

Down-regulation of IL-12 by topical corticosteroids in chronic atopic dermatitis

Nikhil Yawalkar, MD,^{a,b} Stephan Karlen, PhD,^a Fabienne Egli, MD(Dent),^b Christoph U. Brand, MD,^a Hans U. Graber, MD(Vet),^c Werner J. Pichler, MD,^b and Lasse R. Braathen, MD, PhD^a *Bern, Switzerland*

Background: Previous reports indicate that IL-12 may be involved in the development of chronic atopic dermatitis. However, the cellular source of this cytokine in the skin and its expression during successful treatment of the skin lesions are not known.

Objective: Our purpose was to delineate the precise in situ localization of IL-12 and its modulation under topical treatment with corticosteroids.

Methods: Skin biopsy specimens were obtained from nonlesional, lesional, and treated skin of patients with atopic dermatitis and from healthy skin of nonatopic control subjects. IL-12 was investigated by real-time quantitative reverse transcriptase-PCR and immunohistochemistry.

Results: Expression of IL-12 p40 mRNA was significantly enhanced in lesional skin from atopic dermatitis and strongly down-regulated after treatment with topical corticosteroids for 9 to 10 days. In contrast, similar levels of IL-12 p35 transcripts were found in all the samples without any significant differences after treatment. In addition, a strong enhancement of IL-12 immunoreactivity was observed on the mononuclear cell infiltrate in the lesional skin samples, which was also markedly reduced after treatment. IL-12 immunoreactivity was mainly located in the cytoplasm of dermal dendritic cells and macrophages as well as some Langerhans cells.

Conclusion: Our data suggest that the enhanced local production of IL-12 in dendritic cells and macrophages may be responsible for up-regulating production of IFN- γ in chronic lesions and strengthen the idea that IL-12 may have a pivotal role in promoting inflammation in atopic dermatitis.

(*J Allergy Clin Immunol* 2000;106:941-7.)

Key words: Skin, dendritic cells, cytokines, immunohistochemistry, quantitative reverse transcriptase, polymerase chain reaction

Atopic dermatitis (AD) is a chronic inflammatory skin disease that often begins in infancy. It is characterized by

Abbreviations used

AD:	Atopic dermatitis
APAAP:	Alkaline phosphatase-antialkaline phosphatase
DC:	Dendritic cell
LC:	Langerhans cell
LS:	Lesional skin
NL:	Nonlesional skin
NS:	Normal skin
RT:	Reverse transcriptase
SCORAD:	Scoring atopic dermatitis
SDS:	Sequence detection system
TS:	Treated skin

extreme pruritus, typically distributed eczematous skin lesions, and a personal or family history of atopic diseases.¹ Current knowledge suggests that a dysregulation of T cells and an imbalance in the cytokine network are important pathogenic factors in the development of this skin disease.^{2,3} Analysis of the cytokine pattern of AD lesions has demonstrated the predominance of T-helper lymphocytes with a type-2 cytokine profile (IL-4, IL-5, IL-13) during the initial phase of skin inflammation, whereas both type-2 cytokines (ie, IL-5) and type-1 cytokines (ie, IFN- γ) are up-regulated in chronic lesions.³⁻⁶ Although the precise mechanisms leading to the appearance of T-helper cells with a type-1 cytokine in chronic AD have remained unresolved, recent evidence suggests that IL-12 may be involved in this process.^{3,5}

IL-12 is composed of two subunits, a light chain (p35) and a heavy chain (p40), which form the heterodimer p70.⁷ It is predominantly secreted by phagocytic (monocytes-macrophages) and antigen-presenting cells (dendritic cells [DCs]). Furthermore, various other cell types, such as polymorphonuclear leukocytes, mast cells, keratinocytes, and neuronal and glial cells have also been reported to express this cytokine. IL-12 is a key factor in the activation of cytotoxic lymphocytes, the regulation of IFN- γ production, and the generation of T_H1 cell-mediated immune responses.⁷ The expression of IL-12 p35 and p40 transcripts and the precise localization of this cytokine, particularly after treatment of chronic AD lesions, have not been elucidated so far. The current study was therefore designed to investigate the in situ

From the ^aDermatological Clinic and the ^bClinic for Rheumatology and Clinical Immunology/Allergy, ^cDepartment of Clinical Research, Inselspital, University of Bern, Bern, Switzerland.

Supported by the Swiss National Science Foundation grant No. 32-48885.96, SCORE B (N. Y.), and Sandoz-Stiftung grant No. 98B17.

Received for publication Apr 13, 2000; revised June 27, 2000; accepted for publication June 27, 2000.

