Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell–derived cytokines

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Background: Atopic dermatitis (AD) and psoriasis are genetically determined inflammatory skin disorders. Keratinocytes actively participate in cutaneous inflammatory responses by elaborating various chemokines.
Objective: We investigated the capacity of IL-4, IFN-γ, and TNF-α to modulate the expression of CCL and CXCL chemokines in cultured keratinocytes from patients and healthy individuals, as well as chemokine expression in situ.
Methods: Keratinocyte cultures were established from normal-looking skin of adult patients with AD or psoriasis vulgaris and from healthy subjects. Monocyte chemoattractant protein 1 (MCP-1)/CCL2, RANTES/CCL5, IL-8/CXCL8, and IFN-γ–induced protein of 10 kd (IP-10)/CXCL10 production was evaluated at the mRNA and protein levels by using RNase protection assay and ELISA, respectively. The expression of the same chemokines was studied in chronic lesional skin by means of immunohistochemistry or in situ hybridization.
Results: Only IL-8 mRNA was detected in unstimulated keratinocyte cultures. MCP-1 and IP-10 were potently induced by IFN-γ, whereas IL-8 and RANTES were preferentially upregulated by TNF-α and, to a lesser extent, by IFN-γ. IL-4 weakly induced IP-10, RANTES, and IL-8 but not MCP-1. Keratinocytes of patients with AD invariably responded with significantly earlier and higher RANTES expression. By contrast, keratinocytes of patients with psoriasis displayed much higher levels of both constitutive and induced IL-8 and a stronger induction of MCP-1 and IP-10. RANTES and MCP-1 mRNA$^+$ keratinocytes were detected in the basal layer of lesions of patients with AD and psoriasis. IP-10 and IL-8 were consistently upregulated in the epidermis of patients with psoriasis but not in lesions of patients with AD.
Conclusions: Keratinocytes of patients with AD and psoriasis show an intrinsically abnormal and different chemokine production profile and may thus favor the recruitment of distinct leukocyte subsets into the skin. (J Allergy Clin Immunol 2001;107:871-7.)

Key words: *Atopic dermatitis, psoriasis, keratinocytes, inflammation, cytokines, chemokines*

Atopic dermatitis (AD) and psoriasis are common chronic inflammatory disorders in which T cell–mediated mechanisms have a primary role in pathogenesis.[1,2] Patients with AD exhibit exaggerated T_H2 responses, and initiation of AD lesions is thought to be mediated by means of early skin infiltration of T_H2 lymphocytes releasing high levels of IL-4, IL-5, and IL-13.[1] Subsequently, the accumulation of activated monocytes, mature dendritic cells, and eosinophils determines a rise in IL-12 expression and the appearance of a mixed T_H2/T_H1 cytokine pattern, with reduced IL-4 and IL-13 and the presence of IFN-γ.[3] By contrast, psoriasis is characterized by numerous activated T_H1 cells, focal intraepidermal collections of neutrophils, and dermal accumulation of monocytes and dendritic cells. IFN-γ–producing T_H1 clones dominate psoriatic lesions and are primarily responsible for the epidermal changes.[2] Moreover, keratinocytes, mast cells, monocytes, and dendritic cells may be active sources of TNF-α in both diseases.[4,5]

Chemokines are a superfamily of small secreted proteins that regulate cell traffic in both homeostatic and inflammatory conditions, and 2 main subfamilies, recently renamed CCL and CXCL chemokines, have been distinguished.[6] Several studies have suggested a crucial involvement of CCL chemokines in allergic inflammation because they potently attract eosinophils, basophils, monocytes, and T_H2 cells.[7] By contrast, CXCL chemokines attract primarily T_H1 lymphocytes and neutrophils.[8] Keratinocytes are the major cell population of the epidermis and can be activated to produce various chemokines, including the CCL chemokines RANTES/CCL5 and monocyte chemoattractant protein 1 (MCP-1)/CCL2 and the CXCL chemokines IFN-γ–induced protein of 10 kd (IP-10)/CXCL10 and IL-8/CXCL8.[9,10] Upregulated expression of RANTES and MCP-1 has been detected in the epidermis of patients with both AD and psoriasis.[11-14] Moreover, keratinocyte production of IL-8 and IP-10 has been implicated in the patho-

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Abbreviations used

AD: Atopic dermatitis
AP-1: Activator protein 1
IP-10: IFN-γ–induced protein of 10 kd
ISH: In situ hybridization
MCP-1: Monocyte chemoattractant protein 1
PMA: Phorbol myristate acetate

genesis of psoriasis.[15,16] Cytokines released by infiltrating T cells are among the strongest signals for chemokine expression in keratinocytes.[9,10,17-19] A series of previous observations suggest that keratinocytes cultured from nonlesional skin of patients with AD or psoriasis display intrinsic abnormalities in their response to proinflammatory factors[20-26] and that they retain these features for at least 5 culture passages.

