

Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions

Masao Toda, MD, PhD,^a Donald Y. M. Leung, MD, PhD,^b Sophie Molet, PhD,^c

Mark Boguniewicz, MD,^b Rame Taha, MD,^a Pota Christodouloupoulos, MSc,^a

Takeshi Fukuda, MD, PhD,^e Jack A. Elias, MD,^d and Qutayba A. Hamid, MD, PhD^a

Montreal, Quebec, Canada, Denver, Colo, Rennes, France, New Haven, Conn, and Tokyo, Japan

Background: In atopic dermatitis (AD) there is evidence of tissue fibrosis involving a number of structural changes, including papillary dermal fibrosis and epidermal hyperplasia. These changes are suggested to be the result of chronic inflammation of the skin. Several remodeling-associated cytokines, including transforming growth factor (TGF) β 1, IL-11, and IL-17, have been shown to be increased in allergic diseases, including asthma.

Objective: We investigated TGF- β 1, IL-11, and IL-17 expression in skin biopsy specimens recovered from acute and chronic skin lesions from patients with AD, as well as from uninvolved skin of patients with AD and skin from healthy volunteers. We also examined the correlation between the expression of these cytokines and the extent of total, type I, and type III collagen deposition.

Methods: We evaluated the expression of TGF- β 1, IL-11, and IL-17 by means of immunohistochemistry. Collagen deposition was assessed by means of immunohistochemistry and van Gieson staining.

Results: TGF- β 1 expression was markedly enhanced in both acute and particularly chronic lesions ($P < .001$). Although IL-11 expression was significantly increased only in chronic lesions ($P < .0001$), IL-17 was preferentially associated with acute lesions ($P < .005$). Although collagen type III deposition was not significantly different among the groups, type I collagen deposition was significantly increased in chronic AD lesions ($P < .0005$). There was a significant correlation between IL-11 and type I collagen deposition, as well as the number of eosinophils in skin specimens from patients with AD ($r^2 = 0.527$, and $r^2 = 0.622$, respectively; $P < .0001$).

Conclusion: These results suggest that TGF- β 1, IL-11, and IL-17 are involved in the remodeling of skin lesions in patients with AD. However, IL-11 and IL-17 are preferentially expressed at different stages of the disease. Type I collagen

appeared to be the major subtype involved in this repair process. (J Allergy Clin Immunol 2003;111:875-81.)

Key words: Atopic dermatitis, skin biopsy, fibrosis, cytokines, collagen, remodeling

Tissue remodeling has been well documented in asthmatic airways and is characterized by various structural changes, including airway wall thickening, subepithelial fibrosis, mucus overproduction, smooth muscle hyperplasia-hypertrophy, and epithelial hypertrophy.^{1,2} Airway remodeling is a consequence of chronic inflammatory episodes,³ which are associated predominantly with T_H2-type cytokines (IL-4, IL-13, and IL-5). Subepithelial fibrosis is described as the result of an enhanced deposition in the lamina reticularis of extracellular matrix (ECM) components, such as type I, type III, and type V collagens,⁴⁻⁶ that might be regulated by fibrogenic cytokines (eg, transforming growth factor [TGF] β).

Numerous reports demonstrated that among all 3 TGF- β isoforms synthesized by mammalian cells, TGF- β 1 is predominantly associated with fibrosis. TGF- β is the major fibrogenic cytokine that has been shown to act on fibroblasts for the synthesis and secretion of collagens, as well as other mediators.⁷⁻¹² TGF- β has been reported to participate in collagen accumulation within the ECM⁹ and plays a critical role in wound healing and tissue remodeling. Various cell types, including eosinophils,¹³⁻¹⁵ T lymphocytes, fibroblasts, macrophages, and epithelial cells,^{16,17} have all been shown to produce TGF- β .