Reprint requests: N. Yawalkar, MD, Clinic for Rheumatology and Clinical Immunology, Allergy, Inselspital, 3010 Bern, Switzerland.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/1/109825

doi:10.1067/mai.2000.109825

TABLE I. Sequences of primers and TaqMan probes

mRNA	IL-12 p35	IL-12 p40	7S RNA
Sense primer	5'-GTTCCCATGCCTTACCACT-3' (nt305-nt324)	5'-GCCAGCTGCTGAGGAGAGT-3' (nt609-nt628)	5'-ACCACCAGGTTGCCTAAGGA-3' (nt150-nt169)
Reverse primer	5'-TGTCTGGCCTTCTGGAGCA-3' (nt355-nt373)	5'-TGGGTGGGTCAGGTTTGATG-3' (nt709-nt728)	5'-CACGGGAGTTTGTACCTGCT-3' (nt198-nt217)
TaqMan probe	5'-CCAAAACCTGCTGAGGGCCGTC-3' (nt326-nt348)	5'-ACGGCATCCACCATGACCTCAATG-3' (nt657-nt680)	5'-TGAACCGGCCAGGTCGGAAC-3' (nt174-nt195)

expression of IL-12 mRNA and protein in chronic AD skin lesions before and after treatment with topical corticosteroids with use of a real-time quantitative reverse transcriptase (RT)-PCR and immunohistochemistry.

MATERIAL AND METHODS

Subjects and skin specimens

The ethical committee of the medical faculty of the University of Bern approved the study. Eighteen white patients (8 women and 10 men, age range 16-55 years) with moderate to severe chronic AD were included in the study after their informed consent was obtained. AD was diagnosed according to the criteria of Hanifin and Rajka.⁸ The clinical severity of AD was documented by the scoring atopic dermatitis (SCORAD) index as described previously.⁹ The SCORAD index ranged between 30 and 80 in the patients with AD. Punch biopsy specimens of 5 mm diameter were taken from chronic eczematous skin lesions (n = 6) in the large joint flexures and from nonlesional skin (NL) located about 10 cm from a chronic eczematous skin lesion (n = 6). These patients did not receive any systemic corticosteroids or topical treatment with corticosteroids at the site where biopsy specimens were obtained for at least 3 weeks before the investigation. In six additional patients biopsy specimens were taken from a previously chronic eczematous skin lesion in the large joint flexures, which had successfully been treated with a topical corticosteroid (mometasone ointment 0.1%) for 9 to 10 days. These lesions still showed a varying degree of lichenification but no signs of inflammation. The SCORAD index ranged between 15 and 25 in these patients. Healthy skin (NS) from 6 nonatopic control subjects was obtained from patients undergoing reconstructive surgery. The material was snap-frozen with or without tissue-embedding medium with isopentane precooled in liquid nitrogen and stored at -70°C.

Real-time quantitative RT-PCR analysis

Skin biopsy specimens were homogenized and total RNA was extracted from NS of nonatopic control subjects (n = 6), NL (n = 6), lesional skin (LS) (n = 6), and treated AD skin (n = 6) with use of an SV40 total RNA isolation kit (Promega, Madison, Wis). The RNA isolation method included a digestion step for DNA. RNA isolated from EBV-transformed B-lymphocyte cells (kindly provided by K. Frutiger, Institute of Immunology and Allergology, Inselspital, Bern, Switzerland) was used to standardize the amplification reaction. The total amount of RNA was quantitated by spectrophotometry. Equal amounts of RNA (200 ng) were reverse transcribed and cDNA was synthesized with avian myeloblastosis virus RT and random primers (Roche Diagnostics, Basel, Switzerland).

Amplification reactions were performed with the TaqMan method (Perkin Elmer Applied Biosystems, Foster City, Calif). The sequences of the primers and TaqMan probes used in the study are listed in Table I. The primers for IL-12 p35 and p40 were designed on the basis of the published sequences of the human IL-12 cDNA¹⁰ and do not show any significant homology with other human cDNA sequences. Amplification of 7S RNA was monitored as an internal control for quantification.¹¹ Primers and TaqMan probes were syn-

thesized by Perkin Elmer and were used at the final concentration of 300 nmol/L each.

Reaction mixtures of 25 µL were carried out in 1× TaqMan Universal PCR master mix and contained 25 ng of cDNA. Real-time PCR was performed with the ABI Prism 7700 Sequence Detection System (SDS). The PCRs were done in a 96-well MicroAmp optical plate with caps (Perkin Elmer Applied Biosystems), and data were analyzed by the SDS software. Thermal cycler parameters included 2 minutes at 50°C, 10 minutes at 95°C, and 50 cycles of 15 seconds at 95°C, and 1 minute at 60°C. PCR with total RNA as template to check for DNA contamination revealed no amplification. The data obtained were analyzed as described previously.¹² The results are shown as the ratio of the copy number of IL-12 p35 or p40 mRNA divided by that of 7S RNA in each tissue.

