

In vivo expression of cytokine receptor mRNA in atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease with immunopathologic features that vary depending on the duration of the lesion. Acute lesions are associated with a T-cell infiltrate and a high expression of IL-4 mRNA compared with chronic lesions, uninvolved AD skin, or skin from normal control subjects. Chronic lesions are rich in eosinophils and monocyte/macrophages and contain a greater number of IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-12 (p40) mRNA-positive cells. **Objectives:** In this study, we investigated the mRNA expression of the IL-4 receptor (IL-4R α), IL-5R α , GM-CSFR α , and IL-12R β_2 in biopsy specimens from acute and chronic AD lesions, uninvolved AD skin, normal skin, and psoriatic skin lesions. **Methods:** Cytokine receptor mRNA was examined in paraformaldehyde-fixed biopsy specimens with in situ hybridization with specific antisense riboprobes. **Results:** Acute and chronic skin lesions exhibited a significant increase in numbers of IL-5R α and GM-CSFR α mRNA-positive cells compared with uninvolved AD skin and normal skin ($P < .001$). Chronic skin lesions had a significantly greater number of IL-5R α and GM-CSFR α mRNA-positive cells when compared with acute AD skin ($P < .001$). In contrast, IL-4R α mRNA expression was increased in acute but not chronic AD lesions compared with uninvolved and normal skin ($P < .001$). No significant differences were observed in numbers of IL-12R β_2 mRNA-positive cells when comparing acute AD, chronic AD, uninvolved AD, and normal skin. In psoriatic skin, the numbers of GM-CSFR α and IL-12R β_2 mRNA-positive cells were significantly increased compared with acute AD lesions, uninvolved skin, and normal control skin ($P < .01$). **Conclusions:** These results demonstrate that acute AD is associated with a high expression of IL-4R α , whereas IL-5R α and GM-CSFR α mRNA are predominantly increased in chronic AD and to lesser extent in acute lesions. These findings support the biphasic role of IL-4, IL-5, and GM-CSF in the pathophysiology of AD. (*J Allergy Clin Immunol* 1998;102:245-50)

Key words: Atopic dermatitis, skin, allergy, cytokine receptors

Abbreviations used

AD:	Atopic dermatitis
GM-CSFR α	α -Subunit of granulocyte-macrophage colony stimulating factor
IL-4R α	α -Subunit of IL-4R
IL-5R α	α -Subunit of IL-5R
IL-12R β_2 :	β_2 -Subunit of IL-12R
IFN:	Interferon
VCAM:	Vascular cell adhesion molecule

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with peripheral blood eosinophilia and increased serum IgE levels.¹⁻³ Previous studies have shown that a biphasic response in affected AD skin is dependent on the time course of the lesion. Acute erythematous skin lesions are associated with a T-cell infiltrate, whereas monocyte/macrophages and eosinophils are the predominant inflammatory cells in chronic lichenified lesions.^{4, 5} The duration of the lesion is also a critical determinant in the profile of local cytokine expression. Compared with skin from normal control subjects, both acute and chronic AD lesions contain an increased number of cells expressing IL-4, IL-5, and IL-13 mRNA. However, acute lesions exhibit a higher density of IL-4 and IL-13 mRNA-positive cells and less IL-5 mRNA-positive cells compared with chronic lesions in the same patients. In contrast, chronic but not acute lesions are associated with an increased mRNA expression of GM-CSF and IL-12 p40 subunit compared with normal skin.⁵⁻⁷ Taken together, these findings suggest a more prominent role for IL-4 and IL-13 in the initiation of skin inflammation in AD, whereas increased IL-5, GM-CSF, and IL-12 (p40) occur with the chronic inflammatory response.

