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Epidermal Serine Proteases and Their Inhibitors in Atopic Dermatitis

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1. Introduction

The skin and its appendages protect the body from water loss, chemical and physical damages, UV-radiation and infection by pathogenic as well as non-pathogenic microbes. The protective function of both, the physical and the chemical barrier, is provided from epidermal keratinocytes, which are continuously dividing in the *stratum basale* and differentiating towards the surface (Candi et al. 2005). Cells of the uppermost living epidermis layer, the stratum granulosum, are losing their nuclei and other organelles at the transition zone to the stratum corneum (SC), forming now flattened polyhedrons, called corneocytes. These corneocytes contain instead of a cell membrane the cornified envelope (CE) consisting of structural proteins, which are crosslinked by glutaminases (Candi et al. 2005). The intercellular space of the corneocytes is filled with lamellar body-derived lipids, which make the SC more hydrophobic. This mechanism protects skin from water loss and other insults.

Finally, in a tightly regulated process termed desquamation that abolishes the cohesion between corneocytes, these cells are shed into the environment by proteolysis of corneodesmosomal proteins. The formation of stratified epithelia requires a specific differentiation program, which includes a timely and spatially well coordinated proteolytic system to detach the corneocytes from each other without any disturbance of the barrier function. During the last years it became evident that this proteolytic balance is not only important for the physical barrier function of the skin but is also paving the way for immunological responses. In this chapter we want to look at the various proteases in the epidermis, their inhibitors and how they might contribute to the pathogenesis of atopic dermatitis (AD).

2. Proteases

A number of different proteases and their inhibitors have been involved in the desquamation process and to contribute to the skin's barrier function. On the basis of the catalytic domain, proteases are classified into aspartate-, cysteine-, glutamate-, metallo-, serine- and threonine proteases. Particularly serine proteases (SP) have a prominent role in epidermal permeability barrier homeostasis, as acute barrier disruption increases SP-activity

in skin and inhibition by topical SP-inhibitors accelerated recovery of barrier function after acute abrogation (Hachem et al. 2006).

2.1 Cysteine- and aspartate-proteases

Cysteine peptidases represent phylogenetically ubiquitous enzymes, which can be classified into clans of independent proteins (based on the structural organization of the active site). Two of the major clans in mammal genomes are the “CA” clan, where members share an evolutionary and structural history with papain, and the “CB” clan, which includes the caspases and the legumains.

One of the most skin-relevant caspases is the cysteinyl-aspartate protease caspase-14 (Demerjian et al. 2008). In contrast to other ubiquitously expressed members of the caspase family, caspase-14 is rather specifically expressed within the epidermis, where it is of high importance in the formation of the physical skin barrier. It is expressed in keratinocytes of the uppermost stratum granulosum, where it was found to be associated with the nucleus, the keratohyalin granules and the desmosomes. Although its localization suggested a role for nuclear degradation during cornification, in caspase-14-deficient mice nuclear degradation was not affected. The observation that caspase-14 has only been found in terrestrial mammals but not in birds or reptiles and that profilaggrin is a direct substrate of caspase 14, suggests that it is important for formation of a soft stratum corneum. This could indicate a co-evolution of a soft SC and the caspase-14 gene (Denecker et al. 2007). Caspase-14 is produced as procaspase within the stratum granulosum, where it matures in cornifying epithelia. Although it is not clear how it matures, most likely a serine protease with elastase-like properties could be involved (Denecker et al. 2008). Thus, caspase-14 seems to be involved in the correct processing of profilaggrin, preceding its degradation into hygroscopic amino acids as well as the formation of UVB-protective compounds.

Another skin cysteine peptidase is cathepsin C, which represents a lysosomal cysteine peptidase of the papain family CIA, which is important for intracellular degradation and which has a role in the activation of serine proteases in immune cells (Rao et al. 1997). Cathepsin C knockout mice indicated that activation and processing of granzymes A and B, which are important for T-cell-mediated cell-killing, depends on cathepsin C (Pham and Ley 1999). Interestingly, Cathepsin C deficiency in humans leads to a dramatic reduction of both, levels and activities of the neutrophil serine proteases elastase, protease-3 and cathepsin G (Pham et al. 2004), which will be described below.

