

Saveria PASTORE¹
Francesca MASCIA¹
Giampiero GIROLOMONI²

¹ Laboratory of Tissue Engineering and Cutaneous Physiopathology, Istituto Dermatologico dell'Immacolata, IRCCS, via Monti di Creta 104, 00167 Roma, Italy
² Department of Dermatology, University of Verona, Piazzale A. Stefani 1, 37126 Verona, Italy

Reprints: S. Pastore
Fax: (+39) 06 66464705
<pastore@idi.it>

The contribution of keratinocytes to the pathogenesis of atopic dermatitis

Atopic dermatitis (AD) develops from a complex interplay between environmental, genetic, immunologic and biochemical factors. Relevant to the amplification and persistence of inflammatory and immune responses in AD skin are keratinocytes, which can be induced to secrete proinflammatory mediators in response to a variety of stimuli, including epidermal barrier perturbation. Moreover, keratinocytes from AD patients synthesize exaggerated amounts of mediators (*e.g.*, GM-CSF and RANTES/CCL5) important for enhanced recruitment as well as sustained survival and activation of T cells and dendritic cells. AD keratinocytes have a constitutive dysregulated activity of transcription factors that modulate the expression of inflammatory genes, suggesting the existence of predetermined mechanisms targeting atopic inflammation to the skin. Among these, the existence of a defective epidermal barrier, which appears related to decreased ceramide generation and abnormal degradation of corneodesmosomes, certainly plays a central role in the predisposition to AD.

Key words: atopic dermatitis, keratinocytes, epithelial cells, cytokines, chemokines, epidermal differentiation, serine proteinases

Article accepted on 15/11/2005

Atopic diseases are genetically determined disorders affecting exclusively tissues such as the skin, the conjunctiva and the respiratory mucosa, which demarcate the host from the environment. In contrast, the gastrointestinal tract and genital mucosae, which also provide a large interface with the outside, do not undergo atopic disorders, not at least according to their current definition. The reasons why only selected tissues develop atopic diseases are probably very complex, but at least two hypotheses can be put forward. First, the immune system may be altered to react with exaggerated responses to apparently harmless antigens (allergens) that reach the skin and respiratory surfaces. Secondly, these tissues may harbor resident (and thus tissue specific) cells with an abnormal capacity to control inflammatory responses [1]. Atopic diseases are indeed characterized by IgE hyperresponsiveness to environmental allergens and a peculiar hyperreactivity of the target tissues toward a variety of inflammatory stimuli. The latter aspect is always present, whereas the former is not constant. In fact, up to 40% of patients with atopic dermatitis (AD) or bronchial asthma do not show elevated serum IgE or specific IgE [2]. Recent acquisitions have also demonstrated that any perturbation of the epidermal permeability barrier represents *per se* an effective mechanism leading to cutaneous inflammation, since numerous cytokines, chemokines and some of the growth factors released by keratinocytes as autocrine regulators of barrier homeostasis can also favor the development of inflammatory reactions [3]. Furthermore, a defective permeability barrier leads to the penetration of environmental allergens into the epidermis and hence facilitates the initia-

tion of the allergen-specific immunological reactions known to be involved in the pathogenesis of AD [4].

Initiation and amplification of inflammatory skin disorders

Infiltrating leukocytes release cytokines which maximally stimulate keratinocytes to express soluble and membrane mediators with a primary role in the recruitment, retention and activation of T cells and other leukocytes in the skin. Interferon (IFN)- γ is the best-characterized proinflammatory cytokine for keratinocytes. IFN- γ -producing T cell clones dominate psoriasis and allergic contact dermatitis lesions, but also intervene in the establishment of chronic AD lesions [5-7]. After exposure to IFN- γ , keratinocytes express on their surface the intercellular adhesion molecule (ICAM)-1 for T cell retention in the epidermis. Basal and suprabasal keratinocytes of chronic AD lesions express ICAM-1, although not to the extent observed in allergic contact dermatitis or psoriasis, and this expression can be an indicator of the presence of some IFN- γ -releasing T cells in the underlying infiltrate. Moreover, IFN- γ up-regulates MHC class I molecules, induces *de novo* synthesis of mature MHC class II molecules and upregulates Fas expression, thus rendering keratinocytes sensitive to T cell-mediated Fas-dependent apoptosis. During the early phases of keratinocyte apoptosis, E-cadherin is cleaved by caspases. The loss of E-cadherin weakens intercellular contacts between keratinocytes and contributes to the formation of epidermal spongiosis, which characterizes eczema [8]. IFN- γ induces keratinocyte expression of cytokines

with a well-recognized role in skin inflammation, including interleukin (IL)-1 α , IL-1 receptor antagonist (IL-1ra), tumor necrosis factor (TNF)- α and GM-CSF, and a variety of chemokines active in T cell attraction, including CXCR3 ligands and MCP-1/CCL2 [5-7]. In the context of AD inflammation, histamine, which is an essential effector molecule of IgE-mediated allergic responses, may also strengthen the pro-inflammatory behaviour of keratinocytes. These cells constitutively express a functional H1 type receptor on their surface, and stimulation of this receptor concomitant with IFN- γ leads to a stronger expression of numerous mediators including GM-CSF, MCP-1, RANTES/CCL5, MIP-3 α /CCL20 and IP-10/CXCL10 [9]. Among T cell-derived cytokines abundantly released in the skin in the course of AD, IL-4 has been characterized as an active contributor to keratinocyte activation only recently [10]. Cells expressing IL-4 can be detected even in the uninvolved skin of patients with AD, and their number increases prominently in acute and chronic lesions. Keratinocytes express functional IL-4 receptors, and although IL-4 alone has a modest capacity to induce cytokine release by keratinocytes, it effectively reinforces the activity of IFN- γ and TNF- α in the induction of CXCR3 agonistic chemokines, and hence reinforces Th1 lymphocyte attraction into the inflamed skin [10]. Furthermore, IL-4 was recently shown to actively oppose ceramide up-regulation by TNF- α and IFN- γ in keratinocytes of organ skin cultures or *in vitro* reconstructed skin equivalents, and in parallel to increase trans-epidermal water loss (TEWL) in both these models [11]. This mechanism could reasonably contribute to aggravate the disruption of the permeability barrier function in AD lesions.

