

additional determination of ω -5 gliadin-specific IgE levels in wheat-sensitized patients appears not to be helpful. However, our study suggests that for some patients with suspected wheat allergy without increased wheat-specific IgE levels, the determination of ω -5 gliadin-specific IgE levels might be helpful in detecting sensitization to water-insoluble wheat proteins. Importantly, additional studies are necessary to identify the major allergens involved in food allergy to wheat proteins.

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REFERENCES

1. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. *Clin Exp Allergy* 2005;35:268-73.
2. Palosuo K. Update on wheat hypersensitivity. *Curr Opin Allergy Clin Immunol* 2003;3:205-9.
3. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997;100:444-51.
4. Matsuo H, Dahlstrom J, Tanaka A, Kohno K, Takahashi H, Furumura M, et al. Sensitivity and specificity of recombinant omega-5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergy* 2008; 63:233-6.
5. Palosuo K, Varjonen E, Kekki OM, Klemola T, Kalkkinen N, Alenius H, et al. Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. *J Allergy Clin Immunol* 2001;108:634-8.
6. Lauriere M, Pecquet C, Boulenc E, Bouchez-Mahiot I, Snegaroff J, Choudat D, et al. Genetic differences in omega-gliadins involved in two different immediate food hypersensitivities to wheat. *Allergy* 2007;62:890-6.
7. Constantin C, Huber WD, Granditsch G, Weghofer M, Valenta R. Different profiles of wheat antigens are recognised by patients suffering from coeliac disease and IgE-mediated food allergy. *Int Arch Allergy Immunol* 2005;138:257-66.

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Correlation of IL-31 serum levels with severity of atopic dermatitis

To the Editor:

Atopic dermatitis (AD) is a relapsing inflammatory skin disease characterized by eczematous skin lesions and intense pruritus.¹ Pruritus represents one of the major symptoms of AD and has a high effect on quality of life. In AD pruritus is often resistant to H1-antihistamine treatment. Therefore other mediators, including neuropeptides, neurotransmitters, proteinases, arachidonic derivatives, and cytokines, have been suggested for a pathophysiologic role of pruritus in AD.²

Recently, the novel 4-helix-bundle cytokine IL-31 has been described to be involved in the development of chronic dermatitis through the induction of severe pruritus in transgenic mice with lymphocyte-specific overexpression of IL-31.³ The expression

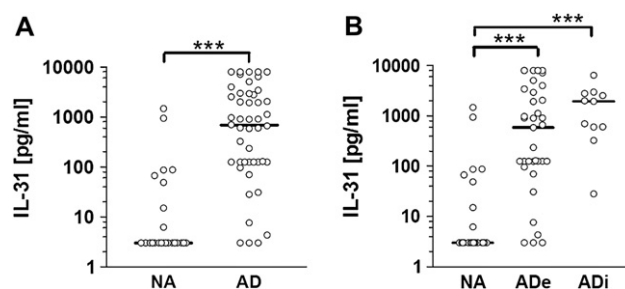


FIG 1. A, IL-31 serum levels of patients with AD (n = 48) compared with those of nonatopic healthy control subjects (NA; n = 24). B, Calculation of IL-31 serum levels according to the extrinsic (ADe; n = 37) and intrinsic (ADi; n = 11) types of AD compared with those seen in nonatopic healthy control subjects (NA; n = 24). Data are presented as scattergrams with medians. **P* < .05, ***P* < .01, and ****P* < .001.

of skin *IL31* mRNA levels was significantly higher in NC/Nga mice with scratching behavior than in mice without scratching behavior.⁴ In addition, scratching counts in mice with long-lasting scratching behavior correlated with skin *IL31* mRNA expression.⁵

In human subjects *IL31* mRNA is overexpressed in pruritic AD but not in nonpruritic psoriatic lesions compared with healthy skin.⁶ Interestingly, increased *IL31* mRNA levels were also found in nonlesional skin of patients with AD compared with those seen in healthy control subjects. Acute allergic contact dermatitis, a skin disease featuring skin inflammation and pruritus, has also been associated with higher *IL31* mRNA levels compared with those seen in healthy skin.⁷ In that study *IL31* mRNA expression correlated with T_H2 cytokines, including IL-4 and IL-13. Possible sources of IL-31 are skin-infiltrating cutaneous lymphocyte antigen (CLA)⁺ T cells and peripheral blood CD45RO CLA⁺ T cells.⁸ Interestingly, staphylococcal superantigens, which represent a general trigger factor for AD, rapidly induced *IL31* mRNA expression in the skin of atopic individuals *in vivo* and in PBMCs *in vitro*, suggesting that superinfection can lead to the sensation of pruritus.⁶ IL-31 signals through a heterodimeric receptor composed of IL-31 receptor A and oncostatin M receptor, which is expressed on epithelial cells, including keratinocytes, and recently has been described on dorsal root ganglia (IL-31 receptor A).⁶

Together, these data provide evidence for a pivotal role of IL-31 in the induction and maintenance of pruritus and chronic skin inflammation in AD. However, data on the correlation between IL-31 protein and AD severity are missing. We therefore thought to investigate whether (1) IL-31 serum levels are increased in AD, (2) IL-31 levels correlate with disease severity in AD, and (3) there is a difference in IL-31 expression between the extrinsic and intrinsic type of AD.

