

No relationship between skin-infiltrating T_{H2} -like cells and allergen-specific IgE response in atopic dermatitis

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More than 500 $CD4^+$ T-cell clones (TCCs) derived from the skin of eight patients with atopic dermatitis (AD), two patients with nonatopic dermatologic disorders, two patients with allergic rhinitis, and one healthy nonatopic donor were analyzed for both their pattern of cytokine production and their antigen specificity. The proportions of TCCs from patients with AD producing interleukin-4 in response to stimulation with phorbol 12-myristate 13-acetate plus anti- $CD3$ antibody were higher, whereas the proportions of interferon- γ -producing TCCs were lower than those of control subjects. In two patients with AD, the majority of TCCs had a T_{H2}/T_{H0} -like phenotype, whereas in six patients with AD a T_{H1}/T_{H0} -like phenotype was prevalent. TCCs with a T_{H2}/T_{H0} -like phenotype were also isolated from the healthy skin of two patients with allergic rhinitis and one nonatopic donor. In contrast, no T_{H2} -like TCCs were derived from the skin of the two patients with dermatologic disorders of nonallergic origin. No unambiguous correlation was found between the proportions of TCCs producing interleukin-4 or interferon- γ (or of TCCs with T_{H2} - or T_{H1} -like profile) and the level of total serum IgE, suggesting that $CD4^+$ T cells infiltrating the atopic skin do not play a major role in the production of serum IgE antibodies. When TCCs from five patients with AD were examined for their specificity, the proportions of allergen-specific (*Dermatophagoides pteronyssinus* and *Lol p 1*) clones were consistently 6% or lower even in patients with high titers of ryegrass- or *D. pteronyssinus*-specific IgE antibodies. Because similar percentages of allergen-specific TCCs were found in skin from two healthy control subjects, the role of aeroallergens in favoring and maintaining skin lesions in patients with AD remains unclear. (*J ALLERGY CLIN IMMUNOL* 1995;96:411-20.)

Key words: Atopic dermatitis, cytokine, *Dermatophagoides pteronyssinus*, IgE, *Lolium perenne*, skin, T-cell subset

Atopic dermatitis (AD) is an inflammatory skin disease associated with atopy, an inherited predisposition to react with environmental and other allergens, leading to IgE production, hypersensi-

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Abbreviations used

AD:	Atopic dermatitis
APCs:	Antigen presenting cells
AR:	Allergic rhinitis
Dp:	<i>Dermatophagoides pteronyssinus</i>
IFN:	Interferon
IL:	Interleukin
mAb:	Monoclonal antibody
PMA:	Phorbol 12-myristate 13-acetate
TCC:	T-cell clone
TCL:	T-cell line

tivity, and typical skin lesions.¹ Patients with AD, as well as atopic individuals in general, show elevated levels of total IgE in the serum.² It has been presumed that a remarkable proportion of

these IgE antibodies is specific for allergens (inhalant and food allergens) because patients with AD have multiple positive immediate skin reactions to them.³ However, a number of patients (about 20%) have total serum IgE levels in the normal range.⁴ On the other hand, although the capability of human Langerhans cells (besides local mast cells and their precursors) to bind IgE has been suggested to play a role in inducing skin lesions,⁵ the meaning of the elevated serum IgE levels in patients with AD remains unclear. Finally, whether infiltrating T cells in lesional skin can recognize aeroallergens, thus directly contributing through cytokine production to the development and maintenance of both serum IgE levels and skin lesions, is also unknown. In this study we have assessed the functional profile of CD4⁺ T-cell clones (TCCs) generated from the in vivo activated skin-infiltrating T cells of patients with AD. A remarkable proportion of CD4⁺ TCCs from patients with AD and allergic rhinitis (AR) showed a T_{H2}-like phenotype, whereas TCCs from patients with nonatopic dermatologic diseases did not. However, variable proportions of CD4⁺ TCCs also produced interferon (IFN)- γ , a characteristic of T_{H1}-like cells. No correlation was seen between the production of interleukin (IL)-4 or IFN- γ and the levels of total serum IgE. Moreover, the overall prevalence of allergen-specific TCCs was fairly low, and the healthy skin of the patient with AR or the healthy donor was also found to harbor allergen-reactive T cells.