Recruitment of inflammatory cells in AD

The inflammatory infiltrate of AD consists predominantly of dendritic cells (DCs) and memory CD4⁺ T cells [12, 13]. Essentially all T cells infiltrating the skin lesions express the cutaneous lymphocyte-associated antigen (CLA), which functions as a skin homing receptor by mediating T lymphocyte rolling over E-selectin expressed by activated endothelial cells. Chemokine receptors are important players in the tissue targeting of T lymphocytes. In line with this concept, it has been shown that skin-seeking CLA⁺ T cells co-express the CCR4 receptor, which is the ligand of TARC/CCL17 and MDC/CCL22. Th2 compared to Th1 lymphocytes preferentially express CCR4. The proportion of CD4⁺ T lymphocytes expressing the CCR4 receptor in the peripheral blood of patients with AD is higher compared to healthy controls. In addition, CCR4⁺CD4⁺ T cells abundantly infiltrate AD lesions, indicating not only increased generation of CCR4⁺ T cells, but also enhanced recruitment into AD skin.

Keratinocytes express numerous chemotactic signals for T lymphocytes, including RANTES, MCP-1, CTACK/CCL27, PARC/CCL18, MIP-3 α /CCL20, IL-16 and TARC/CCL17 [6, 7, 14, 15]. Both RANTES and MCP-1, which are active on both Th1 and Th2 cells, are strongly expressed by keratinocytes in the diseased skin [16], while elevated RANTES can also be found in the serum of AD patients [17]. Interestingly, *in vitro* studies showed that keratinocytes from AD patients produced increased amounts of RANTES, but reduced levels of IP-10, when

compared to keratinocytes from normal controls or patients with psoriasis [16]. Acute and chronic AD lesions also exhibit strong CTACK expression, which correlates with the increased number of CCR10⁺ T cells infiltrating these lesions [18, 19]. Compared to psoriasis, AD lesions present higher levels of PARC transcript [19]. This chemokine, whose receptor is not known, mediates skin homing of memory T cells in AD. Notably, PARC has been recently described as the most highly expressed chemokine in AD, with DCs representing its major producers [20]. A boost in its expression in the skin of AD patients follows the exposure to the relevant allergen or the staphylococcal superantigen enterotoxin B [21]. These observations suggest an important role for PARC in the initiation and amplification of AD skin inflammation. Also the specific transcript of MIP-3 α can be found expressed in AD skin although less abundantly than in psoriasis [19]. Immunostaining localized this chemokine in the basal epidermis and identified its responding (CCR6⁺) cells mainly as DCs and T cells [22]. Interestingly, disruption of the epidermal permeability barrier upregulates MIP-3 α mRNA in the epidermis, revealing an important mechanism for the initial influx of DCs and T cells in AD skin [22]. In acute and, to a lesser extent, chronic AD lesions, enhanced keratinocyte expression of IL-16 mRNA has been associated with increased numbers of skin-infiltrating CD4⁺ cells, while Langerhans cells have been recognized as the most relevant source of this chemokine in this disease [23]. Indeed, IL-16 is strongly chemotactic for different CD4⁺ cells, which include CD4⁺ T cells and CD4-bearing eosinophils as well as DCs, and Fc ϵ RI engagement was shown to upregulate IL-16 production in Langerhans cells derived from atopic donors [24]. The ligands for CCR4 are TARC and MDC, mostly produced by DCs [25, 26]. A strong correlation with disease activity was recently found for TARC serum levels [27]. TARC is found expressed on microvascular endothelial cells in AD lesions, and therefore these cells may be centrally involved in the arrest of circulating CCR4⁺ T cells [26]. Together with mast cells and endothelial cells, DCs are also abundant sources of I-309/CCL1 in AD skin [28]. Contact with significant allergens was shown to induce the expression of this chemokine, which acts through CCR8, in turn expressed on small subsets of circulating T cells, monocytes and DCs. *In vitro*, IgE binding and cross-linking on the surface of mast cells was shown to effectively upregulate the release of this chemokine [28]. Another mediator strongly expressed by the microvascular endothelium is fractalkine/CX3CL1, which thus appears involved in the recruitment of the CX3CR1-positive leukocytes in AD lesions [29]. Finally, in the context of acute and chronic AD lesions, resident cell populations contribute to the attraction of eosinophils mainly through the release of CCR3 binding molecules, including RANTES, MCP-4/CCL13 and eotaxin/CCL11 [19]. *Figure 1* presents a schematic overview of the central role of keratinocyte activation in the cellular and molecular mechanisms underlying AD.