After obtaining informed consent (approved by the local ethics committee), peripheral venous blood was obtained from patients and control subjects at 9 AM to prevent daily variations. Patients with the extrinsic type of AD (n = 37; mean age, 37.8 ± 14.1 years; positive history of intermittent allergic rhinitis; and IgE-mediated sensitization to at least 3/10 common aeroallergens) and the intrinsic type of AD (n = 11; mean age, 31.8 ± 11.2 years; no history of allergic rhinitis or allergic asthma; and no IgE-mediated sensitization to aeroallergens), defined according to the criteria of Hanifin and Rajka, were included in the study.

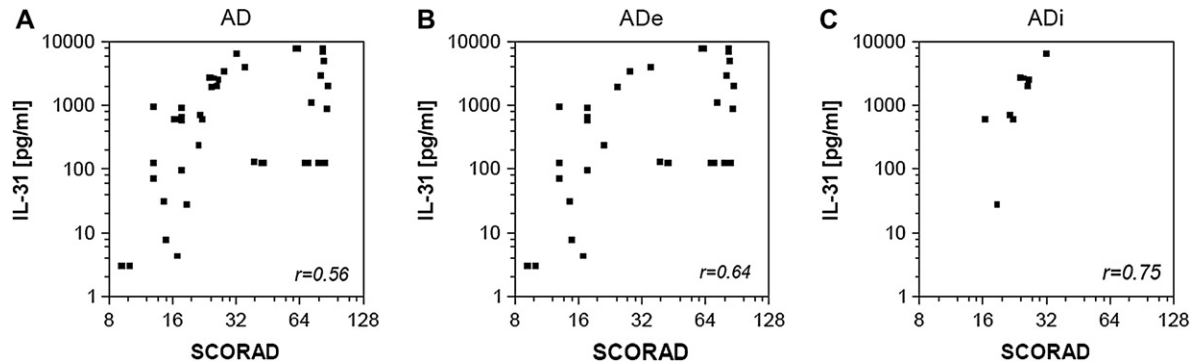


FIG 2. A, Correlation of IL-31 serum levels (*y*-axis) with SCORAD scores (*x*-axis) in patients with AD (Spearman $r = 0.56$, $***P < .0001$, $n = 48$). B and C, Correlation of IL-31 serum levels with SCORAD scores of patients with the extrinsic (ADe; $n = 37$, Spearman $r = 0.64$, $***P < .0001$; Fig 2, B) and intrinsic (ADi; $n = 11$, Spearman $r = 0.75$, $**P < .01$; Fig 2, C) subtypes of AD.

Twenty-four nonatopic subjects with healthy skin (mean age, 35.1 ± 14.0 years) were included as the control group. Subjects did not receive systemic immunosuppressive treatment.

Severity of AD was assessed by using the SCORAD score. Serum IL-31 levels were measured with a commercially available ELISA kit (R&D Systems, Wiesbaden, Germany), according to the manufacturer's instructions. The ELISA detection limit was 126 pg/mL. Statistical analysis was performed with the statistical software package SigmaStat (SigmaStat for Windows; Jandel Scientific Erkrath, Wiesbaden, Germany). Data were analyzed with the nonparametric Mann-Whitney *U* test. The correlation between IL-31 levels and SCORAD scores was assessed with Spearman rank order correlation. A *P* value of less than .05 was considered statistically significant.

Serum IL-31 levels were significantly higher in patients with AD compared with those in nonatopic healthy control subjects ($P < .001$; Fig 1, A). When we subdivided AD into the extrinsic and intrinsic subtypes, we still found significantly higher serum IL-31 levels in the extrinsic ($P < .001$; Fig 1, B) and intrinsic ($P < .001$; Fig 1, B) types of AD compared with those in nonatopic healthy control subjects. There was no difference in IL-31 serum levels between those with the intrinsic and extrinsic types of AD (Fig 1, B).