IL-11 is a hematopoietic cytokine that is produced by a variety of stromal cells, including epithelial cells, fibroblasts, and myocytes.¹⁸⁻²¹ Studies performed in transgenic mice revealed that IL-11 induces some features of remodeling, such as enhanced deposition of type I and type III collagens.^{22,23} IL-17 is a recently described cytokine mainly released by CD4⁺ T cells that induces the release of proinflammatory mediators from macrophages and fibroblasts.²⁴⁻²⁶ We recently demonstrated that IL-17 expression was increased in asthmatic airways, particularly in patients with severe asthma.^{27,28} We also showed that recombinant IL-17 increased the release of IL-6 and IL-11 by fibroblasts isolated from bronchial biopsy specimens in asthmatic subjects.²⁷

From ^aMeakins-Christie Laboratories, Montreal; ^bthe Department of Pediatrics, National Jewish Medical and Research Center, Denver; ^cINSERM U456, Faculté des Sciences Pharmaceutiques et Biologiques, Rennes; ^dthe Department of Internal Medicine, Section of Pulmonary and Critical Care Medicine, Yale University School of Medicine, New Haven; and ^eDokkyo University, Department of Pulmonary Medicine and Clinical Immunology, Mibu-machi, Tochigi, Tokyo.

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Reprint requests: Qutayba A. Hamid, MD, PhD, Meakins-Christie Laboratories, McGill University, 3626 St Urbain, Montreal, QC, H2X 2P2, Canada.

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Abbreviations used

AD: Atopic dermatitis
ECM: Extracellular matrix
HPF: High-power field
TGF: Transforming growth factor

Atopic dermatitis (AD) is a chronic inflammatory skin disease with increased serum IgE levels and tissue eosinophilia.^{29,30} The expression of profibrotic cytokines has yet to be analyzed in AD. In this study we speculate that TGF- β , IL-11, and IL-17 contribute to tissue remodeling in the skin of patients with AD. We therefore assessed the expression of these cytokines and the extent of collagen deposition in skin biopsy specimens recovered from acute and chronic skin lesions, and we compared these results with results from uninvolved skin of patients with AD and skin from healthy volunteers. Furthermore, we investigated whether a particular subtype of collagen might be preferentially expressed or increased at various stages of the disease.

METHODS**Skin biopsy specimens**

Twenty-four skin punch biopsy specimens (2 mm each) were obtained from 8 patients fulfilling the diagnostic criteria for AD.³¹ Three specimens were recovered from each patient, one from acute erythematous AD lesions less than 3 days from onset, one from chronic lichenified AD lesions greater than 2 weeks from onset, and the last from an uninvolved skin area. Punch skin biopsy specimens were also obtained from 8 nonatopic healthy volunteers. Specimens were fixed immediately in 4% paraformaldehyde, embedded in OCT medium and snap-frozen in liquid nitrogen-cooled isopentane for immunohistochemistry.

The age of patients with AD ranged from 19 to 49 years (median, 28 years). All patients had a history of AD dating back to early infancy and had associated respiratory allergy (allergic rhinitis or asthma). Serum IgE levels ranged from 269 to 10,700 IU/mL (median, 5300 IU/mL). None of these patients had other skin pathologies, and none had been previously treated with oral corticosteroids. Topical steroids were withheld for at least 2 weeks before biopsy specimens were taken. The National Jewish Medical and Research Center Institutional Review Board approved the study, and all subjects provided informed written consent before entering the protocol.

Immunohistochemistry

As previously described,³² we used the alkaline phosphatase-antialkaline phosphatase method to identify cytokines, collagen subtypes, and inflammatory cell phenotypes within the skin biopsy sections (5-mm tissue sections). Specific antibodies were used to identify eosinophils (anti-EG2; Kabi Pharmacia Diagnostics AB, Uppsala, Sweden), T cells (anti-CD3; Becton-Dickinson Canada, Mississauga, Ontario, Canada), type I and type III collagens (Medicorp, Montreal, Quebec, Canada), TGF- β 1 (Cedarlane Laboratories, Hornby, Ontario, Canada), and IL-11 and IL-17 (R&D systems, Minneapolis, Minn). For negative control preparations, the primary antibody was replaced with either an irrelevant isotype-matched control immunoglobulin or TRIS-buffered saline solution.

