

Rapid communication

In vivo expression of IL-12 and IL-13 in atopic dermatitis

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Previous studies in atopic dermatitis (AD) have shown that acute and chronic skin lesions are associated with a TH₂-type profile of cytokine expression. IL-12 and IL-13 are recently described cytokines, which possess TH₁- and TH₂-like actions, respectively. We have used the technique of in situ hybridization to examine the expression of IL-12 and IL-13 messenger RNA in skin biopsy specimens of acute and chronic skin lesions and uninvolved skin from patients with AD. When compared with normal control skin, the acute and chronic skin lesions and unaffected skin from patients with AD had significantly greater numbers of cells that were positive for IL-13 mRNA ($p < 0.05$). Acute AD skin lesions expressed a higher number of positive cells than those observed in chronic AD skin lesions ($p < 0.05$) or psoriasis skin lesions ($p < 0.05$). There was a significant increase in the numbers of IL-12 mRNA-positive cells in chronic skin lesions compared with acute lesions and uninvolved skin from patients with AD ($p < 0.05$). These data demonstrate that acute AD skin lesions are associated with an increased expression of IL-13 mRNA. In contrast, the relative increase in IL-12 mRNA in chronic AD skin lesions suggests a possible role for IL-12-producing cells in modulating chronic inflammation. (J Allergy Clin Immunol 1996;98:225-31.)

Key words: Atopic dermatitis, IL-12, IL-13, allergic inflammation, T lymphocytes

Atopic dermatitis (AD) is a chronic inflammatory skin disease of unknown etiology that is associated with elevated serum IgE levels and tissue eosinophilia.¹ Skin biopsy specimens of acute and chronic lesions in AD are characterized by an infiltration with a large number of inflammatory cells, in particular CD4⁺ T lymphocytes.² We and others have shown that there is a high expression of IL-4, IL-5, and IL-10 but not interferon- γ (IFN- γ) in both acute and chronic lesions of AD

Abbreviations used

AD:	Atopic dermatitis
ANOVA:	Analysis of variance
IFN- γ :	Interferon- γ

compared with the uninvolved skin of patients with AD or normal control subjects.^{3,4} These observations suggest the expression of predominantly TH₂-type cytokines in AD, which are critical for IgE synthesis and eosinophil accumulation in skin lesions.

IL-12 and IL-13 are recently described cytokines, which play a critical role in the development of TH₁- versus TH₂-like T cells, respectively. Although the primary source of IL-12 is macrophages or monocytes, because of its spectrum of biologic activity in stimulating the induction of TH₁ cells⁵ and inhibiting IgE production,⁶ IL-12 is considered to be a TH₁-type cytokine. IL-13 is produced mainly by activated T cells of the TH₂ subset,

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which shares a number of biologic activities with IL-4.⁷ In this study we have investigated the expression of IL-12 and IL-13 in AD to compare the expression of these cytokines in acute lesions with chronic lesions, uninvolved skin of patients with AD, psoriatic skin lesions, or skin of normal control subjects by using the technique of in situ hybridization.

METHODS

Skin biopsy specimens

Fifteen punch skin biopsy specimens were obtained from five patients who met the diagnostic criteria for AD.^{1,3} 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 greater than 2 weeks' duration, and a third specimen from uninvolved skin. The clinical severity of each skin lesion was graded according to previously described criteria.³ Control skin biopsy specimens were also obtained from nonatopic normal volunteers ($n = 5$) and affected skin from patients with psoriasis ($n = 4$). Skin biopsy specimens were fixed immediately and prepared for in situ hybridization.³

The age of patients with AD ranged from 19 to 49 years. All patients had a history of AD dating to early infancy and had associated respiratory allergy (allergic rhinitis and/or asthma). Serum IgE levels ranged from 269 to 10,700 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.

In situ hybridization

In situ hybridization was performed as previously described.⁸ Briefly, riboprobes (both antisense and sense) were prepared from complementary DNA and linearized with appropriate enzymes before transcription. The IL-12 probes used were specific for the p35 or the p40 subunits of the IL-12 gene. Transcription was performed in the presence of sulfur 35-labeled uridine triphosphate and the appropriate T7 or SP6 RNA polymerases. Cryostat sections were permeabilized and subsequently treated to prevent nonspecific binding of the ³⁵S-labeled RNA probes.³ Prehybridization was carried out with 50% formamide and 2× standard saline citrate for 15 minutes at 40° C. Dithiothreitol was included in the hybridization mixture to ensure blocking of any further nonspecific binding of the ³⁵S-labeled probes. Posthybridization washing was performed in decreasing concentrations of standard saline citrate at 45° C. Unhybridized single-strand RNAs were removed by RNase A. Hybridization signals were visualized by using autoradiography, and the tissue sections were counterstained with hematoxylin.

