lifethreatening in newborns and infants (Traupe 1989; Ong and Harper 2006; Hovnanian 2012).

In infancy

Newborns affected with NS are classically presented with congenital erythroderma and scaling, with considerable variations in the severity and extension of scaling and redness of the skin (Hausser and Anton-Lamprecht 1996; Weber et al. 2001; Ong and Harper 2006). The initial presentation evolves into persistence of erythroderma in severe cases or into the typical pattern of serpiginous and migratory scaling plaques with a double-edged scale referred as ichthyosis linearis circumflexa (ILC; Fig. 1). ILC is usually not permanent and undergoes flaring interspaced by clearing periods. Skin lesions itch, causing irritability and sleeplessness. During the neonatal period, infants are at high risk of life-threatening complications, including hypernatraemic dehydration, systemic sepsis and malnutrition. Hair, eyebrows and eyelashes can be present, sparse or absent at birth. Hair



Fig. 1 Clinical aspects of Netherton syndrome (NS). **a** Congenital erythroderma with scaling. **b** Erythroderma with transient alopecia and absence of eyebrows. **c** Eczematous-like lesions with fine scaling. **d** Severe skin inflammation with superficial desquamation. **e** Abnormal hair shaft showing protrusion of its distal part into its proximal part

(trichorrhexis invaginata or bamboo hair). **f** Partial alopecia predominating on the occiput, with short, fragile, thin, spiky and broken hair. **g** Ichthyosis linearis circumflexa (ILC) composed of serpiginous and migratory patches with red and double-scaling edges

is usually slow to grow and is classically rare, thin, spiky and fragile. However, striking differences between the hair of patients can be seen, with some patients having hair with a normal appearance. Light microscopy examination almost always shows trichorrhexis invaginata (TI) or bamboo hair, which refers to the protrusion of the distal part of the hair shaft into the cup-shape of its proximal part. TI is specific for NS but usually develops after 1 year of age and might not be present in all hair. Matchstick and golf-tee hairs result from broken TI. Trichorrhexis nodosa can also be seen but is not specific for NS. Systemic and lung infections, persistent and chronic diarrhea, failure to thrive, growth retardation and short stature are common complications. Rare complications include intracranial haemorrhage, staphylococcal scaled skin syndrome (Chao et al. 2005), hypothyroidism (Garty and Nimri 2008), acute pancreatitis (Soreide et al. 2005), bilateral renal vein thrombosis (Pohl et al. 1998), rickets (Andre et al. 2005) and pulmonary hypertension (Macknet et al. 2008).

In adulthood

The skin condition usually improves with age and adults may present localised patches of redness and scaling or ILC. The course of the disease however fluctuates, showing inflammatory flaring with erythroderma and diffuse or localised superficial scaling. Eczematous-like lesions are frequent and are a major cause of discomfort causing pruritus, scratching and infections aggravating skin lesions. Lichenification of the popliteal and antecubital fossae is often present. Rare but severe complications include human papilloma virus (HPV)-positive papillomatous skin lesions of the groin and perineal regions (Folster-Holst et al. 1999; Weber et al. 2001), spinous cell carcinoma (Hintner et al. 1980; Kubler et al. 1987; Saghari et al. 2002; Krasagakis et al. 2003) and giant condyloma of Buschke-Lowenstein (Li et al. 2011).

Atopic manifestations

Atopic features are constant during infancy and adulthood. Eczematous-like lesions, allergic asthma, allergic rhinitis, urticaria and angioedema are found to a variable extent in NS patients (Sun and Linden 2006). Pruritus is a constant feature, often being severe and disabling and causing sleeplessness and scratching, which aggravates skin lesions. Allergies to common food and airborne allergens are frequent, with elevated specific IgE. Blood eosinophils can be elevated and IgE levels are almost always high, sometimes exceeding 10,000 IU/ml.