METHODS

Reagents

Dermatophagoides pteronyssinus extract (*Dp*) was obtained from Lofarma Allergeni (Milan, Italy), and purified *Lolium perenne* group I allergen (Lol p 1) from National Institute of Allergy and Infectious Diseases (Research Resources Branch, National Institutes of Health, Bethesda, Md.). Streptokinase was purchased from Boering (Streptase; Behringwerke, Marburg, Germany). Phytohemagglutinin was purchased from Gibco Laboratories (Grand Island, N.Y.). Anti-CD3 monoclonal antibody (mAb) was purchased from Ortho Pharmaceuticals (Raritan, N.J.). Anti-CD4 and anti-CD8 mAb were purchased from Becton-Dickinson (Mountain View, Calif.). Human recombinant IL-2 was a generous gift of Eurocetus (Milan, Italy), and human recombinant IL-5 was purchased from Amersham (Amersham International, England).

Subjects

Skin biopsy specimens were obtained from the lesional areas of eight patients with AD (AD1 to AD8) with different levels of total serum IgE. The diagnosis of

AD was based on the criteria of Hanifin and Rajka.⁶ Other biopsy specimens were derived from the lesional skin of persons with dermatologic disorders of nonallergic origin (NAD patients 1 and 2), from two subjects with AR (AR patients 1 and 2) and from one healthy donor without any skin disorder. NAD1 was a woman, aged 42, with alopecia areata; NAD2 was a man, aged 65, with human immunodeficiency virus-seronegative Kaposi's sarcoma. AR1 was a man, aged 34, whose skin biopsy was performed when he had symptoms of AR caused by ryegrass pollen, and AR2 was a 24-year-old woman with same symptoms. Her biopsy was performed out of the pollen season. The characteristics, cutaneous and respiratory symptoms, and total and specific IgE serum levels of patients with AD and AR are described in Table I. All of the subjects in the study provided written consent.

Processing of biopsy specimens and generation of TCCs

After the skin biopsy specimens (diameter, 5 mm) were washed with sterile saline solution, subcutis was removed with a scalpel. Then, the biopsy specimens were minced and immediately suspended in complete medium (RPMI 1640 medium supplemented with 2 mmol/L L-glutamine, 20 μ mol/L 2-mercaptoethanol, sodium pyruvate [Gibco], nonessential amino acids [Gibco], 3% human serum, and 10% fetal calf serum [Hyclone Laboratories, Inc., Logan, Utah]). In order to favor the expansion of activated T cells possibly present inside the tissue, biptic specimens were cultured in this medium supplemented with rIL-2 (50 U/ml). Recombinant IL-2 (50 U/ml) was then added three times in a week, and cultures were continued for an additional 9 days. To generate TCCs, T blasts from each T-cell line (TCL) were seeded under limiting dilution conditions (0.3 to 1 cell/well) in round-bottomed microwells containing 3×10^5 irradiated (6000 rad) peripheral blood mononuclear cells (as feeder cells) and phytohemagglutinin (1% vol/vol) in a final volume of 0.2 ml of complete medium supplemented with rIL-2 (20 U/ml) as reported elsewhere.⁷ Growing microcultures were then supplemented, at weekly intervals, with rIL-2 (20 U/ml) and 10^5 irradiated feeder cells.

Phenotypic analysis

The phenotype of blasts of each TCC was examined by flow cytometry on a FACStar machine (Becton Dickinson) by using isothiocyanate- and phycoerythrin-conjugated anti-CD4 and anti-CD8 mAbs.

