BRIEF REPORT

Mutual Antagonism of T Cells Causing Psoriasis and Atopic Eczema

Stefanie Eyerich, Ph.D., Anna T. Onken, M.D., Stephan Weidinger, M.D., Andre Franke, M.D., Francesca Nasorri, Ph.D., Davide Pennino, Martine Grosber, M.D., Florian Pfab, M.D., Carsten B. Schmidt-Weber, Ph.D., Martin Mempel, M.D., Ruediger Hein, M.D., Johannes Ring, M.D., Ph.D., Andrea Cavani, M.D., Ph.D., and Kilian Eyerich, M.D., Ph.D.

SUMMARY

The simultaneous occurrence of psoriasis driven by type 1 helper T (Th1) cells and type 17 helper T (Th17) cells and atopic eczema dominated by type 2 helper T (Th2) cells is rare. Here, we describe three patients with co-occurring psoriasis and atopic eczema with an antagonistic course and distinct T-cell infiltrates in lesions from psoriasis and those from atopic eczema. Sensitized patients with psoriasis had a reaction to epicutaneous allergen challenge, with clinically and histologically verified eczema lesions containing a large number of allergen-reactive T cells. These findings support a causative role for T cells triggered by specific antigens in both psoriasis and atopic eczema. (Supported by the German Research Foundation and others.)

From the Center of Allergy and Environment (ZAUM), Technische Universität and Helmholtz Center Munich (S.E., D.P., C.B.S.-W.), and the Department of Dermatology and Allergy, Technische Universität Munich (A.T.O., M.G., F.P., R.H., J.R., K.E.) — both in Munich; the Department of Dermatology, Allergology, and Venerology (S.W.) and the Institute of Clinical Molecular Biology (A.F.), University Hospital Schleswig–Holstein, Campus Kiel, Kiel; and the Department of Dermatology, Venereology, and Allergology, University Medicine Göttingen, Göttingen (M.M.) — all in Germany; and the Laboratory of Experimental Immunology, Istituto Dermopatico dell'Immacolata, Rome (F.N., A.C.). Address reprint requests to Dr. Kilian Eyerich at the Department of Dermatology and Allergy, Technische Universität Munich, Biedersteiner Straße 29, 80802 Munich, Germany, or at kilian.eyerich@lrz.tum.de.

Drs. S. Eyerich and Onken contributed equally to this article.

N Engl J Med 2011;365:231-8.
Copyright © 2011 Massachusetts Medical Society.

Psoriasis and atopic eczema are prevalent, influence health-related quality of life, are associated with concomitant illness, and pose an economic burden. Whether these diseases are epithelial or immunologic disorders is debated. Both involve complex interactions of hereditary factors and environmental influences. Besides altering the skin barrier, these conditions lead to a systemic T-cell–driven immune response with primary involvement of not only the skin but also other sites such as joints (in psoriatic arthritis) or airways (in asthma and rhinitis). Whereas atopic eczema arises from a systemic Th2-cell–dominated immune shift characterized by frequent elevations of total and allergen-specific IgE levels,[1] psoriasis is caused by an immune response driven by Th1 cells, which secrete high levels of interferon-γ, and by Th17 cells, which secrete high levels of interleukin-17A, interleukin-17F, and interleukin-22.[2] In view of their opposing immune mechanisms, one would expect these diseases to be mutually exclusive. Indeed, cases of concomitant psoriasis and atopic eczema are rare.[3]

METHODS

We evaluated three patients with concomitant psoriasis and atopic eczema and an additional five patients with psoriasis and allergic contact dermatitis to nickel. Diagnoses were made on the basis of clinical presentation, personal history, laboratory findings, and the results of epicutaneous patch testing. In addition, genomic DNA was extracted from leukocyte specimens obtained from the patients and tested for HLA-Cw6 alleles as well as the four most common filaggrin polymorphisms (R501X,

The New England Journal of Medicine
Downloaded from nejm.org at UNIVERSITA TOR VERGATA on July 26, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

2282del4, R2447X, and S3247X), as described previously.[4,5] The clinical presentation of the patients was evaluated by means of the psoriasis area-and-severity index (PASI) and the Scoring Atopic Dermatitis (SCORAD) index. (For details of these grading schemes, see the Supplementary Appendix, available with the full text of this article at NEJM.org.) An epicutaneous challenge to nickel and atopy patch testing of *Dermatophagoides pteronyssinus* were performed according to standardized protocols.[6,7] The study was approved by the local ethics committee, and all patients provided written informed consent.