In this study we examined the capacity of IL-4, IFN-γ, TNF-α, and phorbol myristate acetate (PMA) to modulate the production of RANTES, MCP-1, IP-10, and IL-8 in keratinocytes cultured from nonlesional skin of patients with AD and psoriasis. Furthermore, keratinocyte expression of the same chemokines was investigated in biopsy specimens of chronic lesions of patients with AD and psoriasis.

METHODS

Subjects

Four adult patients with moderate-to-severe chronic AD (2 men and 2 women; age range, 19-45 years), 4 adult patients with moderate-to-severe chronic plaque psoriasis (3 men and 1 woman; age range, 32-49 years), and 4 healthy control subjects (2 men and 2 women; age range, 25-50 years) were included in the study. Patients had definite AD or psoriasis diagnosed according to standard criteria, and they were not receiving any systemic or topical therapy for at least 2 weeks before testing. Informed consent was obtained from all subjects, and the study was approved by the local ethical committee.

Reagents, cytokines, and antibodies

PMA was purchased from Sigma-Aldrich (Milan, Italy). Recombinant human (rh) IL-4, IFN-γ, and TNF-α and rabbit polyclonal anti-human IP-10 (IgG1) or anti-IL-8 (IgG1) antibodies were obtained from R&D Systems (Abingdon, United Kingdom). Biotinylated anti-rabbit IgG was purchased from Vector Laboratories (Burlingame, Calif).

Keratinocyte cultures

Epidermal sheets were procured from the roof of suction blisters raised on normal-appearing skin of the forearms of all patients and healthy subjects, as previously described.[20] Primary cultures were established by seeding epidermal cells on a feeder layer of irradiated 3T3/J2 fibroblasts and cultured according to an optimized Rheinwald and Green culture technique.[20] Keratinocyte cultures were used at the second or third passage and were not contaminated by leukocytes, as assessed by using flow cytometry analysis with mAbs against dendritic cell, monocyte, and T-cell markers (not shown).

RNA preparation and RNase protection assay

Total RNA was extracted with a modified guanidine isothiocyanate-acid phenol protocol by using the Ultraspec RNA isolation system (Biotecx, Houston, Tex), as previously described.[20] For simultaneous RNase protection assay of RANTES, IP-10, MCP-1, and IL-8, the multiprobe template set hCK5 was purchased from PharMingen (San Diego, Calif) and used according to the manufacturer's instructions. Ten micrograms of RNA was used in each assay. The exsiccated gels were exposed to films for 6 hours at –70°C. RNase protection assay bands were quantified by using laser densitometry supported by the Quantity One software (Bio-Rad, Hercules, Calif), with each chemokine mRNA signal normalized to the corresponding L32 mRNA signal. Four separate experiments with keratinocytes from different donors were performed with similar results.

ELISA

RANTES, MCP-1, IP-10, and IL-8 were measured with OptEIA ELISA kits (PharMingen) on cell-free supernatants harvested 48 hours after stimulation. Data are expressed as nanograms per 10^6 cells ± SD of triplicate cultures.

Immunohistochemistry

Four-millimeter punch biopsy specimens were taken from chronic lesions of patients with AD (n = 3) or psoriasis (n = 3) or from normal skin of healthy volunteers (n = 3). Frozen 5-μm skin sections were kept at 30°C for 3 hours, fixed in acetone at –20°C for 20 minutes, permeabilized with 0.05% Triton X-100, and then incubated for 1 hour at room temperature with the appropriate dilution of anti-human IP-10 or IL-8 antibodies or control rabbit Ig. Sections were stained with an avidin-biotin-peroxidase technique (Vector Laboratories) by using 3-amino-9-ethylcarbazole as a substrate. In competition experiments anti-human IP-10 or IL-8 antibody was incubated with excess concentrations of rhIP-10 or rhIL-8 (R&D Systems) overnight at 4°C before application to tissue sections (not shown).

In situ hybridization

Labeled riboprobes were prepared from RANTES and MCP-1 cDNAs. Starting PCR products were subcloned into plasmid pCR^{R}II-TOPO^R (Groningen, The Netherlands) and subsequently transcribed in the presence of ^{35}S-labeled uridine triphosphate. To obtain sense and antisense reaction products, SP6 or T7 polymerase reactions were performed on linearized plasmids, according to standard methods.[14] For hybridization procedure on tissue sections, the protocol by Müller et al[27] was used. For autoradiography, the slides were coated with NTB-2 emulsion (Eastman Kodak, Rochester, NY), exposed at 4°C for 1 to 3 weeks, developed, and finally counterstained with 0.02% toluidine blue. Sections were photographed under bright- and dark-field microscopy. The specific detection of chemokine mRNA by means of in situ hybridization (ISH) was confirmed by using sense riboprobes, which gave only background signals, or by using pretreatment of the tissue sections with DNase-free RNase, which completely abrogated specific signals (not shown).

Statistical analysis

The Mann-Whitney rank sum test was used to compare differences in chemokine release between the different keratinocyte types. The Wilcoxon signed-rank test was applied for within-group comparisons before and after stimulation. Significance was assumed at a P value of .05 or less.

