33 Keratinocytes in Atopic Eczema

G. Girolomoni, F. Mascia, C. Dattilo, A. Giannetti, S. Pastore

33.1 Introduction

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, 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 eczema (AE) or bronchial asthma do not show elevated serum IgE or specific IgE [2]. The preferential development of T-helper 2 (Th2) immune responses in atopic patients has been extensively studied [3], whereas the cellular and molecular bases of the tissue hyperreactivity have been only recently investigated. Accumulating evidence suggests that keratinocytes of AE patients produce higher amounts of certain cytokines and chemokines compared to keratinocytes of nonatopic subjects. Exaggerated release of these factors can be important for enhanced recruitment as well as sustained survival and activation of inflammatory cells, including dendritic cells and T lymphocytes. Moreover, AE keratinocytes may have a dysregulated activity of activator protein (AP)-1 transcription factors, which can help to explain the abnormal expression of granulocyte-macrophage colony stimulating factor (GM-CSF) and other cytokines, and indicating the existence of molecular mechanisms targeting atopic inflammation to the skin of AE patients.

33.2 Keratinocytes Actively Participate in the Initiation and Amplification of Skin Inflammatory Responses

Epithelial cells, including epidermal keratinocytes, are the outermost component of skin and mucous membranes, and they can be activated by diverse factors to produce mediators involved in the initiation and amplification of inflammatory responses. Recent acquisitions have also demonstrated that any perturbation of the epidermal permeability barrier represents per se an effective mechanism leading to cutaneous inflammation, since the cytokines and growth factors released by keratinocytes as autocrine regulators of barrier homeostasis, can also favor the development of inflammatory reactions [4]. Among the environmental factors, ultraviolet radiation, irritants, reactive haptens, as well as bacteria and viruses have been identified as triggers of the inflammatory activities of keratinocytes. Keratinocytes express a number of innate immune-related receptors, including some of the Toll-like receptors [5, 6], and can thus initiate innate response. However, the most thoroughly investigated keratinocyte-activating factors are cytokines released by T lymphocytes. Indeed, resting keratinocytes express functional receptors for and are sensitive to T cell-derived cytokines [7], and actively participate in

the amplification of skin inflammatory reactions initiated by T cells. T lymphocytes play a fundamental role in the pathogenesis of chronic skin disorders such as AE, allergic contact dermatitis to haptens, and psoriasis. In these conditions, infiltrating T lymphocytes release cytokines which 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 AE lesions [66]. After exposure to IFN-γ, keratinocytes express on their surface the intercellular adhesion molecule (ICAM)-1, crucial for T cell retention in the epidermis [8]. Basal and suprabasal keratinocytes of chronic AE 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 upregulates 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 [9, 10]. 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 [11]. IFN-γ induces keratinocyte expression of cytokines with a well-recognized role in skin inflammation, including interleukin (IL)-1α, 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 CXCL10, CXCL11, CXCL9, and CCL2 [12]. The efficiency of IFN-γ in activating keratinocytes is enhanced by cotreatment with other cytokines, such as TNF-α and IL-17 [8]. During chronic inflammatory diseases, TNF-α is released throughout the epidermis by activated keratinocytes and by infiltrating leukocytes, and in turn, TNF-α is very effective in inducing CXCL8 and CCL5 expression in keratinocytes. Among T cell-derived cytokines abundantly released in the skin in the course of AE, IL-4 has been characterized as an active contributor to keratinocyte activation only recently [7, 12]. Cells expressing IL-4 can be detected even in the uninvolved skin of patients with AE, and their number increases prominently in acute and chronic lesions [1]. Keratinocytes express functional IL-4 receptor, 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 elicits T lymphocyte attraction into the inflamed skin [12].

33.3 The Role of Keratinocytes in the Recruitment of Inflammatory Cells in Atopic Eczema

The inflammatory infiltrate of AE consists predominantly of dendritic cells and memory $CD4^+$ T cells. Essentially all T cells infiltrating the skin lesions express the cutaneous lymphocyte-associated antigen (CLA), which functions as a skin homing receptor for T lymphocytes 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 coexpress the CCR4 receptor, the ligand for CCL17 and CCL22. CCR4 is also preferentially expressed by Th2 compared to Th1 lymphocytes. The proportion of $CD4^+$ T lymphocytes expressing the CCR4 receptor in the peripheral blood of patients with AE is higher compared to $CD4^+$ T cells of healthy controls. In contrast, AE patients bear a lower percentage of circulating $CXCR3^+CD4^+$ T cells [13–16]. Moreover, the percentage of blood $CCR4^+CD4^+$ cells correlates positively with disease severity and IL-4 and IL-13 secretion by $CD4^+$ T cells [16, 17]. $CCR4^+CD4^+$ T cells are also positive for the skin-homing receptor, CLA, and infiltrate AE lesions in high numbers [15, 16]), indicating not only increased generation of $CCR4^+$ T cells, but also enhanced recruitment into AE skin.