From each patient, punch-biopsy specimens were obtained simultaneously from eczema and psoriasis lesions while the involved areas were under local anesthesia. Biopsy specimens were immediately divided into two parts. One part was fixed in paraformaldehyde for histologic analysis; from the other part, T-cell lines were isolated and further investigated in vitro, as described previously.[8] The cytokine profile of T-cell lines was determined after stimulation with phorbol myristate acetate–ionomycin for 6 hours in the presence of brefeldin A, and intracellular cytokine accumulation was measured by means of three-color flow cytometry, as described previously.[9,10] In addition, T-cell lines were stimulated with anti-CD3 and anti-CD28 antibody for 72 hours, and secretion of interferon-γ, interleukin-4, interleukin-17, and interleukin-22 levels was quantified by means of commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems).

T-cell specificity was investigated in coculture systems of 10^5 T cells with 3×10^4 autologous monocytes and 5 μg of natural *D. pteronyssinus* per milliliter (in Patients 1, 2, and 3) or 20 μg of nickel sulfate per milliliter (in Patients 4 through 8), as described previously.[8] Two methods were used: the carboxyfluorescein succinimidyl ester diacetate (CFSE-DA) assay and the thymidine-incorporation method. In the former, T cells were marked with 0.5 μM of the fluorescent dye CFSE-DA and subsequently cocultured as described above. (CFSE-DA stains cells and is divided between them after cell division; a decline in CFSE-DA expression reflects T-cell proliferation.) After 5 days, T cells were also stained with CD69 (a marker of activated T cells), and fluorescence intensity was measured by flow cytometry. In the latter method, T cells were cocultured for 72 hours as described above. Cell-free supernatant was obtained for quantification of cytokine secretion by means of ELISA, and subsequently 10 μg per milliliter of ^{3}H-thymidine was added to the coculture for an additional 12 hours. Thymidine incorporation was measured by means of a beta-counting device, as described previously.[8] Results are expressed as the proliferation index (i.e., the counts per minute for stimulated T cells divided by the counts per minute for the negative controls).

RESULTS

Characteristics of the three patients with concomitant psoriasis and atopic eczema are shown in Table 1 and Figure 1. Although the clinical courses of psoriasis and atopic eczema rarely overlapped, indicating independent regulation of the two immune phenotypes, all three patients had periods with active lesions of both diseases. During such periods, skin-biopsy specimens were obtained from psoriasis lesions and atopic eczema lesions at the same time. Histologic analysis of the specimens showed typical features of each disease, such as acanthosis, elongated rete ridges, and neutrophilic microabscesses in psoriasis lesions and spongiosis as well as a mixed infiltrate of T cells, eosinophils, and granulocytes in atopic eczema lesions (Fig. 1A).

Parallel occurrence of antagonistic inflammatory skin reactions may require distinct antigen triggers. In all our patients, the T cells derived from psoriasis lesions and those derived from atopic eczema lesions differed in their cytokine profile. Psoriasis lesions contained a large number of Th1 and Th17 cells, whereas atopic eczema lesions have higher amounts of Th2 and Th22 cells (Fig. 1A and 2A).[11,12] Accordingly, secretion of the Th1 and Th17 cytokines interferon-γ and interleukin-17 was greater in psoriasis-derived T-cell lines, whereas T cells from atopic eczema lesions secreted greater amounts of interleukin-4 in vitro. Interleukin-22 is produced by both Th17 and Th22 cells and was released in similar amounts in the psoriasis lesions and atopic eczema lesions (Fig. 1D and 2C).