Immunohistochemistry

Immunostaining of skin sections from NS of the nonatopic control subjects (n = 6), NL (n = 6), LS (n = 6), and treated skin of the patients with AD (n = 6) was performed by use of the alkaline phosphatase-antialkaline phosphatase (APAAP) method as described previously.¹³ Briefly, 6-µm cryostat tissue sections were fixed in 2% formaldehyde. After application of normal rabbit serum the sections were incubated with one of the following monoclonal anti-human antibodies: anti-IL-12 p35/p70 (50 µg/mL, clone G161-566, PharMingen/Beckon Dickinson, Mountain View, Calif), anti-IL-12 p40/p70 (50 µg/mL, clone B-P24, Diaclone Research, Besançon, France), and anti-IL-12 p70 (50 µg/mL, clone 24945.11, R&D Systems, Minneapolis, Minn). Substitution of the primary antibody with isotype-matched IgG and omission of the primary antibody were used as negative controls. After washing, the sections were incubated with a rabbit anti-mouse antibody (dilution 1:30; Z259; DAKO, Glostrup, Denmark) and the APAAP complexes (dilution 1:50; D651; DAKO). To enhance the signal, the incubation with the rabbit anti-mouse antibody and the APAAP complexes was repeated. Finally, the sections were developed in new fuchsin-naphtol and counterstained with hematoxylin.

Double immunostaining was performed by combining two indirect staining methods with two unlabeled primary antibodies of mouse (IL-12 p40/p70 [clone B-P24] and goat (anti-CD1a [clone C-19, Santa Cruz Biotechnology, Santa Cruz, Calif] or anti-CD68 [clone N-19, Santa Cruz Biotechnology]) origin. Slides were initially incubated with the primary antibodies overnight, followed by alkaline phosphatase-conjugated rabbit anti-mouse (dilution 1:20; DAKO) and peroxidase-conjugated rabbit anti-goat Igs (dilution 1:400; Jackson ImmunoResearch Laboratories, West Grove, Pa). Sections were then developed with new fuchsin-naphtol and diaminobenzidine, which revealed red and brown staining, respectively, and finally were counterstained with hematoxylin. The specificity of the reaction was confirmed by omitting the primary antibodies and by using irrelevant isotype-matched antibodies as negative controls.

Evaluation of sections

The slides were analyzed with a Leitz Dialux 20EB microscope by two independent investigators. In each section staining was assessed on 10 to 15 fields at ×400 magnification. Cells displaying a strong

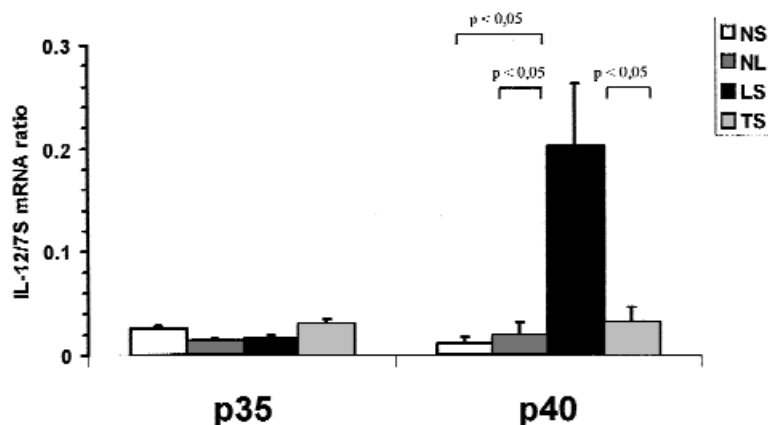


FIG 1. IL-12 p40 mRNA expression is significantly increased in lesional AD skin and decreased after treatment with topical corticosteroids. Total RNA extracted from NS of nonatopic controls ($n = 6$), NL ($n = 6$), LS ($n = 6$), and treated skin (TS) ($n = 6$) of patients with AD and analyzed with use of the p35, p40, and 7S primers described in Table I. IL-12 p35/7S and IL-12 p40/7S RNA ratios were measured by TaqMan RT-PCR analysis as described in Material and methods. Mean values $\pm$ SEM.

cytoplasmic staining were considered to be positive. The positive cells were counted with a 0.063-mm² grid and the number of positive cells (mean $\pm$ SEM) per square millimeter was determined. Only positively stained cells with a nucleus were included in the counting.

Statistical analysis

Statistical analysis was performed by the Kruskal-Wallis non-parametric ANOVA test and subsequently by the Mann-Whitney *U* test (with Bonferroni's correction). A *P* value less than .05 was considered statistically significant.