Keratinocytes offer numerous chemotactic signals for the attraction of T lymphocytes in lesional AE skin. In acute and, to a lesser extent, chronic AE lesions, enhanced keratinocyte expression of IL-16 mRNA has been associated with increased numbers of skin-infiltrating $CD4^+$ cells [18], although Langerhans cells have been recognized as the most relevant source of this chemokine in this disease [19]. IL-16 exerts a strong chemotactic activity towards different $CD4^+$ cells, including $CD4^+$ T cells and CD4-bearing eosinophils as well as dendritic cells [20], and FcεRI engagement has

been shown to upregulate IL-16 production in Langerhans cells derived from atopic donors [21]. Recently, an elevation of circulating IL-16 has been associated to active AE in children [22]. The ligands for CCR4 are CCL17 and CCL22, two chemokines present in high amounts in the plasma of AE patients and whose levels also correlate with disease activity [23–25]. Both TARC and MDC are produced abundantly by dendritic cells in vitro and in vivo in AE lesions [26, 27]. Although keratinocytes can produce small amounts of CCL17 and CCL22 [13, 39], the major source of these chemokines appear to be dendritic cells, which may thus guide not only the activation but also the preferential accumulation of $CCR4^+$ T cells in AE skin. CCL17 is also expressed on microvascular endothelial cells in AE lesions, and therefore may be primarily involved in the arrest of $CCR4^+$ T cells [27].

Other chemokines that participate in the accumulation of T cells in AE include CCL5, CCL2, CCL20, CCL27, CCL11, and CCL13. CCL5 and CCL2, which attract both Th1 and Th2 cells, are expressed by infiltrating leukocytes but especially by keratinocytes in diseased skin [28], although only CCL5 is elevated in the serum of patients [29]. Via the interaction with CCR3, CCL5 may play a role in the early recruitment of Th2 cells and eosinophils, but it is also a powerful chemoattractant for dendritic cells and monocytes; however, in chronic lesions, CCL5 can also attract Th1 cells through CCR5. Noteworthy, keratinocytes cultured from nonlesional skin of AE patients responded to stimulation with IFN-γ, TNF-α, or phorbol esters (PMA) with significant higher levels of CCL5 secretion, when compared to keratinocytes from healthy controls or psoriatic patients [28]. In line with the evidence that keratinocytes are committed to an increased synthesis of this chemokine is the observation that AE patients carry a functional mutation, responsible for a much higher transcriptional activity of CCL5 promoter [30, 31]. CCL2 is another chemokine strongly expressed by basal keratinocytes of lesional AE skin, and effective towards T cells, monocytes, and dendritic cells [28]. Similarly to psoriasis, acute and chronic AE lesions exhibit strong CCL27 expression in the epidermis and numerous $CCR10^+$ T cells [32]. CCL27 is constitutively produced by keratinocytes, can be potently induced by stimulation with TNF-α and IL-1β in synergism, and preferentially attracts a subset of $CCR10^+CLA^+$ memory T cells. CCL20 mRNA is also expressed in AE skin, although less abundantly than in psoriasis [33], with immunostaining localizing the chemokine in the basal epidermis, and $CCR6^+$ cells being mainly dendritic cells and T cells [34]. Interestingly, disruption of the epidermal permeability barrier upregulates epidermal CCL20 mRNA, revealing an important mechanism for the initial influx of dendritic cells and T cells in AE skin, which constitutively presents epidermal permeability barrier dysfunction [33]. In acute and chronic AE lesions, keratinocytes have been reported to synthesize CCL11 and CCL13, particularly active in eosinophil attraction and activation [35]. However, no significant staining for eotaxin could be found in the keratinocytes of AE skin in a previous work, while its expression was observed in mononuclear cells and eosinophils, as well as in fibroblasts [36]. Moreover, in vitro studies indicated that cytokine-activated fibroblasts are major sources of eotaxin and CCL13 in the lesional AE skin [37, 38]. In contrast to psoriasis, CXCL8 and CXCL10 are only weakly expressed in some limited areas of the epidermis in AE lesions. Keratinocytes may contribute relevantly to the partial Th2-to-Th1 lymphocyte switch observed in the transition from acute to chronic AE via the release of chemokines attracting Th1 cells [39].