The actions of these cytokines are thought to have a key role in the pathogenesis of AD. IL-4 is involved in the switching of antigen-specific CD4⁺ cells to a T_{H2}-like phenotype of cytokine expression.⁸ IL-4 also induces immunoglobulin heavy chain gene rearrangement to IgE production⁹ and upregulates vascular cell adhesion molecule (VCAM)-1 expression on endothelial cells to facilitate eosinophil and lymphocyte recruitment.¹⁰ IL-13 has functional similarities to IL-4,¹¹ including VCAM-1 upregulation¹² and antibody class switching to IgE.¹³ These shared activities may be explained on the basis of a common receptor component.¹⁴ In contrast to IL-4, IL-12 favors the development of interferon (IFN)- γ -producing T_{H1}-like T lymphocytes, which promote cytotoxic macrophage responses.^{15, 16} IL-5 is an essential activation, differentiation, survival, and growth factor for eosinophils,¹⁷ whereas GM-CSF enhances the survival and proliferation of

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neutrophils, eosinophils, macrophages, and their progenitors.^{18, 19} Recent work has implicated high levels of GM-CSF in the inhibition of monocyte apoptosis in chronic AD.⁷

The activity of these cytokines is critically dependent on the expression of their receptors. IL-4R, IL-5R, and GM-CSFR each consist of a high-affinity specific α subunit in conjunction with one or more common signaling subunits.²⁰⁻²² IL-12R consists of β_1 and β_2 subunits²³; the latter is responsible for signaling and was recently shown to be selectively expressed on murine and human T_{H1} - but not T_{H2} -cytokine-producing $CD4^+$ T cells.²⁴ In this study, we therefore examined the expression of IL-4R α , IL-5R α , GM-CSFR α , and IL-12R β_2 mRNA in biopsies of acute lesions, chronic lesions, and uninvolved skin of patients with AD compared with skin from normal control subjects and psoriatic skin specimens.

METHODS

Skin biopsy specimens

This study was performed with institutional review board approval, and all subjects gave informed written consent before entry into the protocol. A total of 15 punch skin biopsy specimens (2 mm each) were obtained from 5 patients meeting the diagnostic criteria for AD.^{2, 6} Three biopsy specimens were obtained from each patient: one specimen from acute, erythematous AD lesions of less than 3 days' onset, a second specimen from chronic lichenified AD lesions of more than 2 weeks duration, and a third specimen from uninvolved skin. The clinical severity of each skin lesion was graded in accordance with previously described criteria.⁵ Punch skin biopsy specimens were also obtained from five nonatopic normal volunteers and the affected areas of four patients with psoriasis.

In the patients with AD, age ranged from 19 to 49 years (median age 28 years). All patients had a history of AD dating back to early infancy and had associated respiratory allergy (allergic rhinitis, asthma, or both). Serum IgE levels ranged from 269 to 10,700 IU/mL (median IgE level, 5300 IU/mL). None of these patients had other skin conditions and none had previously received oral steroids. Topical steroids were withheld for at least 2 weeks before biopsies were performed.

Tissue preparation

Skin biopsy specimens were fixed immediately in freshly prepared 4% paraformaldehyde for 2 hours, then washed twice (1 hour each) with 15% sucrose in 0.1 mol/L phosphate-buffered saline (pH 7.4). Tissues were blocked with ornithine carbamyl transferase compound and kept at -80°C .

In situ hybridization

In situ hybridization was performed as previously described.²⁵ Briefly, riboprobes, both antisense (complementary to mRNA) and sense (having an identical sequence to mRNA) were prepared from cDNA encoding the membrane-bound isoform of IL-4R α , the membrane-bound isoform of IL5R α , GM-CSFR α , and IL-12R β_2 . The cDNAs were inserted into different pGEM vectors and linearized with appropriate enzymes before transcription. Transcription was performed in the presence of ^{35}S -UTP and the appropriate T7 or SP6 RNA polymerases. Cryostat sections were permeabilized with Triton X-100 and proteinase K (1 $\mu\text{g/mL}$) in 0.1 mol/L Tris containing 50 mmol/L ethylenediamine tetraacetic acid for 20 minutes at 37°C . To prevent nonspecific binding of ^{35}S -labeled RNA probes, the preparations were incubated with 10 mmol/L *n*-ethyl