RESULTS

Chemokine expression in cultured keratinocytes

Chemokine mRNA and protein expression was investigated in keratinocyte cultures obtained from the nonlesional skin of patients with AD and psoriasis and from healthy subjects. Induction of specific mRNA signals was examined after 1 to 24 hours' stimulation with IL-4

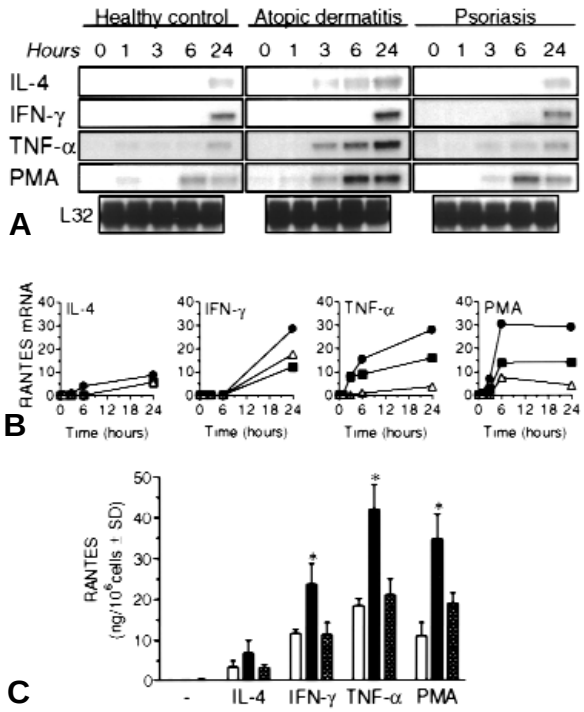

FIG 1. Keratinocytes from patients with AD produce higher amounts of RANTES. **A**, RANTES RNase protection assay of untreated keratinocytes or keratinocytes stimulated with IL-4, IFN-γ, TNF-α, or PMA. **B**, Quantitation of RNase protection assay bands by means of laser densitometry (arbitrary units) in keratinocytes from healthy control subjects *(triangles)*, patients with AD *(circles)*, and patients with psoriasis *(squares)*. **C**, ELISA on supernatants of cells cultured from healthy donors *(open bars)*, patients with AD *(filled bars)*, or patients with psoriasis *(hatched bars)*. $*P < .01$ versus keratinocytes from patients with psoriasis or healthy control subjects.

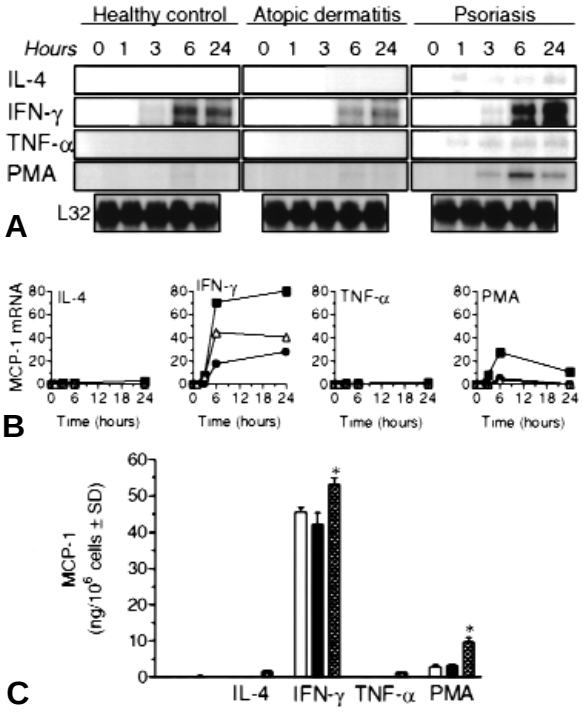

FIG 2. Keratinocytes from patients with psoriasis display stronger MCP-1 induction than keratinocytes from patients with AD or control subjects. **A**, MCP-1 RNase protection assay of keratinocytes stimulated as indicated in the legend to Fig 1. **B**, Quantitation of RNase protection assay bands by means of laser densitometry (arbitrary units) in keratinocytes from healthy control subjects *(triangles)*, patients with AD *(circles)*, and patients with psoriasis *(squares)*. **C**, ELISA on supernatants of cells cultured from healthy donors *(open bars)*, patients with AD *(filled bars)*, or patients with psoriasis *(hatched bars)*. $*P < .03$ versus keratinocytes from patients with AD or healthy control subjects.

(50 ng/mL), IFN-γ (100 U/mL), TNF-α (50 ng/mL), or PMA (10 ng/mL). RANTES mRNA was not detected in unstimulated keratinocytes, and it was weakly induced by IL-4 and markedly upregulated by IFN-γ, TNF-α, or PMA (Fig 1, *A*). RANTES induction was especially evident in keratinocytes from patients with AD, which responded to TNF-α with 7-fold and 2-fold higher mRNA levels than cells from healthy control subjects and patients with psoriasis, respectively (Fig 1, *B*). Measurement of RANTES protein accumulation in the culture medium over 48 hours showed that the strongest release followed TNF-α or PMA treatment and that it was more conspicuous in keratinocytes from patients with AD than in cells from control subjects or patients with psoriasis (Fig 1, *C*).