Currently, there is an increasing interest in defining the role of the prominent overexpression of epidermal growth factor receptor (EGFR) and its ligands (TGF-α and HB-EGF) in the epithelia affected by atopic disorders [40] The EGFR-ligand system plays a fundamental role in self-protection and repair to injury in epithelial tissues, and its activation has been associated to accelerated cell regeneration and reduced inflammatory infiltrate following mechanical, chemical, or ischemic tissue damage [41–44]. By contrast, its marked activation in both intact and damaged bronchial epithelium in severe asthma has been recently correlated with the high levels of CXCL8 and consequently with the strong neutrophilia found in the broncho-alveolar lavage fluid of these patients [45, 46]. The persistence of a massive neutrophilic infiltrate cooperates to the perpetuation of epithelial cell proinflammatory activation and tissue damage in asthma. Indeed, EGFR activation is a valid stimulus to induce CXCL8 expression in all epithelial cells [32, 40, 45]. Recently, however, a deeper investigation into the effects of EGFR activation unveiled its complex role in the control of chemokine expression (at least) in skin keratinocytes, where EGFR-driven signaling downregulated the expression of a cluster of chemokines, including CCL5, CCL2, and CXCL10, con-

comitant to a promotion of CXCL8 induction [40, 47]. In the mouse model of contact hypersensitivity to 2, 4-dinitro fluorobenzene, pharmacological abrogation of EGFR signaling induced a deranged expression of these chemokines and consequently an amplification of both irritant and immune-specific inflammation in response to hapten painting [40]. These observations indicate that targeting EGFR should not be invariably considered an attractive therapy in the inflammatory skin disorders accompanied by epithelial hyperproliferation, and that further analyses are necessary to better define its specific involvement in atopic diseases.

33.4 Keratinocytes from Atopic Eczema Patients Produce Increased Amounts of GM-CSF and Other Proinflammatory Cytokines

GM-CSF is readily produced by epithelial cells in response to autocrine IL-1α and TNF-α, and to the T cell-derived cytokines IFN-γ, IL-4, and IL-17 [7, 8]). GM-CSF promotes the proliferation and survival of keratinocytes, T cells, eosinophils, monocytes, and dendritic cell precursors. In addition, GM-CSF favors the recruitment and activation of monocytes, basophils, eosinophils, and dendritic cells. Finally, GM-CSF, together with IL-4, induces differentiation of dendritic cells from monocyte precursors, a phenomenon that may be particularly relevant to the pathophysiology of AE. Indeed, lesional skin of AE patients exhibits an increased number of cells belonging to the dendritic cell lineage, including epidermal Langerhans cells, dermal dendritic cells, and a unique population of $CD1a^+$ dendritic cells expressing CD1b and/or CD36, which closely resemble dendritic cells generated in vitro by culturing monocytes in the presence of GM-CSF and IL-4. Such dendritic cells can efficiently present IgE-bound allergens to T lymphocytes, since they display an upregulated expression of the high affinity (FcεRI) IgE receptor [48, 49]. In the context of atopic diseases, a prominent 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 AE patients (reviewed in [50]). We have shown that GM-CSF is overexpressed in keratinocytes of AE lesions, and that keratinocytes cultured from nonlesional skin of adult AE patients produce higher levels of GM-CSF, both basally and in response to IL-1α, IFN-γ, or phorbol esters (PMA), when compared to keratinocytes from nonatopic individuals [50, 51]. In addition, supernatants from atopic keratinocytes are able to strongly stimulate mononuclear cell proliferation in a GM-CSF-dependent manner, and conditioned medium from PMA-treated AE keratinocytes, together with exogenous IL-4, can support phenotypical and functional differentiation of peripheral blood monocytes into dendritic cells. These findings could explain the persistence of a heavy infiltrate of „inflammatory" dendritic cells in AE skin [50]. 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 AE [52]. Recent studies have shown that AE keratinocytes express in vivo high levels of thymic stromal lymphopoietin, a factor which activates myeloid dendritic cells to a high production of chemokines attracting $CCR4^+$ Th2 lymphocytes and increased stimulation of T cell responses [53, 54]. Moreover, resting and activated AE keratinocytes release higher amounts of CCL5 compared to keratinocytes from psoriatic patients and healthy controls [28], and more abundant TNF-α, IL-1α, and IL-1 receptor antagonist following IFN-γ stimulation in vitro [55], although TNF-α expression in lesional AE skin is hardly detectable compared to psoriasis (unpublished observation), possibly in relation to the limited amount of IFN-γ available locally in AE skin. At any rate, in the context of chronic AE lesions, keratinocyte overresponse to IFN-γ may serve as a further amplification mechanism to enhance disease severity [56]. The triggers that activate keratinocytes in the very early phases may include the altered epidermal permeability barrier functions [4, 33]. In contrast to bronchial epithelial cells, environmental allergens such as those of the house dust mite do not seem to stimulate keratinocyte production of chemokines or cytokines [57].