Quantification

Slides were coded and counted blindly by using 100× magnification with an eyepiece graticule. The results were expressed as the mean number of positive cells per field. The number of fields per section counted was 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 intra-observer coefficient of variation for repeated measures was less than 5%.⁹

For the negative controls, skin biopsy specimens were hybridized with sense probes. In addition, sections were treated with RNase A solution before the prehybridization step with antisense probes. Positive cells were only observed when the antisense probes were used; preparations treated with the sense probes or pretreated with RNase were negative with only baseline background signals.

Statistical analysis

Statistical comparison of the results was performed by using an analysis of variance (ANOVA), and subsequent post hoc comparisons were made by using a Tukey HSD multiple comparison test (Systat v 5.1; Systat Inc., Evanston, Ill.). A p value of less than 0.05 was considered statistically significant.

RESULTS

Biopsy specimens from acute AD lesions demonstrated varying degrees of epidermal hyperplasia with focal spongiosis and epidermal inflammatory infiltrate. Perivascular infiltrates were also prominent. In contrast, chronic AD lesions were characterized by acanthosis with minimum epidermal infiltration. All biopsy specimens from acute and chronic AD skin lesions showed positive hybridization signals for IL-13 mRNA (Fig. 1). Biopsy specimens from uninvolved skin obtained from patients with AD demonstrated a few IL-13 mRNA-positive cells. Only two of five of the normal skin biopsy specimens and two of four of the psoriasis skin biopsy specimens were positive for IL-13 mRNA, but the numbers of positive cells were very few. There was no significant difference in the numbers of IL-13 mRNA-positive cells between patients with psoriasis and normal control subjects ($p > 0.05$). The number of IL-13 mRNA-positive cells was significantly higher in acute lesions compared with chronic lesions ($p < 0.05$), uninvolved skin ($p < 0.001$), normal skin ($p < 0.001$), or skin lesions from patients with psoriasis ($p < 0.05$). However, the number of IL-13 mRNA-positive cells in chronic lesions was still higher than the number of IL-13 mRNA-positive cells in uninvolved skin and normal skin ($p < 0.01$) and in

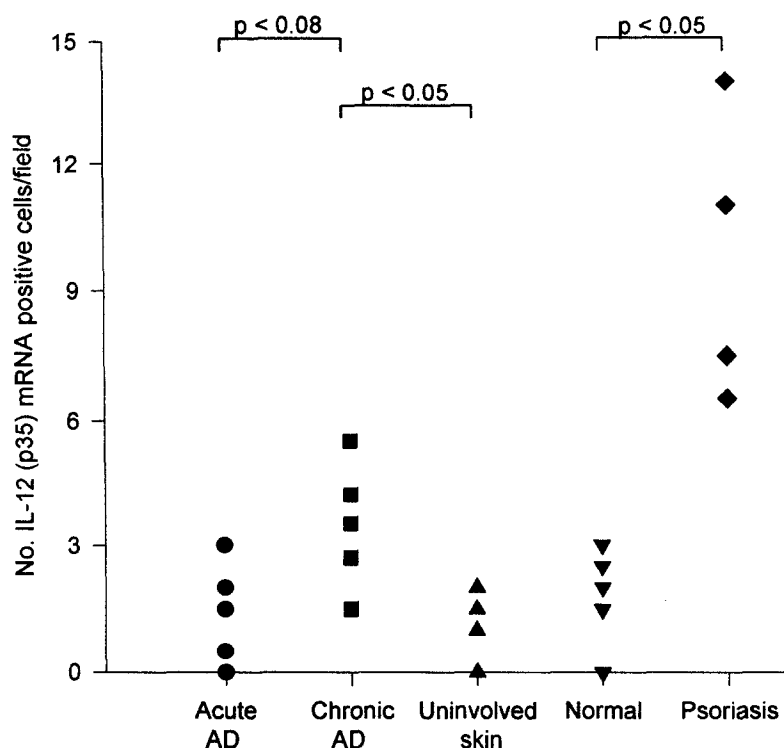


FIG. 2. In situ hybridization of AD skin lesions, uninvolved skin, normal skin, and skin biopsy specimens from patients with psoriasis when the p35 riboprobe for IL-12 was used. Results are expressed as the number of positive cells per high-power field (0.202 mm²). Comparisons were made by using a nonparametric ANOVA test. IL-12 (p35) mRNA-positive cells were significantly increased in chronic AD skin lesions when compared with uninvolved skin ($p < 0.05$) and in biopsy specimens from patients with psoriasis compared with normal skin ($p < 0.05$).

sense probe, confirming the specificity of the probes and signals.