Variability in disease expression

In addition to the classical clinical presentation, a wide range of disease severity can be seen between patients

(Ong and Harper 2006; Hovnanian 2012). These differences can occur in infancy and/or in adulthood. In particular, NS neonates can be presented with erythroderma with limited scaling confined to the fingers and toes. In contrast, NS infants can show diffuse, sometimes continuous, sheets of peeling with minor or no erythroderma. Scales are usually thin but thick seborrheic-like hyperkeratosis is possible, in particular on the scalp and forehead. TI might be not detectable because of complete alopecia or because of the young age of the patient. Clinical differences can also be seen between adults with respect to the presence and/or intensity of redness of the skin and scaling, hair abnormalities, eczematous-like lesions and the aspect and extent of ILC. In particular, in some cases, hair can have a normal appearance with no TI, which should not formally exclude the diagnosis of NS. The use of powerful diagnostic tests such as LEKTI immunodetection in the skin and *SPINK5* sequence analysis have facilitated the recognition of these “non-classical” forms of NS.

Immune abnormalities

Only a few immunological studies have been reported in NS (Judge et al. 1994; Stryk et al. 1999; Van Gysel et al. 2001; Renner et al. 2009). The most frequent finding is elevated specific IgE levels to common allergens, although a Th2 skew has not been consistently found. Lymphocyte count and subphenotypes and immunoglobulin levels are usually normal. Two studies have described a selective antibody deficiency to bacterial polysaccharide antigens (Stryk et al. 1999; Renner et al. 2009) and a recent study has reported reduced natural killer (NK) cell function with a normal count of NK cells (Renner et al. 2009). These results are consistent with a functional immune defect that could contribute to the frequent infections seen in NS.

Pathology

Skin sections of NS patients show epidermal hyperplasia (acanthosis) with elongation of the rete ridges, partial or complete lack of the granular layer, persistence of nuclei in the stratum corneum (parakeratosis) and stratum corneum detachment (Hausser and Anton-Lamprecht 1996). A mild to moderate inflammatory dermal infiltrate can be seen. In a majority of cases, LEKTI immunostaining shows the complete absence of LEKTI detection in the granular layer and in the inner root sheath (IRS) of the hair follicles, a specific characteristic of NS (Bitoun et al. 2003; Ong et al. 2004). In rare cases, LEKTI staining is markedly reduced in the granular layer and IRS; this can be seen patients with *SPINK5* “leaky” splice site mutations allowing for the synthesis of some LEKTI polypeptides. In contrast, a patchy LEKTI

staining pattern in the epidermis is seen in atopic dermatitis (AD) and poriasis but not in NS (Bitoun et al. 2003).

***SPINK5* encodes a multidomain serine protease inhibitor**

SPINK5 is located on chromosomal region 5q33.1 within a cluster of other *SPINK* genes including *SPINK6* and *SPINK9* (Meyer-Hoffert et al. 2009, 2010). *SPINK5* comprises 33 exons spanning 61 kb. It is transcribed into a 3.7-kb cDNA encoding 15 inhibitory domains interspaced by linker regions and preceded by a signal peptide sequence (Magert et al. 1999). The large number of encoded inhibitory domains distinguishes *SPINK5* from the other *SPINK* genes, which encode a unique inhibitory domain. In addition, LEKTI is transcribed into three different transcripts that differ in their C-terminal region. This results in the synthesis of three LEKTI precursors: a 145-kDa protein corresponding to full-length LEKTI (D1-D15); a 125-kDa isoform referred to as short LEKTI (D1-D12) composed of the first 13 domains as a result of alternative polyadenylation; and a long isoform (148 kDa) corresponding to the insertion of an additional exon between exons 28 and 29, encoded by a 90-bp sequence from intron 28, which extends linker region 13 (Tartaglia-Polcini et al. 2006). LEKTI is a Kazal-type-related inhibitor, the Kazal motif being defined by the presence of six cysteine residues positioned at a precise distance, allowing three disulphide bonds to form in a 1-5, 2-4 and 3-6 pattern (Magert et al. 1999, 2002a). Two of the LEKTI domains (D2 and D15) share this classical motif, whereas the other domains share only four cysteine residues. Nevertheless, the four disulphide bonds produced allow for a rigid inhibitory loop to form, which is thought to mimic the substrate of the target protease and to inactivate its catalytic site. Of note, sequence alignment of the LEKTI 15 inhibitory domains points to an arginine residue at position P1 in all domains, except for domains D1, D2 and D15, suggesting that LEKTI preferentially inhibits serine proteases with trypsin activity. This prediction has been largely confirmed by *in vitro* inhibition studies.