Psoriasis lesions regularly develop after mechanical trauma or skin irritation (Koebner's phenomenon).[13] To evaluate whether nonspecific responses such as Koebner's phenomenon or antigen-specific T-cell responses predominate, we performed a challenge with the major house-dust mite allergen, *D. pteronyssinus* (atopy patch test).

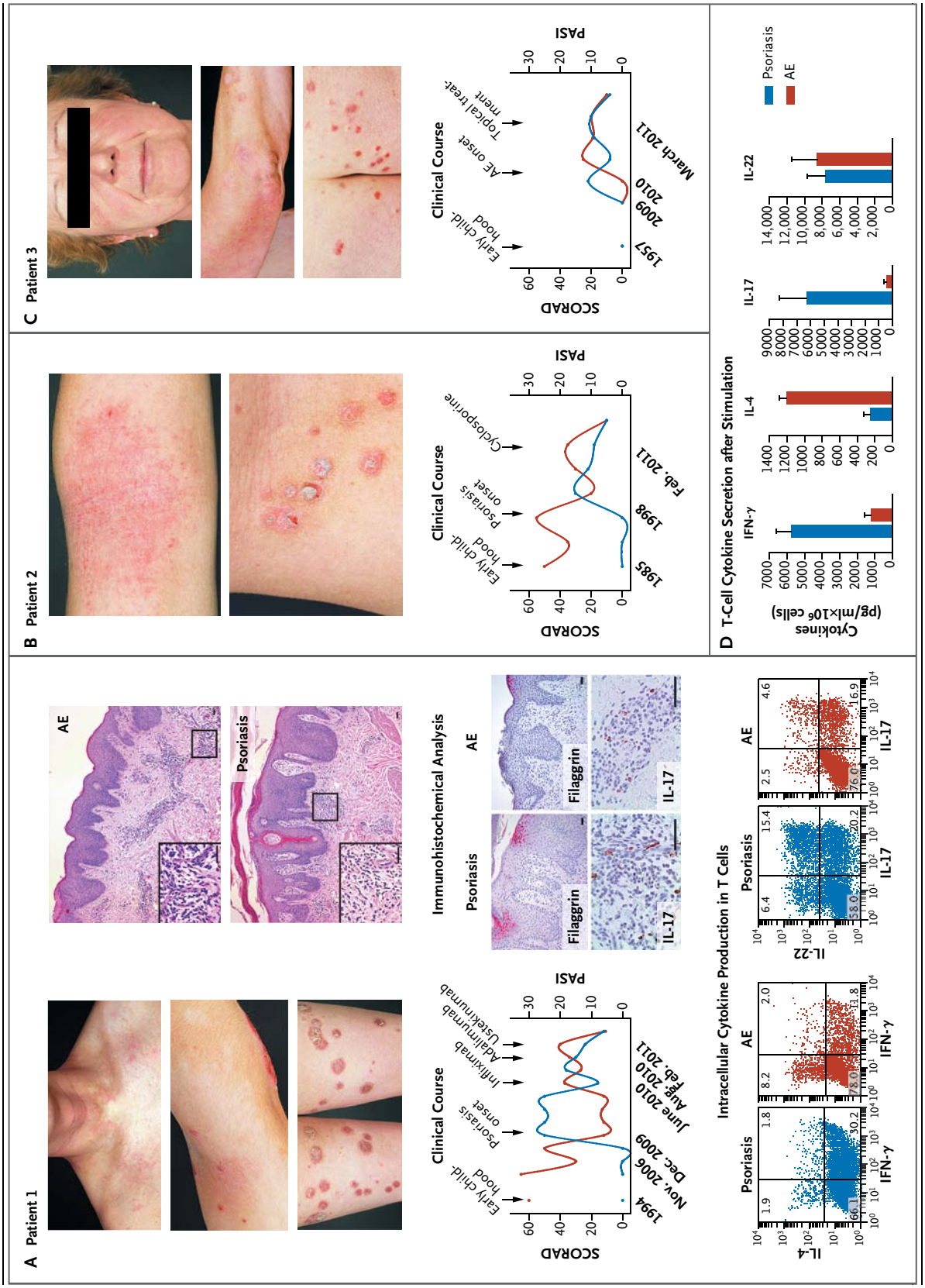
The New England Journal of Medicine
Downloaded from nejm.org at UNIVERSITA TOR VERGATA on July 26, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