Histochemistry

Total collagen was identified on skin biopsy sections by using van Gieson staining, which allowed collagen fibers to appear as red fibers.

Quantification

Slides were coded, and positive cells were counted blindly under a 200 $\times$ magnification with an eyepiece graticule. Two to 6 fields were counted per section, depending on the size of the biopsy specimens and the pattern of alignment of the grid covering an intact area of epithelial and subepithelial areas. Immunoreactive cells were quantified as the mean cell count expressing the appropriate positive marker per high-power field (HPF; 0.202 cm²). The severity of dermal fibrosis was scored on a 1 to 4 scale according to the extent of fibrosis within the dermis, with a score of 4 representing extensive fibrosis that extended to the whole papillary dermis. The within-observer coefficient of variation for repeated measures was less than 5%. Data are expressed in the text as means $\pm$ SD.

Statistical analysis

Statistical analysis of the results was performed by using Kruskal-Wallis nonparametric ANOVA, and subsequently, post hoc comparisons were made by using the Scheffe F and Dunn multiple comparison tests. The coefficients of correlation were calculated from the Pearson moment coefficient and corrected for multiple comparisons by using the Bonferroni correction factor (Systat version 8.0; SASS, Chicago, Ill). Results were considered as statistically significant when the *P* value was less than .05.

RESULTS**Phenotype of inflammatory cells**

Skin biopsy specimens from acute AD lesions characteristically showed epidermal hyperplasia with intracellular edema and inflammatory cell infiltration in the epidermis. Mononuclear cell infiltrates were prominent in perivascular areas. In particular, CD3⁺ T cells were markedly accumulated in acute lesions compared with in chronic lesions, uninvolved skin from patients with AD, and normal skin (*P* < .0001, Table I). Although the number of CD3⁺ T cells was also significantly greater in chronic lesions than in uninvolved skin from patients with AD and normal skin (*P* < .0001), chronic lichenified lesions showed hyperkeratosis with minimal spongiosis. Epidermal and particularly dermal infiltrates showed increased numbers of eosinophils. The EG2⁺ cell population was significantly greater in chronic lesions than in acute lesions, uninvolved skin from patients with AD, and normal skin (*P* < .0001). No significant difference was seen in the number of eosinophils in normal skin versus that in uninvolved skin from patients with AD.

TGF- β 1, IL-11, and IL-17 expression

TGF- β 1 expression was significantly increased in chronic and acute lesions (13.5 ± 1.7 and 9.0 ± 3.1 cells/HPF, respectively) compared with that in uninvolved areas (3.8 ± 2.4 cells/HPF; *P* < .0001 and *P* < .001 vs chronic and acute lesions, respectively) and normal skin (1.5 ± 1.4 cells/HPF; *P* < .0001 and *P* < .005 vs chronic and acute lesions, respectively; Fig 1). Moreover, the number of TGF- β 1⁺ cells was higher in chronic than