maleimide and 10 mmol/L iodoacetamide for 30 minutes at 37°C and then in 0.5% acetic anhydride and 0.1 mol/L triethanolamine for 10 minutes at 37°C . Prehybridization was carried out with 50% formamide and $2 \times$ standard saline citrate for 15 minutes at 40°C . Dithiothreitol (100 mmol/L) was included in the hybridization mixture to ensure blocking of any further nonspecific binding of the ^{35}S -labeled probes. Posthybridization washing was performed in decreasing concentrations of standard saline citrate at 45°C . Unhybridized single-stranded RNA was removed by RNase A. Hybridization signals were visualized by autoradiography, and sections were counterstained with hematoxylin. Positive hybridization signals were recognized as dense deposits of silver grains overlaying cells. For negative controls, skin biopsy specimens were hybridized with sense probes for each cytokine receptor. In addition, sections were treated with RNase-A solution before the prehybridization step with antisense probes. Positive cells were only observed with an antisense probe; preparations treated with sense probes or pretreated with RNase were negative.

Quantification

Slides were coded and positive cells counted blindly at $100\times$ magnification with an eyepiece graticule. The results were expressed as mean number of positive cells per field (0.202 mm^2) $\pm$ SEM. The number of fields per section counted were two to six, depending on the size of the biopsy specimen and the pattern of alignment of the grid covering an intact area of epithelial and subepithelial tissue. The within-observer coefficient of variation for repeated measures was less than 5%.

Statistics

Statistical comparison was performed with an analysis of variance, and subsequent post hoc comparisons were made with a Tukey honestly significant differences multiple comparison test (Systat v 5.1; Systat Inc., Evanston, Ill.). Values of $P < .05$ were considered statistically significant.

RESULTS

Skin biopsy specimens from acute AD lesions were characterized by epidermal hyperplasia with intracellular edema and inflammatory cell infiltration in the epidermis. Perivascular mononuclear cell infiltrates were also prominent. Chronic lichenified skin lesions were associated with hyperkeratosis with minimal spongiosis and epidermal infiltrate.

Most of the signal for cytokine receptor mRNA was localized to inflammatory cells in the subepithelial layer. Skin from normal control subjects and uninvolved AD skin did not differ significantly in the number of cells expressing IL-4R α , IL-5R α , GM-CSFR α , or IL-12R β_2 mRNA. As shown in Fig. 1, the numbers of IL-4R α mRNA-positive cells were significantly increased in acute AD skin compared with chronic lesions, uninvolved skin, normal skin, and psoriatic lesions ($P < .001$; Fig. 2). There was no significant difference in the presence of IL-4R α mRNA-positive cells in chronic lesions versus uninvolved and normal skin. However, IL-4R α mRNA expression was significantly higher in chronic lesions compared with psoriatic lesions ($P < .05$). The number of cells expressing IL-4R α transcripts was not different in psoriatic skin compared with normal and uninvolved AD skin.

IL-5R α mRNA-positive cells were strikingly increased in chronic AD lesions compared with all other specimens