There is currently an increasing interest in defining the role of the over-expression of epidermal growth factor receptor (EGFR) and its ligands (TGF- α and HB-EGF) in the epithelia affected by chronic inflammatory disorders. The EGFR-ligand system plays a fundamental role in self-protection and response to injury [30]. By contrast, EGFR activation was correlated with sustained IL-8/CXCL8 expression and strong neutrophilia in the broncho-alveolar

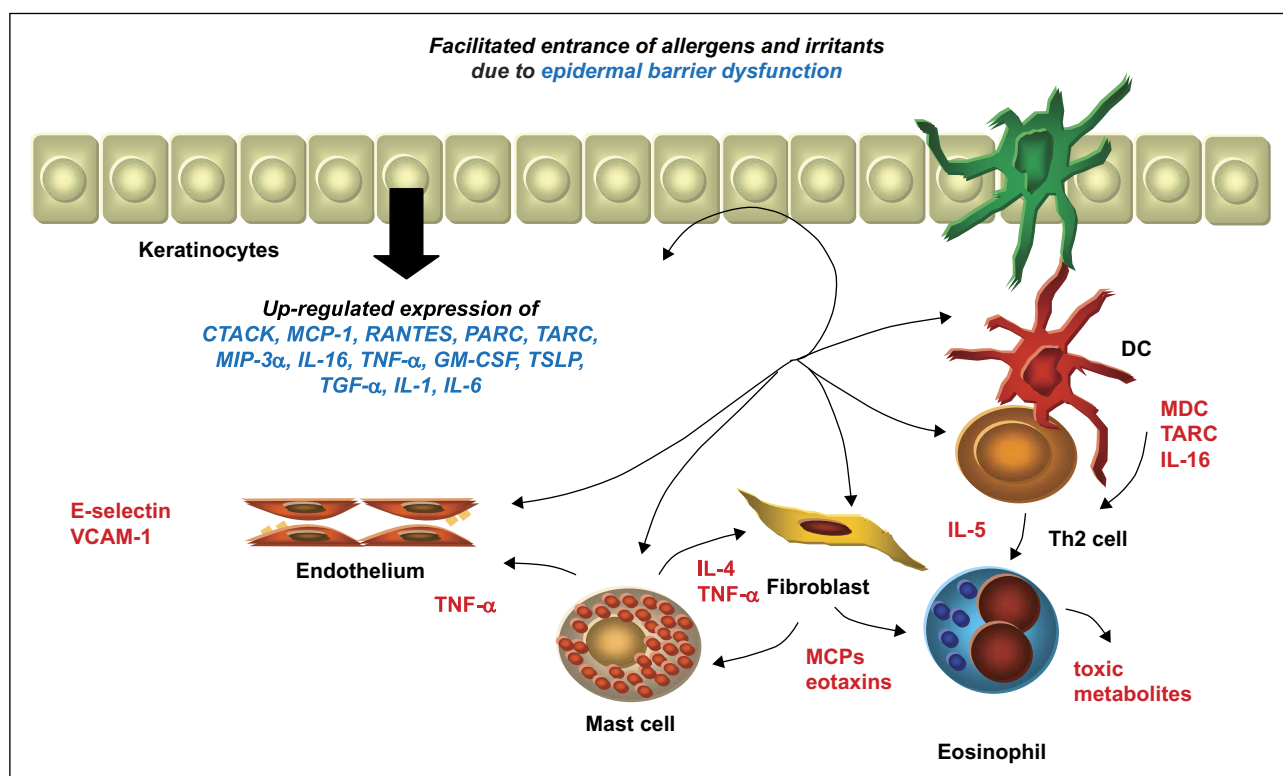


Figure 1. Keratinocytes actively contribute to sustain inflammatory cell recruitment and activation in AD. The natural epidermal barrier against penetration of exogenous irritants and allergens is strongly impaired in the skin of AD patients. As a consequence, chemical *noxa* from the environment can easily provoke sustained keratinocyte expression of numerous cytokines, chemokines and growth factors. In addition, barrier perturbation *per se* induces keratinocyte pro-inflammatory activation, as part of the process aimed to restore cutaneous homeostasis. Under the effect of the local keratinocyte-driven cytokine milieu, infiltrating cell populations mainly represented by DCs, Th2 cells, mast cells and eosinophils, and underlying dermal fibroblasts undergo their own functional activation and further reinforce pro-inflammatory circuits in AD through their massive release of potent cytokines and chemokines.

lavage fluid of asthma patients [31, 32]. EGFR activation is a valid stimulus to induce IL-8 expression in all epithelial cells, including keratinocytes [31-34]. Recently, however, a deeper investigation into the effects of EGFR activation unveiled its complex role in the control of chemokine expression in skin keratinocytes both *in vitro* and *in vivo*. In particular, we could observe that EGFR-driven signaling down-regulates the expression of a cluster of chemokines implicated in the leukocyte recruitment into the epidermis, including MCP-1, RANTES and IP-10 [33, 34]. Activation of extracellular-signal regulated kinase 1,2 (ERK1/2) mediates these regulatory events, which take place at the post-transcriptional level, via the control of chemokine transcript half-life [35]. These observations indicate that targeting EGFR should not invariably be considered an attractive therapy in the inflammatory skin disorders associated with epithelial hyperproliferation, and that further analyses are necessary to better define its specific involvement in atopic diseases.

Keratinocytes from AD patients produce increased amounts of GM-CSF and other pro-inflammatory cytokines

GM-CSF is readily produced by epithelial cells in response to autocrine IL-1 α and TNF- α , and to T cell-derived cyto-

kines such as IFN- γ , IL-4, and IL-17 [10]. It promotes the proliferation and survival of keratinocytes, T cells, eosinophils, monocytes and DC precursors. In addition, GM-CSF favors the recruitment and activation of monocytes, basophils, eosinophils and DCs. Finally, GM-CSF together with IL-4 induces differentiation of DCs from monocyte precursors, a phenomenon that may be particularly relevant to the pathophysiology of AD. Of note, lesional skin of AD patients exhibits an increased number of cells belonging to the DC lineage, including epidermal Langerhans cells, dermal DCs, and a unique population of CD1a⁺ DCs expressing CD1b and/or CD36, which closely resemble DCs generated *in vitro* by culturing monocytes in the presence of GM-CSF and IL-4. Such DCs can efficiently present IgE-bound allergens to T lymphocytes, since they display an upregulated expression of the high affinity (Fc ϵ RI) IgE receptor [36]. In the context of atopic diseases, increased expression of GM-CSF has been documented in nasal and bronchial epithelial cells of rhinitis and asthma patients, respectively, as well as in peripheral blood mononuclear cells of AD patients [13]. Furthermore, we have shown that GM-CSF is overexpressed in keratinocytes of AD lesions, and that keratinocytes cultured from nonlesional skin of adult AD patients produce higher levels of GM-CSF both basally and in response to IL-1 α , IFN- γ or phorbol esters, when compared to keratinocytes from nonatopic individuals [37, 38]. In addition, supernatants from atopic keratinocytes are able

