

Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis

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Background: IL-31 is produced by activated T lymphocytes, preferentially by T_H2 cells. Transgenic mice overexpressing IL-31 have a phenotype resembling allergic dermatitis in human subjects.

Objective: We sought to evaluate the potential importance of IL-31 in the pathogenesis of human T cell-mediated skin diseases.

Methods: We analyzed total RNA taken from 149 skin biopsy specimens from patients with atopic dermatitis (AD), allergic contact dermatitis (ACD), or psoriasis in comparison with specimens taken from patients with healthy skin (n = 13) by using quantitative real-time PCR for the expression of T_H1/T_H2 cytokines.

Results: We found statistically increased mRNA levels of IL-31 in biopsy specimens taken from patients with AD, irrespective of the severity of the disease and serum IgE levels. Moreover, IL-31 mRNA levels were strongly increased in many biopsy specimens taken from patients with ACD. However, no increased transcription of IL-31 could be detected in biopsy specimens taken from psoriatic plaques. A comparison of mRNA levels of IL-31 with T_H1 or T_H2 cytokines demonstrates a correlation of the expression of IL-31 with IL-4 and IL-13 but not with IFN- γ . No significant increase of IL-31 receptor mRNA could be detected in any disease, whereas the second receptor subunit of IL-31, the oncostatin M receptor, seems to be enhanced transcribed in patients with psoriasis.

Conclusion: IL-31 expression is not only increased in patients with AD but also in those with ACD, 2 pruritic skin disorders. In both types of eczema, expression of IL-31 is associated with the expression of the T_H2 cytokines IL-4 and IL-13.

Clinical implications: IL-31 might contribute not only to the development of AD but also to ACD-provoked skin inflammation. (J Allergy Clin Immunol 2006;118:930-7.)

Key words: Atopy, allergy, T_H1/T_H2, IgE, cytokines, inflammation

Inflammation plays a major role in the development of most skin diseases; prominent examples include atopic dermatitis (AD) and allergic contact dermatitis (ACD), as well as psoriasis. It is well established that T cells contribute substantially to the pathogenesis of all these diseases.¹⁻⁴ However, the T lymphocytes themselves are not able to establish and maintain a chronic inflammatory disease; instead, a complex interplay of immune cells with resident cells at the site of inflammation is required. Histologic analyses of skin biopsy specimens from all 3 inflammatory skin disorders have revealed that the mononuclear infiltrate contains substantial amounts of T lymphocytes belonging to the CD4⁺ T_H subset.^{3,5,6}

Recently, a novel cytokine, IL-31, has been cloned and found to be mainly produced by CD4⁺ T cells,⁷ in particular by skin-homing CD45RO⁺ (memory) cutaneous lymphocyte-associated antigen (CLA)-positive T cells.⁸ In patients with AD, the number of CLA⁺ T cells in the periphery is increased in comparison with that seen in healthy individuals.⁹ Because these cells respond more strongly to allergens than CLA⁻ cells, it has been postulated that this T-cell population might be crucial for the initiation and maintenance of AD.¹⁰ Transgenic mice overexpressing IL-31 either with a lymphocyte-specific promoter (E μ -Lck) or a ubiquitous promoter (EF1 α) experience a skin phenotype closely resembling AD in human subjects.⁷ Interestingly, in mice IL-31 seems to be preferably produced by T cells skewed toward a T_H2 phenotype; however, T_H1-skewed T cells also produce substantial amounts of this cytokine.⁷

IL-31 is most closely related to the family of IL-6-type cytokines known to be involved in many immunomodulatory functions, particularly the acute-phase response, but also in the proliferation of B and T cells.¹¹ However, IL-31

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Abbreviations used

ACD: Allergic contact dermatitis
AD: Atopic dermatitis
CLA: Cutaneous lymphocyte-associated antigen
IL-31R: IL-31 receptor
OSMR: Oncostatin M receptor
STAT6: Signal transducer and activator of transcription 6

is clearly distinct from the IL-6-type cytokines because it does not signal through glycoprotein 130, the common signaling receptor subunit. In contrast, it uses an earlier described orphan receptor, the so-called glycoprotein 130-like monocyte receptor¹² or glycoprotein 130-like receptor,¹³ in combination with the oncostatin M receptor (OSMR).⁷

To corroborate the involvement of IL-31 in several major inflammatory skin diseases, we analyzed the IL-31 mRNA expression levels in skin biopsy specimens taken from patients with AD as an example of a biphasic allergic reaction in the acute phase dominated by T_H2 lymphocytes, ACD as an example of a delayed-type allergic disease, and psoriasis as a mainly T_H1-dependent inflammatory disease.