33.5 Dysregulated Activation of AP-1 Transcription Factors May Be Implicated in the Enhanced Expression of Inflammatory Genes by Atopic Eczema Keratinocytes

The biochemical mechanisms underlying excessive production of certain proinflammatory mediators by epithelial cells are probably multiple. For instance,

functional polymorphisms in the regulatory/coding regions of clusters of cytokine/chemokine genes, including RANTES, have been found in AE patients, which could be implicated in overproduction by keratinocytes. However, apart from genes coding for Th2 cytokines, polymorphisms for other inflammatory genes have not been confirmed in other studies [58]. Indeed, the genes that contribute to complex diseases are difficult to identify because they typically exert small effects on disease risk; in addition, the magnitude of their effects is likely to be modified by other unrelated genes as well as environmental factors. Thus, susceptibility loci for complex diseases identified in one study may not be replicated in other populations.

More interestingly, an altered response to inflammatory stimuli could confer specific tissue targeting of the atopic syndromes. In searching for a molecular mechanism underlying abnormal cytokine production in AE keratinocytes, we have examined GM-SCF expression following PMA stimulation [51]. Similar GM-CSF mRNA decay kinetics in keratinocytes from both nonatopic and AE subjects indicated that GM-CSF mRNA overexpression in AE keratinocytes was not due to reduced mRNA degradation. Conversely, GM-CSF gene transcriptional activity was significantly stronger in AE keratinocytes, both in unstimulated and in PMA-stimulated conditions, and it was correlated with higher nuclear levels of functional activator protein-1 (AP-1) complexes. A higher expression level of c-Jun, and a more pronounced PMA-induced phosphorylation of JunB and c-Fos were observed. Although the activity of AP-1 depends on complex promoter- and tissue-specific cooperation with other transcription factors, an amplification of its function could seriously affect a variety of AP-1-mediated processes. AP-1 is activated by various cytokines, including IL-4, IFN-γ, and TNF-α, as well as oxidative stress, and AP-1 binding sites are located in the promoters of a vast array of cytokines and chemokines, including IL-1, TNF-α, and RANTES.

The mechanisms that underlie the selective, excessive activation of c-Jun, JunB, and c-Fos in AE keratinocytes are presently unknown. However, it is possible that abnormal function of diacylglycerol (DAG)-dependent protein kinase C (PKC) isoforms contributes to enhanced AP-1 activation [59]. In fact, the epidermis of AE patients is characterized by a marked decrease in the content of ceramides, which causes a dysfunction in the cutaneous permeability barrier [60]. Intracellularly, ceramides can compete with the activating binding of DAGs on distinct PKC isozymes, and interfere with PKC functions [61]. A defect in ceramide generation could therefore result in enhanced PKC activation, leading to an excessive AP-1 activation, and, eventually, to hyperproduction of GM-CSF and other proinflammatory cytokines by AE keratinocytes.

An important role of AP-1 has been indicated also in bronchial asthma. Higher levels of AP-1 DNA binding activity, secondary to increased generation of c-Fos or phosphorylation of JNK, have been documented respectively in peripheral blood mononuclear cells and in tuberculin-induced skin inflammation of corticosteroid-resistant patients with atopic asthma [62, 63]. In addition, a selective inhibitor of Ref-1/AP-1 proved therapeutically effective in a mouse asthma model [64].

33.6 Concluding Remarks

Keratinocytes participate in the pathogenesis of AE through the production of numerous inflammatory signals, which amplify and sustain skin inflammation. It is likely that genetic abnormalities affect the constitutive and induced production of mediators by AE keratinocytes along complex patterns involving inflammatory genes themselves and/or signal transduction pathways. These alterations can modulate initiation, amplification, and persistence of skin inflammation in AE patients, and possibly direct the specific tissue expression of the atopic state [65]. A better understanding of the molecular bases of this abnormal behavior may ultimately afford the identification of novel targets for specific and effective therapeutic intervention.