MCP-1 mRNA signal was absent in baseline conditions. IFN-γ efficiently induced MCP-1 mRNA, which was less represented in keratinocytes from patients with AD than keratinocytes from control subjects or patients with psoriasis (Fig 2, *A*). Keratinocytes from patients with psoriasis displayed 4-fold higher MCP-1 mRNA levels than cells from patients with AD at 6 hours' stimulation (Fig 2, *B*). PMA caused a significant MCP-1 mRNA induction only in keratinocytes from patients with psoriasis, whereas IL-4 and TNF-α appeared ineffective. Analogous behavior could be observed when examining MCP-1 protein release. Compared with keratinocytes from control subjects or patients with AD, higher levels of MCP-1 were secreted from cells from patient with psoriasis after treatment with IFN-γ or PMA (Fig 2, *C*). After stimulation with IL-4 or TNF-α, very modest levels of MCP-1 were measured only in the supernatants of keratinocytes from patients with psoriasis.

Unstimulated keratinocytes did not express detectable IP-10 mRNA. IFN-γ was a potent stimulus for its induction, whereas IL-4, TNF-α, and PMA caused only a weak and transient upregulation (Fig 3, *A*). Of note, IP-10 mRNA levels at 6 hours' stimulation were 1.4-fold and 3-fold higher in keratinocytes from patients with psoriasis than in those from healthy control subjects or patients with AD, respectively (Fig 3, *B*). Similar data were obtained by measuring IP-10 protein secretion, with significantly higher release from keratinocytes of patients with psoriasis after IFN-γ, TNF-α, or PMA treatment (Fig 3, *C*). Furthermore, keratinocytes from patients with AD responded to the different stimuli with reduced IP-10 release compared with cells from healthy control subjects.

In contrast to the previous chemokines, IL-8 mRNA was

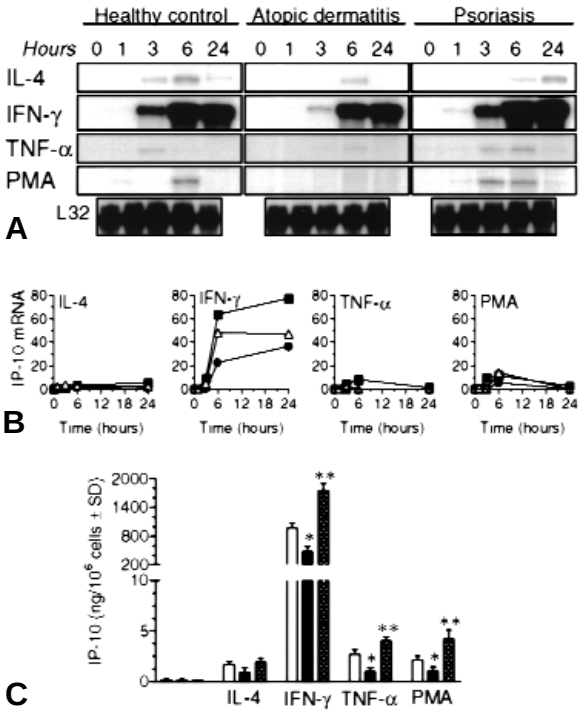

FIG 3. Keratinocytes from patients with psoriasis express higher levels of IP-10 in response to IFN-γ, TNF-α, or PMA. **A**, IP-10 RNase protection assay of keratinocytes stimulated as indicated in the legend to Fig 1. **B**, Quantitation of RNase protection assay bands by means of laser densitometry (arbitrary units) in keratinocytes from healthy control subjects *(triangles)*, patients with AD *(circles)*, and patients with psoriasis *(squares)*. **C**, ELISA on supernatants of cells cultured from healthy donors *(open bars)*, patients with AD *(filled bars)*, or patients with psoriasis *(hatched bars)*. $*P < .02$ versus keratinocytes from healthy control subjects; $**P < .03$ versus keratinocytes from patients with AD or healthy control subjects.