METHODS

Patients

Punch biopsy specimens from lesional skin of patients with psoriasis (n = 60), AD (n = 33), or ACD (n = 56), as well as from control individuals without any history of atopic diseases (n = 13), were collected after obtaining informed consent in the Departments of Dermatology of the Universities of Aachen, Bonn, and Cologne, Germany, for Miltenyi Biotec GmbH as part of a study supported by the Technologie- und Innovationsprogramm NRW, Germany. In all cases the diagnosis was not only based on the typical clinical signs and symptoms but was confirmed by including the SCORAD score and total IgE levels (AD), the PASI score (psoriasis), patch test results (ACD), and histologic examination of biopsy specimens. None of the patients had received local treatment with corticosteroids for at least 1 week and systemic treatment for at least 3 weeks before biopsy. The mean age of the patients was 47.4 years (SD, 16.7 years), with 86 male patients and 76 female patients. The mean age ($\pm$ SD) and number of male and female patients for each disease group is shown in Table E1 in the Online Repository at www.jacionline.org.

RNA extraction

Isolation of total RNA from human skin biopsy specimens was performed with TriReagent (Sigma, St Louis, Mo) and subsequently with the NucleoSpin II RNA Kit (Macherey & Nagel, Düren, Germany). The RNA was quantified by means of photometric measurement (Nanodrop), and the integrity was proved by using the 2100 Bioanalyzer (Agilent Technologies, Palo Alto, Calif). RNA samples for quantitative real-time PCR analyses were amplified with T7 polymerase (Ambion, Austin, Tex) by using a modified protocol according to the method of Van Gelder et al.¹⁴

Quantitative real-time PCR

Purified RNA was reverse transcribed with the TaqMan Reverse Transcription Reagents kit (Applied Biosystems, Weiterstadt,

Germany), with random hexamers as primers. TaqMan experiments were carried out on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems) by using Assay-on-Demand gene expression products for IL-13 (Hs00174379), according to the manufacturer's recommendations. The primer/probe set for human IL-4 and IFN- γ was designed by using Primer Express 2.0.0 software (Applied Biosystems) on the basis of published sequences. For human IL-31, human IL-31 receptor (IL-31R), and human OSMR, the primer/probe sets described by Dillon et al⁷ were used and labeled with carboxyfluorescein (FAM) and Dark Quencher (Eurogentec, Liege, Belgium). An Assay-on-Demand product for human hypoxanthine phosphoribosyl-transferase (Hs99999909) was used as an internal reference to normalize the target transcripts. Because of low amounts of total RNA, the amplification could only be performed once for each cytokine and patient. Real-time PCR efficiencies were determined for each primer/probe set from standard curves generated from serial dilutions of a cDNA sample of phorbol myristate acetate/PHA-stimulated PBMCs. The relative differences between the cytokine expression of biopsy specimens taken from lesional skin versus healthy skin biopsy specimens were calculated by using the mathematic model by Pfaffl,¹⁵ including the PCR efficiencies.

Statistical methods

Cytokine mRNA expression levels were summarized by quantiles (particularly median and quartiles) and displayed graphically by using parallel box plots. The complete statistical evaluation is shown in Tables E2 and E3 in the Online Repository at www.jacionline.org. Because of the asymmetric and nonnormal distribution of the cytokine mRNA, the nonparametric Wilcoxon rank sum test was used to determine statistically significant differences of expression levels of the 4 cytokines (IL-4, IL-13, IL-31, and IFN- γ) between the various patient groups. All statistical tests were conducted on a prespecified global significance level of an α value of 5%. Because this is an explorative pilot study investigating a relatively small amount of patients, all statistical test results were interpreted in a clearly explanatory manner; that is, no α adjustment for multiple testing was carried out. Thus test results with *P* values of .05 or less could be interpreted as statistically significant. Degrees of correlation between SCORAD score/IgE serum level/itch score versus IL-31 mRNA expression was evaluated by calculating the Spearman rank correlation coefficient. Moreover, hierarchic clustering using the Euclidian distance as a dissimilarity measure and the "average linkage" method was conducted to classify the 4 cytokines into classes with similar mRNA expression patterns. The Spearman rank correlation coefficient was calculated to assess the degree of association between mRNA expression levels of any combination of 2 of the 4 cytokines analyzed separately for the 3 skin disorders.