Table 1. Characteristics of the Three Patients with Atopic Eczema and Psoriasis and Five Additional Patients with Psoriasis and Allergic Contact Dermatitis (from Nickel).*

Characteristic	Patients with Atopic Eczema and Psoriasis			Patients with Psoriasis and Allergic Contact Dermatitis				
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age (yr)	18	25	59	35	45	43	55	39
Sex	M	F	F	F	F	F	F	F
Diagnosis								
Atopic eczema	Yes	Yes	Yes	No	No	No	No	No
Psoriasis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Age at disease onset (yr)								
Atopic eczema	1	1	58					
Psoriasis	16	14	56	15	38	14	49	15
Associated disease								
Atopic eczema	Allergic rhinoconjunctivitis	Allergic rhinoconjunctivitis	No					
Psoriasis	No	No	No	No	No	No	Psoriatic arthritis	No
Family history								
Atopic eczema	Negative	Negative	Negative					
Psoriasis	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Negative
IgE antibody								
IU/ml	270	3944	411	43	59	63	18	9
Antigen (IgE class)†	*D. pteronyssinus* (1), cat (3), *P. pratense* (3), *B. pendula* (3), hazelnut (3)	*D. pteronyssinus* (6), *A. artemisiifolia* (5), *P. pratense* (4), cat (4), *B. pendula* (2)	*D. pteronyssinus* (2)	None	None	None	None	None
APT grade‡	*D. pteronyssinus* (+++)	*D. pteronyssinus* (++)	Negative					
EPT grade‡				Nickel (+++)	Nickel (++)	Nickel (+++)	Nickel (+++)	Nickel (+++)
Genomic DNA testing								
Filaggrin mutation§	No	No	No	No	No	No	No	No
HLA-Cw6	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Negative
Skin colonization								
Atopic eczema	*S. aureus*	*S. aureus*	*S. aureus*					
Allergic contact dermatitis				Negative	Negative	Negative	Negative	Negative
Psoriasis	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

* *A. artemisiifolia* denotes *Ambrosia artemisiifolia*, APT atopy patch test, *B. pendula Betula pendula*, *D. pteronyssinus Dermatophagoides pteronyssinus*, EPT epicutaneous patch test, *P. pratense Phleum pratense*, and *S. aureus Staphylococcus aureus*.
† IgE class indicates the level of sensitization, with scores ranging from 0 (no sensitization) to 6 (very strong sensitization).
‡ Plus signs indicate the degree of reaction (+++ denotes strong reaction, and ++ moderate reaction).
§ Filaggrin represents the four most common filaggrin polymorphisms (R501X, 2282del4, R2447X, and S3247X).

The New England Journal of Medicine
Downloaded from nejm.org at UNIVERSITA TOR VERGATA on July 26, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.



The New England Journal of Medicine
Downloaded from nejm.org at UNIVERSITA TOR VERGATA on July 26, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

Figure 1 (facing page). Patients with Concomitant Psoriasis and Atopic Eczema (AE).