The overall mean SCORAD score ($\pm$ SD) was 37.53 ± 27.7 for patients with AD ($n = 48$). Analyzing AD subtypes showed a SCORAD score of 42.9 ± 29.3 for patients with the extrinsic type of AD ($n = 37$) and 19.5 ± 9.4 for patients with the intrinsic type of AD ($n = 11$), respectively. There was no significant difference in SCORAD score between patients with the extrinsic and intrinsic types of AD. The SCORAD score significantly correlated with serum IL-31 levels for all patients with AD (Spearman $r = 0.56$, $P < .0001$; Fig 2, A). The correlation was still significant when we calculated it for the group of patients with the extrinsic and intrinsic types of AD, respectively. IL-31 serum levels significantly correlated with SCORAD scores in those with the extrinsic type of AD (Spearman $r = 0.64$, $P < .0001$; Fig 2, B). In addition, SCORAD scores positively correlated with IL-31 serum levels in those with the intrinsic type of AD (Spearman $r = 0.75$, $P < .01$; Fig 2, C).

In summary, our data provide evidence that, irrespective of the atopic phenotype, serum IL-31 levels correlate with disease activity in AD. In this regard recent data provide strong support for a pathophysiologic role of IL-31 in the nonatopic type of

eczema, as shown by Schulz et al.⁹ In their study, performed in 3 independent European populations including 690 affected families, they assessed a significant association of a common *IL31* haplotype with the nonatopic type of eczema.

IL-31 expression is largely restricted to CD45RO⁺ memory effector T cells.⁸ Furthermore, IL-31 protein in culture supernatants and *IL31* mRNA have been found in higher levels in CLA⁺ T cells compared with CLA⁻ subsets in patients with AD, with a trend higher than that of healthy individuals. Therefore increased serum levels of IL-31 might also account for other cell types than T cells, including eosinophil granulocytes, a possibility that is currently under investigation.

Taken together, our findings provide evidence that IL-31 has a role in AD pathophysiology with regard to pruritus and skin inflammation, as assessed by the positive correlation with AD severity. In patients with AD, pruritus is often resistant to H1-antihistamine treatment. Therefore IL-31 might present a novel target for antipruritic and anti-inflammatory options in the treatment of AD.

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REFERENCES

1. Boguniewicz M, Schmid-Grendelmeier P, Leung DY. Atopic dermatitis. *J Allergy Clin Immunol* 2006;118:40-3.
2. Ständer S, Steinhoff M. Pathophysiology of pruritus in atopic dermatitis: an overview. *Exp Dermatol* 2002;11:12-24.
3. Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat Immunol* 2004;5:752-60.

4. Takaoka A, Arai I, Sugimoto M, Yamaguchi A, Tanaka M, Nakaike S. Expression of IL-31 gene transcripts in NC/Nga mice with atopic dermatitis. *Eur J Pharmacol* 2005; 516:180-1.
5. Takaoka A, Arai I, Sugimoto M, Honma Y, Futaki N, Nakamura A, et al. Involvement of IL-31 on scratching behavior in NC/Nga mice with atopic-like dermatitis. *Exp Dermatol* 2006;15:161-7.
6. Sonkoly E, Muller A, Lauerma AI, Pivarsci A, Soto H, Kemeny L, et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol* 2006;117:411-7.
7. Neis MM, Peters B, Dreuw A, Wenzel J, Bieber T, Mauch C, et al. Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis. *J Allergy Clin Immunol* 2006;118:930-7.
8. Bilsborough J, Leung DY, Maurer M, Howell M, Boguniewicz M, Yao L, et al. IL-31 is associated with cutaneous lymphocyte antigen-positive skin homing T cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 2006;117:418-25.
9. Schulz F, Marenholz I, Folster-Holst R, Chen C, Sternjak A, Baumgrass R, et al. A common haplotype of the IL-31 gene influencing gene expression is associated with nonatopic eczema. *J Allergy Clin Immunol* 2007;120:1097-102.

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Alefacept (lymphocyte function-associated molecule 3/IgG fusion protein) treatment for atopic eczema

To the Editor:

Atopic eczema (AE) is a chronic inflammatory skin disease characterized by a predominantly T_H2 cell-mediated immune response. T-cell activation is mediated by the interaction between T-cell receptor and peptide-MHC on antigen-presenting cells but also requires costimulation through accessory molecules, such as CD2/lymphocyte function-associated molecule (LFA) 3.¹ Alefacept (Amevive; Biogen Dompé, Zug, Switzerland) is a fusion protein composed of the first extracellular domain of LFA-3 (CD58) and the human IgG1 fragment crystallizable (Fc) domain.² Binding of the LFA-3 fragment to CD2 blocks costimulation and the subsequent activation of T cells.² Furthermore, by binding to CD2 and Fcγ receptor III (CD16), alefacept mediates cognate interaction between T cells and natural killer cells, resulting in T-cell apoptosis.³