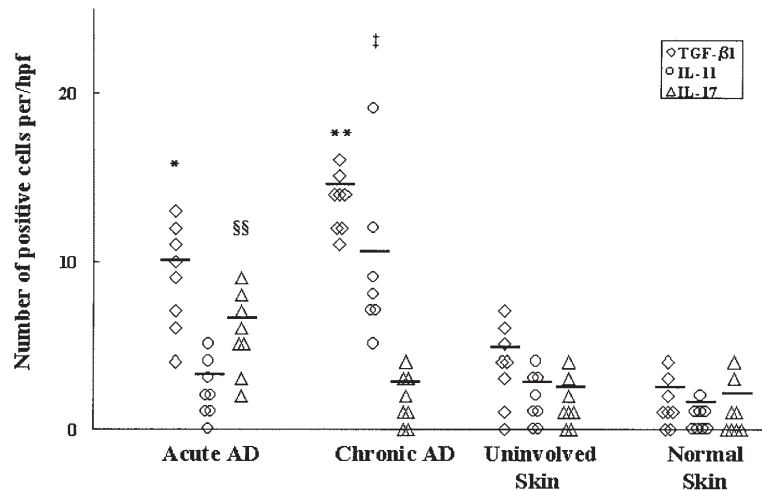


FIG 1. TGF- β 1, IL-11, and IL-17 immunostainings in acute chronic lesions and uninvolved skin biopsy specimens of patients with AD and in skin of healthy volunteers. Bars represent means. * $P < .001$ and $P < .0001$ compared with uninvolved skin from patients with AD and normal skin, respectively; §§ $P < .005$, $P < .001$, and $P < .0005$ compared with chronic lesions, uninvolved skin from patients with AD, and normal skin, respectively; ** $P < .005$, $P < .0001$, and $P < .0001$ compared with acute lesions, uninvolved skin of patients with AD, and normal skin, respectively; † $P < .0001$ compared with acute lesions, uninvolved skin from patients with AD, and normal skin.

TABLE I. Phenotype of inflammatory cells accumulated in skin biopsy specimens

	Acute lesions	Chronic lesions	Uninvolved skin	Normal skin
CD3 ⁺ cells (/HPF)	99.4 ± 22.4*	60.1 ± 8.3†	15.0 ± 7.1	9.8 ± 4.3
EG2 ⁺ cells (/HPF)	3.9 ± 2.4	12.4 ± 5.1‡	0.1 ± 0.4	0.1 ± 0.4

Values are presented as means ± SD.

* $P < .0001$ compared with chronic lesions, uninvolved skin of patients with AD, and normal skin.

† $P < .0001$ compared with uninvolved skin of patients with AD and normal skin.

‡ $P < .0001$ compared with acute lesions, uninvolved skin of patients with AD, and normal skin.

in acute lesions ($P < .005$). No significant difference was seen between uninvolved and normal skin.

IL-11 expression was only significantly increased in chronic lesions (9.6 ± 4.7 cells/HPF, $P < .0001$), whereas it reached similarly low levels in acute lesions and in uninvolved and normal skin (2.3 ± 1.7 , 1.8 ± 1.5 , and 0.6 ± 0.7 cells/HPF, respectively; Fig 1). In contrast, a significant increase in IL-17 expression was only detectable in acute lesions (5.6 ± 2.4 cells/HPF) compared with in the 3 other groups, which had low levels of IL-17 expression (1.8 ± 1.5 [$P < .005$], 1.5 ± 1.4 [$P < .001$], and 1.1 ± 1.6 cells/HPF [$P < .0005$] in chronic lesions, uninvolved skin, and normal skin, respectively; Fig 1).

Deposition of total, type I, and type III collagens

Total collagen deposition, as assessed by means of van Gieson staining, was not different among acute and chronic skin lesions (2.5 ± 1.2 and 2.4 ± 1.3 , respectively), uninvolved skin and normal skin (1.5 ± 0.9 and 1.3 ± 1.0 , respectively; Fig 2). However, the score of type I collagen, as assessed by means of immunostaining, was significantly higher in chronic skin lesions (3.1 ± 0.8) compared with that in acute lesions (1.3 ± 0.7 , $P < .001$),

uninvolved skin (1.3 ± 0.7 , $P < .001$), and normal skin (0.6 ± 0.7 , $P < .0001$). Collagen type I deposition was observed particularly in the upper dermis of skin lesions (Fig 3). Regarding type III collagen expression, no significant difference was seen among the 4 groups (1.6 ± 1.1 , 2.0 ± 1.3 , 2.1 ± 1.6 , and 1.3 ± 0.7 in acute lesions, chronic lesions, uninvolved skin, and normal skin, respectively).

Correlation studies between eosinophils, fibrogenic cytokines, and type I collagen deposition

The results obtained in acute and chronic AD lesions and uninvolved skin specimens were analyzed together. We found a significant correlation among the number of EG2⁺ cells (Fig 4, A), the number of IL-11⁺ cells (Fig 4, B), and the deposition of type I collagen ($r^2 = 0.622$ [$P < .0001$] and $r^2 = 0.527$ [$P < .0001$], respectively).