LEKTI proteolytic processing and inhibitory functions

An important aspect of LEKTI biology is the finding that LEKTI precursors require proteolytic activation for physiological inhibition of target proteases *in vivo*. Recently, the precise sequence of LEKTI proteolytic steps has been described by Fortugno et al. (2012) by combining *in silico* analysis and site-directed mutagenesis (Fortugno et al. 2011; Furio and Hovnanian 2011). This elegant work has shown that four arginine residues located within linker

regions 5, 9, 6 and 7 corresponding to Arg 355, Arg 625, Arg425 and Arg 489, respectively, are sequentially used. This proteolytic cascade cleaves the full-length pro-form (D1-D15) into domains D1-D5 and D6-D15, the latter being further cleaved into D6-D9 and D10-D15 and then into D6, D7-D9 and D7, D8-D9 and D8. This process generates multiple LEKTI domains containing one to six domains with different inhibitory functions. Initial inhibitory studies used the full-length LEKTI and reported the inhibition of trypsin, plasmin, subtilisin A, cathepsin G and human neutrophil elastase (Mitsudo et al. 2003). Subsequent studies with various LEKTI fragments showed different inhibitory profiles, with KLK5, KLK7 and KLK14 being major target proteases of several LEKTI polypeptides (Mitsudo et al. 2003; Jayakumar et al. 2004; Kreutzmann et al. 2004; Egelrud et al. 2005; Schechter et al. 2005; Borgono et al. 2007; Deraison et al. 2007). This was confirmed by the inhibition profile of physiological LEKTI fragments, with fragments D6-D9, D7-D9 and D8-D9 showing the strongest inhibition towards KLK5 and KLK14 in the nanomolar range (Fortugno et al. 2011). Recently, Bennett et al. (2010, 2012) have reported the inhibition of caspase 14 by LEKTI and the interaction of LEKTI with dermcidin and cathepsin G. These results implicate LEKTI in epidermal hydration through the regulation of the final steps of filaggrin degradation and suggest a role for LEKTI in antimicrobial defence through the defective trafficking of dermcidin. By extending the spectrum of LEKTI inhibition to cysteine proteases, these results suggest that LEKTI is a multifunctional protease inhibitor.

***SPINK5* mutations**

More than 70 *SPINK5* mutations have now been reported in NS patients (Sprecher et al. 2001, 2004; Bitoun et al. 2002a, 2002b; Chao et al. 2003; Raghunath et al. 2004; Mizuno et al. 2005, 2006; Shimomura et al. 2005; Descargues et al. 2006; Lin et al. 2007; Fong et al. 2010; Goujon et al. 2010; Alpigiani et al. 2012; Lacroix et al. 2012; see also <http://www.hgmd.org>). Molecular findings can be summarised as follows:

- i. *SPINK5* mutations are usually small defects (substitutions, insertions or deletions of a small number of nucleotides) leading to nonsense or frameshift mutations resulting in premature termination codons (PTCs). Other frequent mutations are splice-site mutations, intronic or exonic, which lead to complete or partial abolition of normal splicing. Of special interest is the description of a frequent Cys297Cys synonymous mutation within exon 11 that leads to the skipping of exon 11 resulting in a frameshift and a PTC (Lacroix et al. 2011). This mutation is, so far, the most frequent mutation identified in patients of

European origin and corresponds to a founder effect in patients of Greek or Mediterranean ancestors. A recurrent mutation corresponding to founder effects is also found in patients from Turkey.

- ii. A majority of *SPINK5* mutations are therefore null-mutations predicting the complete absence of LEKTI synthesis, which is confirmed by negative LEKTI immunodetection on skin sections. However, some “leaky” splice-site mutations allow for some residual LEKTI polypeptide to be expressed, as is detectable on skin sections and by Western-blot analysis and accounts for a milder phenotype (Lacroix et al. 2011)
- iii. Some rare patients have larger rearrangements involving several exons or, in contrast, have no detectable mutation at the genomic level but display deep intronic mutations leading to abnormal splicing. These patients require specific diagnostic strategies from genomic DNA or *SPINK5* transcripts.
- iv. *SPINK5* is a highly polymorphic gene, with a majority of single nucleotide polymorphisms showing a high frequency of heterozygosity (Bitoun et al. 2002b). Of these genetic variations, E420K has been associated with AD in several studies and was recently shown to have a functional effect on LEKTI proteolytic processing and activity (Fortugno et al. 2012).