DISCUSSION

We and others have recently reported that acute AD, in common with other forms of atopic inflammation, is characterized by infiltration of the skin by cells expressing predominantly TH₂-like cytokines, such as IL-4, IL-5, and IL-10.^{3, 10} To extend our investigations into the pathogenesis of AD and assess further differences in cytokine expression between acute and chronic skin lesions, we examined mRNA for IL-12 and IL-13 in skin biopsy specimens from patients with AD and compared them with skin biopsy specimens from patients with psoriasis and nonatopic normal skin.

Our results provide direct evidence for the predominant expression of IL-13 in AD and support the role of TH₂-type cytokines in the pathogenesis of this disease. This study demonstrates that both acute and chronic AD lesions are associated with an increased expression of IL-13 mRNA when compared with skin biopsy specimens from normal control subjects. These results cannot be attributed

to a nonspecific accumulation of lymphocytes within the skin because biopsy specimens from patients with psoriasis did not show enhanced numbers of IL-13 mRNA-positive cells.

Similar elevated levels of IL-13 mRNA expression have recently been reported by our laboratory in bronchial biopsy specimens from patients with allergic asthma.¹¹ These observations support an active role for IL-13 in the pathogenesis of allergic disorders. Interestingly, in patients with allergic asthma, levels of mRNA for IL-13 are subsequently reduced after steroid therapy in patients who showed a clinical improvement in response to steroids.¹¹ This further strengthens the association between the development of atopic diseases and the synthesis of IL-13.

In this study we did not attempt to identify the phenotype of cells responsible for the synthesis of mRNA for IL-13. Both CD4⁺ and CD8⁺ T cells have been reported as having the capacity to produce IL-13.⁷ However, it has recently been demonstrated that activated mast cells, and possibly basophils, also possess the capacity to secrete IL-13.¹² Although we did not attempt to quantify

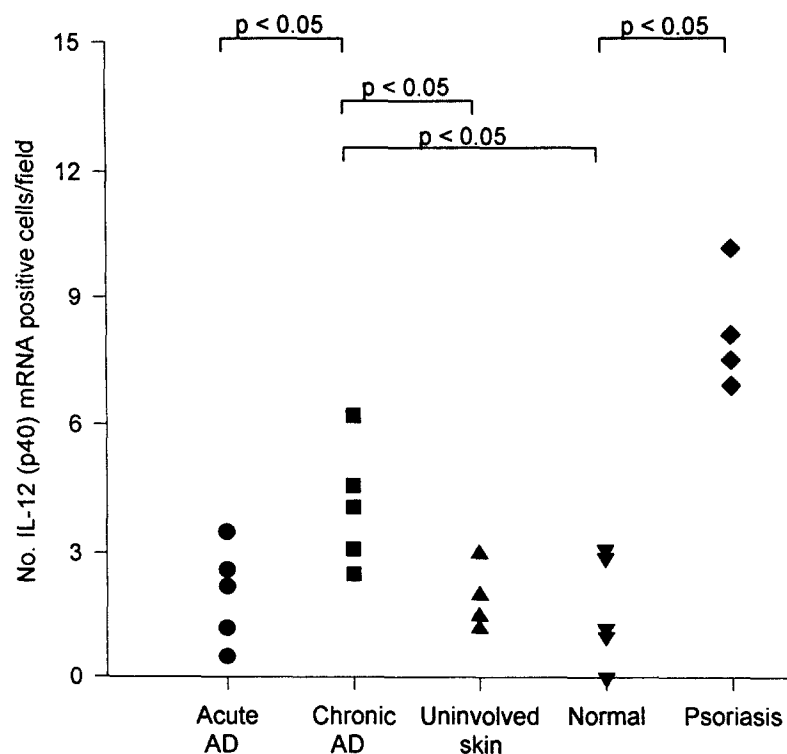


FIG. 3. In situ hybridization of AD skin lesions, uninvolved skin, normal skin, and skin biopsy specimens from patients with psoriasis when the p40 riboprobe for IL-12 was used. Results are expressed as the number of positive cells per high-power field (0.202 mm²). Comparisons were made by using a nonparametric ANOVA test. IL-12 (p40) mRNA-positive cells were significantly increased in chronic AD skin lesions when compared with acute skin lesions ($p < 0.05$), uninvolved skin ($p < 0.05$) and in skin biopsy specimens taken from normal individuals ($p < 0.05$). IL-12 mRNA expression was also increased in biopsy specimens from patients with psoriasis when compared with normal skin ($p < 0.05$).