DISCUSSION

Structural changes, including papillary dermal fibrosis, epidermal hyperplasia, and diffuse intercellular and

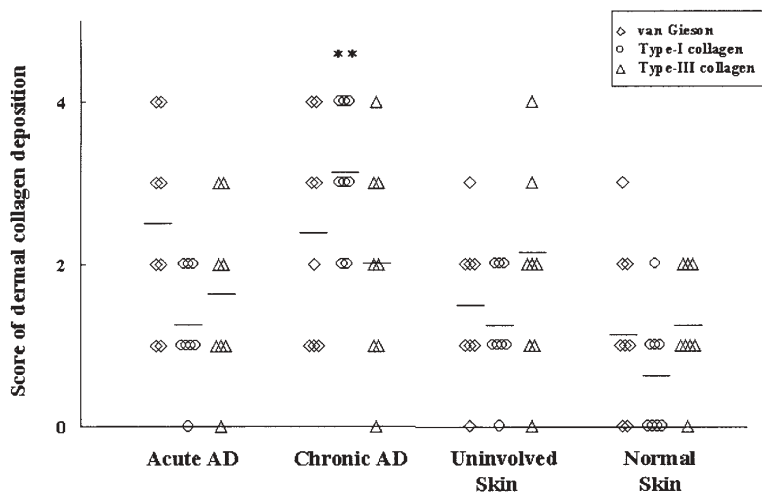


FIG 2. Papillary dermal fibrosis, as assessed by the extent of collagen deposition in acute chronic lesions and uninvolved skin biopsy specimens of patients with AD and in skin of healthy volunteers. ** $P < .0005$, $P < .0005$, and $P < .0001$ compared with acute lesions, uninvolved skin of patients with AD, and normal skin, respectively.

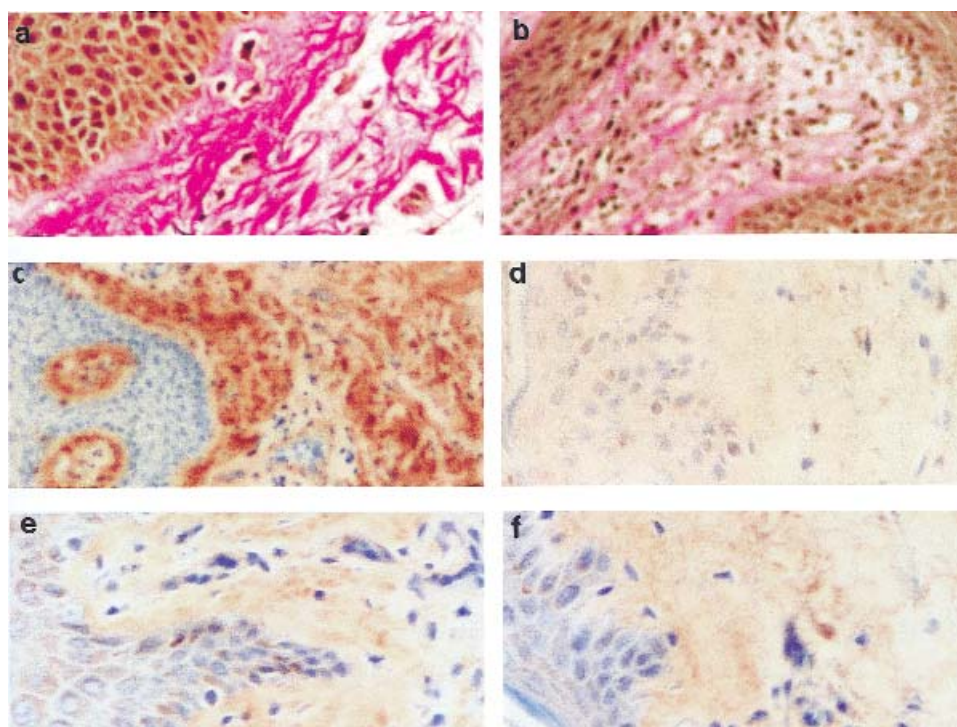


FIG 3. Representative examples of total collagen deposition, as detected by using van Gieson staining in chronic (a) and acute (b) AD; immunostaining for type I collagen in chronic AD (c) and in uninvolved skin from patients with AD (d); and immunostaining for type III collagen in chronic (e) and acute (f) AD.