Cathepsin D represents the main aspartic protease of endolysosomes. It is active at the physiological acidic pH of healthy skin and is of relevance in the desquamation process (Horikoshi et al. 1999). Cathepsin D knockout mice showed reduced levels of involucrin and loricrin and lower transglutaminase 1 activity, which indicates that cathepsin D contributes indirectly to the barrier function of human skin (Egberts et al. 2004).

2.2 Serine proteases

Serine proteases represent a family of enzymes which use a catalytic triad in the substrate-binding pocket (Ser, His, Asp) to cleave peptide bonds. On the basis of their substrate specificity these proteases can be subdivided into trypsin-like enzymes (cleaves C-terminally of Arg and Lys), chymotryptic enzymes (cleave behind aromatic or bulky, hydrophobic amino acids, and the elastase-like enzymes (cleave behind small or medium size non-polar amino acids. These enzymes play an important role in the terminal differentiation process and desquamation.

2.2.1 Matriptase and prostasin

One of the most important players in epidermal barrier function is profilaggrin. It is processed at the stratum granulosum/stratum corneum interphase to filaggrin monomers. These are crosslinked to form macrofibrils and eventually “natural moisturizing factors, NMFs), which are important for maintaining the hydration of the SC (reviewed in (Ovaere et al. 2009). The importance of profilaggrin proteolysis to maintain epidermal structure and hydration has been underscored by human genetic studies: These have shown that loss-of-function mutations in profilaggrin cause ichthyosis vulgaris and are strongly predisposing to atopic dermatitis and asthma, possibly due to a disturbed epidermal barrier function, which allows entry of allergens and infectious agents (Sandilands et al. 2006).

Major proteases required for initiating profilaggrin processing are the type II transmembrane serine protease matriptase and prostasin, a glycosylphosphatidylinositol-anchored membrane serine protease. There is now evidence that the autoactivating protease matriptase acts upstream of prostasin in a zymogen activation cascade that regulates terminal epidermal differentiation and is required for prostasin zymogen activation (Netzel-Arnett et al. 2006). A reduced matriptase expression was shown to be associated with incomplete terminal differentiation of epidermis, epidermal appendages, and oral epithelium (Bugge et al. 2007). Matriptase gene mutations lead to ichthyosis as recently reported (Alef et al. 2008). Matriptase is immediately activated by exposure to an acidic pH, as it occurs in skin, suggesting that matriptase activation may be a direct response to proton exposure (Tseng et al. 2010). Recent evidence showed that during epidermal differentiation, the matriptase-prostasin proteolytic cascade is tightly regulated by two mechanisms, either by prostasin activation temporally coupled to matriptase autoactivation or by the hepatocyte growth factor activator-inhibitor-1(HAI-1), which is rapidly inhibiting not only active matriptase but also active prostasin, resulting in an extremely brief window of opportunity for both active matriptase and active prostasin to act on their substrates (Chen et al. 2010). So far these two membrane-bound proteases are thought to be mainly involved in skin homeostasis. However, the ability of matriptase to activate Kallikrein-related proteases in the skin points to a regulatory role of matriptase in inflammatory skin diseases (Sales et al. 2010).

2.2.2 Kallikrein-related peptidases

Kallikrein-related proteases (KLKs) are the largest family of tryptic and chymotryptic serine proteases, which are encoded by 15 genes on chromosome 19q13.4. In skin, KLKs are produced by keratinocytes of the stratum granulosum (SG), where they are released into interstices of the upper SG and lower SC. To date, SC serine protease activity is attributed to human tissue KLKs (Borgono et al. 2007). At least eight KLKs have been reported to be expressed in healthy skin, of which KLK5, KLK7, KLK8, and KLK14 seem to be the most important (reviewed in (Lundwall and Brattsand 2008)). Their putative function has been extensively studied (reviewed in (Eissa and Diamandis 2008; Lundwall and Brattsand 2008). A wealth of literature revealed proteolytic function of the two serine proteases, KLK5 and KLK7, in SC. These proteases, previously termed ‘stratum corneum tryptic enzyme, SCTE’ (KLK5) and ‘stratum corneum chymotryptic enzyme, SCCE’ (KLK7), have an important role in the desquamation process, as it was shown that serine protease inhibitors were able to inhibit corneocyte shedding from human plantar skin *ex vivo* (Lundstrom and Egelrud 1988). Both enzymes are maximally expressed in the stratum granulosum, where they are released from lamellar bodies and located within stratum corneum interstices. Here they are