References

1. Girolomoni G, Pastore S (2001) Epithelial cells and atopic diseases. Curr Allergy Asthma Rep 1:481 – 482
2. Schmid-Grendelmeier P, Simon D, Simon HU, Akdis CA, Wuthrich B (2001) Epidemiology, clinical features, and immunology of the „intrinsic“ (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis) Allergy 56: 841 – 849
3. Murphy KM, Reiner SL (2002) The lineage decisions of helper T cells. Nat Rev Immunol 2:933 – 944

4. Elias PM, Wood LC, Feingold KR. Epidermal pathogenesis of inflammatory dermatoses (1999) Am J Contact Dermatitis 10:119 – 126
5. Curry JL, Qin JZ, Bonish B, Carrick R, Bacon P, Panella J, Robinson J, Nickoloff BJ (2003) Innate immune-related receptors in normal and psoriatic skin. Arch Pathol Lab Med 127:178 – 186
6. Pivarcsi A, Bodai L, Rethi B, Kenderessy-Szabo A, Koreck A, Szell M, Beer Z, Bata-Csorgoo Z, Magocsi M, Rajnavolgyi E, Dobozy A, Kemeny L (2003) Expression and function of Toll-like receptors 2 and 4 in human keratinocytes. Int Immunol 15:721 – 730
7. Albanesi C, Scarponi C, Cavani A, Federici M, Nasorri F, Girolomoni G (2000a) Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon-γ- and interleukin-4-induced activation of human keratinocytes. J Invest Dermatol 115:81 – 87
8. Albanesi C, Cavani A, Girolomoni G (1999) IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic and antagonist effects with IFN-γ and TNF-α. J Immunol 162:494 – 502
9. Traidl C, Sebastiani S, Albanesi C, Merk HF, Puddu P, Girolomoni G, Cavani A (2000) Disparate cytotoxic activity of nickel-specific CD8+ and CD4+ T cell subsets against keratinocytes. J Immunol 165:3058 – 3064
10. Trautmann A, Akdis M, Kleemann D, Altznauer F, Simon HU, Graeve T, Noll M, Brocker EB, Blaser K, Akdis CA (2000) T cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. J Clin Invest 106:25 – 35
11. Trautmann A, Akdis M, Brocker EB, Blaser K, Akdis CA (2001) New insights into the role of T cells in atopic dermatitis and allergic contact dermatitis. Trends Immunol 22: 530 – 532
12. Albanesi C, Scarponi C, Sebastiani S, Cavani A, Federici M, De Pità O, Puddu P, Girolomoni G (2000b) IL-4 enhances keratinocyte expression of CXCR3 agonistic chemokines. J Immunol 165:1395 – 1402
13. Vestergaard C, Bang K, Gesser B, Yoneymana H, Matsushima K, Larsen CG (2000) A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA^+CCR4^+ lymphocytes into lesional atopic dermatitis skin. J Invest Dermatol 115:640 – 646
14. Yamamoto J, Adachi Y, Onoue Y, Adachi YS, Okabe Y, Itazawa T, Toyoda M, Seki T, Morohashi M, Matsushima K, Miyawaki T (2000) Differential expression of the chemokine receptors by the Th1- and Th2-type effector populations within circulating $CD4^+$ T cells. J Leukoc Biol 68:568 – 574
15. Nakatani T, Kaburagi Y, Shimada Y, Inaoki M, Takehara K, Mukaida N, Sato S (2001) $CCR4^+$ memory $CD4^+$ T lymphocytes are increased in peripheral blood and lesional skin from patients with atopic dermatitis. J Allergy Clin Immunol 107:353 – 358
16. Wakugawa M, Nakamura K, Kakinuma T, Onai N, Matsushima K, Tamaki K (2001) CC chemokine receptor 4 expression on peripheral blood $CD4^+$ T cells reflects disease activity of atopic dermatitis. J Invest Dermatol 117:188 – 196