intracellular edema, are features of AD.^{30,33} Previous studies have shown that matrix metalloproteinases and their specific inhibitors contribute to inflammation-induced tissue destruction and subsequent remodeling in AD.³⁴ We have established that remodeled asthmatic airways are associated with increased numbers of TGF- β 1⁺, IL-11⁺, and IL-17⁺ cells.^{18,23,27,28} As such, we hypothe-

sized that the expression of fibrogenic and fibrosis-associated cytokines, such as TGF- β 1, IL-11, and IL-17, might be enhanced within AD skin lesions.

In this study we investigated the expression of TGF- β 1, IL-11, and IL-17 within acute and chronic AD skin lesions by means of immunohistochemistry. To our knowledge, this is the first report demonstrating that the

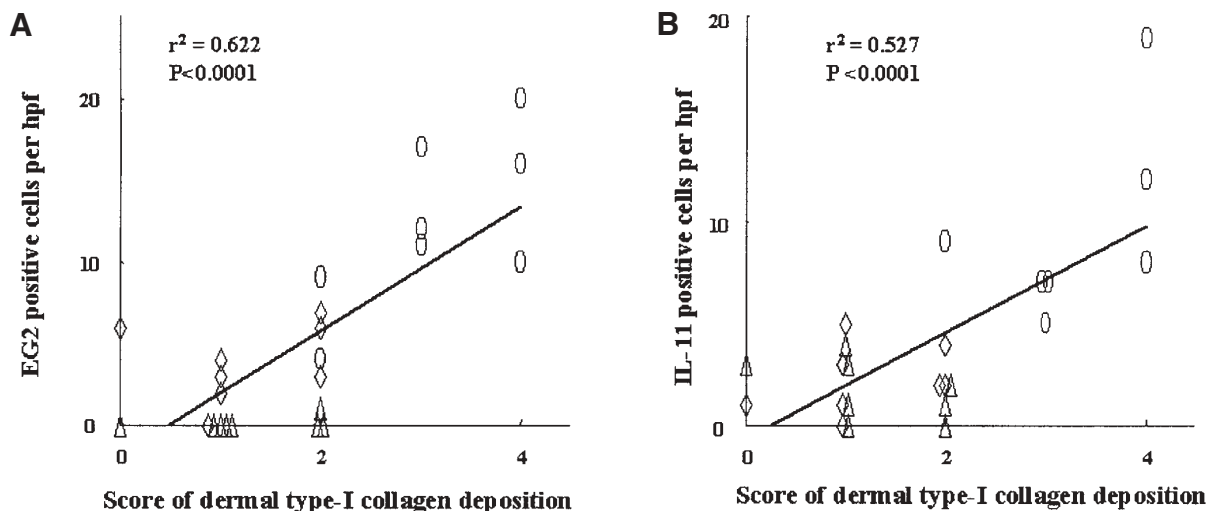


FIG 4. Correlation analysis. Significant correlation was found between type I collagen deposition and the number of EG2⁺ eosinophils ($r^2 = 0.622$, $P < .001$; **A**) and the number of IL-11⁺ cells ($r^2 = 0.527$, $P < .0001$; **B**) in patients with AD. Acute and chronic lesions and uninvolved skin of patients with AD are represented by diamonds, circles, and triangles, respectively.

number of cells expressing TGF- β 1, IL-11, and IL-17 are increased in AD lesions compared with in uninvolved skin from the same individual and from normal control subjects. Moreover, acute skin lesions are associated predominantly with the expression of IL-17 and, to a lesser extent, with TGF- β 1. In contrast, chronic lesions are predominantly associated with TGF- β 1 and IL-11 expression. Morphologic analysis of our tissue sections revealed that the majority of IL-17 was T cells and, to a lesser extent, eosinophils. However, the main cellular source of IL-11 and TGF- β was eosinophils and, to a lesser extent, T cells.