RESULTS

IL-12 p40 mRNA is significantly enhanced in AD skin lesions and down-regulated after topical corticosteroid treatment

To analyze the expression of IL-12 p35 and p40 mRNA in the skin of nonatopic control subjects and patients with AD, real-time quantitative TaqMan RT-PCR analysis was performed. The results are summarized in Fig 1. Low levels of mRNA encoding the p40 subunit were measured in NS of the nonatopic controls and in NS from patients with AD. A significant enhancement in the expression of p40 was observed in LS samples, which was reduced to normal levels on treatment with topical corticosteroids for 9 to 10 days. In contrast, the level of p35 expression was similar in all of the samples without any significant differences after treatment.

IL-12 immunoreactivity is mainly located in macrophages and DCs

Immunostaining for IL-12 was performed with use of 3 mAbs recognizing different epitopes of IL-12 (ie, p35/p70, p40/p70, and p70). Representative stainings with these antibodies and quantification of the positive cells in NS of the nonatopic control subjects, NL, LS, and treated skin of the patients with AD are shown in Figs 2 and 3. In contrast to NS and nonlesional AD skin, a significant enhancement of IL-12 immunoreactivity

was found in lesional AD skin, which was again significantly diminished in the treated lesions. The staining pattern was similar with all of the antibodies, with the difference that the antibody-recognizing p70 was expressed weaker than the other two. No positive staining was observed when the primary antibody was substituted with an isotype-matched IgG (not shown).

IL-12 immunoreactivity was predominantly observed in the cytoplasm of mononuclear cells scattered throughout the dermis and particularly in the dense perivascular infiltrate. The staining pattern was mostly granular. The morphologic features and location of the stained cells as shown in Fig 4, *a* and *b*, strongly suggest that macrophages, dermal DCs, and Langerhans cells (LCs) express IL-12 in AD skin lesions. In addition, double immunostaining experiments confirmed that CD68⁺ macrophages as well as CD1a⁺ DCs express IL-12 (Fig 5, *a* and *b*). Furthermore, as shown in Fig 4, *a* and *c*, keratinocytes focally also stained positive for IL-12. Immunostaining was located in the cytoplasm of the keratinocytes and showed a more diffuse staining pattern.

DISCUSSION

In this study we demonstrate that the in situ expression of IL-12 is significantly increased in chronic AD lesions and markedly reduced after successful treatment with topical corticosteroids. By use of in situ hybridization techniques, expression of IL-12 has previously also been reported to be enhanced, particularly in chronic AD lesions.⁵ Our data confirm and extend these results on the expression of IL-12 in AD by use of real-time quantitative RT-PCR and immunostaining techniques. In accordance with Hamid et al we were also able to verify an increased expression of IL-12 p40 in chronic compared with acute AD lesions (data not shown).

Bioactive IL-12 p70 is composed of two covalently linked subunits, p35 and p40.¹⁴ Previous studies have