All statistical analyses were conducted with the R statistical analysis software package, version 2.1.0.

RESULTS

IL-31 expression in the skin of patients with AD does not correlate with serum IgE levels or severity of the disease

Quantitative real-time PCR analyses were performed of IL-31 mRNA in comparison with IL-4 and IL-13 (as classical T_H2-type cytokines) and IFN- γ (as a T_H1-type cytokine) to evaluate the role of IL-31 in the process of T_H1/T_H2-mediated inflammatory skin diseases.

AD is described as a biphasic inflammatory disease, in which antigen-specific activated T cells produce large

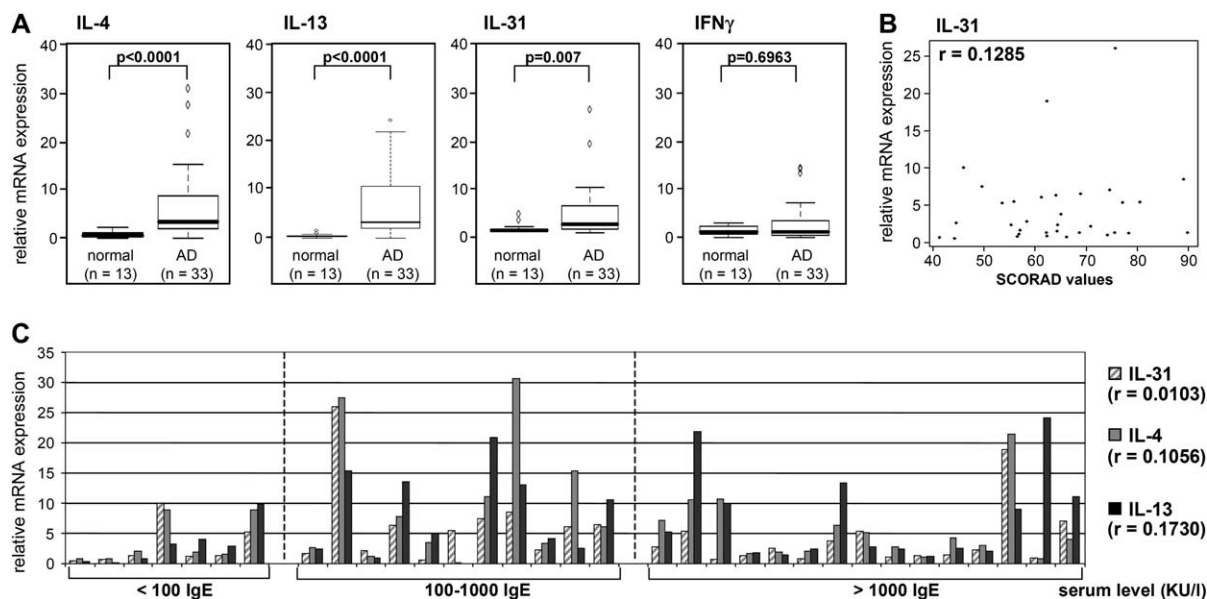


FIG 1. IL-31 is enhanced in biopsy specimens from patients with AD. **A**, Parallel box plots comparing cytokine mRNA levels normalized to hypoxanthine phosphoribosyl-transferase (*HPRT*) presented as medians (ranges) in biopsy specimens from patients with healthy skin and patients with AD. $\diamond$, Outliers. **B** and **C**, Relative IL-31 mRNA normalized to hypoxanthine phosphoribosyl-transferase was compared with the SCORAD values (scatterplot; Fig 1, **B**), as well as IL-31, IL-4, and IL-13 mRNA to serum IgE levels (Fig 1, **C**) for each patient.

amounts of T_H2 cytokines (IL-4 and IL-13) shortly after manifestation. After 48 to 72 hours, however, the initial T_H2 response switches to a T_H1 response characterized by IFN- γ -secreting T cells.¹⁶⁻¹⁹

As expected, the expression levels of the T_H2 cytokines IL-4 and IL-13 were statistically significantly upregulated ($P < .0001$; Fig 1, **A**) in patients with AD. Interestingly, we could also detect statistically significant amounts of IL-31 mRNA in many skin samples ($P = .007$; Fig 1, **A**). Even though the mRNA levels vary among individual patients, 13 (39.4%) of 33 patients show an upregulation of IL-31 mRNA exceeding the median of IL-31 transcripts in healthy skin by 5-fold. This is comparable with IL-4 (18 [54.5%] of 33 patients) and IL-13 (24 [72.7%] patients). In contrast, no significant increase in IFN- γ transcripts could be detected ($P = .6963$; Fig 1, **A**), and only 5 (15.2%) patients showed a 5-fold increase in mRNA levels. These patients might have been in a more advanced state of the disease.