Findings for Patients 1, 2, and 3 are shown in Panels A, B, and C, respectively. Clinical photographs are shown, as well as the clinical course, assessed over time by means of the psoriasis area-and-severity index (PASI, with scores ranging from 0 to 72 and higher scores indicating more severe disease) (blue lines) and the Scoring Atopic Dermatitis (SCORAD) index for AE (with scores ranging from 0 to 103 and higher scores indicating more severe disease) (red lines). Note that dates along the x axes of the clinical-course plots are not evenly spaced according to date. Panel A also shows light-microscopical images of representative lesion-infiltrate samples stained for visualization (hematoxylin and eosin, top) and immunohistochemically stained with for filaggrin and interleukin-17 (bottom), with the small squares corresponding to the insets (and all scale bars representing 20 μm). Intracellular cytokine accumulation, measured in stimulated T-cell lines derived from psoriasis and atopic eczema lesions, is shown at the bottom of Panel A; the value in each quadrant indicates the relative frequency among 20,000 cells. Panel D shows T-cell cytokine secretion after stimulation (mean of at least three independent experiments per patient ±SE). IFN denotes interferon, and IL interleukin.

Clinical and histologic signs of eczema developed in Patients 1 and 2. Eczema-invading T cells were mostly Th2 cells,[7,14] a large number of which reacted to *D. pteronyssinus* allergen challenge in vitro after antigen presentation by autologous monocytes (Fig. 2A, 2D, and 2E).

To determine whether psoriasis and eczema are caused by opposing T-cell subsets or antigen-specific T cells, we expanded testing to include eczema-inducing agents that typically elicit a Th1- and Th17-cell immune response, such as nickel. On epicutaneous challenge with the hapten nickel, five additional patients with psoriasis and a known sensitization to nickel (allergic contact dermatitis) (Table 1) had an eczema reaction (Fig. 2B). Histologic analysis of biopsy specimens from fresh psoriasis lesions and from patch test–induced eczema, obtained at the same time, confirmed the clinical diagnosis. Whereas the cellular infiltrate in atopic eczema lesions was dominated by Th2 cells, the infiltrate in allergic contact dermatitis lesions, like the infiltrate in psoriasis lesions, was dominated by Th1 and Th17 cells (Fig. 2C). This finding may explain why psoriasis in combination with allergic contact dermatitis is much more common than psoriasis in combination with atopic eczema. A large percentage of T cells derived from allergic contact dermatitis lesions reacted to nickel in vitro, whereas few T cells were reactive to nickel in psoriasis lesions (Fig. 2D and 2E).

Besides the differences in their cellular infiltrates, atopic eczema lesions and psoriasis lesions could also be distinguished according to skin colonization with microorganisms and filaggrin expression. Namely, all atopic eczema lesions, but not psoriasis lesions, were colonized with *Staphylococcus aureus*, as determined by smear-test culture. Furthermore, filaggrin expression, measured immunohistochemically, was higher in psoriasis lesions than in atopic eczema lesions (Fig. 1A).