IL-4 mRNA-positive cells in these biopsy samples, we have previously reported the numbers of cells positive for IL-4 mRNA in acute and chronic AD.³ The results presented in this article suggest that a similar number of cells positive for IL-4 and IL-13 mRNA is observed in both groups. Furthermore, in a recent study on bronchial biopsy specimens from patients with allergic asthma, we have shown that IL-13 is colocalized with IL-4, and both are expressed in CD3⁺ cells.¹³ We therefore believe that most of the IL-13 mRNA-positive cells in AD are resident or infiltrating T cells.

The highest number of IL-13 mRNA-positive cells was observed in the acute skin lesions, and this number was significantly greater than that seen in chronic skin lesions. A differing profile of cytokine gene expression has previously been documented in acute and chronic AD lesions,³ and these observations may be related to the differences in histopathology noted in this study and reported previously.¹⁴ IL-13 shares many biologic properties with IL-4, including the ability to act as

a switch factor inducing the production of IgE via a common signalling pathway.^{15, 16} IL-13 has also been shown to downregulate the production of IL-12 and IFN- γ ,⁷ and the presence of this cytokine may be one explanation for the relative paucity of IL-12 mRNA-positive cells in acute AD. In contrast, chronic lesions were found to be associated with relatively few cells expressing mRNA for IL-4 and significantly greater numbers of IL-5 mRNA-positive cells and activated eosinophils. Because IL-13 has recently been shown to selectively induce vascular cell adhesion molecule-1 (an adhesion molecule associated with eosinophil accumulation) expression on human endothelial cells,¹⁷ it is likely that this cytokine plays a role in the recruitment of these cells to the sites of AD skin lesions. Whether acute and chronic lesions represent different stages of an ongoing allergic inflammatory response with a common pathogenesis or whether they each have a distinct immunologic basis remains to be determined.

A significantly elevated number of cells express-

ing mRNA for IL-13 was also observed in uninvolved skin from patients with AD compared with normal control subjects. Thus enhanced synthesis of IL-13 may reflect an atopic disposition of the skin, possibly being primed for the production of IgE antibodies. Whether this observation is restricted to patients with allergic cutaneous responses or is evident in atopic subjects in general is presently unknown.

There was considerable variability in the numbers of IL-12 mRNA-positive cells in all skin biopsy specimens studied, although there was a similar pattern of mRNA expression when both p35 and p40 subunits of the IL-12 molecule were examined. It has been previously shown that the p35 subunit of IL-12 is expressed constitutively by cells, whereas the p40 subunit is inducible.¹⁸ The expression of both of these subunits is required for biologic activity. Because the expression of both subunits was elevated in chronic AD skin lesions, our data suggest that the biologically active heterodimer form of IL-12 was being produced. There was no significant difference in the number of IL-12 mRNA-positive cells in biopsy specimens from patients with acute AD compared with specimens from normal control subjects or uninvolved skin. The number of IL-12 mRNA-positive cells was significantly higher in chronic AD lesions, when compared with acute AD lesions, uninvolved skin, and biopsy specimens from normal subjects. Biopsy specimens from patients with psoriasis demonstrated large numbers of IL-12 mRNA-positive cells. We believe that most of the IL-12 mRNA-positive cells are macrophages, because the positive cells were relatively large. Moreover, we have recently reported the phenotype of IL-12 mRNA-positive cells in cutaneous late-phase reactions after immunotherapy, where we demonstrated that 80% of the cells positive for IL-12 mRNA were macrophages.¹⁹ The relative increase in the IL-12 mRNA-positive cells in chronic lesions might reflect the ability of macrophages to regulate and control the inflammatory response in AD. IL-12 has the ability to suppress IgE production in vitro by both IFN- γ -dependent and IFN- γ -independent mechanisms⁶ and can switch TH₀ cells to a TH₁-like phenotype.⁵ These data are consistent with our finding of IL-12 expression after successful treatment with steroids¹¹ or after immunotherapy.¹⁹ IL-12 is also a potent inducer of IFN- γ production,²⁰ although in chronic AD lesions no significant increase in the production of IFN- γ has been observed.³ This lack of IFN- γ production may be explained by the increased expression of

IL-4, IL-10, and prostaglandin E₂ observed in AD,^{3, 21, 22} all of which serve to limit the synthesis of IFN- γ .

In summary, we have demonstrated enhanced expression of mRNA for IL-13 in acute and chronic skin lesions in patients with AD and, to a lesser extent, in the uninvolved skin from these individuals. The numbers of IL-12 mRNA-positive cells were primarily elevated in chronic skin lesions. These data suggest that IL-13 might play a role in the pathogenesis of AD and support the hypothesis that inflammation in acute AD is mediated by TH₂-type cytokines. It also opens a new avenue for the design of new therapeutic approaches in this common skin disease.

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