The SCORAD value of each patient was compared with the expression level of IL-31 to evaluate whether the expression level of IL-31 is associated with the severity of the disease. Patients included in this study displayed medium-to-severe AD, with SCORAD values between 41.3 and 89.9. As shown in Fig 1, **B**, no correlation between the severity of AD and the expression level of IL-31 could be observed (Spearman rank correlation coefficient, $r = 0.1285$). Similarly, neither IL-4 nor IL-13 mRNA levels were related to the severity of the disease (not shown). We then compared the amount of IL-31 mRNA with the measured IgE serum levels. Again, we did not find a correlation of IL-31 ($r = 0.0103$; Fig 1,

C), IL-4 ($r = 0.1056$; Fig 1, **C**), or IL-13 ($r = 0.1730$; Fig 1, **C**) mRNA with normal (<100 KU/L) or increased (>100 KU/L) serum IgE levels.

IL-31, as well as IL-13 and IL-4, mRNA levels are strongly upregulated in biopsy specimens from patients with ACD

Biopsy specimens taken from patients with ACD, mostly patch tests (75%), turned out to be particularly interesting. We detected high mRNA levels of IL-31 in a substantial number of patients (24 [42.9%] of 56 patients showed a more than 5-fold increase). Even more impressive is the high expression of IL-4 (50 [89.3%] of 56 patients showed a more than 5-fold increase) and IL-13 (48 [85.7%] of 56 patients showed a more than 5-fold increase). For IL-4 and IL-31, mRNA levels in some biopsy specimens turned out to be several 100-fold increased (Fig 2, **A** and **D**, right panels). For better graphic representation of the significant increase in transcripts in the majority of patients, these outliers were excluded from the comparative figures (Fig 2, **A** and **D**, left panels). Nevertheless, all values were included in the statistical evaluation, which reveals a significant mRNA upregulation for all 3 cytokines in ACD biopsy specimens in comparison with healthy skin (Fig 2, right columns; IL-31, $P = .0024$; IL-4, $P < .0001$; IL-13, $P < .0001$).

In addition to the fact that ACD, classified as a delayed hypersensitivity reaction IV, has been mainly associated with a T_H1 phenotype, higher increased levels of the principal T_H1 cytokine IFN- γ are only found in a rather small number of patients (15 [26.8%] of 56 patients showed a more than 5-fold increase), and the median mRNA level

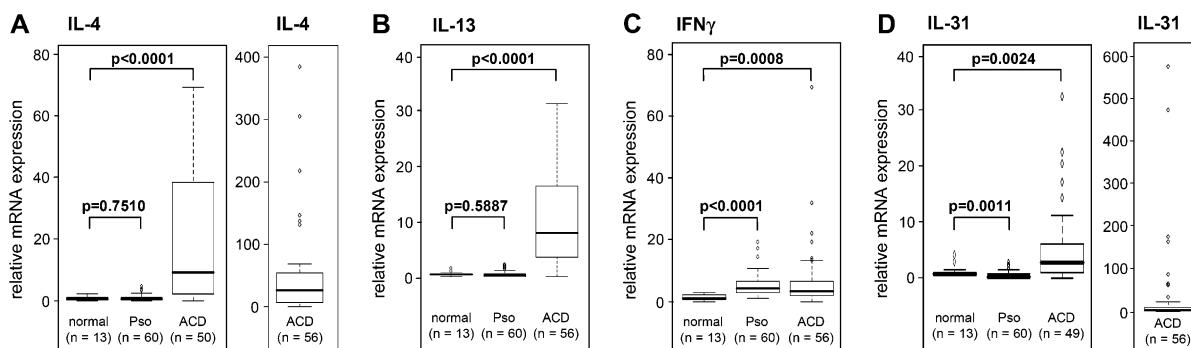


FIG 2. IL-31 is enhanced in biopsy specimens from patients with ACD but not in biopsy specimens from patients with psoriasis. **A-D**, Parallel box plots comparing cytokine mRNA levels normalized to hypoxanthine phosphoribosyl-transferase (*HPRT*) presented as medians (ranges) in biopsy specimens from patients with healthy skin, psoriasis (*Pso*), or ACD. $\diamond$, Outliers. The 6 (IL-4) and 7 (IL-31) most extreme cytokine mRNA levels in biopsy specimens from patients with ACD were excluded from Fig 2, **A** and **D** (right panels), to achieve suitable graphic representations and shown in separate box plots (left panels) with a different scale. *P* values correspond to the Wilcoxon test including all patients.