Genotype-phenotype correlations

Moderate NS phenotypes have been associated with LEKTI residual expression in several cases (Hachem et al. 2006; Lacroix et al. 2012). Interestingly, the level of LEKTI expression inversely correlates with the intensity of thymic stromal lymphopoietin (TSLP) and tumour necrosis factor (TNF)- α expression, providing further evidence for a functional link between LEKTI defective expression and the induction of these pro-allergic and pro-inflammatory cytokines (Lacroix et al. 2011). In other patients, however, no residual LEKTI is detectable in skin sections or Western-blot analysis, despite striking differences in disease severity, suggesting the involvement of modifier genes and/or environmental factors. In this respect, several other serine protease inhibitors are expressed in the skin. They include other members of the Kazal-type inhibitor family, e.g., *SPINK6* and *SPINK9* (Meyer-Hoffert et al. 2009, 2010), members of the trappin family such as skin-derived antileucoprotease (SKALP, elafin; Schalkwijk et al. 1990) and secretory leukocyte protease inhibitor (SLPI; Wingens et al. 1998) and SP inhibitors of Kunitz-type-1 (*SPINT1*; Szabo et al. 2009) and type-2 (*SPINT2* encoding bikunin; Cui et al. 1999). Although none of these inhibitors are able to compensate for LEKTI deficiency, they might modulate some of the effects of loss of LEKTI expression.

Pathogenesis

The pathogenesis of NS remained unknown until *SPINK5* was identified by positional cloning as being the causative gene (Chavanas et al. 2000). The development of murine models has been essential to unravelling the consequences of the loss of Lektin function in the skin.

What have we learned from murine models?

Spink5 knock-out mice were the first to be developed. *Spink5*-defective embryos show a severe skin barrier defect at day 17.5 as revealed by dye penetration assay (Yang et al. 2004; Descargues et al. 2005; Hewett et al. 2005; Bonnart et al. 2010b). At birth, *Spink5* knock-out pups display superficial peeling caused by stratum corneum detachment and die within the next 10 h from dehydration secondary to enhanced transepidermal water loss. Proteolytic activities of kallikrein 5, kallikrein 7 and a 28-kDa protease, which was subsequently identified as elastase 2 (ELA2; Bonnart et al. 2010a), are significantly enhanced in the epidermis. Ultrastructural analysis shows detachment of the stratum corneum from the granular layer, secondary to desmosomal cleavage. Differentiation is abnormal, with hyperkeratinisation, persistence of nuclei and lamellar bodies in corneocytes, impaired intercorneocyte lipid lamellae and lipid droplets. Loricirin expression is increased and filaggrin processing is enhanced with the disappearance of large-molecular-weight filaggrin precursors and increased levels of filaggrin monomers (Descargues et al. 2005). These results suggest KLK5, KLK7 and ELA2 as LEKTI targets and establish that Lektin deficiency has profound effects on several aspects of epidermal homeostasis, including desquamation, epidermal differentiation, profilaggrin processing and lipid metabolism (Hovnanian 2012).

However, because these animals died rapidly and in the absence of an inducible *Spink5* knock-out murine model, we developed *Spink5* knock-out skin grafts onto nude mice to analyse the disease phenotype in adult skin (Briot et al. 2009). The skin grafts developed hairless, inflammatory and crusty lesions, with mast cell and eosinophil infiltration in the dermis and the epidermis. Trypsin activity and KLK5, KLK7 and ELA2 activities were also enhanced. Moreover, the skin grafts showed enhanced expression of pro-inflammatory cytokines, such as interleukin-1 β (IL-1 β), TNF- α , IL-8 and intercellular adhesion molecule-1 (ICAM1) and pro-allergic mediators, such as TSLP, thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC). These results revealed that Lektin-deficient skin was able to induce severe skin inflammation and a pro-allergic environment through the attraction and activation of inflammatory cells in the absence of T lymphocytes from the recipient mouse.