DISCUSSION

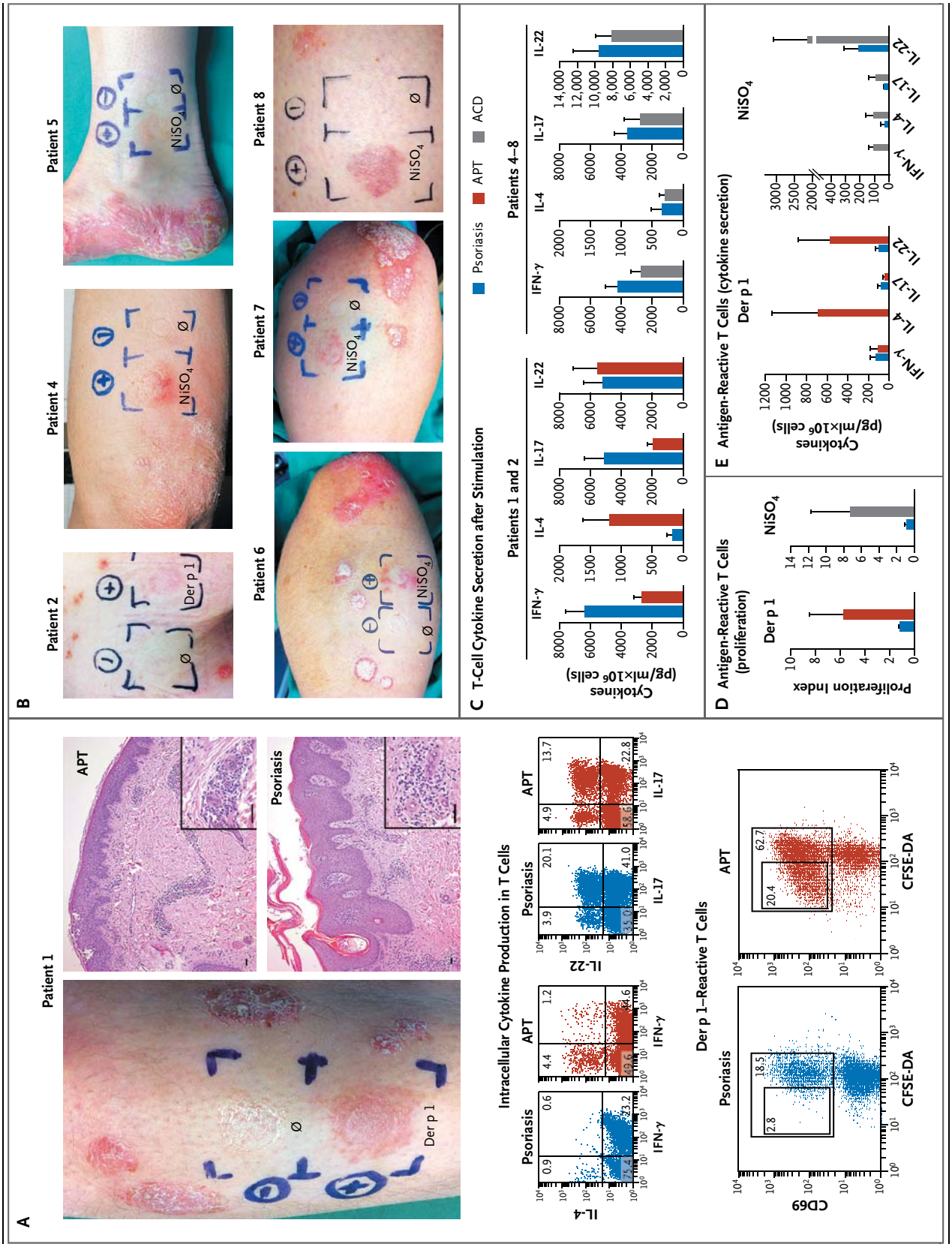
Our findings suggest that an intrinsic epithelial abnormality is not the basis of the pathogenesis of psoriasis or atopic eczema. Rather, specific T cells migrate into the skin in response to distinct antigen triggers and determine the outcome of an inflammatory skin disease — that is, atopic eczema or psoriasis. Thus, antigens differ in the skin reactions they elicit. The specific antigen exposures that lead to psoriasis are undefined.

Besides indicating antigen dependence, our findings shed light on how the local cytokine microenvironment directs cutaneous immunity. The fact that atopic eczema lesions, but not psoriasis plaques, were colonized with *S. aureus* confirms that Th17 and Th1 cells induce an innate immune response in the skin that is partially antagonized by Th2 cells.[8,15] Furthermore, the lower filaggrin expression in atopic eczema lesions is consistent with the finding that Th2 cytokines inhibit expression of the filaggrin gene in vitro.[16]

Formerly, topical glucocorticoids and systemic immunosuppressive drugs were the only therapies for both psoriasis and atopic eczema. An understanding of the molecular basis of both diseases has led to the development of more targeted biologic therapies. The diversity of psoriasis and atopic eczema is reflected by the responses to these targeted biologic agents.