thought to form a proteolytic cascade in which KLK5 activates itself as well as KLK7 (Ovaere et al. 2009). Once active, both enzymes are believed to digest *in vivo* corneodesmosin, DSG1 and desmocollin-1, as these substrates have been shown to be digested *in vitro*. There is now evidence that also other KLKs participate in desquamation: It was recently shown, that KLK14 is responsible for 50% of the total trypsin-like serine protease activity in the stratum corneum. Because KLK14 can activate and be activated by KLK5, it is very likely that it also participates in the cascade pathway.

Apart from these three KLKs also KLK8 seems to be involved in a proteolytic activation cascade regulating skin desquamation: KLK8 is abundantly expressed and co-localized with other KLKs in human epidermis and sweat glands. It is also transported and exocytosed by lamellar bodies into the stratum granulosum/stratum corneum interface and thus may play a role in SC barrier functions. Very recent studies showed that recombinant KLK8 is optimally active at pH 8.5 suggesting that it plays a role in the upper stratum granulosum where the pH is rather neutral (Eissa et al. 2011). Active KLK8 has been found in SC extracts and in sweat, where until recently only KLK1 and kininase II were identified as active serine proteases. This raises its potential functional involvement in skin desquamation, although the physiological substrates need to be identified.

2.3 Proteases of bacterial, fungal and parasite origin

Apart from proteases produced by keratinocytes during differentiation and desquamation, the stratum corneum might also contain extracellular proteases originating from microbes and/or parasites residing at the skin surface. Bacterial proteases are often accessory proteins which are not fundamental for cell growth and division, but are considered to be virulence factors, which are often associated with mobile genetic elements such as plasmids, integrated phages and pathogenic islands. The clustering of bacterial protease genes in operons allows their coordinated expression, which in turn may imply a cooperation of the produced proteins (Wladyka and Pustelny 2008).

Staphylococci produce a number of extracellular proteases, among them epidermolytic toxins, staphylococcal serine proteases like a glutamyl endopeptidase referred to as V8, a cysteine protease in *S. aureus*. Similar and other proteases are produced by skin-relevant bacteria, including commensal *S. epidermidis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and others (Wladyka and Pustelny 2008). Among the proteases, serine proteases, cysteine proteases and metalloproteases represent the most abundant bacterial proteases. These affect the host's innate immune system in a bacterial species-specific manner by targeting phagocytes, cytokines and cytokine receptors, inflammatory signaling pathways, complement, contact activation as well as antimicrobial peptides (Potempa and Pike 2009).

Apart from bacteria also fungi represent an important source of proteases. Upon fungal infections, which are mostly seen at mucosal surfaces, *Candida albicans* represents the most common fungal pathogen. *Candida* species are ubiquitous commensal yeasts that reside as part of the normal mucosa microflora without causing infections. Suitable predisposing conditions will let *C. albicans* change to a 'pathogenic' stage, in which proteases represent major virulence factors. These are exclusively secreted aspartyl proteases (SAPs) (Naglik et al. 2004). It is believed that extracellular proteases of saprophytic microorganisms are primarily secreted to get nutrients from decomposition of complex materials. There is, however, strong evidence, that SAPs are also needed for invasion of the host, thereby interacting with several important host defense functions eventually causing inflammation (Naglik et al. 2004).

Proteolysis is also a vital element for survival of parasites, which enables them to digest resistant structural proteins. E. g. house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*) produce cysteine proteases and serine proteases (Donnelly et al. 2006), which are well known as 'group 1 house dust mite allergens' to induce allergic reactions. Several reports have shown that these proteases interact with pathways of the innate defense system suggesting that these might be also directly involved in inflammatory skin reactions.