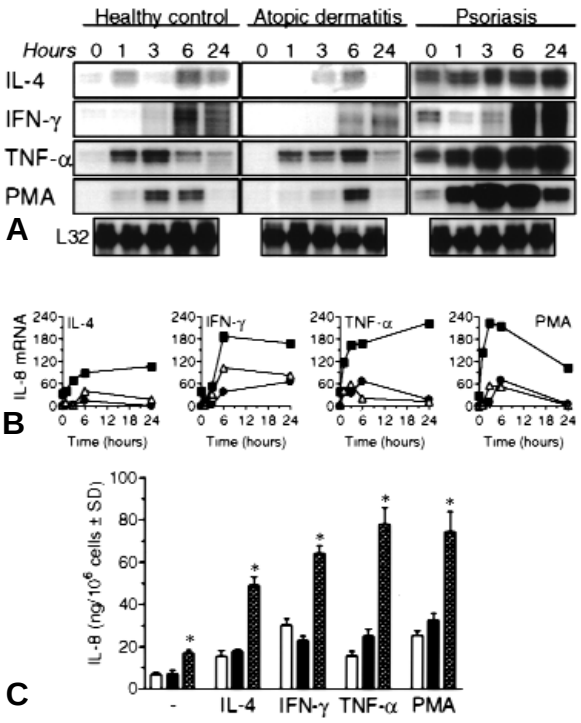

FIG 4. Keratinocytes from patients with psoriasis display abnormal levels of basal and induced IL-8 expression. **A**, IL-8 RNase protection assay of keratinocytes stimulated as indicated in the legend to Fig 1. **B**, Quantitation of RNase protection assay bands by means of laser densitometry (arbitrary units) in keratinocytes from healthy control subjects *(triangles)*, patients with AD *(circles)*, and patients with psoriasis *(squares)*. **C**, ELISA on supernatants of cells cultured from healthy donors *(open bars)*, patients with AD *(filled bars)*, or patients with psoriasis *(hatched bars)*. $*P < .01$ versus keratinocytes from patients with AD or healthy control subjects.

expressed constitutively in unstimulated keatinocytes, with the specific IL-8 doublet detected at high levels in keratinocytes from patients with psoriasis (Fig 4, *A*). Longer exposures (>15 hours) were necessary to reveal the signals in unstimulated cells from normal control subjects or patients with AD (not shown). IL-8 mRNA induction in response to cytokines or PMA was invariably earlier, stronger, and more persistent in psoriatic keratinocytes than in keratinocytes from control subjects or patients with AD (Fig 4, *B*). ELISA elicited further evidence of an exaggerated IL-8 production by keratinocytes from patients with psoriasis in response to the different stimuli, which was significantly higher compared with that of cells from control subjects or patients with AD (Fig 4, *C*). The amounts of chemokines measured in supernatants of activated keratinocyte cultures were in a functionally relevant range.[10,28]

Chemokine expression in lesional epidermis in situ

Keratinocyte expression of chemokines was examined in chronic lesions of patients with AD and psoriasis, as well as in normal skin from healthy subjects. By using ISH, specific RANTES mRNA signals were detected in the basal epidermis of patients with both AD and psoriasis, especially at the tips of the rete ridges. In contrast, no hybridization signal was observed in healthy skin (Fig 5, *A-C*). Similar findings were obtained for MCP-1 (Fig 5, *D-F*), with mRNA$^+$ keratinocytes more evident in the basal epidermal layers of lesions from patients with AD and psoriasis. Psoriasis consistently showed a more intense MCP-1 reactivity in the epidermis, as well as in the dermal papillae, with the latter likely related to focal accumulations of leukocytes (Fig 5, *F*). By means of immunohistochemical analysis, no IL-8 immunoreactivity could be detected in normal skin from healthy control subjects (Fig 5, *G*). Lesional AD skin showed some IL-8–reactive leukocytes in the dermis but not keratinocyte staining (Fig 5, *H*), whereas epidermis of patients with psoriasis presented a diffuse staining and areas of strong positivity in the stratum corneum (Fig 5, *I*), as previously described.[4] IP-10 reactivity was almost absent in healthy skin (Fig 5, *J*), whereas it was limited to infiltrating leukocytes in AD lesional skin, with the epidermis showing no or weak staining in some areas (Fig 5, *K*). In con-

trast, epidermis of patients with psoriasis displayed a diffuse cytoplasmic staining, which was more intense in the upper, subcorneal keratinocyte layers (Fig 5, *L*). Nonlesional skin from patients with AD or psoriasis did not show a different chemokine expression compared with skin from healthy control subjects (data not shown).