to strongly stimulate mononuclear cell proliferation in a GM-CSF-dependent manner, and conditioned medium from phorbol myristate acetate (PMA)-treated AD keratinocytes together with exogenous IL-4, can support phenotypical and functional differentiation of peripheral blood monocytes into DCs [37]. These findings could explain the persistence of a heavy infiltrate of “inflammatory” DCs in AD skin. The relevant role of GM-CSF overexpression is emphasized by a rat compartmentalized transgene model, where a prolonged skin expression of GM-CSF induced changes commonly observed in AD [39]. Recent studies have shown that AD keratinocytes express high levels of thymic stromal lymphopoietin (TSLP), a factor that activates myeloid DCs to increased expression of chemokines active towards CCR4⁺ Th2 lymphocytes [40, 41]. Skin-restricted overexpression of TSLP in a transgenic mouse results in an AD-like phenotype, with the development of eczematous lesions, a dramatic increase in Th2 T cells expressing cutaneous homing receptors and elevated serum levels of IgE [42]. Moreover, as we previously mentioned, resting and activated AD keratinocytes release higher amounts of RANTES compared to keratinocytes from psoriatic patients and healthy controls [16].

The distinct propensity of keratinocytes to produce higher than normal levels of growth factors (GM-CSF), chemokines (RANTES), and cytokines (TSLP) may greatly stimulate DC differentiation from precursors, and recruit as well as activate DCs in AD skin (figure 2). The biochemical mechanisms underlying excessive production of certain

proinflammatory mediators by epithelial cells are probably multiple. Numerous functional polymorphisms in the regulatory/coding regions of clusters of cytokine/chemokine genes, including RANTES, have been found in AD patients, which could be implicated in an overproduction by keratinocytes. However, apart from genes coding for Th2 cytokines, polymorphisms for other inflammatory genes were not confirmed in other studies [43, 44]. More interestingly, an altered response to inflammatory stimuli could confer specific tissue targeting of the atopic syndrome. In searching for a molecular mechanism underlying abnormal cytokine production in AD keratinocytes, we have examined GM-CSF expression following PMA stimulation [38]. GM-CSF gene transcriptional activity was significantly stronger in AD keratinocytes, both in unstimulated and in PMA-stimulated conditions, and it could be correlated with higher nuclear levels and DNA binding activity of activator protein-1 (AP-1) complexes. More recently, abnormal AP-1 activation was also confirmed in peripheral blood mononuclear cells isolated from AD patients following *in vitro* stimulation with IL-4 [45]. AP-1 is critically implicated in fundamental processes of cell physiology, including keratinocyte proliferation and differentiation [46].

The mechanism underlying enhanced AP-1 activation in cells from AD donors is presently uncharacterized. However, it is possible that abnormal ceramide expression in AD keratinocytes plays some role in this process. Intracellularly, ceramides can compete with the activating binding of