2.4 Neutrophil serine proteases

Upon skin infection or at conditions causing 'neutrophilic dermatoses', the primary cell infiltrate consists of neutrophils. A massive infiltrate in the epidermis can lead to pustule formation. Upon infection, neutrophils phagocytose microbes and then kill these microbes within the phagolysosome by oxygen-radical-generating systems, the alpha-defensins as well as proteases which are released from primary ('azurophilic') as well as secondary ('specific') granules (Faurschou and Borregaard 2003). Only primary granules contain high amounts of the serine proteases human leukocyte elastase (HLE), cathepsin G and protease 3 (PR3). These enzymes are not released upon phagocytosis. But upon 'frustrating phagocytosis' (attempts to phagocytose particles, which are bigger than leukocytes) as well as formation of "neutrophil extracellular traps" (NETs) consisting of neutrophil-derived DNA, where these cationic enzymes are bound (Brinkmann et al. 2004), a release of these enzymes can occur. Indeed, HLE activity is present at the surface of lesional skin of patients with psoriasis, a neutrophilic dermatosis (Wiedow et al. 1992). Neutrophil serine proteases have been identified as important innate immune regulators (Meyer-Hoffert 2009; Meyer-Hoffert and Wiedow 2010). Thus, neutrophil-derived enzymes may further determine the outcome of an inflammatory skin lesion – independent of possible homeostasis of keratinocyte-derived proteases and protease-inhibitors.

3. Protease inhibitors

Proteolytic activity in the skin, which is often restricted to a few target proteins, its tissue localization and its enzymatic activity, needs to be properly controlled in the tissue. Although gene expression and zymogen-activation are important regulatory elements to restrict enzymatic activity, the most important one is the expression of more or less specific protease inhibitors within the skin. These inhibitors regulate more or less protease-specifically in a timely and concentration-dependent fashion the activity of diverse proteases. This review will summarize the current knowledge on the most important epithelial protease-inhibitors.

3.1 Kazal-type-related protease inhibitors

The 'lympho-epithelial Kazal-type related inhibitor' (LEKTI, today named LEKTI-1) is an effective inhibitor of multiple serine proteases (Roelandt et al. 2009). Processing of this multidomain protease inhibitor into fragments or single domains restricts the inhibitory properties to serine proteases such as trypsin, plasmin, subtilisin A, cathepsin G and human neutrophil elastase. LEKTI-1 consists of 15 complete or incomplete Kazal domains. *In vitro*, recombinant LEKTI-1 fragments or single domains inhibit the keratinocyte-derived serine proteases KLK5, -6, -7, -13 and -14. LEKTI-1 is expressed in various stratified epithelia as three splice variants. In the epidermis LEKTI-1 is expressed in the stratum granulosum, where LEKTI-1 protein is located in lamellar bodies – separate from KLKs, but secreted into the

extracellular space together (Ishida-Yamamoto et al. 2004; Ishida-Yamamoto et al. 2005). The 145 kDa form comprises all 15 potential inhibitory Kazal-domains, but it is cleaved rapidly into multidomain fragments, which might be cleaved further to produce single domains and complexes with KLK5 and KLK7 in the SC. These complexes dissociate at acidic pH, which due to a pH gradient within the SC may lead to a controlled homeostatic desquamation. Mutations in *Spink5* (which encodes LEKTI-1) generating premature termination codons, as seen in Netherton Syndrome (Chavanas et al. 2000), result in expression of truncated LEKTI forms lacking several protease-inhibiting domains. This rare ichthyosiform skin disease is characterized by dry skin, increased desquamation, hair abnormalities ('bamboo hair') and atopy. A decreased level of functional LEKTI correlates inversely with serine protease activity in SC, a decrease physical barrier function and severity of the disease.