DISCUSSION

Keratinocytes can participate in skin inflammation through the regulated expression of chemokines attracting various leukocyte subsets, including monocytes, dendritic cells, and T lymphocytes. Inflammatory cells express different chemokine receptors according to the differentiation and activation state. In particular, T_H1 cells express high levels of CCR5 and CXCR3 and migrate preferentially to macrophage inflammatory proteins and IP-10, respectively. In contrast, T_H2 cells express high CCR3, CCR4, and CCR8 levels and are efficiently attracted by their respective ligands, eotaxin, thymus and activation-regulated chemokine (CCL17)/macrophage-derived chemokine (CCL22), and I-309.[8,28] In this study we could observe that basal keratinocytes are actively committed to neosynthesizing RANTES in both AD and psoriasis. RANTES is chemotactic for monocytes, dendritic cells, and T_H1 lymphocytes by means of interaction with CCR5 and also attracts T_H2 cells through CCR3.[8] ISH also showed that basal keratinocytes strongly express MCP-1 in patients with AD and at higher levels in patients with psoriasis. MCP-1 potently attracts monocytes and dendritic cells in vivo,[29] but it can induce migration of both T_H1 and T_H2 cells.[28] In the case of AD, RANTES and MCP-1 can contribute relevantly to the mixed T_H1/T_H2 infiltrate that characterizes the chronic phase. Moreover, MCP-1 has been shown to stimulate IL-4 expression in T cells and the development of T_H2-polarized responses in mice.[30,31] IP-10 was markedly expressed in the epidermis of patients with psoriasis vulgaris but only weakly and limited to some areas in AD lesions. The higher expression of MCP-1 and especially IP-10 in epidermis of patients with psoriasis compared with that of patients with AD likely reflects the presence of more numerous T_H1 cells in the former because IFN-γ is the most potent inducer of these chemokines.[9,10] In turn, the elevated expression of IP-10 in keratinocytes from patients with psoriasis can support an amplification circuit of polarized T_H1 responses.[8,28] We also confirmed keratinocyte-associated IL-8 production in psoriasis[4] but not in skin of patients with AD or healthy skin. High levels of IL-8 expression have already been implicated in multiple aspects of the pathogenesis of psoriasis, including T-cell and neutrophil chemotaxis and activation, as well as keratinocyte hyperproliferation.[2] In vitro studies have shown that IL-8 was the only chemokine constitutively produced by keratinocytes, with unstimulated cells from patients with psoriasis expressing the highest levels. TNF-α was the most potent inducer of RANTES and IL-8. By contrast, IFN-γ was by far the most effective stimulus for MCP-1 and IP-10 production, as discussed above. IL-4 also induced some RANTES, IP-10, and IL-8 expression, but

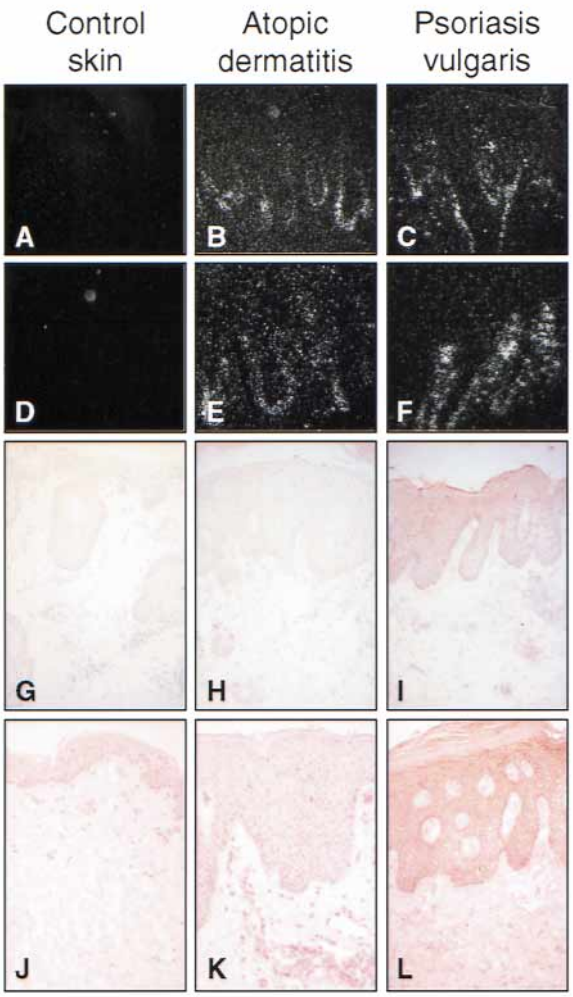

FIG 5. Distinct chemokine upregulation in the epidermis of chronic lesional skin from patients with AD and psoriasis. Representative examples of dark-field photomicrographs of ISH detection of RANTES **(A-C)** and MCP-1 **(D-F)** mRNA and representative examples of immunohistochemical analysis of IL-8 **(G-I)** and IP-10 **(J-L)** are shown.

not MCP-1 expression, which is consistent with the notion that keratinocytes express functional IL-4 receptor.[19] This observation, together with the capacity of IL-4 to reinforce the activity of TNF-α and IFN-γ in promoting keratinocyte expression of CXCR3 agonistic chemokines,[10] indicates that IL-4 might be implicated in the recruitment of T_H1 cells in chronic AD. Finally, ke-ratinocytes can also release low amounts of thymus and activation-regulated chemokine, macrophage-derived chemokine, and I-309 but only after IFN-γ stimulation (unpublished observation),[32] suggesting that kerat-inocytes can attract T_H2 cells but only after prior activation by T_H1 cells.