Specific T-cell proliferative response

The proliferative response of TCCs to allergens (Lol p 1: 5 to 10 μ g/ml or *Dp*: 20 μ g/ml) or antigen (streptokinase: 200 U/ml) was assessed by measuring tritiated-thymidine uptake after 60-hour stimulation under major histocompatibility complex-restricted conditions (in

TABLE I. Characteristics, symptoms, and serum IgE levels of patients with AD and AR

Patient	Age (yr)	Sex	Disease activity*	Itching†	Respiratory symptoms‡	Total IgE (IU/ml)	Dp-specific IgE (AU/ml)	Ryegrass-specific IgE (AU/ml)
AD1	50	F	++	+	No	15	<0.35	<0.35
AD2	17	M	+++	+++	Yes	1,685	56.8	9.3
AD3	25	F	+	+++	No	1,915	14.8	<0.35
AD4	21	M	++	++	Yes	2,129	2.2	32.0
AD5	22	M	+++	+++	No	20,133	>100	>100
AD6	40	F	+	++	No	1,413	<0.35	1.4
AD7	38	M	+	+++	No	773	<0.35	>100
AD8	28	M	+++	++	Yes	5,800	<0.35	<0.35
AR1	34	M	—	—	Yes	24	<0.35	14.1
AR2	24	F	—	—	Yes	152	<0.35	28.6

*Overall clinical estimation of disease activity of skin made by the dermatologist treating the patients (scale 0 to +++).

†Subjective estimation (scale 0 to +++).

‡Patients AD2, AD4, AD8, AR1, and AR2 had symptoms of allergic rhinoconjunctivitis.

the presence of autologous antigen-presenting cells [APCs]), as reported elsewhere.⁸ Cultures were performed in triplicate; appropriate controls (phytohemagglutinin stimulation and autologous irradiated peripheral blood mononuclear cells plus unrelated antigen) were also included. When stimulation index (ratio between the mean counts per minute obtained in cultures stimulated with APCs plus antigen and the mean counts per minute obtained in cultures with APCs alone) was greater than 5, responses were considered positive.

Induction of cytokine production by TCCs and quantitation of IL-2, IFN- γ , IL-4, and IL-5 in TCC supernatants

To induce cytokine production, 10^6 T blasts from each TCC were cultured in 1 ml of complete medium in the presence of phorbol 12-myristate 13-acetate (PMA) (10 ng/ml) plus anti-CD3 mAb (100 ng/ml) for 48 hours, after which culture supernatants were collected and stored in aliquots at -70°C until use. To evaluate the ability of TCCs to produce IL-2, 0.2 ml of supernatant was added at different dilutions (1:2 to 1:40) to 4×10^3 indicator cells (CTLL-2 murine cell line), as previously described.⁹ A semiquantitative estimate of IL-2 produced was obtained by a standard curve of rIL-2. The quantitative determinations of IFN- γ and IL-4 were performed in duplicate by a commercial radioimmunoassay (Centocor Inc., Malvern, Pa.) and ELISA (Quantikine R & D Systems, Minneapolis, Minn.), respectively. For the measurement of IL-5, the murine LyH7.B13 cell line was used as a source of indicator cells (a kind gift of Dr. R. Palacios, Basel, Switzerland). A semiquantitative estimate of IL-5 produced was obtained by a standard curve of human IL-5.¹⁰ TCC supernatants showing IFN- γ , IL-2, IL-4, or IL-5 levels 3 standard deviations above the mean levels in control supernatants derived from irradiated stimulated APCs

alone were regarded as positive. For statistical analyses of frequencies of cytokine-producing TCCs, the chi square test was used.

RESULTS

Most activated T lymphocytes infiltrating the lesional skin of patients with AD have a CD4⁺ phenotype

To induce the proliferation of skin-derived activated T cells, biopsy specimens were incubated with IL-2 (50 U/ml). After 4 to 6 days, abundant migration of T lymphocytes could be observed from the biopsy material in all cases. The distribution of CD4 and CD8 surface marker expression in TCLs was examined by flow cytometry. As shown in Table II, TCLs from patients with AD showed percentages of CD4⁺ and CD8⁺ T cells ranging from 53% to 70% and from 7% to 27%, respectively, whereas in control TCLs CD4⁺ T cells ranged from 25% to 90%, and CD8⁺ T cells from 9% to 57%. The TCLs generated from the patient with Kaposi's sarcoma (NAD2) showed a low number of CD4⁺ T cells (25%), the majority being CD8⁺ cells. After the cloning procedure, the proportion of CD4⁺ and CD8⁺ TCCs in each case roughly correlated with the phenotype distribution of the TCL (Table II).