For psoriasis, molecules inhibiting the proinflammatory molecule tumor necrosis factor α (TNF-α) are highly effective. In our study, while Patient 1 was receiving TNF-α antibodies (infliximab, which was discontinued after two injections because of a severe adverse event [temperature up to 39°C, arthritis symptoms, weakness, and vomiting] and adalimumab), his psoriasis lesions

The New England Journal of Medicine
Downloaded from nejm.org at UNIVERSITA TOR VERGATA on July 26, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.



The New England Journal of Medicine
Downloaded from nejm.org at UNIVERSITA TOR VERGATA on July 26, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

Figure 2 (facing page). Antigen-Specific Eczematous Reactions on Epicutaneous Challenge in Patients with Psoriasis and Allergic Sensitization.

Photographs of eczema lesions (within marked area) in close proximity to psoriasis lesions (outside marked area) are shown for Patient 1 in Panel A (top) and for Patient 2, as well as five patients with psoriasis and allergic contact dermatitis (ACD) (Patients 4 through 8), in Panel B (top). The eczema was induced by Der p 1 (the major allergen of *Dermatophagoides pteronyssinus*) in Patients 1 and 2 and by nickel sulfate ($NiSO_4$) in Patients 4 through 8. The null symbol indicates the negative control. Also shown for the lesions in Patient 1 are the histologic features of skin-biopsy specimens (Panel A, top; all scale bars indicate 20 μm), cytokine staining of T cells (Panel A, middle), and the results of flow-cytometric analysis of T cells stained with the fluorescent dye carboxyfluorescein succinimidyl ester diacetate (CFSE-DA) and CD69 (Panel A, bottom; the value in each quadrant indicates the relative frequency among 20,000 cells). Panel C shows T-cell cytokine secretion in psoriasis lesions (blue bars), after atopy patch testing (APT) (red bars), and in allergic contact dermatitis lesions (gray bars). The proliferation index and cytokine secretion of antigen-reactive T cells are shown in Panels D and E, respectively. Values are the means for at least three independent experiments. All T bars are standard errors of the mean. IFN denotes interferon, and IL interleukin.

cleared, but his atopic eczema lesions at other sites were exacerbated (Fig. 1A, clinical course). Thus, blocking a Th1-mediated and Th17-mediated immune disease with a specifically targeted agent may result in a flare of Th2-mediated disease.

Another promising therapy for psoriasis is the Th2 cytokine interleukin-4,[17] which counteracts the effects of interferon-γ and interleukin-17 on keratinocytes.[8,15] However, since Th2 cytokines are overproduced by the cellular infiltrate of atopic eczema lesions, interleukin-4 would not be expected to ameliorate skin symptoms of atopic eczema. In fact, biologic drugs specifically inhibiting one T-cell subset appear to be ineffective for the treatment of co-occurring psoriasis and atopic eczema. Instead, our patients benefited from less specific therapy, aimed at general T-cell suppression: in Patient 1, psoriasis and atopic eczema lesions cleared after receipt of the anti–interleukin-12 p40-subunit antibody ustekinumab, which targets Th1 and Th17 cells (Fig. 1A). Ustekinumab is effective in treating psoriasis,[18] whereas evidence-based data regarding its effect on atopic eczema are lacking. However, since there are reports that Th1 and Th17 respond to microbial antigens (e.g., the *S. aureus* infecting our patients with atopic eczema) and to self-antigens released after cell damage, during the chronic phase of atopic eczema,[1,8] ustekinumab could partially improve atopic eczema lesions. Cyclosporine suppresses all T-cell subpopulations through calcineurin inhibition and was a highly effective treatment for both psoriasis and atopic eczema lesions in Patient 2 (Fig. 1B, clinical course).

In summary, we describe the rare simultaneous occurrence of two supposedly antagonistic diseases, psoriasis and atopic eczema. Within the same patients, distinct T-cell subpopulations were found to infiltrate the same organ: predominantly Th1 and Th17 cells in psoriasis and Th2 cells in atopic eczema. On epicutaneous challenge with eczema-inducing antigens, patients with psoriasis and sensitization against these antigens do not react with an unspecific triggering of psoriasis plaques (Koebner's phenomenon), but typical eczematous lesions containing antigen-reactive T cells do develop. Our observations suggest that distinct, antigen-specific T-cell subsets determine the pathogenesis of psoriasis and atopic eczema.