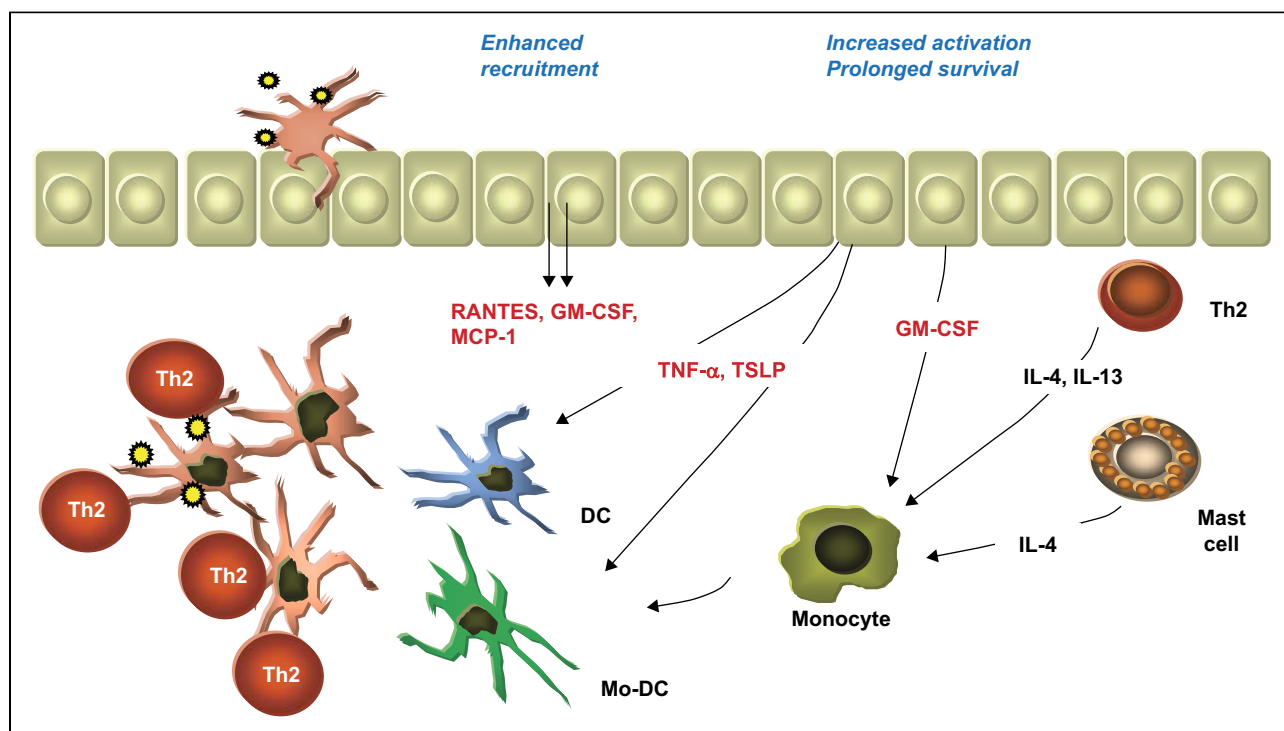


Figure 2. Keratinocytes have a primary role in the promotion of DC functions in AD. In AD lesional skin, up-regulated RANTES, MCP-1 and GM-CSF provide strong recruitment signals for monocytes and DC precursors. In addition, strong epidermal expression of GM-CSF, TSLP, and TNF-α contributes to increase DC maturation from precursors as well as DC functional activation and survival. In addition, IgE hyperresponsiveness to environmental allergens is a well-known flare factor in the majority of AD patients. DCs can bind IgE through high affinity membrane receptors and efficiently present IgE-bound allergens to T lymphocytes, causing the preferential expansion and effector functions of Th2 cells, and a simultaneous boost to their own activation.

diacyl glycerols on distinct protein kinase C (PKC) isozymes, and interfere with PKC functions [47]. A defect in ceramide generation could therefore result in enhanced PKC activation, leading to a dysregulated AP-1 activation and eventually to hyperproduction of GM-CSF and other pro-inflammatory cytokines by AD keratinocytes. An important role of AP-1 has been indicated also in bronchial asthma. Recently, a selective inhibitor of AP-1 function proved therapeutically effective in a mouse asthma model [48].

Mechanisms involved in the alteration of epidermal barrier function

Much of the barrier function of human epidermis against the environment is provided by the cornified cell envelope (CE), an assembly of several structural proteins and lipids that forms the endpoint of keratinocyte differentiation and death [49]. The CE replaces the plasma membrane of differentiating keratinocytes and consists of keratins that are enclosed within an insoluble matrix of proteins, which in turn are crosslinked by transglutaminases and surrounded by a ceramide-rich lipid envelope. Indeed, both protein and lipid components are essential for an optimal barrier function, as demonstrated by genetic defects underlying several human diseases and mouse models [49, 50]. In particular, recent findings have provided evidence that a disturbed protease-antiprotease balance could cause faulty differentiation processes in the epidermis [50, 51].

The existence of a defective permeability barrier function in the skin of AD patients is well accepted, and the epidermal abnormality, generally viewed in the past as a consequence of the inflammatory phenotype, is now considered the outcome of a pre-existing defect of epidermal differentiation [4]. TEWL is found increased both in dry non-eczematous skin and in apparently normal skin of patients with AD, although a further barrier permeability loss correlates with the degree of inflammation in lesional skin. In the lesion, a contribution to this defect could come from IL-4-induced down-regulation of ceramide expression by keratinocytes, as previously commented [11]. However, a complex defect of total lipids, sterol esters and phospholipids including sphingomyelin, as well as an increase in free fatty acids and sterols compared to normal controls, characterizes the skin of AD patients even in the absence of inflammation [4]. Furthermore, evidence exists that both non-lesional and lesional skin of AD patients contains elevated levels of a sphingomyelin deacylase, with a consequent reduction of ceramide synthesis due to the preferential hydrolysis of sphingomyelin into free fatty acids and phosphorylcholine [52]. More recently, the activities of the enzymes crucially involved in ceramide generation in the skin, namely acidic and neutral sphingomyelinase (A- and N-SMase), were found prominently reduced in both non-lesional and lesional AD skin when compared to healthy controls, and reduced A-SMase activity was correlated with decreased ceramide content [53]. Being ceramide content in the stratum corneum closely correlated with its barrier function, these enzymatic defects may represent major predisposition factors to AD inflammation.

Dysregulated serine proteinase activity

Cornification requires a massive activation of epidermal proteases, although for most of these their precise role remains elusive [49-51]. Cysteine- and serine-protease inhibitors are abundantly expressed in the epidermis, suggesting an important role in the control of protease activity. Extracellular proteases are particularly abundant in the CE and are involved in the control of desquamation. Corneodesmosomal proteins that are degraded during desquamation include desmoglein-1, desmocollin-1, plakoglobin and corneodesmosin. The enzymes that are responsible for their degradation are not yet fully identified, although the major candidates include stratum corneum chymotryptic enzyme (SCCE), stratum corneum tryptic enzyme (SCTE) and stratum corneum cathepsin-L-like enzyme (SCCL). The presence of these enzymes is strictly confined to the differentiating suprabasal layers of the epidermis.

The clinically asymptomatic skin of AD patients typically shows a thinner CE as well as a reduced mean corneocyte area. In addition, AD patients release more corneocyte clumps from the skin surface in forced desquamation studies, strongly suggesting that abnormal desquamation is an underlying feature in AD [4]. Independent groups have recently suggested the involvement of a dysregulated serine protease activity in the development of AD. Hansson and coworkers found that a transgenic mouse model over-expressing human SCCE exhibited severe symptoms of chronic itchy dermatitis resembling AD [54]. In addition, an association was recently found between a genetic variant (AACC insertion) in the coding region of SCCE and AD [55], which is now under further investigation for a possible pathophysiological meaning. Being SCCE active also in the proteolytic degradation of distinct lipid processing enzymes including A-SMase [56], enhanced SCCE function could reasonably provide a direct link between enhanced serine protease activity and reduced ceramide expression in atopic skin. The importance of regulated proteolysis in epithelia is well demonstrated by the discovery of the Kazal-type 5 serine protease inhibitor, *Spink5* as the defective gene in Netherton syndrome [57]. The defective inhibitory regulation of the protein product of *Spink5* LEKTI results in increased protease activity in the stratum corneum, accelerated degradation of desmoglein-1 and over-desquamation of corneocytes [58]. LEKTI is strongly expressed in differentiated keratinocytes in normal skin, and the lamellar granule system has been shown to transport and secrete LEKTI earlier than SCCE and SCTE, reasonably in order to prevent improper loss of CE integrity [59]. Previously, Walley and coworkers identified six polymorphisms in *Spink5* and found that a Glu420Lys variant in LEKTI shows significant association with atopy including AD in two independent panels of families [60].