Another Kazal-type inhibitor is LEKTI-2 (*Spink9*), which has been originally discovered in palmar and plantar SC extracts (Brattsand et al. 2009; Meyer-Hoffert et al. 2009). LEKTI-2/SPINK9 is mainly expressed in palmar and plantar skin, close to KLK5. Apart from skin, expression was seen in the thymus (thus referred as LEKTI-2). All other tissues showed a very low transcription level of *Spink9*. LEKTI-2/SPINK9 selectively inhibited KLK5, but not other proteases including chymotryptic KLK7 and tryptic KLK14 or several serine proteases like trypsin and chymotrypsin. The LEKTI-2/SPINK9 activity differs in this respect from that of LEKTI-1: The K_i of LEKTI-2 was found in the range of 60 – 250 nM. LEKTI-1-domains have been reported to inhibit KLK5 in the range of 3 nM (domain 8-11) to 120 nM (domain 9-15). Further, the LEKTI-1 domains exhibit a more or less broad activity spectrum. It remains to be determined whether LEKTI-2/SPINK9 plays a role in skin diseases. Considering the specific expression of LEKTI-2/SPINK9 at palmar and plantar sites as well as its specific activity to inhibit KLK5, it is intriguing to speculate that it could be a relevant factor in hand and foot eczema.

By following the hypothesis that likely more Kazal-type inhibitors are present in human skin, we identified SPINK6 as a selective inhibitor of KLKs in the skin (Meyer-Hoffert et al. 2010). Unlike LEKTI-1 but similar to LEKTI-2, SPINK6 possesses only one typical Kazal domain. SPINK6 is strongly expressed, unlike LEKTI-2, in skin from various locations and can be purified from human plantar SC extracts. At low levels it is expressed in many other tissues and is induced during keratinocyte differentiation. While immunohistochemical analyses revealed SPINK6 expression in the stratum granulosum of healthy human skin at various anatomical localizations and in the skin appendages, including sebaceous glands and sweat glands, SPINK6 expression was found to be decreased in lesions of atopic dermatitis. Recombinant SPINK6 inhibited KLK4, KLK5, KLK6, KLK7, KLK12, KLK13 and KLK14 but not KLK1, KLK3 and KLK11, suggesting a tissue KLK-selective inhibitory activity, since thrombin, trypsin, plasmin, matriptase, prostatic, mast cell chymase, cathepsin G, neutrophil elastase, and chymotrypsin were not inhibited (Meyer-Hoffert et al. 2010; Kantyka et al. 2011). The finding that SPINK6 inhibited desquamation of human plantar callus in an *ex vivo* model suggests that SPINK6 plays a role in modulating the activity of KLKs in human skin. Interestingly, SPINK6 exhibited some proteolytic inhibitory activity against caspase-14 and is so far the only reversible inhibitor of caspase-14 in human skin (Kantyka et al. 2011).

3.2 Trappins and serpins of human skin

Apart from LEKTIs keratinocytes produce a number of additional protease inhibitors. Members of one group are termed 'trappins' (acronym for transglutaminase substrate,

WAP-domain-containing proteins) (Schalkwijk et al. 1999). Human epidermis contains two, secretory leukocyte protease inhibitor (SLPI) and elafin. Both are efficient inhibitors of neutrophil serine proteases: SLPI inhibits cathepsin G and elastase, elafin inhibits elastase and protease-3. This suggests that these protease inhibitors are important at inflammatory conditions to protect the tissue from damage caused by neutrophil serine proteases. Whereas SLPI is constitutively expressed in the epidermis, elafin is present at a low level in healthy skin, but highly up-regulated at inflammatory conditions such as psoriasis (Wiedow et al. 1990). Elafin is stored in lamellar bodies and thus secreted as precursor at the interphase between stratum granulosum and stratum corneum, where it is crosslinked to proteins of the CE via transglutamination.

Another group of serine protease inhibitors are SERPINs, which encompass nearly 40 members, of which many have been implicated in cancer and inflammation (Meyer-Hoffert 2009). These protease inhibitors have a unique mechanism to inhibit enzymatic activity: SERPINs cause a conformational change of the protease and then covalently bind to it. A few members of the SERPIN family have been reported to be expressed in human skin, among them SERPINB3 (squamous cell carcinoma antigen-1), SERPINB4 (squamous cell carcinoma antigen-2), and SERPINB13 (headpin/hurpin). SERPINs have possibly a role in protecting tissue from proteolysis by bacterial proteases: SERPINB8 and SERPINB9 are inhibiting subtilisin A. SERPINA1 inactivates some microbial proteases including protease K. Further, a C-terminal fragment of SERPINA1 inhibits HIV-1 entry by interaction with the gp41 fusion protein.