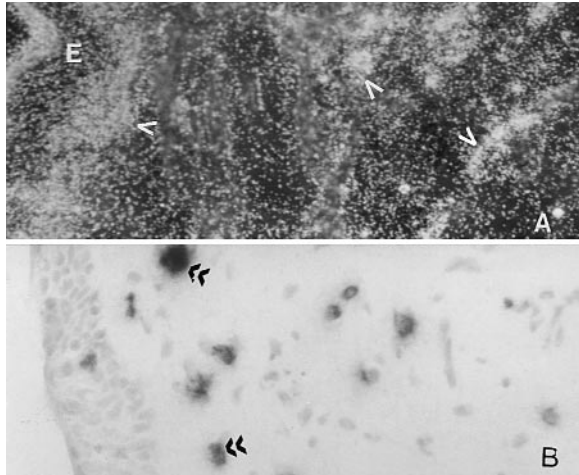


FIG. 1. Representative autoradiographs showing in situ hybridization for cytokine receptor mRNA in AD lesions. **A**, Dark-field illumination of IL-4Rα mRNA expression in an acute AD lesion. IL-4Rα mRNA-positive cells (arrowheads) were observed in the epidermis (E) and dermis. **B**, Bright-field illumination of IL-5Rα mRNA expression in a section from a chronic AD lesion. Cells expressing IL-5Rα mRNA were observed predominantly in infiltrating cells in the dermis (double arrowheads).

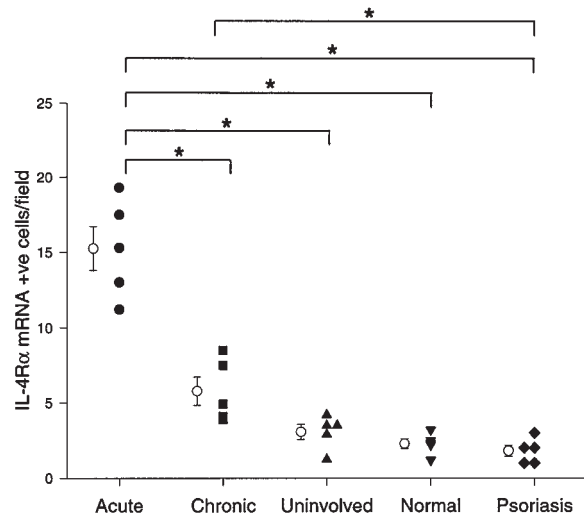


FIG. 2. In situ expression of IL-4Rα mRNA. Specific antisense radiolabeled riboprobes were used to identify IL-4Rα mRNA-positive cells in skin biopsy specimens from acute and chronic AD lesions, uninvolved AD skin, normal skin, and skin from psoriatic lesions by in situ hybridization. Results are expressed as numbers of positive cells per field (0.202 cm²). Filled symbols represent data for individual patients; open circles denote mean $\pm$ SEM for each group. * $P < .05$.

examined ($P < .001$; Figs. 1 and 3). Acute lesions demonstrated a significant increase in cells expressing IL-5Rα mRNA in comparison with uninvolved and normal skin ($P < .05$), but this increase did not achieve statistical significance when compared with psoriatic lesions. Psoriatic, normal, and uninvolved skin did not differ significantly in IL-5Rα mRNA expression.

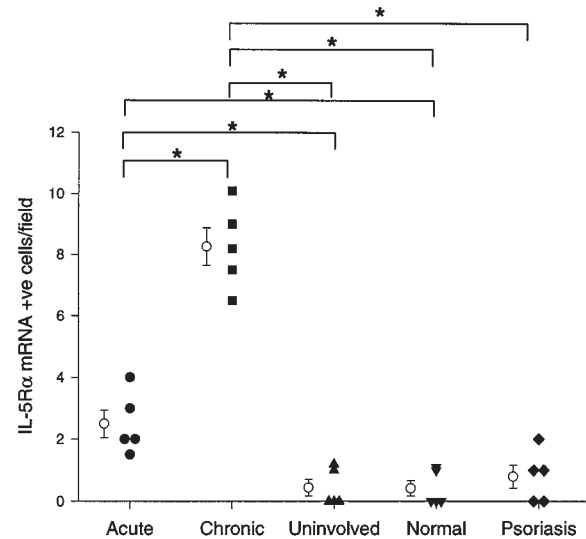


FIG. 3. In situ expression of IL-5Rα mRNA. In situ hybridization with IL-5Rα antisense radiolabeled riboprobes was used to examine and compare the density of IL-5Rα mRNA-positive cells in acute AD lesions, chronic AD lesions, uninvolved AD skin, and normal skin. Results are expressed as numbers of positive cells per field (0.202 cm²). Filled symbols represent data for individual patients; open circles denote mean $\pm$ SEM for each group. * $P < .05$.