Because T cell/antigen-presenting cell interactions, as well as T-cell activation and proliferation, are key pathomechanisms in AE, we aimed to investigate the effects of alefacept in AE. In this investigator-initiated, open-label pilot study ClinicalTrials.gov identifier: NCT 00376129, 10 patients with moderate-to-severe AE not adequately responding to topical corticosteroid therapy, calcineurin inhibitor therapy, or both (6 female patients; age, 19-51 years) were enrolled. The study was approved by the local ethics committee. Written informed consent was obtained from all patients before participation in the study. There was a 2-week washout period for topical and a 4-week washout period for systemic AE therapy. Two weeks before and during the study, patients were allowed to exclusively take moderately potent topical corticosteroids and antihistamines. Low CD4⁺ cell numbers and severe infections had been ruled out before alefacept treatment. Patients received 12 weekly intramuscular injections of 15 mg of alefacept.

Clinical parameters (eczema area and severity index [EASI], pruritus score, and concomitant medication), differential white blood cell analysis (including immunophenotyping), skin histology, immunofluorescence analysis, and cytokine expression analysis of PBMCs and skin-infiltrating cells on mRNA and protein levels, were monitored (for methods, see Simon et al⁴).

Data are presented as means ± SEMs. For statistical analyses, the paired *t* test and 1-way repeated-measures analysis, followed by the Tukey test or the corresponding nonparametric tests, were applied. A *P* value of less than .05 was considered statistically significant.

All patients experienced a significant improvement on alefacept therapy. The EASI levels continuously decreased over the treatment period, starting after the fourth injection, except in one patient who had an acute exacerbation at week 8. After treatment, EASI levels remained low or decreased further during the observation period until week 22, suggesting a sustained effect of alefacept (Fig 1, A and B). Mean percentage EASI improvement was 78% at week 12 and 86% at week 22. In parallel, the pruritus (patient assessment: 0, no; 1, mild; 2, moderate; and 3, severe) significantly decreased (2.5 ± 0.17 before therapy, 0.85 ± 0.18 at week 12, and 1.05 ± 0.19 at week 22; *P* < .001). Moreover, with the improvement of the skin lesions, all patients could drastically reduce the application of concomitant medication. Before therapy, topical corticosteroids were applied on 5.6 ± 0.6 days per week, and after therapy, they were applied on 1.1 ± 0.3 days per week (*P* < .001). In parallel with the reduction of the EASI, the area of affected skin requiring corticosteroid therapy decreased. Three patients were able to stop the use of any concomitant AE medication. The therapy with alefacept was well tolerated, and no severe adverse events were observed.

Total and differential white blood cell counts, including CD4⁺ and CD8⁺ T-cell counts, were monitored before and every second week during the treatment period. We observed nonsignificant reductions in the numbers of leukocytes, lymphocytes, CD4⁺ and CD8⁺ T cells, neutrophils, and eosinophils. Immunophenotyping revealed decreases in the numbers of CD4⁺ and CD8⁺ cells expressing CD25, HLA-DR, and CD95 after therapy, suggesting reduced activation of T cells. Statistically significant differences were determined for CD4⁺CD95⁺ (*P* < .001), CD8⁺CD95⁺ (*P* < .001), and CD8⁺CD25⁺ (*P* = .018) cells.

Alefacept therapy had no effect on the spontaneous cytokine production of PBMCs, but on stimulation with anti-CD3 mAb, we observed a significant decrease in the production of IL-4 (*P* = .009), IL-5 (*P* = .012), and IL-13 (*P* = .043), indicating reduced numbers of T_H2 effector memory cells in the blood. Moreover, the production of IFN-γ after LPS stimulation decreased with alefacept therapy (*P* = .027).

The histopathologic alterations, such as hyperkeratosis, acanthosis, spongiosis, and dermal inflammatory cell infiltrate, that were observed in lesional skin markedly decreased on alefacept therapy toward findings usually seen in nonlesional skin. Before therapy, the dermal infiltrate was composed mainly of CD4⁺ and CD8⁺ T cells. With alefacept therapy, the numbers of T cells, but also B cells (CD21⁺) and eosinophils (eosinophil cationic protein positive), were significantly reduced (Fig 1, C).

Measuring mRNA expression of cytokines in the skin of patients with AE revealed a 75% decrease of *IL5* and a 50% decrease of *IL13* expression with alefacept therapy. The mRNA expression of *IFNG* and *IL10* decreased by approximately 25% (data not shown). The decrease of cytokine expression was confirmed by means of double-immunofluorescence staining and confocal microscopy. In parallel with the decrease of inflammatory cell numbers, the numbers of CD4⁺ and CD8⁺ T cells expressing the cytokines IL-5, IL-10, IL-13, or IFN-γ were significantly reduced (Fig 1, D and E).