17. Okazaki H, Kakurai M, Hirata D, Sato H, Kamimura T, Onai N, Matsushima K, Nakagawa H, Kano S, Minota S (2002) Characterization of chemokine receptor expression and cytokine production in circulating $CD4^+$ T cells from patients with atopic dermatitis: up-regulation of C-C chemokine receptor 4 in atopic dermatitis. Clin Exp Allergy 32:1236 – 1242
18. Laberge S, Ghaffar O, Boguniewicz M, Center DM, Leung DJ, Hamid Q (1998) Association of increased CD4+ T-cell infiltration with increased IL-16 gene expression in atopic dermatitis. J Allergy Clin Immunol 102:645 – 650
19. Reich K, Hugo S, Middel P, Blaschke V, Heine A, Gutgesell C, Williams R, Neumann C (2002) Evidence for a role of Langerhans cell-derived IL-16 in atopic dermatitis. J Allergy Clin Immunol 109:681 – 687
20. Stoitzner P, Ratzinger G, Koch F, Janke K, Scholler T, Kaser A, Tilg H, Cruikshank WW, Fritsch P, Romani N (2001) Interleukin-16 supports the migration of Langerhans cells, partly in a CD4-independent way. J Invest Dermatol 116: 641 – 649
21. Reich K, Heine A, Hugo S, Blaschke V, Middel P, Kaser A, Tilg H, Blaschke S, Gutgesell C, Neumann C (2001) Engagement of FcεRI stimulates the production of IL-16 in Langerhans cell-like dendritic cells. J Immunol 167:6321 – 6329
22. Frezzolini A, Paradisi M, Zaffiro A, Provini A, Cadoni S, Ruffelli M, De Pita O (2002) Circulating interleukin 16 (IL-16) in children with atopic/eczema dermatitis syndrome (AEDS): a novel serological marker of disease activity. Allergy 57:815 – 820
23. Fujisawa T, Fujisawa R, Kato Y, Nakayama T, Morita A, Katsumata H, Nishimori H, Iguchi K, Kamiya H, Gray PW, Chantry D, Suzuki R, Yoshie O (2002) Presence of high contents of thymus and activation-regulated chemokine in platelets and elevated plasma levels of thymus and activation-regulated chemokine and macrophage-derived chemokine in patients with atopic dermatitis. J Allergy Clin Immunol 110:139 – 146
24. Horikawa T, Nakayama T, Hikita I, Yamada H, Fujisawa R, Bito T, Harada S, Fukunaga A, Chantry D, Gray PW, Morita A, Suzuki R, Tezuka T, Ichihashi M, Yoshie O (2002) IFN-γ-inducible expression of thymus and activation-regulated chemokine/CCL17 and macrophage-derived chemokine/CCL22 in epidermal keratinocytes and their roles in atopic dermatitis. Int Immunol 14:767 – 773
25. Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, Torii H, Komine M, Asahina A, Tamaki K (2002) Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. Clin Exp Immunol 27:270 – 273
26. Vulcano M, Albanesi C, Stopacciaro A, Bagnati R, D'Amico G, Struyf S, Transidico P, Bonecchi R, Del Prete A, Allavena P, Ruco LP, Chiabrando C, Girolomoni G, Mantovani A, Sozzani S (2001) Dendritic cells as a major source of macrophage-derived chemokine/CCL22 in vitro and in vivo. Eur J Immunol 31:812 – 822
27. D'Ambrosio D, Albanesi A, Lang R, Girolomoni G, Sinigaglia F, Laudanna C (2002) Quantitative differences in chemokine receptor engagement generate diversity in integrin-dependent lymphocyte adhesion. J Immunol 169: 2303 – 2312