An interesting observation of this study was that keratinocytes from patients with AD produced increased amounts of RANTES but reduced levels of IP-10 in response to IFN-γ, TNF-α, or PMA when compared with keratinocytes from normal control subjects or patients with psoriasis (Fig 6). By contrast, keratinocytes from

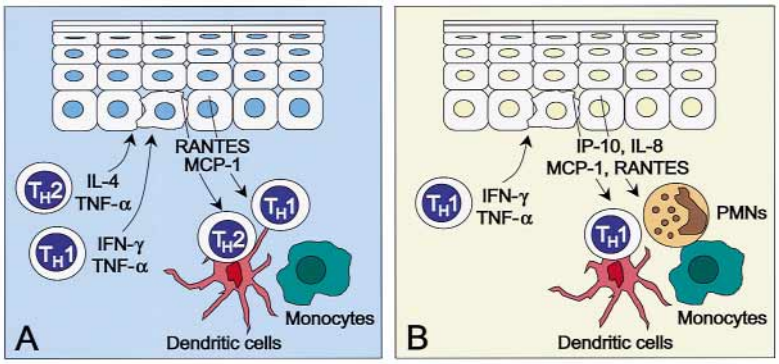

FIG 6. Interplay between TH cells and keratinocytes may favor the recruitment of different leukocyte types in chronic atopic dermatitis **(A)** and psoriasis **(B)**.

patients with psoriasis responded to the same stimuli with an exaggerated expression of IL-8, MCP-1, and IP-10 production. These results may indicate the existence of genetically determined defects in the constitutive and induced chemokine production by keratinocytes of patients with AD and psoriasis. Moreover, this abnormal production appeared under a complex chemokine-associated, rather than stimulus-specific, control. RANTES is not the only proinflammatory factor the expression of which has been found upregulated in AD keratinocytes because they displayed overproduction of spontaneous, as well as PMA-, IL-1α– and IFN-γ–induced, GM-CSF release when compared with healthy control keratinocytes.[20,21] Numerous functional polymorphisms in the regulatory-coding regions of clusters of cytokine-chemokine genes, including RANTES, have been found in patients with AD,[1,33] which could be implicated in overproduction by keratinocytes. In searching for a molecular mechanism underlying abnormal GM-CSF production, we have found that keratinocytes from patients with AD express higher constitutive or induced levels of members of the activator protein 1 (AP-1) family of transcription factors, including c-Jun, JunB, and c-Fos, compared with keratinocytes from normal control subjects.[22] AP-1 is prominently activated by phorbol esters but also by various cytokines, including IL-4, IFN-γ, and TNF-α, and AP-1–binding sites are strategically located in the promoters of a vast array of cytokines and chemokines, including RANTES.[34] By contrast, no clues can be proposed to explain downregulation of IP-10 production in keratinocytes from patients with AD in response to cytokines and PMA. The increased production of MCP-1, IP-10, and IL-8 observed in psoriatic keratinocytes suggests a dysregulated response to a series of proinflammatory stimuli also in these cells. A previous report on psoriatic keratinocyte response to IFN-γ documented a reduced activation of IFN regulatory factor 1 and signal transducer and activator of transcription 1α[25] and thus provided a novel mechanism to explain the reduced antiproliferative and apoptotic effects of this cytokine on epidermis of patients with psoriasis.[23,26]

In conclusion, our data support the hypothesis that contribution of keratinocytes to the pathogenesis of AD and psoriasis is linked to the presence of distinct, intrinsic alterations in their capacity to respond to proinflammatory stimuli and that these abnormalities can modulate amplification and persistence of skin inflammation.

REFERENCES

1. Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic interventions. J Allergy Clin Immunol 2000;105:860-76.
2. Bos JD, De Rie MA. The pathogenesis of psoriasis: immunological facts and speculations. Immunol Today 1999;20:40-6.
3. Grewe M, Bruijnzel-Koomen CAFM, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, et al. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today 1998;19:359-61.
4. Nickoloff BJ, Karabin GD, Barker JN, Griffiths CE, Sarma V, Mitra RS, et al. Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. Am J Pathol 1991;138:129-40.
5. Ackermann L, Harvima IT. Mast cells of psoriatic and atopic dermatitis skin are positive for TNF-alpha and their degranulation is associated with expression of ICAM-1 in the epidermis. Arch Dermatol Res 1998;290:353-9.
6. Zlotnik A, Yoshie O. Chemokines: a new classification and their role in immunity. Immunity 2000;12:121-7.
7. Homey B, Zlotnik A. Chemokines in allergy. Curr Opin Immunol 1999;11:626-34.
8. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol 2000;18:593-620.
9. Albanesi C, Cavani A, Girolomoni G. IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effects with IFN-γ and TNF-α. J Immunol 1999;162:494-502.
10. Albanesi C, Scarponi C, Sebastiani S, Cavani A, Federici M, De Pità O, et al. IL-4 enhances keratinocyte expression of CXCR3 agonistic chemokines. J Immunol 2000;165:1395-402.
11. Niwa Y. Elevated RANTES levels in plasma or skin and decreased plasma IL-10 levels in subsets of patients with severe atopic dermatitis. Arch Dermatol 2000;136:125-6.
12. Raychaudhuri SP, Jiang W-Y, Farber EM, Schall TJ, Ruff MR, Pert CB. Upregulation of RANTES in psoriatic keratinocytes: a possible pathogenic mechanism for psoriasis. Acta Derm Venereol 1999;79:9-11.
13. Zweiman B, Kaplan AP, Tong L, Moskovitz AR. Cytokine levels and inflammatory responses in developing late-phase allergic reactions in the skin. J Allergy Clin Immunol 1997;100:104-9.
14. Gillitzer R, Wolff K, Tong D, Müller C, Yoshimura T, Hartmann AA, et al. MCP-1 mRNA expression in basal keratinocytes of psoriatic lesions. J Invest Dermatol 1993;101:127-31.
15. Gillitzer R, Berger R, Mielke V, Müller, Wolff K, Stingl G. Upper keratinocytes of psoriatic skin lesions express high levels of NAP-1/IL-8 mRNA in situ. J Invest Dermatol 1991;95:73-9.
16. Gottlieb AB, Luster AD, Posnett DN, Carter DM. Detection of a γ interferon–induced protein IP-10 in psoriatic plaques. J Exp Med 1988;168:941-8.