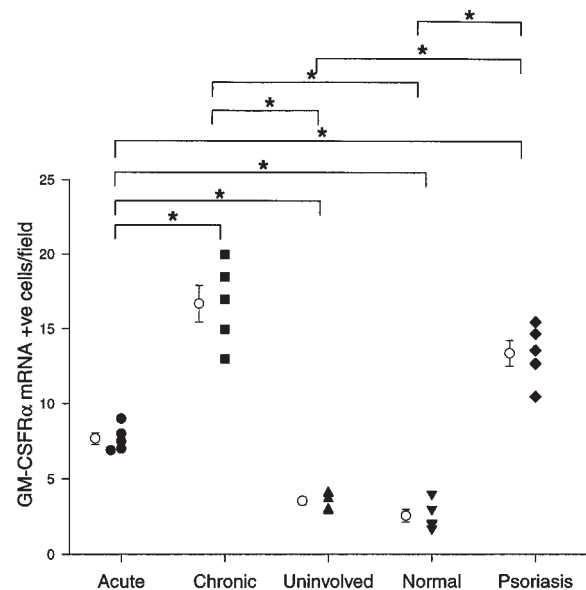


FIG. 4. Expression of GM-CSFRα mRNA in skin biopsy specimens from acute AD lesions, chronic AD lesions, uninvolved skin with AD, and normal skin by in situ hybridization. Results are expressed as numbers of positive cells per field (0.202 cm²). Filled symbols represent data for individual patients; open circles denote mean $\pm$ SEM for each group. * $P < .01$.

The numbers of cells expressing positive hybridization signals for GM-CSFRα mRNA were markedly increased in both chronic AD and psoriatic skin lesions compared with acute AD, uninvolved, and normal skin ($P < .001$; Fig. 4). There was no significant difference in chronic

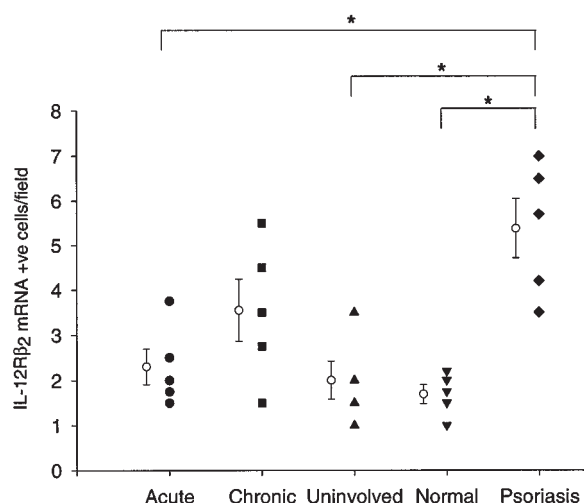


FIG. 5. IL-12R β_2 mRNA expression in skin biopsy specimens from acute AD lesions, chronic AD lesions, uninvolved AD skin, and normal skin by in situ hybridization. Results are expressed as numbers of positive cells per field (0.202 cm²). Filled symbols represent data for individual patients; open circles denote mean $\pm$ SEM for each group. * $P < .01$.

versus psoriatic lesions. Acute lesions contained a greater number of GM-CSFR α mRNA-positive cells compared with normal and uninvolved skin ($P < .01$).

The expression of IL-12R β_2 mRNA was observed in all biopsy specimens studied. The numbers of IL-12R β_2 mRNA-positive cells were significantly increased in psoriasis compared with acute AD, uninvolved, and normal skin ($P < .01$; Fig. 5) but were not statistically different from chronic AD lesions.

Expression of IL-4R α , IL-5R α , GM-CSFR α , and IL-12R β_2 mRNA localized predominantly in infiltrating cells in the dermis. In contrast to the other cytokine receptor mRNAs however, prominent IL-4R α mRNA-positive signals were observed in keratinocytes and endothelial cells as well.