TGF- β is a potent fibrogenic cytokine that stimulates fibroblasts to produce ECM components and protease inhibitors, to inhibit protease synthesis and release, and to promote fibroblast chemoattraction.^{10,35,36} Among TGF- β 1, TGF- β 2, and TGF- β 3 isoforms, TGF- β 1 is predominantly expressed during fibrosis development.¹⁹ Our data provide evidence for the first time that the expression of TGF- β 1 occurs in the skin of patients with AD. The fact that this expression was particularly increased in chronic lesions suggests that this cytokine might be associated with more persistent lesions. Moreover, when all the AD biopsy specimens were examined, there was a significant correlation between the number of accumulated eosinophils and the number of TGF- β 1⁺ cells, which is consistent with our previous study in asthma.¹⁸ Although we previously demonstrated that TGF- β 1 expression was related to the severity of asthma,¹⁸ its precise relationship in AD remains to be clarified.

The assumption that IL-11 plays a role in the development of subepithelial fibrosis and airway remodeling comes from studies done in IL-11 transgenic mice.²² In vitro studies have also demonstrated that TGF- β 1 is a potent stimulator of IL-11 production, accumulation, and

gene transcription in human fibroblasts, alveolar and bronchial epithelial cells, and smooth muscle cells.³⁷⁻⁴⁰ Our results are in agreement with these data because both TGF- β 1 and IL-11 expression were increased in chronic lesions. In contrast, the presence of TGF- β 1 and the absence of IL-11 in acute lesions might suggest that TGF- β 1 initiates the upregulation of IL-11 observed in chronic stages of the disease. In addition, we have recently demonstrated that IL-11 mRNA expression was more dramatic as asthma became increasingly severe.²³ Moreover, this expression was related to increased type I and type III collagen deposition.

Because its expression was enhanced only in chronic skin AD lesions, we speculate that IL-11 is associated with the persistence of AD. In support of this is the significant correlation we found in our previous study²³ between the number of eosinophils and IL-11⁺ cells. These results suggest that the accumulated eosinophils at the site of chronic lesions might be the major producers of IL-11 through TGF- β 1 stimulation in an autocrine or paracrine manner. In addition, eosinophil-derived major basic protein has also been described to participate in the production of IL-11 by lung fibroblasts.⁴¹ However, other IL-11-producing cells should be considered, such as structural cells, including stromal cells, fibroblasts, endothelial cells, and epithelial cells.^{1,21} The evidence that TGF- β 1 and IL-11 are expressed by eosinophils further supports the eventuality that these cells might be involved in the development of structural remodeling in the injured skin of patients with AD.

IL-17 is a novel cytokine apparently secreted preferentially by CD4⁺ activated memory T cells.²⁴⁻²⁶ Although we found no significant correlation between the expression of IL-17 and the number of T cells in our study, the increase in IL-17 in acute lesions paralleled the