Additional evidence for the intrinsic property of the Lekti-deficient epidermis to trigger skin inflammation came from the investigation of *Spink5* knock-out embryos at 19.5 days (Briot et al. 2009, 2010). These embryos showed an enhanced and perinuclear location of protease-activated receptor-2 (Par-2), indicative of receptor activation. Par-2 is a transmembrane receptor highly expressed at the surface of keratinocytes and becomes activated upon cleavage of its aminoterminal region by serine proteases such as KLK5, tryptase and trypsin. Similarly to Lekti-deficient skin grafts, *Spink5* knock-out embryos showed strong TSLP expression in the epidermis, a cytokine not detected in wild-type embryos at age 19.5 and expression for several inflammatory and allergic cytokines. These observations clearly indicated that the KLK5-Par2-TSLP cascade was active in *Spink5* null embryos as soon as embryonic age 19.5 and developed independently from the external environment and challenges.

Despite this significant progress in the understanding of NS pathogenesis, the role of ELA2, a new epidermal protease identified by mass spectrometry (MS)/MS from LEKTI-deficient epidermal extracts, remained unknown (Bonnart et al. 2010a). ELA2 is not directly inhibited by LEKTI but is efficiently inhibited by SLPI and elafin (or SKALP), two serine protease inhibitors produced by keratinocytes and neutrophils. In contrast, ELA2 is activated by KLK5 and by KLK7 to a lesser extent. The development of a TgELA2 overexpressing murine ELA2 has been instrumental to dissecting the role of ELA2 in NS (Bonnart et al. 2010b). TgELA2 mice show a transient desquamative phenotype, with enhanced filaggrin proteolytic degradation as shown by immunostaining and the ultrastructure of skin sections and by the Western-blot analysis of epidermal extracts. ELA2 overexpression has also a significant impact on lipid metabolism, with the presence of lipid droplets and disorganisation of intercorneocyte lipid lamella in the stratum corneum. Lipid composition is abnormal with decreased free fatty acids and abnormalities in immediate ceramide precursors (increased total glucosylceramide and reduced sphingomyelin). This work has identified ELA2 as a major actor of profilaggrin processing and lipid metabolism in the epidermis, whose activation by unopposed KLK5 and KLK7 activities contributes to epidermal barrier dysfunction in NS (Bonnart et al. 2010a, 2010b).

In NS patients

Patient investigations have confirmed the relevance of abnormalities seen in *Spink5* knock-out mice (Descargues et al. 2006; Hachem et al. 2006). Immunostaining of NS patient skin sections shows a reduction of desmoglein 1, desmocollin 1 and desmoplakin, which is associated with the redistribution of KLK5 and KLK7. In situ zymography of skin sections reveals enhanced trypsin-like, chymotrypsin-like and ELA2 activities. Ultrastructural analysis shows stratum

corneum detachment secondary to desmosomal cleavage at the interface between the last granular layer and the stratum corneum. Epidermal differentiation is also abnormal, with increased loricrin expression, reduced keratohyalin granules and decreased filaggrin immunostaining in the granular layer. Lipid abnormalities are also observed: lipid droplets, abnormal lipid lamellae, premature fusion and unprocessed lamellar bodies in the extracellular spaces are also seen in patients (Descargues et al. 2006; Bonnart et al. 2010b). These observations support the notion that, in NS patients, KLK5 and KLK7 unrestrained activities lead to desmosomal cadherin degradation and to stratum corneum detachment, with a profound effect on epidermal differentiation and lipid metabolism.