DISCUSSION

We have previously shown that the initiation of skin inflammation in AD is associated with a predominance of IL-4 and IL-13 mRNA expression, whereas increased numbers of cells expressing IL-5, GM-CSF, and to a lesser extent, IL-12 p40 transcripts occur with the maintenance of chronic inflammation.⁵⁻⁷ In this study, we investigated the expression of mRNA encoding membrane-bound receptors for IL-4, IL-5, GM-CSF, and IL-12. Our results complement and extend earlier investigations by demonstrating that acute AD lesions are mainly associated with an increased number of cells expressing IL-4R α mRNA, whereas increased IL-5R α and GM-CSFR α mRNA-positive cells are observed in chronic lesions. This suggests that local actions of IL-4, IL-5, and GM-CSF may be amplified not only by enhanced cytokine expression but also by the presence of a greater number of responsive cells in skin with AD.

The IL-4R complex includes an IL-4-specific subunit (IL-4R α) and at least one other subunit that may be shared between a number of cytokine receptors.²⁰ IL-13 has many functional similarities to IL-4 including VCAM-1 upregulation¹² and the induction of immunoglobulin isotype switching to IgE.¹³ Unlike IL-4, however, T cells do not respond to IL-13.¹¹ Functional studies and receptor blocking experiments have suggested that IL-13 also utilizes IL-4R α in its receptor complex.¹⁴

IL-4R α expression has been described in unstimulated T and B lymphocytes, and its expression is markedly upregulated by IL-4.^{26, 27} Mononuclear cells from the peripheral blood of patients with AD express higher levels of IL-4 and IL-4R mRNA than normal control subjects, and this was inhibited with the in vitro addition of IFN- γ .²⁸ Also, anti-IL-4 antibodies were effective in inhibiting the increased spontaneous production of IgE in lymphocytes from patients with AD.²⁹ Compared with uninvolved AD and normal skin, we found that the expression of IL-4R α mRNA was markedly increased in acute lesions. These results cannot be attributed to a nonspecific accumulation of lymphocytes within the skin because biopsy specimens from patients with psoriasis did not show increased numbers of IL-4R α mRNA-positive cells. Augmented IL-4R α expression could explain the presence of a greater number of T cells in acute AD compared with chronic lesions, given the proliferative effect of IL-4.³⁰ IL-4 has also been shown to increase the expression of the α subunit of Fc ϵ RI in eosinophils³¹ and Fc ϵ RII³² in monocytes, which predominate in chronic AD.⁴ Barata et al.³³ have recently shown a correlation between local Fc ϵ RI expression on eosinophils and the magnitude of the late-phase cutaneous response, suggesting a role for high-affinity IgE receptor-expressing eosinophils in vivo in the skin. Consistent with a role for IL-4 in the modulation of endothelial adhesion molecule expression,¹⁰ abundant hybridization signals for IL-4R α were observed in the endothelium of all groups. Interestingly, IL-4R α mRNA-positive cells were sparse in the epithelium in the skin. This is in contrast to high expression of this receptor that we have observed in the bronchial epithelium of asthmatic patients.³⁴ In the bronchial subepithelium, CD3-positive T cells and, to a lesser extent, tryptase-positive mast cells were shown to comprise significant proportions IL-4R α mRNA-positive cells in asthmatic subjects.³⁴

Although IL-4 and IFN- γ have both been shown to be increased in chronic AD lesions,^{5, 35} successful treatment with topical corticosteroids was associated with an attenuated expression of IFN- γ but not IL-4. This links IFN- γ to the clinical course of the chronic disease, in which it has been suggested to upregulate adhesion molecules such as intracellular adhesion molecule-1. In support of this, intracellular adhesion molecule-1 expression was demonstrated to decrease with treatment of chronic AD in parallel to IFN- γ .³⁵ Studies by our group and others have led to the proposal of a biphasic model of AD in which T_{H2}-predominated responses are crucial for the initiation of AD, whereas T_{H1}-predominated responses may be more important in the chronic disease.^{5, 6, 35}