of IFN- γ detected in ACD (median, 3.569; see Table E2 in the Online Repository at www.jacionline.org) is lower than that found in biopsy specimens from patients with psoriasis (median, 4.356; see Table E2 in the Online Repository at www.jacionline.org). Nevertheless, a statistically significant increase in IFN- γ mRNA levels was determined for patients with ACD ($P = .0008$).

In contrast to AD and ACD, none of the 60 patients with psoriasis displayed a significant upregulation of IL-31 mRNA (Fig 2, **D**, middle column). This is in line with the low expression levels for IL-4 and IL-13 (Fig 2, **A** and **B**, middle columns) in this disease and in contrast to IFN- γ (19 biopsy specimens showed a more than 5-fold increase in mRNA; Fig 2, **C**). Indeed, a statistically relevant increase in mRNA levels compared with healthy skin could only be detected for IFN- γ ($P < .0001$).

Nevertheless, the mRNA expression levels for IL-31 in psoriatic plaques (median, 0.517; see Table E2 in the Online Repository at www.jacionline.org) were found to differ significantly from the expression levels in healthy skin (median, 1.0; see Table E2 in the Online Repository at www.jacionline.org), but remarkably, the IL-31 mRNA levels were lower in patients with psoriasis ($P = .0011$; Fig 2, **D**).

mRNA levels of IL-31 and the T_H2 cytokine IL-4 show a high degree of correlation

Hierarchic cluster analyses applied to the cytokine mRNA expression levels revealed that the mRNA expression profile of IL-31 is quite similar to the expression pattern of the T_H2 cytokine IL-4 for all 3 skin diseases (Fig 3). Moreover, in psoriatic skin samples quite similar low mRNA expression profiles could be found for IL-31 and both of the T_H2 cytokines IL-4 and IL-13 (Fig 3, right panel).

Correlation analyses (Spearman rank correlation coefficient) revealed that IL-31 mRNA expression levels were moderately positively correlated with the expression levels of the 2 T_H2 cytokines in AD (IL-31/IL-4, $r =$

0.6594; IL-31/IL-13, $r = 0.4533$) and ACD (IL-31/IL-4, $r = 0.5739$; IL-31/IL-13, $r = 0.6285$), confirming the results of the hierarchic cluster analyses. The highest correlations were found for the coexpression of IL-4 and IL-13 in AD and ACD ($r = 0.6862$ and $r = 0.7163$, respectively). This finding is not surprising because it is well known that levels of these cytokines are increased in T_H2-mediated diseases. Indeed, their genes are located in close proximity on the same chromosome, and cotranscription has been suggested. However, a recent study demonstrated that monoallelic expression of both cytokines is a rather rare event.²⁰

Analysis of IL-31R and OSMR expression levels in biopsy specimens from patients with AD, psoriasis, or ACD

IL-31 signals through a receptor complex consisting of the specific IL-31R and the OSMR. It has been shown recently that keratinocytes express both receptors.⁷ Additionally, the IL-31R was detected in skin-infiltrating macrophages.⁸ Immunohistochemical analyses of biopsy specimens taken from a limited number of patients with AD ($n = 5$) revealed that IL-31R protein levels seem to be increased in patients with AD.⁸ To determine whether this is due to enhanced transcription and also true for the second receptor subunit, the OSMR, we analyzed the mRNA levels for both receptors in the 3 different skin diseases in comparison with the mRNA levels in healthy skin (Fig 4). The mRNA expression levels for both the IL-31R and the OSMR vary only moderately, and for the IL-31R, no statistically significant changes in mRNA could be detected (median values = 1.328 for healthy skin, 1.896 for AD, 0.9605 for psoriasis, and 0.909 for ACD). In contrast, statistically significant increases in OSMR mRNA levels were detected in all 3 diseases compared with those seen in healthy skin (see Table E3 in the Online Repository at www.jacionline.org) but particularly in patients with psoriasis ($P < .0001$;