28. Giustizieri ML, Mascia F, Frezzolini A, De Pità O, Chinni ML, Giannetti A, Girolomoni G, Pastore S (2001) 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 107:871 – 877
29. Kaburagi Y, Shimada Y, Nagaoka T, Hasegawa M, Takehara K, Sato S (2001) Enhanced production of CC-chemokines (RANTES, MCP-1, MIP-1α, MIP-1β, and eotaxin) in patients with atopic dermatitis. Arch Dermatol Res 293: 350 – 355
30. Nickel RG, Casolaro V, Wahn U, Beyer K, Barnes KC, Plunkett BS, Freidhoff LR, Sengler C, Plitt JR, Schleimer RP, Caraballo L, Naidu RP, Levett PN, Beaty TH, Huang SK (2000) Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. J Immunol 164:1612 – 1616
31. Elliot K, Forrest S (2002) Genetics of atopic dermatitis. In: Bieber T, Leung DYM (eds) Atopic dermatitis. Marcel Dekker, New York, pp 81 – 110
32. Homey B, Alenius H, Muller A, Soto H, Bowman EP, Yuan W, McEvoy L, Lauerma AI, Assmann T, Bunemann E, Lehto M, Wolff H, Yen D, Marxhausen H, To W, Sedgwick J, Ruzicka T, Lehmann P, Zlotnik A (2002) CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat Med 8:157 – 165
33. Schmuth M, Neyer S, Rainer C, Grassegger A, Fritsch P, Romani N, Heufler C (2002) Expression of the C-C chemokine MIP-3α/CCL20 in human epidermis with impaired permeability barrier function. Exp Dermatol 11:135 – 142
34. Nakayama N, Fujisawa R, Yamada H, Horikawa T, Kawasaki H, Hieshima K, Izawa D, Fujiie S, Tezuka T, Yoshie O (2000) Inducible expression of a CC chemokine liver- and activation-regulated chemokine (LARC)/macrophage inflammatory protein (MIP)-3α/CCL20 by epidermal keratinocytes and its role in atopic dermatitis. Int Immunol 13:95 – 103
35. Taha RA, Minshall EM, Leung DY, Boguniewicz M, Luster A, Muro S, Toda M, Hamid QA (2000) Evidence for increased expression of eotaxin and monocyte chemotactic protein-4 in atopic dermatitis. J Allergy Clin Immunol 105:1002 – 1007
36. Yawalkar N, Uguccioni M, Scharer J, Braunwalder J, Karlen S, Dewald B, Braathen LR, Baggiolini M (1999) Enhanced expression of eotaxin and CCR3 in atopic dermatitis. J Invest Dermatol 113:43 – 48
37. Mochizuki M, Bartels J, Mallet AI, Christophers E, Schröder JE (1998) IL-4 induces eotaxin: a possible mechanism of selective eosinophils recruitment in helminth infection and atopy. J Immunol 160:60 – 68
38. Petering H, Höchstetter R, Kimming D, Smolarski R, Kapp A, Elsner J (1998) Detection of MCP-4 in dermal fibroblast and its activation of the respiratory burst in human eosinophils. J Immunol 160:555 – 558
39. Albanesi C, Scarponi C, Sebastiani S, Cavani A, Federici M, Sozzani S, Girolomoni G (2001) A cytokine-to-chemokine axis between T lymphocytes and keratinocytes can favor Th1 cell accumulation in chronic inflammatory skin diseases. J Leukoc Biol 70:617 – 623
40. Mascia F, Mariani V, Girolomoni G, Pastore S (2003) Blockade of the EGF receptor induces a deranged chemokine expression in keratinocytes leading to enhanced skin inflammation. Am J Pathol 163:303 – 312
41. Madtes DK, Busby HK, Strandjoford TP, Clark JG (1994) Expression of transforming growth factor-α and epidermal growth factor receptor is increased following bleomycin-induced lung injury in rats. Am J Respir Cell Mol Biol 11:540 – 551
42. Tokumaru S, Higashiyama S, Endo T, Nakagawa T, Miyagawa JI, Yamamori K, Hanakawa Y, Ohmoto H, Yoshino K, Shirakata Y, Matsuzawa Y, Hashimoto K, Taniguchi N (2000) Ectodomain shedding of epidermal growth factor receptor ligands is required for keratinocyte migration in cutaneous wound healing. J Cell Biol 151:209 – 220
43. Hardie WD, Prows DR, Piljan-Gentle A, Dunlavy MR, Wesselkamper SC, Leikauf GD, Korfhagen TR (2002) Dose-related protection from nickel-induced lung injury in transgenic mice expression human transforming growth factor-α. Am J Respir Cell Mol Biol 26:430 – 437
44. Berlanga J, Prats P, Remirez D, Gonzalez R, Lopez-Saura P, Aguiar J, Ojeda M, Boyle JJ, Fitzgerald AJ, Playford RJ (2002) Prophylactic use of epidermal growth factor reduces ischemia/reperfusion intestinal damage. Am J Pathol 161:373 – 379
45. Hamilton LM, Torres-Lozano C, Puddicombe SM, Richter A, Kimber I, Dearman RJ, Vrugt B, Aalbers R, Holgate ST, Djukanovic R, Wilson SJ, Davies DE (2003) The role of the epidermal growth factor receptor in sustaining neutrophil inflammation in severe asthma. Clin Exp Allergy 33:233 – 240
46. Lukacs NW, Miller AL, Hogaboam CM (2003) Chemokine receptors in asthma: searching for the correct immune targets. J Immunol 171:11 – 15
47. Pastore S, Mascia F, Mariani V, Girolomoni G (2002) Epidermal growth factor receptor ligands and tumor necrosis factor-α coregulate chemokine expression in human keratinocytes. Ann N Y Acad Sci 973:210 – 213
48. Wollenberg A, Kraft S, Hanau D, Bieber T (1996) 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 106:446 – 453
49. Novak N, Tepel C, Koch S, Brix K, Bieber T, Kraft S (2003) Evidence for a differential expression of the FcεRIγ chain in dendritic cells of atopic and nonatopic donors. J Clin Invest 111:1047 – 1056
50. Pastore S, Fanales-Belasio E, Albanesi C, Chinni ML, Giannetti A, Girolomoni G (1997) Granulocyte macrophage colony-stimulating factor is overproduced by keratinocytes in atopic dermatitis. Implications for sustained dendritic cell activation in the skin. J Clin Invest 99:3009 – 3017
51. Pastore S, Giustizieri M, Mascia F, Giannetti A, Kaushansky K, Girolomoni G (2000) Dysregulated activation of activator protein 1 in keratinocytes of atopic dermatitis patients with enhanced expression of granulocyte/macrophage-colony stimulating factor. J Invest Dermatol 115: 1134 – 1143
52. Xing Z, Gauldie J, Tremblay GM, Hewlett BR, Addison C (1997) Intradermal transgenic expression of granulocyte-macrophage colony-stimulating factor induces neutrophi-