IL-12 plays a key role in the regulation of IFN- γ production and the generation of CD4⁺ T lymphocytes expressing a T_{H1} profile of cytokines.^{15, 16} Biologically active IL-12 consists of a heterodimeric molecule composed of p40 and p35 subunits. IL-12 p40 was shown to be increased in chronic AD lesions; however, expression of the p35 subunit did not differ significantly from acute lesions, uninvolved skin, or normal control subjects.⁶ Recently, IL-18 has been shown to strikingly enhance IFN- γ production in the presence of small amounts of IL-12.³⁶ In addition, epidermal keratinocytes have been described as a source of constitutive and contact allergen-induced IL-18 in mice.³⁷ This raises the interesting possibility that IL-18 participates with IL-12 in the induction of IFN- γ in chronic AD; however, this remains to be determined. The signaling component of the IL-12R, IL-12R β_2 , has recently been identified as a cell surface receptor specific for T_{H1} cells.²⁴ It was shown to be expressed on human T_{H1} but not T_{H2} clones and was induced during differentiation of human naive cells along the T_{H1} but not the T_{H2} pathway. Contrary to the notion that progression of acute AD lesions to chronic AD lesions represents a transition in T-cell populations from "T_{H1}-like" to "T_{H2}-like," we found that IL-12 β_2 was not increased in chronic AD lesions compared with acute lesions, uninvolved skin, or normal skin. However, the possibility that the specific localization of IL-12R β_2 to T cells differs between acute and chronic AD lesions cannot be excluded. Notably, IL-12R β_2 mRNA-positive cells were increased in psoriatic lesions, which are characterized by a high expression of both p35 and p40 subunits of IL-12 and IFN- γ and a low expression of IL-4.^{5, 6}

In keeping with the presence of elevated eosinophils and monocytes/macrophages in chronic AD,^{4, 5, 7} cells expressing IL-5R α and GM-CSFR α mRNA were highest in these lesions compared with acute lesions, uninvolved skin, and skin from normal control subjects. In addition, numbers of IL-5R α and GM-CSFR α mRNA-positive cells were significantly increased in acute lesions versus normal and uninvolved skin. In the bronchial mucosa of asthmatic subjects, the majority (>90%) of cells expressing IL-5R α are eosinophils.³⁸ Indeed, IL-5 plays a central role in the recruitment, terminal differentiation, activation, and prolonged survival of eosinophils at sites of allergic inflammation.^{17, 39} Compared with normal control subjects or patients with psoriasis, a lower incidence of apoptosis in peripheral blood monocytes from individuals with chronic AD has been attributed to the increased production of GM-CSF, because the effect could be inhibited with anti-GM-CSF neutralizing antibodies.⁷ GM-CSF may play a similar role in the inhibition of monocyte/macrophage apoptosis in the tissue and thereby contribute to the persistence of these cells in diseases associated with a high local expression of this cytokine, such as chronic AD and psoriasis. Psoriatic lesions also contained an increased density of GM-CSFR α mRNA-positive cells compared with acute AD lesions, uninvolved AD skin, and skin from normal control subjects. In asthmatic airways, the

expression of GM-CSFR α is predominantly localized to monocyte/macrophages.⁴⁰

Interestingly, the expression of IL-4 and IL-13, but not IL-5, IL-12, or IFN- γ , has been shown to be significantly different between uninvolved AD skin and normal skin.⁵⁻⁷ The increased expression of these cytokines in the skin may represent features that predispose to AD. None of the cytokine receptors we studied showed a significant difference in the number of mRNA-positive cells between uninvolved and normal skin.

In conclusion, the results of this study demonstrate that acute AD is associated with a high expression of IL-4R α , whereas IL-5R α and GM-CSFR α mRNA are predominantly increased in chronic AD and to lesser extent in acute lesions. These findings support the local activity of IL-4, IL-5, and GM-CSF in the pathophysiology of AD.

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