CD4⁺ TCCs generated from skin lesions of patients with AD produce high amounts of IL-4 and no or low amounts of IFN- γ (T_H2-like cells)

The proportion of skin-derived CD4⁺ TCCs from patients with AD producing IL-4 in response

TABLE II. Phenotypic distribution of T-cell subsets in TCLs and clones derived from the skin of patients with AD and of control subjects

Source of cells	TCLs			No. of TCCs tested	TCCs		
	CD4 (%)	CD8 (%)	CD4/CD8		CD4	CD8	CD4/CD8
Patients with AD							
AD1	63	27	2.3	18	12	6	2.0
AD2	53	21	2.5	66	56	17	3.3
AD3	69	20	3.5	42	30	12	2.5
AD4	69	14	4.9	13	9	4	2.3
AD5	59	23	2.6	44	38	6	6.3
AD6	67	7	9.6	73	66	7	9.4
AD7	70	27	2.6	111	75	36	2.1
AD8	55	17	3.2	26	20	6	3.3
Control subjects							
NAD1	78	17	4.6	51	43	8	5.4
NAD2	25	57	0.4	140	35	105	0.3
HD	90	10	9.0	59	54	5	10.8
AR1	61	30	2.0	66	52	14	3.7
AR2	89	9	9.9	37	33	4	8.3

CD4⁺ and CD8⁺ cells were identified by flow cytometry analysis as described in Methods.

NAD, Nonatopic dermatologic disorder; HD, healthy donor.

to 48-hour stimulation with PMA plus anti-CD3 antibody was significantly higher than that found in control specimens ($p < 0.0001$), whereas the percentage of IFN- γ -producing CD4⁺ TCCs from patients with AD was significantly lower ($p < 0.0001$) (Table III). No significant differences were found among the two series of TCCs with regard to the production of both IL-2 (data not shown) and IL-5 (Table III). Of note is that IL-4 and IL-5 were produced independently by several skin-derived CD4⁺ TCCs (including control TCCs) (Fig. 1). This finding is in contrast with the results of previous reports showing associated IL-4 and IL-5 production by CD4⁺ TCCs generated from peripheral blood.¹⁰ For this reason, the categorization of T-helper subtypes into T_{H1}, T_{H0}, and T_{H2}^{8, 10} cannot be easily adapted to skin-derived human TCCs; however, when T_{H1}, T_{H0}, and T_{H2} cells were defined on the basis of IFN- γ and/or IL-4 production only, the proportions of T_{H1} and T_{H2} TCCs from patients with AD were significantly decreased and increased, respectively, in comparison with control subjects ($p < 0.0001$) (Table IV). On the other hand, a significantly higher proportion of IL-5-producing TCCs from patients with AD synthesized IL-4 in comparison with control TCCs ($p < 0.0001$) (Fig. 1). Conversely, a significantly lower number of IL-5-producing TCCs synthesized IFN- γ ($p < 0.0001$), suggesting that in patients

with AD IL-5 was mainly produced by T_{H2}-like cells.

Because TCCs producing IL-4, but little or no IFN- γ , are able to induce B cells to synthesize IgE, we tried to correlate the IL-4 production with serum IgE levels in each patient. Indeed, no direct correlation between serum IgE levels and the proportion of IL-4-producing TCCs ($r = -0.02$, $p = 0.9$) or the proportion of TCCs with T_{H2} profile ($r = -0.5$, $p = 0.2$) or the mean values of IL-4 amounts produced in each patient ($r = -0.15$, $p = 0.3$) was observed. Similarly, no inverse correlation between serum IgE levels and the proportion of IFN- γ -producing TCCs, or the proportion of T_{H1} TCCs or the mean values of IFN- γ produced by TCCs in each patient was found (data not shown).