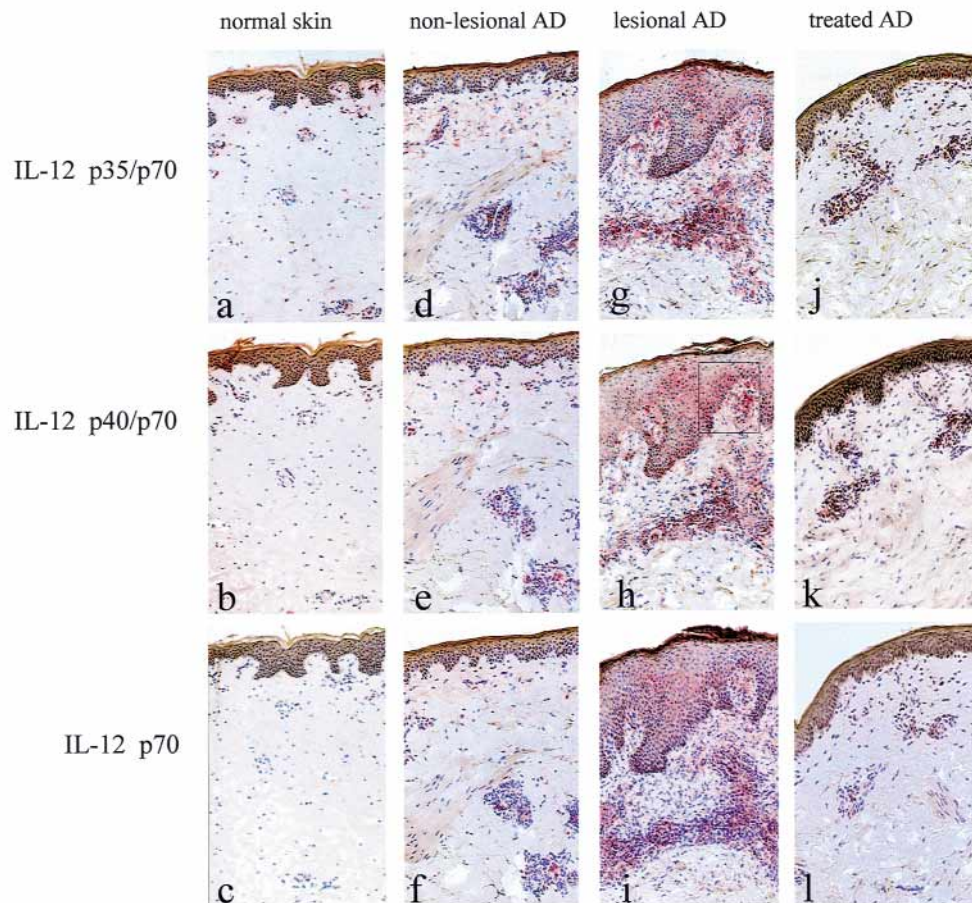


FIG 2. IL-12 immunoreactivity in NS and AD. Representative examples of NS from nonatopic control (**a-c**), nonlesional (**d-f**), lesional (**g-i**), and treated skin (**j-l**) from AD are shown. Immunostaining was performed with three different antibodies recognizing IL-12 p35/p70 (clone G161-566), IL-12 p40/p70 (clone B-P24), and IL-12 p70 (clone 24945.11). IL-12-positive cells were visualized with new-fuchsin (red). Box in **h** indicates region shown in Fig 4 **a**. (Original magnification $\times 100$.)

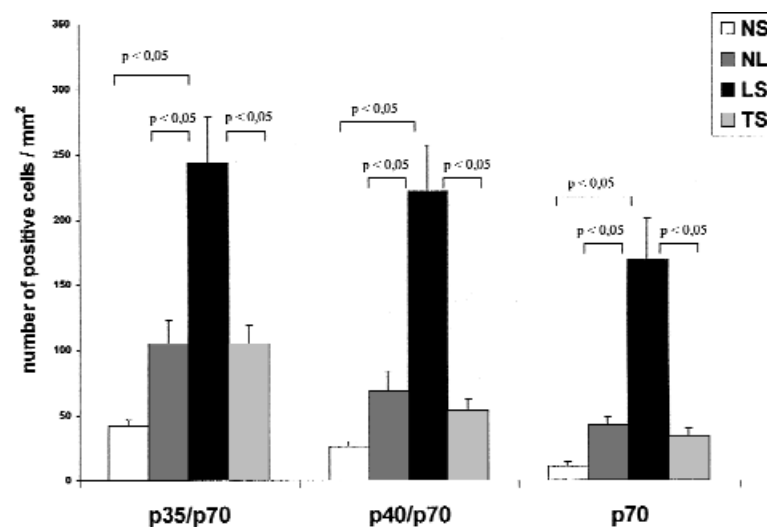


FIG 3. Quantification of IL-12-positive cells in NS ($n = 5$), NL ($n = 6$), LS ($n = 6$), and treated skin (TS) ($n = 6$). Immunostaining was performed with three different antibodies recognizing IL-12 p35/p70, IL-12 p40/p70, and IL-12 p70. Mean values $\pm$ SEM.