Supported by grants from Deutsche Forschungsgemeinschaft (EY97/1-2 and WE2678/4-1), Kommission Klinische Forschung of the Klinikum Rechts der Isar Munich, Hochschulwissenschaftsprogramm of the Technische Universität Munich, Bayerische Forschungsstiftung, the National Genome Research Network (01GS0809 and 01GS0818), and the Christine Kühne Center of Allergy Research and Education.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Juliette Kranz for technical assistance.

REFERENCES

1. Bieber T. Atopic dermatitis. N Engl J Med 2008;358:1483-94.
2. Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med 2009;361:496-509.
3. Henseler T, Christophers E. Disease concomitance in psoriasis. J Am Acad Dermatol 1995;32:982-6.
4. Weidinger S, O'Sullivan M, Illig T, et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol 2008;121(5):1203.e1-1209.e1.
5. Ellinghaus E, Ellinghaus D, Stuart PE, et al. Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2. Nat Genet 2010;42:991-5.
6. Darsow U, Vieluf D, Ring J. Atopy patch test with different vehicles and allergen concentrations: an approach to standardization. J Allergy Clin Immunol 1995;95:677-84.
7. Eyerich K, Huss-Marp J, Darsow U, et al. Pollen grains induce a rapid and biphasic eczematous immune response in atopic eczema patients. Int Arch Allergy Immunol 2008;145:213-23.
8. Eyerich K, Pennino D, Scarponi C, et al. IL-17 in atopic eczema: linking aller-

The New England Journal of Medicine
Downloaded from nejm.org at UNIVERSITA TOR VERGATA on July 26, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

gen-specific adaptive and microbial-triggered innate immune response. J Allergy Clin Immunol 2009;123(1):59.e4-66.e4.
9. Eyerich K, Foerster S, Rombold S, et al. Patients with chronic mucocutaneous candidiasis exhibit reduced production of Th17-associated cytokines IL-17 and IL-22. J Invest Dermatol 2008;128:2640-5.
10. Pennino D, Eyerich K, Scarponi C, et al. IL-17 amplifies human contact hypersensitivity by licensing hapten nonspecific Th1 cells to kill autologous keratinocytes. J Immunol 2010;184:4880-8.
11. Eyerich S, Eyerich K, Pennino D, et al. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. J Clin Invest 2009; 119:3573-85.
12. Nograles KE, Zaba LC, Shemer A, et al. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. J Allergy Clin Immunol 2009; 123(6):1244.e2-1252.e2.
13. Weiss G, Shemer A, Trau H. The Koebner phenomenon: review of the literature. J Eur Acad Dermatol Venereol 2002;16: 241-8.
14. Grewe M, Walther S, Gyufko K, Czech W, Schöpf E, Krutmann J. Analysis of the cytokine pattern expressed in situ in inhalant allergen patch test reactions of atopic dermatitis patients. J Invest Dermatol 1995; 105:407-10.
15. Albanesi C, Fairchild HR, Madonna S, et al. IL-4 and IL-13 negatively regulate TNF-alpha- and IFN-gamma-induced beta-defensin expression through STAT-6, suppressor of cytokine signaling (SOCS)-1, and SOCS-3. J Immunol 2007;179:984-92.
16. Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol 2009;124:Suppl 2:R7-R12.
17. Ghoreschi K, Thomas P, Breit S, et al. Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. Nat Med 2003;9:40-6.
18. Griffiths CE, Strober BE, van de Kerkhof P, et al. Comparison of ustekinumab and etanercept for moderate-to-severe psoriasis. N Engl J Med 2010;362:118-28.
Copyright © 2011 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org at UNIVERSITA TOR VERGATA on July 26, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.