3.3 Cystatins

Apart from serine proteases also cysteine protease activity is under the control of inhibitors in skin: These include members of the cystatine gene family (Zeeuwen et al. 2009). Cystatins represent polypeptides which are members of a superfamily of evolutionarily-related proteins that can be divided in three subgroups, and which are widely expressed in several human tissues and secretions. They effectively inhibit various cysteine proteases, such as cathepsins B, L, H, K, and S, at micromolar to picomolar concentration in a competitive and reversible manner. Whereas cystatin A and cystatin C were reported to act as epidermal protease inhibitor with antimicrobial properties – possibly by inhibiting microbial cysteine proteases – cystatin M/E regulates in the epidermis crosslinking of structural proteins by transglutaminase 3 in the cornification process by controlling cathepsin L and legumain activities (Meyer-Hoffert 2009). Cathepsin L has been shown to activate transglutaminase 3, an epidermis-specific enzyme that is important in the cornification process where it is responsible for crosslinking of small proline-rich proteins and loricrin. A deregulation of this pathway by uncontrolled cysteine protease activity leads to abnormal stratum corneum and disturbance of skin barrier function (Zeeuwen et al. 2009).

3.4 Other regulating factors of protease activity

It should not be overlooked that the proteolytic activity of proteinases depends on factors like pH and ion-concentration. All serine proteases including KLKs decrease their proteolytic activity in acidic environments. The physiological pH of around 5.5 already results in more than 90% less active compared to optimal in vitro conditions. Patients with atopic dermatitis often show an elevated pH at the skin surface, which might likely contribute to observed elevated serine protease levels in these patients (Voegeli et al. 2009).

Interestingly, the inhibitory activity of LEKTI-1 depends on the pH, too, which might enhance proteolytic deregulation, when the epidermal pH is elevated. Moreover, Zn^{2+} inhibits KLK5 (Debela et al. 2007). This might have clinical consequences, when Zinc levels are low as in acrodermatitis enteropathica. The exfoliation and inflammation observed in this disease might be a result of decreased KLK5 inhibitions.

4. Protease-activated receptors (PARs)

PARs represent seven membrane-spanning G-protein coupled receptors, which are activated by serine proteases, which cleave a 'tethered' receptor-activating ligand at the N-terminus (Steinhoff et al. 2005). To date, four PARs (PAR1-4) have been characterized. PAR-1, PAR-3 and PAR-4 are activated by thrombin, and PAR-2 by trypsin and chymotrypsin. In human skin, PAR-2 is abundantly expressed by keratinocytes (Steinhoff et al. 1999), where it plays a role in regulating permeability barrier homeostasis, inflammation, pruritus, pigmentation, and wound healing upon activation by various endogenous and exogenous serine proteases. Whereas during skin-inflammation, PAR-2 is activated by neutrophil elastase and mast cell tryptase, upon infection or skin barrier defects proteases originating from certain bacteria, house dust mites, cockroaches or parasites can activate this receptor (Shpacovitch et al. 2007). Activation by *Propionibacterium acnes* protease causes induction of certain proinflammatory proteins, matrix metalloproteinases and antimicrobial peptides, including hCAP18/LL-37 (Lee et al. 2010).

5. Epidermal serine proteases and their inhibitors in atopic dermatitis

Our understanding of the pathogenesis of Atopic Dermatitis has increased by the discovery of pro-filaggrin (*FLG*) mutations as the major predisposing factor of AD and the genodermatose Netherton Syndrome, which shows similarities to AD. *FLG* mutations lead to decreased or missing filaggrin, which leads to dry skin as its cleavage and processing product, the skin moisturizing factor, is decreased. The skin barrier defect leads to increased antigen penetration, which finally leads to specific sensitization and allergic inflammation. Here we have learnt from the ND model that missing LEKTI-1 leads to increased KLK activity, which activate PAR-2, thus resulting in increased thymic stromal lymphopoietin (TSLP) (Briot et al. 2009). TSLP finally leads to a Th2 imbalance. Therefore, both events, the Filaggrin defect and the KLK-PAR2-TSLP axis, seem to be crucial for inducing AD.