lia, epidermal hyperplasia, Langerhans' cell/macrophage accumulation, and dermal fibrosis. Lab Invest 77:615 – 622
53. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan T, de Waal-Malefyt Rd R, Bazan F, Kastelein RA, Liu YJ (2002) Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 3:673 – 680
54. Gilliet M, Soumelis V, Watanabe N, Hanabuchi S, Antonenko S, de Waal-Malefyt R, Liu YJ (2003) Human dendritic cells activated by TSLP and CD40L induce proallergic cytotoxic T cells. J Exp Med 197:1059 – 1063
55. Pastore S, Corinti S, La Placa M, Didona B, Girolomoni G (1998) Interferon-γ promotes exaggerated cytokine production in keratinocytes cultured from patients with atopic dermatitis. J Allergy Clin Immunol 101:538 – 544
56. Klunker S, Trautmann A, Akdis M, Verhagen J, Schmid-Grendelmeier P, Blaser K, Akdis CA (2003) 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 171:1078 – 1084
57. Mascia F, Mariani V, Giannetti A, Girolomoni G, Pastore S (2002) House dust mite allergen exerts no direct proinflammatory effects on human keratinocytes. J Allergy Clin Immunol 109:532 – 538
58. Kozma GT, Falus A, Bojszko A, Krikovszky D, Szabo T, Nagy A, Szalai C (2002) Lack of association between atopic eczema/dermatitis syndrome and polymorphisms in the promoter region of RANTES and regulatory region of MCP-1. Allergy 57:160 – 163
59. Rutberg SE, Saez E, Glick A, Dlugosz AA, Spiegelman BM, Yuspa SH (1996) Differentiation of mouse keratinocytes is accompanied by PKC-dependent changes in AP-1 proteins. Oncogene 13:167 – 176
60. Ishibashi M, Arikawa J, Okamoto R, Kawashima M, Takagi Y, Ohguchi K, Imokawa G (2003) Abnormal expression of the novel epidermal enzyme, glucosylceramide deacylase, and the accumulation of its enzymatic reaction product, glucosylsphingosine, in the skin of patients with atopic dermatitis. Lab Invest 83:397 – 408
61. Jones MJ, Murray AW (1995) Evidence that ceramide selectively inhibits protein kinase C-α translocation and modulates bradykinin activation of phospholipase D. J Biol Chem 270:5007 – 5013
62. Lane SJ, Adcock IM, Richards D, Hawrylowicz C, Barnes PJ, Lee TH (1998) Corticosteroid-resistant bronchial asthma is associated with increased c-fos expression in monocytes and T lymphocytes. J Clin Invest 102:2156 – 2164.
63. Sousa AR, Lane SJ, Soh C, Lee TH (1999) In vivo resistance to corticosteroids in bronchial asthma is associated with enhanced phosphorylation of JUN N-terminal kinase and failure of prednisolone to inhibit JUN N-terminal kinase phosphorylation. J Allergy Clin Immunol 104:565 – 574
64. Nguyen C, Teo JL, Matsuda A, Eguchi M, Chi EY, Henderson WR Jr, Kahn M (2003) Chemogenomic identification of Ref-1/AP-1 as a therapeutic target for asthma. Proc Natl Acad Sci 100:1169 – 1173
65. Cookson WO, Moffatt MF (2002) The genetics of atopic dermatitis. Curr Opin Allergy Clin Immunol 2:383 – 387
66. Girolomoni G, Sebastiani S, Albanesi C, Cavani A (2001) T-cell subpopulations in the development of atopic and contact allergy. Curr Opin Immunol 13:733 – 737