17. Li J, Ireland GW, Farthing PM, Thornhill MH. Epidermal and oral keratinocytes are induced to produce RANTES and IL-8 by cytokine stimulation. J Invest Dermatol 1996;106:661-6.
18. Yamada H, Matsukura M, Yudate T, Chinara J, Stingl G, Tezuka T. Enhanced production of RANTES, a eosinophil chemoattractant factor, by cytokine-stimulated epidermal keratinocytes. Int Arch Allergy Immunol 1997;114(Suppl 1):28-32.
19. Albanesi C, Scarponi C, Federici M, Nasorri F, Cavani A, Girolomoni G. 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 2000;115:81-7.
20. Pastore S, Fanales-Belasio E, Albanesi C, Chinni LM, Giannetti A, Girolomoni G. Granulocyte macrophage colony-stimulating factor is overproduced by keratinocytes in atopic dermatitis. J Clin Invest 1997;99:3009-17.
21. Pastore S, Corinti S, La Placa M, Didona B, Girolomoni G. Interferon-γ promotes exaggerated cytokine production in keratinocytes cultured from patients with atopic dermatitis. J Allergy Clin Immunol 1998;101:538-44.
22. Pastore S, Giustizieri ML, 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.
23. Baker BS, Powles AV, Valdimarsson H, Fry L. An altered response by psoriatic keratinocytes to gamma interferon. Scand J Immunol 1988;28:735-40.
24. Nickoloff BJ, Mitra RS, Varani J, Dixit VM, Polverini PJ. Aberrant production of interleukin-8 and thrombospondin-1 by psoriatic keratinocytes mediates angiogenesis. Am J Pathol 1994;144:820-8.
25. Jackson M, Howie SEM, Weller R, Sabin E, Hunter JA, McKenzie RC. Psoriatic keratinocytes show reduced IRF-1 and STAT-1α activation in response to gamma-IFN. FASEB J 1999;13:495-502.
26. Chen S-H, Arany I, Apisarnthanarax N, Rajaraman S, Tyring SK, Horikoshi T, et al. Response of keratinocytes from normal and psoriatic epidermis to interferon-γ differs in the expression of zinc-α2-glycoprotein and cathepsin D. FASEB J 2000;14:565-71.
27. Müller C, Gershenfeld HK, Lobe CG, Okada CY, Bleakley RC, Weissman IL. A high proportion of T lymphocytes that infiltrate H-2 incompatible heart allografts in vivo express genes encoding cytotoxic cell-specific serine-proteases, but do not express the MEL-14-defined lymph node homing receptor. J Exp Med 1988;167:1124-36.
28. Sebastiani S, Allavena P, Albanesi C, Nasorri F, Bianchi G, Traidl C, et al. Chemokine receptor expression and function in $CD4^+$ T lymphocytes with regulatory activity. J Immunol 2001;116:996-1002.
29. Nakamura K, Williams IR, Kupper TS. Keratinocyte-derived monocyte chemoattractant protein 1 (MCP-1): analysis in a transgenic model demonstrates MCP-1 can recruit dendritic and Langerhans cells to skin. J Invest Dermatol 1995;105:635-43.
30. Karpus WJ, Lukacs NW, Kennedy KJ, Smith WS, Hurst SD, Barrett TA. Differential CC chemokine-induced enhancement of T helper cell cytokine production. J Immunol 1997;158:4129-36.
31. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. Nature 2000;404:407-11.
32. Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG. A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA^+CCR4^+ lymphocytes into lesional atopic dermatitis skin. J Invest Dermatol 2000;115:640-6.
33. Nickel RG, Casolaro V, Wahn U, Beyer K, Barnes KC, Plunkett BS, et al. Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. J Immunol 2000;164:1612-6.
34. Foletta VC, Segal DH, Cohen DR. Transcriptional regulation in the immune system: all roads lead to AP-1. J Leukoc Biol 1998;63:139-52