With regard to LEKTI-targeted enzymes, serine proteinases can mediate pro-inflammatory effects via the proteinase-activated receptor-2 (PAR-2), one of the four members of a new subfamily of G-protein receptors known to be highly expressed on epidermal keratinocytes and dermal endothelial cells. These cell populations respond to PAR-2 signalling with hyper-proliferation and enhanced expression of pro-inflammatory cytokines and chemokines [61]. PAR-2 over-activation could also be importantly involved in the development of pruritus [62]. Endogenous PAR-2 activators may include mast cell-derived tryptase

and other trypsin-like enzymes such as SCTE, whereas exogenous activators could be tryptic enzymes released by *Staphylococcus aureus* and house dust mites. Indeed, several constituents of house dust mites can sustain a non-immune specific inflammatory reaction via their serine proteinase activity in parallel with the elicitation of an allergen-specific response.

Chymase is a chymotrypsin- and cathepsin G-like serine proteinase released by mast cells. Due to their strong expression of the high-affinity IgE receptor, mast cells are well-recognized crucial effectors of IgE-dependent reactions. Upon IgE receptor aggregation via IgE binding, mast cells release a plethora of preformed and newly synthesized mediators, including large quantities of chymase. Apart from its possible direct involvement in fibrosis and tissue remodeling, chymase exhibits chemotactic activity for human polymorphonuclear leukocytes *in vitro* and on eosinophils *in vivo* [63]. Recent data have confirmed previous observations of a significant association between polymorphism in the coding region of chymase promoter and AD but no association with serum IgE levels, and support the hypothesis that it may serve as a candidate gene for this disease [64].

Conclusion

Keratinocytes participate to the pathogenesis of AD through the production of numerous inflammatory signals, which initiate, amplify and sustain skin inflammation. It is likely that genetic abnormalities in the homeostatic mechanisms controlling keratinocyte differentiation affect the constitutive and induced production of mediators by AD keratinocytes along complex patterns involving inflammatory genes themselves and/or signal transduction pathways. Together, these alterations direct the specific expression of the atopic state to the skin. A further advancement in the understanding of the complex molecular bases of abnormal keratinocyte behavior in AD may ultimately lead to the identification of novel targets for specific and effective therapeutic intervention. ■