Investigation of skin inflammation in NS patients shows a mild to moderate infiltrate of mast cells and eosinophils (Briot et al. 2009). PAR-2 expression is increased and shows a perinuclear location suggestive of receptor activation. TSLP is also strongly expressed in the upper layers of the epidermis and is not detectable in controls. To investigate further the consequences of unopposed KLK5 activity on keratinocyte functions, we analysed the effects of KLK5 addition on the expression levels of pro-inflammatory and pro-allergic molecules by normal primary human keratinocytes in culture (Briot et al. 2009). KLK5 addition induces the expression of TSLP, TNF- α , IL-8 and ICAM-1 transcripts, an effect that is prevented by PAR-2 short interfering RNAs (si-RNAs). si-RNA against KLK5 leads to a significant reduction of the expression level of these cytokines in NS keratinocytes in culture. Taken together, these results provide evidence that the KLK5-PAR-2-TSLP pathway is active in NS patients and is an autonomous event in LEKTI-defective keratinocytes.

In parallel to this cascade, Hosomi et al. (2008) have reported increased capase 1 activity in the stratum corneum of NS patients and elevated levels of IL-18, a member of the IL-1 family. As caspase 1 is crucial for pro-IL-1 β activation into IL-1 β , this implies that inflammasome activation also participates in skin inflammation in NS.

Proposed disease mechanism in NS

On the basis of these observations from murine models and NS patients, a dual mechanism can be proposed for NS, with multiple layers of interplay (Fig. 2; Magert et al. 2002b; Descargues et al. 2006; Briot et al. 2009, 2010; Hovnanian 2012). LEKTI deficiency leads to unopposed KLK5 and KLK7 activity and ELA2-enhanced activation, all of which have complex effects on the essential aspects of skin homeostasis. Deregulated protease activity impacts on skin desquamation and epidermal permeability through impaired epidermal differentiation and lipid metabolism, leading to a defective skin barrier. In parallel, unopposed KLK5 activity triggers PAR-2-dependent and non-PAR-2-dependent pathways of inflammation and allergy aggravating the skin barrier



TARC and MDC expression, which recruits Th2 cells in the skin. Th2 cytokines such as IL-4, IL-5 and IL-13 induce a B cell switch to IgE and activate eosinophils and mast cells. These cells produce pro-inflammatory and pro-allergic cytokines that aggravate the skin barrier defects (outside-inside-outside mechanism). TNF- α , IL-8 and I-CAM1 cytokines also contribute to skin inflammation by attracting and activating inflammatory cells.

Link with AD

Several studies have reported a genetic association between AD and the LEKTI E420K variant, in some but not all populations studied (Walley et al. 2001; Kato et al. 2003; Nishio et al. 2003; Kabesch et al. 2004; Kusunoki et al. 2005; Weidinger et al. 2008; Zhao et al. 2011). Recent functional analyses by Fortugno et al. (2012) have demonstrated that the E420K variant creates a new furine cleavage site in linker 6 of the molecule, leading to abnormal LEKTI proteolytic processing, with an absence of D6-D9 fragments. As a result, trypsin-like epidermal proteolytic activity is enhanced and staining for desmoglein 1 and filaggrin is reduced. Overall, TSLP expression is enhanced in E420K skin, providing a direct link with a predisposition to atopy (Fortugno et al. 2012). This work, together with previous studies reporting enhanced protease activity in AD skin, supports the notion that protease regulation plays a role in predisposition to AD (Elias and Schmuth 2009). In particular, excessive proteolysis of filaggrin would result in the reduced production of urocanic acid resulting in acidification and decreased hydration of the stratum corneum. The finding that LEKTI E420K, which is a frequent variation (50%), shows only a weak association with AD, suggests that E420K takes part in a complex network involving multiple genes and/or interactions with the environment.

Treatment

NS is an orphan disease with multisystemic and severe complications, with currently no specific treatment available. Therefore, an unmet need exists for effective treatment. Recent reports concerning the successful use of intravenous immunoglobulins (IVIG) and anti-TNF- α appear to represent new therapeutic options (Renner et al. 2009; Fontao et al. 2011).