To date, there is no evident point demonstrating that either intrinsic or extrinsic signals are promoting cutaneous allergic-type inflammation in AD. The fact that only 37–50% of patients with *Ichthyosis vulgaris* develop atopic manifestations (Kuokkanen 1969; Smith et al. 2006), supports the notion that in NS in which all patients develop allergic manifestations, additional mechanisms to primary skin barrier defect have a key role in immune response polarization toward a Th2 response. Since serine protease activity is elevated in AD patients (Voegeli et al. 2009), one might speculate that similar mechanisms are involved in AD as in ND. It was reported recently that KLK11 and KLK7 are elevated in lesions of AD patients (Voegeli et al. 2011). Some genetic studies have suggested that proteases and inhibitors are involved in the genetic predisposition of AD patients. In a case-control study on 103 AD patients and 261 matched controls, a significant association was found between a 4-bp insertion in the 3'-untranslated region of the *KLK7* gene, encoding KLK7 and AD (Vasilopoulos et al. 2004). This association of the 4-bp insertion mutation with AD could not

be confirmed in two independent studies (Hubiche et al. 2007; Weidinger et al. 2008). *SPINK5* gene mutations are linked with AD, when maternally inherited (Walley et al. 2001; Kato et al. 2003; Nishio et al. 2003; Weidinger et al. 2008). It is worth noting that the association was weaker than in the case of *FLG* mutations, in part, owing to a high prevalence in the control population. In a separate study on a French population, an association between *SPINK5* and AD was not found; however, there was an association between carriers and raised IgE serum levels (Hubiche et al. 2007). The association of *SPINK5* mutations with raised IgE serum levels and with other atopic conditions, such as asthma, led to the suggestion that they are risk factors for general atopy (Walley et al. 2001; Nishio et al. 2003). In addition to *SPINK5*, a mutation has been identified in the *CSTA* gene encoding the cysteine protease inhibitor, cystatin A, which associates with AD. The cystatin A gene maps to chromosome 3q21, which has been identified as a major susceptibility locus for AD (Lee et al. 2000).

6. Conclusion

Until today the complex pathogenesis of AD is not fully understood. But there is more and more evidence that epidermal proteases and their inhibitors are involved in the pathogenesis of AD. Their expression can be altered by genetic mutations and their activity is influenced by environmental factors like the pH, which is increased in AD patients (Sparavigna et al. 1999). Taken together there seems to exist a feedback regulation system important for skin barrier homeostasis in AD especially for kallikrein activity involving filaggrin mutations, PAR2 and LEKTI expression Tanaka 2011. It will be interesting to see whether inhibition of elevated protease activity will improve severity of AD lesions. Clinical trials are currently on their way with promising preliminary results (<http://clinicaltrials.gov>).

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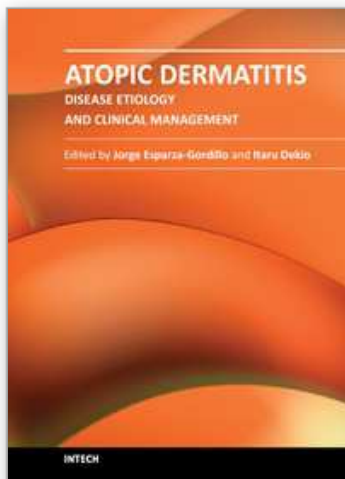
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Atopic Dermatitis is a common disease characterized by inflamed, itching and dry skin. This relapsing allergic disorder has complex etiology and shows a remarkably high clinical heterogeneity which complicates the diagnosis and clinical management. This book is divided into 4 sections. The first section (Disease Etiology) describes some of the physiological mechanisms underlying Atopic Dermatitis, including alterations in the immune system and the skin-barrier function. The important role of host-microorganism interactions on the pathophysiology of Atopic Dermatitis is discussed in the second section (Microorganisms in Atopic Dermatitis). An overview of the clinical diagnostic criteria and the disease management protocols commonly used is given in the third section (Diagnosis and Clinical Management). The last section (New Treatments) describes new therapeutic approaches that are not widely used but are currently being studied due to preliminary evidence showing a clinical benefit for Atopic Dermatitis.

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