References

1. Girolomoni G, Pastore S. Epithelial cells and atopic diseases. *Curr Allergy Asthma Rep* 2001; 1: 481-2.
2. Schmid-Grendelmeier P, Simon D, Simon HU, Akdis CA, Wüthrich B. Epidemiology, clinical features, and immunology of the "intrinsic" (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 2001; 56: 841-9.
3. Elias PM, Wood LC, Feingold KR. Epidermal pathogenesis of inflammatory dermatoses. *Am J Contact Dermatitis* 1999; 10: 119-26.
4. Elias PM. Stratum corneum defensive functions: an integrated view. *J Invest Dermatol* 2005; 125: 183-200.
5. Klunker S, Trautmann A, Akdis M, Verhagen J, Schmid-Grendelmeier P, Blaser K, et al. A second step of chemotaxis after transendothelial migration: keratinocytes undergoing apoptosis release IFN- γ -inducible protein 10, monokine induced by IFN- γ , and IFN- γ -inducible α -chemoattractant for T cell chemotaxis toward epidermis in atopic dermatitis. *J Immunol* 2003; 171: 1078-84.
6. Pastore S, Mascia F, Mariotti F, Dattilo C, Girolomoni G. Chemokine networks in inflammatory skin diseases. *Eur J Dermatol* 2004; 14: 203-8.
7. Pastore S, Cavani A, Albanesi C, Girolomoni G. Chemokines of human skin. In: Bos JD, ed. *Skin Immune System*. 3rd ed. Boca Raton: CRC Press, 2005: 373-92; (CA).
8. Trautmann A, Altnauer F, Akdis M, Simon HU, Disch R, Brocker EB, et al. The differential fate of cadherins during T cell-induced keratinocyte apoptosis leads to spongiosis in eczematous dermatitis. *J Invest Dermatol* 2001; 117: 927-34.
9. Giustizieri ML, Albanesi C, Fluhr J, Gisondi P, Norgauer J, Girolomoni G. H1 histamine receptor mediates inflammatory responses in human keratinocytes. *J Allergy Clin Immunol* 2004; 114: 1176-82.
10. Albanesi C, Scarponi C, Giustizieri ML, Girolomoni G. Keratinocytes in inflammatory skin diseases. *Curr Drug Targets Inflamm Allergy* 2005; 4: 329-34.
11. Hatano Y, Terashi H, Arakawa S, Katagiri K. Interleukin-4 suppresses the enhancement of ceramide synthesis and cutaneous permeability barrier functions induced by TNF- α and IFN- γ in human epidermis. *J Invest Dermatol* 2005; 124: 786-92.
12. Girolomoni G, Sebastiani S, Albanesi C, Cavani A. T-cell subpopulations in the development of atopic and contact allergy. *Curr Opin Immunol* 2001; 13: 733-7.
13. Leung DYM, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New Insights into atopic dermatitis. *J Clin Invest* 2004; 113: 651-7.
14. Sicherer SH, Leung DYM. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. *J Allergy Clin Immunol* 2005; 116: 153-63.
15. Zheng X, Nakamura K, Tojo M, Akiba H, Oyama N, Nishibu A, et al. Ultraviolet A irradiation inhibits thymus and activation-regulated chemokine (TARC/CCL17) production by a human keratinocyte HaCaT cell line. *Eur J Dermatol* 2003; 13: 348-53.
16. Giustizieri ML, Mascia F, Frezzolini A, De Pittà O, Chinni ML, Giannetti A, et al. Keratinocytes from patients with atopic dermatitis and psoriasis show a different chemokine production profile in response to T cell-derived cytokines. *J Allergy Clin Immunol* 2001; 107: 871-7.
17. Kaburagi Y, Shimada Y, Nagaoka T, Hasegawa M, Takehara K, Sato S. Enhanced production of CC-chemokines (RANTES, MCP-1, MIP-1 α , MIP-1 β , and eotaxin) in patients with atopic dermatitis. *Arch Dermatol Res* 2001; 293: 350-5.
18. Homey B, Alenius H, Muller A, Soto H, Bowman EP, Yuan W, et al. CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. *Nat Med* 2002; 8: 157-65.
19. Nomura I, Boguniewicz GB, Darst MA, Travers JB, Leung DYM. Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* 2003; 112: 1195-202.
20. Gunther C, Bello-Fernandez C, Kopp T, Kund J, Carbadillo-Perrig N, Hinteregger S, et al. CCL18 is expressed in atopic dermatitis and mediates skin homing of human memory T cells. *J Immunol* 2005; 174: 1723-8.
21. Pivarcsi A, Gompert M, Dieu-Nosjean MC, Lauerma A, Kubitzka R, Meller S, et al. CC chemokine ligand 18, an atopic dermatitis-associated and dendritic cell-derived chemokine, is regulated by staphylococcal products and allergen exposure. *J Immunol* 2004; 173: 5810-7.
22. Schmuth M, Neyer S, Rainer C, Grassegger A, Fritsch P, Romani N, Heufner C. Expression of the C-C chemokine MIP-3 α /CCL20 in human epidermis with impaired permeability barrier function. *Exp Dermatol* 2002; 11: 135-42.
23. Reich K, Hugo S, Middel P, Blaschke V, Heine A, Gutgesell C, et al. Evidence for a role of Langerhans cell-derived IL-16 in atopic dermatitis. *J Allergy Clin Immunol* 2002; 109: 681-7.
24. Reich K, Heine H, Hugo S, Blaschke V, Middel P, Kaser A, et al. Engagement of the Fc ϵ RI stimulates the production of IL-16 in atopic dermatitis. *J Immunol* 2001; 167: 6321-9.
25. Vulcano M, Albanesi C, Stopacciaro A, Bagnati R, D'Amico G, Struyf S, et al. Dendritic cells as a major source of macrophage-derived chemokine/CCL22 *in vitro* and *in vivo*. *Eur J Immunol* 2001; 31: 812-22.
26. D'Ambrosio D, Albanesi C, Lang R, Girolomoni G, Sinigaglia F, Laudanna C. Quantitative differences in chemokine receptor engagement generate diversity in integrin-dependent lymphocyte adhesion. *J Immunol* 2002; 169: 2303-12.
27. Hijnen D, Bruin-Weller M, Oosting B, Lebre C, De Jong E, Bruijnzeel-Koomen C, Knol E. Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell-attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. *J Allergy Clin Immunol* 2004; 113: 334-40.