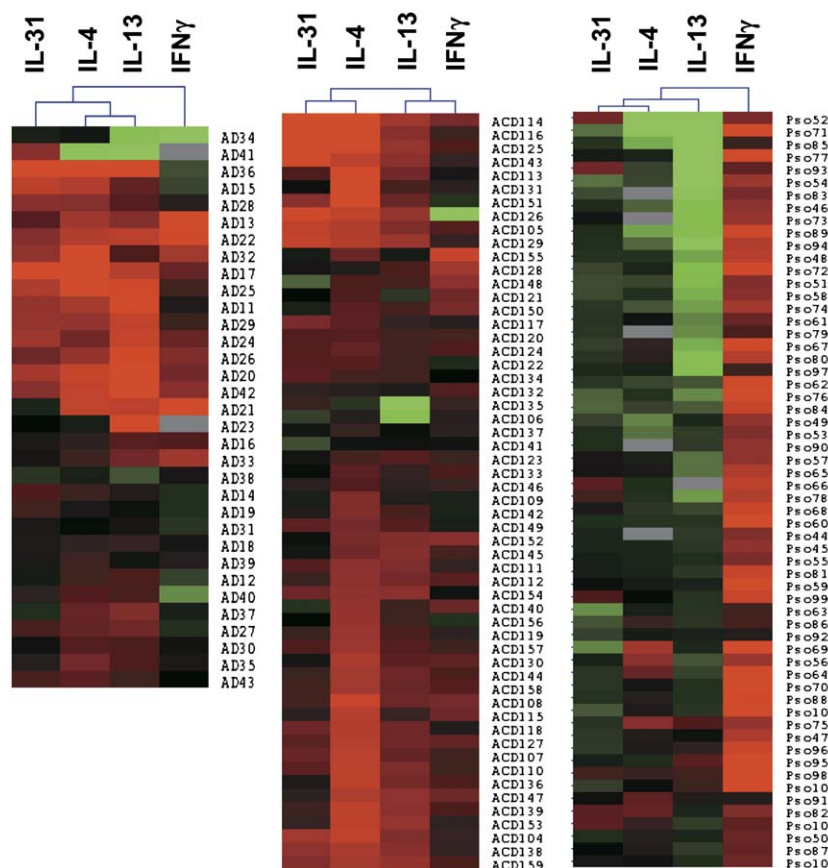


FIG 3. Hierarchic cluster analysis of IL-4, IL-13, IL-31, and IFN- γ in biopsy specimens from patients with AD (left), ACD (middle), or psoriasis (right) relative to healthy skin. Members with similar expression patterns appear under the same node. According to the amount of mRNA levels for each cytokine from high to low, the colors change from red to green, respectively. Black, Cytokine mRNA unchanged; gray, mRNA not detectable.

median, 1.965; Fig 4; see Table E3 in the Online Repository at www.jacionline.org).

DISCUSSION

IL-31 was recently identified as a novel 4-helix-bundle cytokine, and overexpression in transgenic mice led to a phenotype closely resembling AD in human patients.⁷ Analyzing 33 skin biopsy specimens taken from human AD lesions, we could demonstrate a statistically significant increase in IL-31 mRNA in comparison with that seen in healthy skin. This finding is in line with the recent publication by Sonkoly et al,²¹ in which the authors analyzed lesional and nonlesional skin of patients with AD, and extend the findings of Bilsborough et al,⁸ who also analyzed patients with AD but could not find a significant statistical increase in IL-31 mRNA because of the limited number of patients ($n = 6$). The most likely candidate cells for the production of IL-31 in inflamed skin are CD4⁺ T cells, in particular the skin-homing CD45RO⁺ CLA⁺ T cells,⁸ which have been shown to be major contributors to the development of AD.^{10,22}

Interestingly, inbred NC/Nga mice, which spontaneously experience an AD-like skin disease and have been proposed as an animal model for this human disease,²³ also express high mRNA levels for IL-31.²⁴ A recent publication suggested that this type of dermatitis in NC/Nga mice develops in the absence of signal transducer and activator of transcription 6 (STAT6). The transcription factor STAT6 is the major downstream signal transducer in the pathway of both IL-4 and IL-13. Therefore the presence of AD-like skin lesions in STAT6-deficient NC/Nga mice indicates that neither IL-4 nor IL-13 might be required for the disease establishment²⁵ and further implies an important role for IL-31 in the pathogenesis of AD.