Current options

NS treatment is symptomatic and the therapeutic objectives differ with patient age. NS newborns and infants are at high risk of dehydration because of their severe skin barrier defect. They are also at high risk of infections and failure to thrive, which can lead to life-threatening complications. Therefore,

young patients should benefit from a multidisciplinary medical team to prevent and treat severe complications that could require hospitalisation in intensive care units (Sun and Linden 2006). Patient-appropriate fluid, electrolyte and nutritional support with a hypoallergic diet are essential to prevent dehydration and growth retardation. A gastrostomic tube might be required in cases of severe defective food uptake. Skin infections should be prevented by strict asepsis and bacterial colonisation of the infant by *Staphylococcus aureus* should be regularly monitored. The frequent and regular use of emollients is essential to counteract the skin barrier defect. “Eczematous-like lesions” can be treated by a short course of topical corticosteroids of moderate strength on limited body areas, because of the high risk of systemic absorption at this age. Histamine H1 receptor antagonists are helpful for treating itching and allergic manifestations. Allergic asthma requires specific treatment. Food allergies necessitate strict food avoidance and/or desensitisation strategies in cases of documented hypersensitivity. The condition of NS patients often partially improves with age. Children are still at risk of severe dehydration and infections but adults are not exposed to life-threatening complications, although they can be disabled by persistent skin inflammation and severe itching. Children and adults can benefit from topical calcineurin inhibitors (tacrolimus or pimecrolimus), although these might induce irritation and their use should be limited to a restricted body area, because of significant absorption arising from the skin barrier defect, which exposes the patient to the risk of immune suppression (Allen et al. 2001; Oji et al. 2005; Saif and Al-Khenaizan 2007; Yan et al. 2010). Antisepsis, the frequent use of emollients and the prevention and treatment of skin infections are essential aspects of the treatment. In a few cases, topical calcipotriol has shown effectiveness, whereas the use of topical recombinant alpha1-antitrypsin, an elastase inhibitor, has brought no significant improvement (Mazereeuw-Hautier et al. 2006). Recently, IVIG treatment (0.4 g/kg per month) has demonstrated impressive effectiveness on the disease course in five children with severe NS (Renner et al. 2009). Patients showed reduced skin inflammation and infections and a remarkably improved clinical course, with the correction of growth retardation. The mechanism(s) of action of IVIG is (are) not well known but IVIG might counteract a functional immune defect. Monthly infusions were required to prevent relapse of the disease. Another trial has reported spectacular improvements of skin inflammation by anti-TNF- α monoclonal antibodies (infliximab) in an adult patient with a severe form of NS (Fontao et al. 2011). This study has also identified TSLP as a biological marker that correlates with disease activity. After induction, infliximab infusions were delivered every 8 weeks to maintain clinical improvement. These two successful trials encourage the inclusion of a larger number of NS patients into controlled IVIG or anti-TNF α therapeutic trials in order to evaluate the long-term risk-benefit ratios of these new therapeutic options.

New therapeutic developments

The significant progress made in the understanding of NS pathogenesis allows the development of new therapeutic strategies aimed at targeting specific biological pathways of the disease (Hovnanian 2012; Xiang and Di 2012). Protein or polypeptide replacement therapy with selected LEKTI fragments or modified recombinant inhibitors, despite their large molecular weight, could ameliorate the severe skin barrier defect that characterises NS. The development of small protease inhibitors specifically to block unopposed kallikrein activity is also a promising approach. Alternative options such as targeting PAR-2 or more downstream events such as TSLP-, TNF- α - or IL-1 β -enhanced expression or high IgE levels, are also worth considering. Gene addition by means of a *SPINK5* lentiviral or adenoviral vector has also been proposed by using autologous grafts of genetically corrected patient keratinocytes (Di et al. 2011a, 2011b; Roedl et al. 2011). Rather than the correction of localised areas of the skin, this approach would test the possibility of full-body correction through LEKTI secretion by the corrected graft (Xiang and Di 2012).

Concluding remarks and perspectives

The improved understanding of the natural history of NS will facilitate the establishment of standardised protocols for the clinical and biological assessment of patients in order to allow personalised medication. The emerging knowledge on NS pathogenesis will foster the development of new therapeutic approaches. Such progress has placed the control of serine protease regulation at the centre of skin barrier formation, inflammation and allergy. Moreover, it has built a link with the pathogenesis of AD. Despite this progress, several aspects of NS remain to be better understood, such as the impact of LEKTI deficiency on T cell development and on innate immunity, the reasons for the failure to thrive in infants and for a possible predisposition for HPV tumors in adults and the identification of modifier genes. Further basic and clinical research is thus required to address these issues, although some new therapeutic options are already available.

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