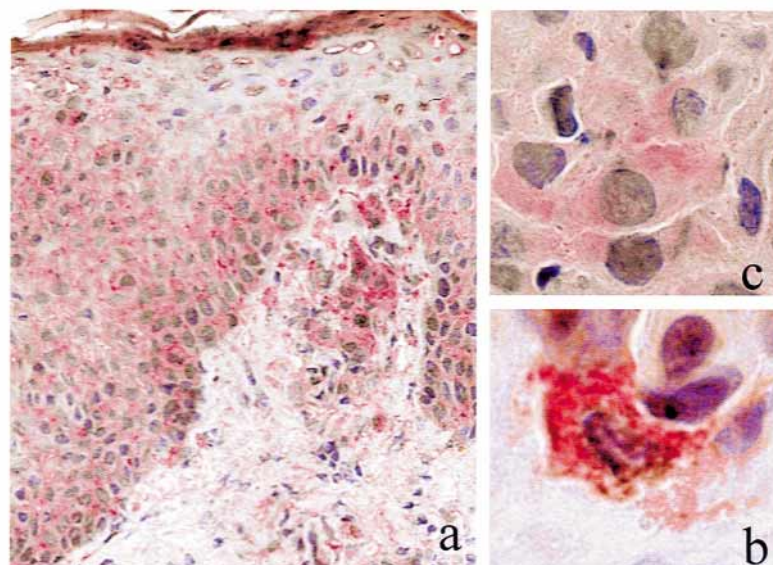


FIG 4. Localization of IL-12 immunoreactivity in chronic AD skin lesions. Immunostaining with use of an antibody recognizing IL-12 p40/70 (clone B-P24) is shown as a representative example. The morphologic features of the stained cells suggest that IL-12 is mainly expressed in macrophages and dendritic cells (**a** and **b**). Positive immunoreactivity was also observed focally in the cytoplasm of the keratinocytes (**c**). IL-12-positive cells were visualized with new-fuchsin (red). (**a**, Original magnification $\times 250$; **b** and **c**, original magnification $\times 1000$.)

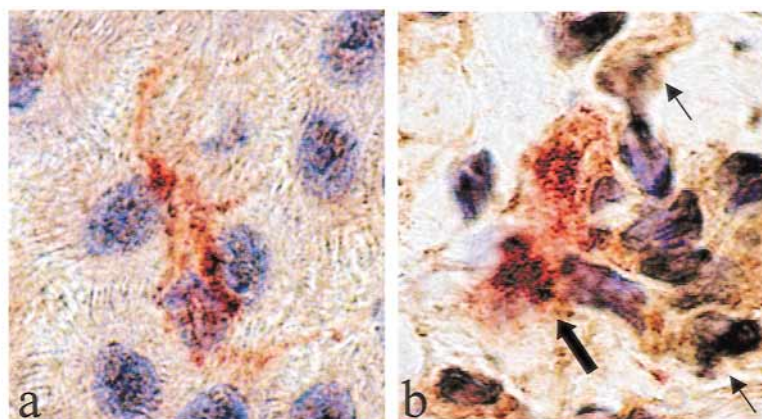


FIG 5. Double immunostaining confirmed that IL-12 is located in CD68⁺ macrophages and CD1a⁺ LCs. CD68⁺ (**a**) and CD1a⁺ (**b**) positive cells were determined with diaminobenzidine (brown), IL-12 with new-fuchsin (red). *Thin arrow*, Single staining; *thick arrow*, double staining. (Original magnification $\times 1000$.)

demonstrated that p35 is constitutively expressed in various cells, whereas p40 is the inducible subunit.^{15,16} Thus secretion of IL-12 appears to be predominantly regulated at the level of p40 chain transcription, which correlates with the ability of the cells to produce the biologically active p70 heterodimer.^{7,17,18} Accordingly, up-regulation of IL-12 mRNA in this study was particularly found for the p40 subunit, whereas messengers encoding the p35 subunit did not show any significant differences. These findings are in agreement with previous studies demonstrating that p40 mRNA is expressed in considerable excess to that of p35.^{7,18,19} Interestingly, in contrast to the low p35 mRNA levels, a signifi-

cantly stronger immunoreactivity was observed for IL-12 p35/p70 in lesional AD skin compared with the other samples. Such differences between mRNA and protein levels may be a consequence of transcriptional and posttranscriptional modifications and result, for example, from differences in their degradation. Indeed, several copies of the adenine- and uracil-rich motif UUAU-UUAU are present within the 3' untranslated region of the p35 transcripts and to a markedly lesser degree within that of p40 mRNA. Such adenine- and uracil-rich motifs have been shown to direct a rapid decay of several cytokine messengers, including GM-CSF, TNF- α , and IL-13²⁰⁻²² and may explain the short half-life of

the p35 mRNA. Thus the p35 subunit may also be a rate-limiting factor in determining the level of IL-12 p70 production and thereby help controlling the secretion of bioactive IL-12.^{23,24}

IL-12 production has been reported to be mediated by various pathways, including T-cell independent and dependent mechanisms.⁷ T cell-independent mechanisms include stimulation by bacteria such as *Staphylococcus aureus* cowan I strain and bacterial products such as lipopolysaccharides and superantigens. *S aureus* and its exotoxins are thought to be an important pathogenic factor in AD.²⁵⁻²⁷ Because the skin of patients with AD is highly colonized with *S aureus*, it is feasible that these factors may have contributed to the up-regulation of IL-12 in situ. Furthermore, superantigens have also been reported to induce the expression of cutaneous leukocyte antigen on T cells by an IL-12-dependent mechanism.²⁸ Thus these factors may sustain cutaneous inflammation by increasing the population of memory T cells capable of migrating into the skin. Production of IL-12 is also up-regulated by a T cell-dependent mechanism involving CD40-CD40L interactions between macrophages or DCs and T cells. As ascertained by the morphologic features of the cells and double immunostaining, CD68⁺ macrophages and CD1a⁺ DCs were found to be the major source of IL-12 in the dermis and epidermis of the skin lesions. Interactions between macrophages or DCs and T cells in the cutaneous infiltrate could therefore also explain the enhanced in situ expression of IL-12 in AD. Such interactions may also be important in other inflammatory skin diseases such as psoriasis and contact dermatitis, in which an enhanced expression of IL-12 has been reported previously.^{5,13,19}