Even though transgenic mice overexpressing IL-31 show the hallmarks of AD, the serum levels of IgE were found to be normal.⁷ Therefore the mechanism by which IL-31 promotes the development of AD seems to be distinct from that of IL-4 because IL-4 transgenic mice clearly show increased levels of IgE.²⁶⁻²⁸ The absence of high serum IgE levels can also be observed in 20% to 30% of patients (21.2% in this study), which have been subclassified as patients with intrinsic AD.² Dillon et al⁷ therefore suggested that the lack of enhanced IgE levels in IL-31

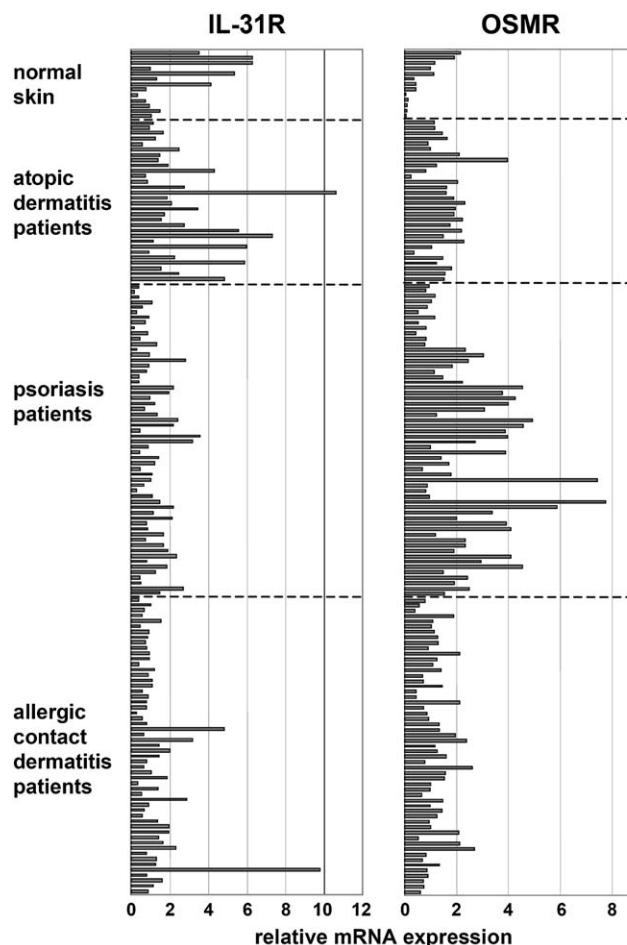


FIG 4. IL-31R and OSMR expression levels in inflammatory skin disorders. Quantitative real-time PCR analysis of IL-31R and OSMR mRNA normalized to hypoxanthine phosphoribosyl-transferase in patients with healthy skin (n = 13), AD (n = 30), psoriasis (n = 58), or ACD (n = 55) are shown. Statistical analysis was done with the nonparametric Wilcoxon rank sum test.

transgenic mice might indicate a role of IL-31 in the intrinsic form of AD. However, in NC/Nga mice the high expression levels of IL-31 are associated with increased levels of serum IgE, a finding clearly distinct from the findings by Dillon et al.⁷ In this study we cannot correlate increased levels of IL-31, IL-4, or IL-13 with enhanced serum IgE levels (>100 KU/L) in patients with AD.

In contrast to a recent publication by Bilsborough et al,⁸ we did not find a statistically significant increase in IL-31R transcripts in skin biopsy specimens from patients with AD. Because we analyzed mRNA levels and Bilsborough et al performed immunohistochemistry analyses, this discrepancy might be explained by a disturbed receptor trafficking, internalization, or degradation in patients with AD. Future studies will have to clarify this hypothesis.