augmentation of T cells. Our previous study showed that IL-17 expression was increased in bronchoalveolar lavage fluid, sputum, bronchial biopsy specimens, and peripheral blood eosinophils recovered from asthmatic patients.^{27,28} Moreover, the pattern of IL-17 expression in asthmatic airways was predominantly associated with subepithelial fibrosis lesions.²⁸ Thus we hypothesized that IL-17 might be expressed and associated with dermal fibrosis in AD skin lesions. In fact, the increase in IL-17 expression was observed only in acute, but not in chronic, AD lesions. This observation is of interest because we have demonstrated that IL-17 increased the basal production of IL-11 by normal and asthmatic bronchial fibroblasts.²⁷ Therefore we might hypothesize that IL-17 initiates the development of tissue fibrosis in AD skin lesions indirectly through the release of IL-11. To further support our hypothesis, IL-17 stimulates epithelial and endothelial cells, fibroblasts, and macrophages for the release of IL-6, IL-1 β , TNF- α , and GM-CSF,²⁴⁻²⁶ a number of which are increased in AD skin lesions. Studies done in transgenic mice demonstrated that IL-6 is a potent fibrogenic cytokine, leading to the development of a subepithelial fibrosis in the airways.⁴² IL-1, TNF- α , and GM-CSF also stimulate fibroblasts to induce the proliferation and synthesis of ECM components. We have shown also that IL-17 enhanced in vitro the basal production of IL-6 by fibroblasts isolated from asthmatic bronchial biopsy specimens.²⁷ Consequently, IL-17 might play a role in skin remodeling in AD and especially in dermal fibrosis but more probably in an indirect manner through the release of profibrotic cytokines, such as IL-11 and IL-6.

Regarding collagen, papillary dermal fibrosis corresponding to lichenified lesions has been well accepted as a characteristic feature of chronic AD.³⁰ Our results showed that type I collagen deposition was increased in chronic AD lesions, whereas total and type III collagen accumulation was not modulated among the groups. Of interest, when all the AD biopsy specimens were examined, the extent of type I collagen deposition was also found to be significantly correlated with the number of eosinophils and IL-11 expression. The lack of correlation between type I collagen and IL-17 expression was not surprising because our previous in vitro studies demonstrated that IL-17 was not able to stimulate bronchial fibroblasts to upregulate their production of type I and type III collagens.²⁷ On the other hand, type I collagen deposition and TGF- β expression did correlate; however, this failed to achieve statistical significance. Although we expected to find a significant relationship between these 2 parameters, this observation is in agreement with reports suggesting that some of the effects attributed to TGF- β 1 might be in fact due to IL-11 because we do have a significant correlation between type I collagen and TGF- β 1 expression. Because type I collagen is the predominant ECM component in fibrotic diseases,⁴³ our results can be regarded as reasonable findings. Although several studies showed a simultaneous increase in the deposition of type I and type III collagens in lesions in

asthma^{4,23} and in scleroderma,⁴⁴ we did not find any modulation in the extent of total and type III collagen in any of the skin biopsy specimens used in this study. Nevertheless, according to our results, we might hypothesize that the evaluation of type I collagen deposition is the most sensitive parameter for detection of dermal fibrosis in AD. In asthma, airway remodeling changes, such as airway wall thickening, might lead to a persistent reduction in airway lumen diameter and thus to bronchial hyperresponsiveness.^{18,45,46} Indeed, several studies showed a link between asthma severity and subepithelial fibrosis,^{18,23,45,46} as assessed by the thickness of basement membrane. Whether the extent of type I collagen deposition in the dermis might account for the severity of AD is not known and should be further investigated.

In summary, the increase in IL-17 and IL-11 expression was polarized toward acute and chronic AD lesions, respectively, whereas TGF- β 1 expression was increased in both acute and chronic skin lesions, although at higher levels in the chronic situation. The finding that chronic AD skin lesions are associated with a predominance of TGF- β 1- and IL-11-expressing cells and with type I collagen accumulation is also a new and interesting finding. On the basis of these results, we can speculate that in addition to TGF- β 1, IL-17 initiates the development of tissue fibrosis in skin lesions in AD, whereas IL-11 is involved in the development and maintenance of this process. This process of tissue remodeling can have many clinical consequences, such as depigmentation or diminished responsiveness to topical agents. Studies, however, need to be done to investigate this further. According to the literature, T cells and macrophages contribute to the release of TGF- β 1, IL-11, and IL-17 in the acute initial phase of the fibrosis process, whereas eosinophils are the major cellular source of these cytokines involved in the progression and perpetuation of fibrosis. Whether these cytokines are responsible for the characteristic lichenification and dermal fibrosis that accompanies chronic AD skin lesions requires further investigation.

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