A further notable finding of this study was the focal localization of IL-12 immunoreactivity in the cytoplasm of some keratinocytes. Positive immunostaining was particularly enhanced when the antibodies recognizing p35/p70 and p40/p70 were used and to a lesser degree with those recognizing p70. Because low amounts of IL-12 have been reported to be produced by keratinocytes in vitro,^{29,30} it is possible that keratinocytes indeed also produce this cytokine in vivo. However, the focal localization may suggest that further stimulatory factors such as the presence of other proinflammatory cytokines such as IFN- γ are necessary for the optimal production of IL-12 by keratinocytes in situ.

In recent years a biphasic model in the generation and maintenance of AD lesions has been proposed.³ Thereby T_H2-predominated responses have been suggested to be crucial for the initiation of AD, whereas T_H1-predominated responses are thought to be more important in the chronic phase of the disease. The increased penetration of bacteria and bacterial products during the eczematous skin reaction together with the enhanced influx and presence of DCs and macrophages capable of IL-12 production could induce a change in the local skin microenvironment and favor IFN- γ production by T cells. Because IFN- γ , in turn, also augments IL-12 secretion, these cytokines may comprise a positive feedback system that could play an essential part in maintaining chronic cutaneous inflammation. Furthermore,

our observations showing a significant down-regulation of IL-12 together with the remission of the skin lesions after successful treatment add further evidence for a significant role of this cytokine in the development of AD.

Besides stimulation of IFN- γ production, IL-12 is also known to be a potent stimulatory and maturational factor for cytotoxic lymphocytes.⁷ Interestingly, it has been suggested that cytotoxic lymphocytes are mandatory for the elicitation of contact hypersensitivity reactions.³¹ In view of the enhanced in situ production of IL-12 in AD and as also shown previously in contact dermatitis,¹³ this cytokine may therefore play a decisive role in activating cytotoxic lymphocytes in inflammatory skin diseases. Further evidence for this is provided by the increased presence of perforin- and granzyme B-containing cells in AD lesions and contact dermatitis (manuscript in preparation). Activation of such cytotoxic lymphocytes in the skin may contribute to tissue damage and again act to maintain chronic skin inflammation. Finally, the enhanced IL-12 expression in AD skin lesions may also initiate a cascade of dysregulated immune responses (ie, failure of peripheral tolerogenic mechanisms or even generation of autoimmune inflammatory processes). These assumptions are substantiated by recent data showing an enhancement of peripheral tolerance after neutralization of endogenous IL-12.³² Such mechanisms may particularly perpetuate AD lesions, even when the initiating allergens are absent.

In conclusion, our data show that the expression of IL-12 is up-regulated in AD skin lesions and significantly reduced during improvement of skin inflammation. These findings provide further evidence that IL-12 may represent a key cytokine in the generation of inflammatory skin diseases and suggest IL-12 receptor antagonists as potential candidates especially for therapy of chronic AD.

We thank E. Seger and F. Halim-Forrer for excellent technical help. We also thank Dr Gillmann, Institute of Social and Preventive Medicine, Bern, for his help with statistical analysis.

REFERENCES

1. Beltrani VS. The clinical spectrum of atopic dermatitis. *J Allergy Clin Immunol* 1999;104:87-98.
2. Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000;105:860-76.
3. Grewe M, Bruijnzeel-Koomen C, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka, et al. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 1998;19:359-61.
4. Hamid Q, Boguniewicz M, Leung DYM. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;94:870-6.
5. Hamid Q, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DYM. In vivo expression of IL-12 and IL-13 in atopic dermatitis. *J Allergy Clin Immunol* 1996;98:225-31.
6. Grewe M, Gyufko K, Czech W, Krutmann J. Lesional expression of interferon- γ in atopic eczema. *Lancet* 1994;343:24-6.
7. Ma X, Aste-Amezaga M, Gri G, Gerosa F, Trinchieri G. Immunomodulatory functions and molecular regulation of IL-12. *Chem Immunol* 1997;68:1-22.
8. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venerol* 1980;92:44-7.
9. Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997;195:10-9.