ACD has been classified as a delayed hypersensitivity type IV reaction, although the signs and symptoms, including itching, of both eczemas, ACD and AD, are quite similar and differ mainly in the eliciting antigen. Because of the ability of T-cell clones to produce IFN- γ and additionally their ability to produce local passive

transfer *in vivo*, the disease appeared as mainly T_H1 cytokine driven.^{29,30} In the past years, it has become clear, however, that this exclusiveness might not reflect the physiologic situation. Ten years ago, the group of Asherson et al^{31,32} pointed out that many T_H1 cell clones were unable to evoke contact hypersensitivity when administered intravenously (ie, no systemic transfer was possible). However, treatment of the recipient mice with low doses of IL-4 before injection allowed a systemic transfer, whereas treatment of the mice with IL-4 mAbs abrogated the systemic response.³¹ Therefore it was postulated that IL-4 might play a critical role in the effector phase of contact sensitivity.³² Interestingly, the large majority of biopsy specimens analyzed in this study displayed high expression levels (>5-fold median increase) of IL-4 and IL-13 (89.3% and 85.7%, respectively), whereas a more than 5-fold increase in IFN- γ mRNA levels was only detected in a limited number of patients (26.8%). As in AD, mRNA levels of IL-31 were significantly increased in a high number of patients (42.9% showed a more than 5-fold increase). All patients with ACD were controlled

for their atopy status, and only 1 of the 56 individuals turned out to have a history of an atopic disease (asthma). This patient, however, displayed only a strong increase in IL-4 mRNA levels, whereas the number of IL-13, IL-31, and IFN- γ transcripts was only slightly changed.

IL-4 and IL-13 share one common receptor subunit (IL-4R α), and both cytokines have been shown to signal through the transcription factor STAT6. Recent studies with STAT6-deficient mice have shown that the contact hypersensitivity to 2,4,6-trinitro-1-chlorobenzene is strongly reduced,³³ and the same group has also used decoy STAT6 binding elements to prevent binding of STAT6 to its endogenous target promoters. Importantly, this therapeutic approach in mice did not only ameliorate the response of ACD but also of AD, indicating that these 2 diseases might be more closely related than initially assumed.^{34,35} Indeed, the cytokine profiles of AD and ACD in this study appear quite similar, except in ACD we can also detect a statistically significant increase in IFN- γ , even though the relative increase was not as prominent as for the T_H2-like cytokines. Thus at least in some patients a mixed T_H1/T_H2 phenotype can be observed in the course of ACD. This phenomenon is probably best known for the contact allergen nickel, for which both a predominant T_H1 response,³⁶ as well as a predominant T_H2 response,³⁷ have been described. Recently, a similar observation was made for the organic compounds methylchloroisothiazolinone and methylisothiazolinone, the active ingredients of the preservative Kathon CG (Sigma, St Louis, Mo). PBMCs from sensitized patients produced both T_H1- and T_H2-like cytokines on restimulation with methylchloroisothiazolinone/methylisothiazolinone.³⁸ Our results indicate that in addition to IL-4 and IL-13, IL-31 might play a crucial role in the course of ACD. Interestingly, Dillon et al⁷ pointed out that the increased IL-31 formation was associated with very strong itching, and Sonkoly et al²¹ found the highest relative increase in IL-31 mRNA production in biopsy specimens from patients with prurigo nodularis, one of the most pruritic chronic skin disorders. Because IL-31R mRNA levels are particularly high in dorsal root ganglia, the authors hypothesized that IL-31 might be crucially involved in the development of pruritus. Because itch is a prominent symptom not only in AD and prurigo nodularis but also in ACD,³⁹ the high expression levels of IL-31 found in these biopsy specimens might further strengthen this hypothesis. However, we have to note here that the itch score is one of the criteria included in the case history of patients with AD, and we could not find a correlation of a subjective high itch score and a particularly high IL-31 expression ($r = 0.0709$). Interestingly, Takaoka et al⁴⁰ could link the expression of IL-31 to long-lasting scratching behavior in NC/Nga mice. In this model no itch was observed in 2,4,6-trinitro-1-chlorobenzene-induced eczema formation associated with a lack in IL-31 mRNA increase, which is not the case in human ACD. Whether this difference is due to a difference in the observed types of diseases or dependent on different phases of the same or similar disease remains open.

In summary, we found high expression levels of IL-31 not only in biopsy specimens taken from patients with AD but also in specimens from patients with ACD. Although murine and human T cells skewed *in vitro* toward a T_H1 phenotype secrete substantial amounts of IL-31,⁷ we cannot associate this cytokine with the pathogenesis of psoriasis, a T_H1-mediated human inflammatory skin disease.³ In correlation analyses we could find a positive correlation of IL-31 with IL-4 expression levels in AD and ACD. How IL-31 acts in concert with known mediators of pruritus/dermatitis, such as IL-4, IL-7, histamines, and neuropeptides, remains to be determined, and a precise understanding of the involvement of this novel cytokine in the development of these diseases is critical for more effective management strategies.

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