28. Gompert M, Dieu-Nosjean MC, Winterberg F, Bünnemann E, Kubitz RC, Da Cunha L, et al. CCL1-CCR8 interactions: an axis mediating the recruitment of T cells and Langerhans-type dendritic cells to sites of atopic skin inflammation. *J Immunol* 2005; 174: 5082-91.
29. Echigo T, Hasegawa M, Shimada Y, Takehara K, Sato S. Expression of fractalkine and its receptor, CX3CR1, in atopic dermatitis: possible contribution to skin inflammation. *J Allergy Clin Immunol* 2004; 113: 940-8.
30. Jost M, Kari C, Rodeck U. The EGF receptor – an essential regulator of multiple epidermal functions. *Eur J Dermatol* 2000; 10: 505-10.
31. Hamilton LM, Torres-Lozano C, Puddicombe SM, Richter A, Kimber I, Dearman RJ, et al. The role of the epidermal growth factor receptor in sustaining neutrophil inflammation in severe asthma. *Clin Exp Allergy* 2003; 33: 233-40.
32. Lukacs NW, Miller AL, Hogaboam CM. Chemokine receptors in asthma: searching for the correct immune targets. *J Immunol* 2003; 171: 11-5.
33. Pastore S, Mascia F, Mariani V, Girolomoni G. Epidermal growth factor receptor ligands and tumor necrosis factor- α coregulate chemokine expression in human keratinocytes. *Ann N Y Acad Sci* 2002; 973: 210-3.
34. Mascia F, Mariani V, Girolomoni G, Pastore S. Blockade of the EGF receptor induces a deranged chemokine expression in keratinocytes leading to enhanced skin inflammation. *Am J Pathol* 2003; 163: 303-12.
35. Pastore S, Mascia F, Mariotti F, Dattilo C, Mariani V, Girolomoni G. ERK1/2 regulates epidermal chemokine expression and skin inflammation. *J Immunol* 2005; 174: 5047-56.
36. Wollenberg A, Kraft S, Hanau D, Bieber T. Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. *J Invest Dermatol* 1996; 106: 446-53.
37. Pastore S, Fanale-Belasio E, Albanesi C, Chinni ML, Giannetti A, Girolomoni G. 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: 3009-17.
38. Pastore S, Giustizieri M, Mascia F, Giannetti A, Kaushansky K, Girolomoni G. 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: 1134-43.
39. Xing Z, Gauldie J, Tremblay GM, Hewlett BR, Addison C. Intra-dermal transgenic expression of granulocyte-macrophage colony-stimulating factor induces neutrophilia, epidermal hyperplasia, Langerhans cell/macrophage accumulation, and dermal fibrosis. *Lab Invest* 1997; 77: 615-22.
40. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; 3: 673-80.
41. Gilliet M, Soumelis V, Watanabe N, Hanabuchi S, Antonenko S, de Waal-Malefyt R, et al. Human dendritic cells activated by TSLP and CD40L induce proallergic cytotoxic T cells. *J Exp Med* 2003; 197: 1059-63.
42. Yoo J, Omori M, Gyarmati D, Zhou B, Aye T, Brewer A, Corneau MR, Campbell DJ, Ziegler SF. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. *J Exp Med* 2005; 202: 541-9.
43. Kozma GT, Falus A, Bojszko A, Krikovszky D, Szabo T, Nagy A, Szalai C. Lack of association between atopic eczema/dermatitis syndrome and polymorphisms in the promoter region of RANTES and regulatory region of MCP-1. *Allergy* 2002; 57: 160-3.
44. Cookson W. The immunogenetics of asthma and eczema: a new focus on the epithelium. *Nat Rev Immunol* 2004; 4: 978-88.
45. Yamazaki F, Aragane Y, Maeda A, Matsushita K, Ueno K, Yudate T, Kawada A, Tezuka T. Overactivation of IL-4-induced activator protein-1 in atopic dermatitis. *J Dermatol Sci* 2002; 28: 227-33.
46. Iversen L, Johansen C, Kragballe K. Signal transduction pathways in human epidermis. *Eur J Dermatol* 2005; 15: 4-12.
47. Jones MJ, Murray AW. Evidence that ceramide selectively inhibits protein kinase C- α translocation and modulates bradykinin activation of phospholipase D. *J Biol Chem* 1995; 270: 5007-13.
48. Nguyen C, Teo JL, Matsuda A, Eguchi M, Chi EY, Henderson Jr. WR, Kahn M. Chemogenomic identification of Ref-1/AP-1 as a therapeutic target for asthma. *Proc Natl Acad Sci USA* 2003; 100: 1169-73.
49. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 2005; 6: 328-40.
50. Zeeuwen PJM. Epidermal differentiation: the role of proteases and their inhibitors. *Eur J Cell Biol* 2004; 83: 761-73.
51. Mägert HJ, Drögemüller K, Raghunath M. Serine proteinase inhibitors in the skin: role in homeostasis and disease. *Curr Protein Pept Sci* 2005; 6: 241-54.
52. Ishibashi M, Arikawa J, Okamoto R, Kawashima M, Takagi Y, Ohguchi K, et al. Abnormal expression of the novel epidermal enzyme, glucosylceramide deacylase, and accumulation of its enzymatic reaction product, glucosylsphingosine, in the skin of patients with atopic dermatitis. *Lab Invest* 2003; 83: 397-408.
53. Jensen J-M, Fölster-Holst R, Baranowsky A, Schunck M, Winoto-Morbach, Neuman C, Schütze S, Proksch E. Impaired sphingomyelinase activity and epidermal differentiation in atopic dermatitis. *J Invest Dermatol* 2005; 122: 1423-31.
54. Hansson L, Backman A, Ny A, Edlund M, Ekholm E, Ekstrand Hammarstrom B, et al. Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. *J Invest Dermatol* 2002; 118: 444-9.
55. Vasilopoulos Y, Cork MJ, Murphy R, Williams HC, Robinson DA, Duff GW, et al. Genetic association between an AACC insertion in the 3'UTR of the stratum corneum chymotryptic enzyme gene and atopic dermatitis. *J Invest Dermatol* 2004; 123: 62-6.
56. Hachem J-P, Man M-Q, Crumrine D, Uchida Y, Brown BE, Rogiers V, 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: 510-20.
57. Bitoun E, Chavanas S, Irvine AD, Lonie L, Bodemer C, Paradisi M, et al. Netherton syndrome: disease expression and spectrum of SPINK5 mutations in 21 families. *J Invest Dermatol* 2002; 118: 352-61.
58. Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A, et al. Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat Genet* 2005; 37: 56-65.
59. Ishida-Yamamoto A, Deraison C, Bonnart C, Bitoun E, Robinson R, O'Brien T, Wakamatsu K, et al. LEKTI is localized in lamellar granules, separated from KLK5 and KLK7, and is secreted in the extracellular spaces of the superficial stratum granulosum. *J Invest Dermatol* 2005; 124: 360-6.
60. Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubhi B, Lawrence R, et al. Gene polymorphism in Netherton and common atopic disease. *Nat Gen* 2001; 29: 175-8.
61. Steinhoff M, Buddenkotte J, Shpacovitch V, Rattenholl A, Moormann C, Vergnolle N, et al. Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response. *Endocr Rev* 2005; 26: 1-43.
62. Steinhoff M, Neisius U, Ikoma A, Fartasch M, Heyer G, Skov PS, Luger TA, Schmelz M. Proteinase-activated receptor 2 mediates itch: a novel pathway for pruritus in human skin. *J Neurosci* 2003; 23: 6176-80.
63. Crivellato E, Ribatti D. Involvement of mast cells in angiogenesis and chronic inflammation. *Curr Drug Targets Inflamm Allergy* 2005; 4: 9-11.
64. Weidinger S, Rümmler L, Klopp N, Wagenpfeil S, Baurecht HJ, Fischer G, et al. Association study of mast cell chymase polymorphisms with atopy. *Allergy* 2005; 60: 1256-61.