10. Wolf SF, Temple PA, Kobayashi M, Young D, Diczg M, Lowe L, et al. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J Immunol* 1991;146:3074-81.
11. Korc M, Chandrasekar B, Yamanaka Y, Friess H, Buchier M, Beger HG. Overexpression of the epidermal growth factor receptor in human pancreatic cancer is associated with concomitant increases in the levels of epidermal growth factor and transforming growth factor alpha. *J Clin Invest* 1992;90:1352-60.
12. Maeda I, Takano T, Furnio M, Matsuzuka T, Maruyama T, Higashiyama T, et al. Rapid screening of specific changes in mRNA in thyroid carcinomas by sequence specific-differential display: decreased expression of acid ceramidase mRNA in malignant and benign thyroid tumors. *Int J Cancer* 1999;81:700-4.
13. Yawalkar N, Egli F, Brand CU, Pichler WJ, Braathen LR. Antigen-presenting cells and keratinocytes express interleukin 12 in allergic contact dermatitis. *Contact Dermatitis* 2000;42:18-22.
14. Gubler U, Chua AO, Schoenhaut DS, Dwyer CM, McComas W, Motyka R, et al. Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. *Proc Natl Acad Sci U S A* 1991;88:4143-7.
15. Bette M, Jin SC, Germann T, Schafer MK, Weihe E, Rude E, et al. Differential expression of mRNA encoding interleukin-12 p35 and p40 subunits in situ. *Eur J Immunol* 1994;24:2435-40.
16. Stanley F, Sieburth D, Sypek J. Interleukin 12: a key modulator of immune function. *Stem cells*. 1994;12:154-68.
17. Ma X, Chow JM, Gri G, Carra G, Gerosa F, Wolf SF, et al. Interleukin-12 p40 gene promoter is primed by interferon gamma in monocytic cells. *J Exp Med* 1996;183:147-57.
18. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* 1993;178:1041-8.
19. Yawalkar N, Karlen S, Brand CU, Hunger RE, Braathen LR. Expression of IL-12 in normal and psoriatic skin. *J Invest Dermatol* 1998;111:1053-7.
20. Wang XY, Hoyle PE, McCubrey JA. Characterization of proteins binding the 3' regulatory region of the IL-3 gene in IL-3-dependent and autocrine-transformed hematopoietic cells. *Leukemia* 1998;12:520-31.
21. Maurer F, Medcalf RL. Plasminogen activator inhibitor type 2 gene induction by tumor necrosis factor and phorbol ester involves transcriptional and post-transcriptional events: identification of a functional non-amer AU-rich motif in the 3'-untranslated region. *J Biol Chem* 1996;271:2674-80.
22. Zubiaga AM, Belasco JG, Greenberg ME. The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol Cell Biol* 1995;15:2219-30.
23. Yamane H, Kato T, Nariuchi H. Effective stimulation of IL-12 p35 mRNA accumulation and bioactive IL-12 production of antigen-presenting cells interacted with Th cells. *J Immunol* 1999;162:6433-41.
24. Snijders A, Hilken CM, van der Pouw Kraan TC, Engel M, Aarden LA, Kapsenberg ML. Regulation of bioactive IL-12 production in lipopolysaccharide-stimulated human monocytes is determined by the expression of the p35 subunit. *J Immunol* 1996;156:1207-12.
25. Nomura I, Tanaka K, Tomita H, Katsunuma T, Ohya Y, Ikeda N, et al. Evaluation of the staphylococcal exotoxins and their specific IgE in childhood atopic dermatitis. *J Allergy Clin Immunol* 1999;104:441-6.
26. Bunikowski R, Mielke M, Skarabis H, Herz U, Bergmann RL, Wahn U, et al. Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. *J Allergy Clin Immunol* 1999;103:119-24.
27. Bunikowski R, Mielke ME, Skarabis H, Worm M, Anagnostopoulos I, Kolde G, et al. Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* 2000;105:814-9.
28. Leung DY, Gatly M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ. Bacterial superantigens induce T-cell expression of the skin selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. *J Exp Med* 1995;181:747-53.
29. Müller G, Saloga J, Germann T, Bellinghausen I, Mohamadadeh M, Knop J, et al. Identification and induction of human keratinocyte-derived IL-12. *J Clin Invest* 1994;94:1799-805.
30. Yawalkar N, Limat A, Brand CU, Braathen LR. Constitutive expression of both subunits of interleukin-12 in human keratinocytes. *J Invest Dermatol* 1996;106:80-3.
31. Kehren J, Desvignes C, Krasteva M, Ducluzeau MT, Assossou O, Horand F, et al. Cytotoxicity is mandatory for CD8(+) T cell-mediated contact hypersensitivity. *J Exp Med* 1999;189:779-86.
32. Marth T, Strober W, Kelsall BL. High dose oral tolerance in ovalbumin TCR-transgenic mice: systemic neutralization of IL-12 augments TGF-beta secretion and T cell apoptosis. *J Immunol* 1996;157:2348-57.