Low numbers of CD4⁺ TCCs infiltrating the lesional skin from patients with AD are specific for allergens

The specificity of skin-infiltrating CD4⁺ TCCs was evaluated in TCCs from five patients with AD and two control subjects (HD and AR1). As shown in Table V, the proportions of Dp- or Lol p 1-specific TCCs from the skin of patients with AD were similar to those of control subjects and did not show any correlation with the allergen-specific serum IgE levels. In fact, no difference was seen in the proportion of Dp-specific TCCs in subjects

TABLE III. Profile of cytokine production by CD4⁺ TCCs derived from the skin of patients with AD and control subjects

	No. of TCCs tested	No. of cytokine-producing TCCs (%)		
		IL-4	IFN- γ	IL-5
Patients with AD				
AD1	12	10 (83)	3 (25)	12 (100)
AD2	56	37 (66)	21 (38)	49 (88)
AD3	30	13 (43)	24 (80)	18 (60)
AD4	9	5 (56)	6 (67)	5 (56)
AD5	38	24 (63)	33 (87)	9 (24)
AD6	66	40 (61)	47 (71)	43 (65)
AD7	71	38 (54)	29 (41)	66 (93)
AD8	20	10 (50)	10 (50)	12 (60)
All TCCs	302	177 (59)*	173 (57)†	214 (71)‡
Control subjects				
NAD1	43	13 (30)	43 (100)	29 (67)
NAD2	35	18 (51)	35 (100)	11 (31)
HD	53	19 (36)	37 (70)	39 (74)
AR1	52	9 (17)	28 (54)	34 (65)
AR2	33	10 (30)	16 (48)	27 (82)
All TCCs	216	69 (32)*	159 (74)†	140 (65)‡

10⁶ T blasts were stimulated for 48 hours with PMA (10 ng/ml) plus anti-CD3 mAb (100 ng/ml). Cell-free supernatants were then assessed for their cytokine content as described in Methods. Supernatants containing cytokine concentrations at least 3 SDs higher than background values (production by stimulated feeder cells alone) were regarded as positive.

NAD, Nonatopic dermatologic disorder; HD, healthy donor.

* $p < 0.0001$.

† $p < 0.0001$.

‡ $p = \text{NS}$.

displaying high (patients AD2, AD3, and AD5) or undetectable (patients AD6 and AD7) *Dp*-specific IgE serum levels. Moreover, a similar proportion of *Dp*-specific TCCs (4%) was found in TCCs derived from the skin of a healthy control subject without any skin lesions. Accordingly, no correlation between the proportion of Lol p 1-specific TCCs in lesional skin and Lol p 1-specific serum IgE levels in patients with AD was found.

When the 13 allergen-specific TCCs of patients with AD were classified into subtypes according to their cytokine secretion profile (IL-4 and/or IFN- γ), four (three *Dp*- and one Lol p 1-specific TCCs) showed a T_{H2}-like profile, seven (four *Dp*- and three Lol p 1-specific TCCs) showed a T_{H0}-like profile, and two (one *Dp*- and one Lol p 1-specific TCC) synthesized only IL-5 but not IL-4 or IFN- γ . On the contrary, of the four allergen-specific TCCs derived from control subjects, one showed a T_{H2}-like, one a T_{H0}-like, and two a T_{H1}-like profile of cytokine secretion (Table VI). Finally, a small proportion (2%) of TCCs derived from the lesional skin of patients with AD (one TCC from patient AD6 and four TCCs from patient AD7), but none from control subjects, were

specific for streptokinase (Table V). One streptokinase-specific TCC displayed a T_{H1}-like profile, and four displayed a T_{H0}-like profile of cytokine secretion, respectively (data not shown).

DISCUSSION

During the past few years, clear-cut evidence that IgE synthesis is regulated by a network of cytokines has been provided.^{11, 12} In particular, two cytokines appear to play a major role in this regulation: IL-4, which is known to be the essential mediator for IgE synthesis, and IFN- γ , which exerts a negative regulatory activity on IL-4-driven IgE synthesis. Recently, on the basis of different profiles of cytokine production of TCCs, two subsets of helper T cells have been described in both the mouse and human beings.^{9, 10, 13-15} T_{H1}, but not T_{H2}, cells are able to produce IL-2, IFN- γ , and tumor necrosis factor- β ; whereas T_{H2}, but not T_{H1}, cells secrete IL-4 and IL-5. T_{H1}-like cells are responsible for the phagocyte-dependent response, leading to the classical delayed-type hypersensitivity reaction, granuloma formation, and clearance of pathogens such as intracellular bacteria or

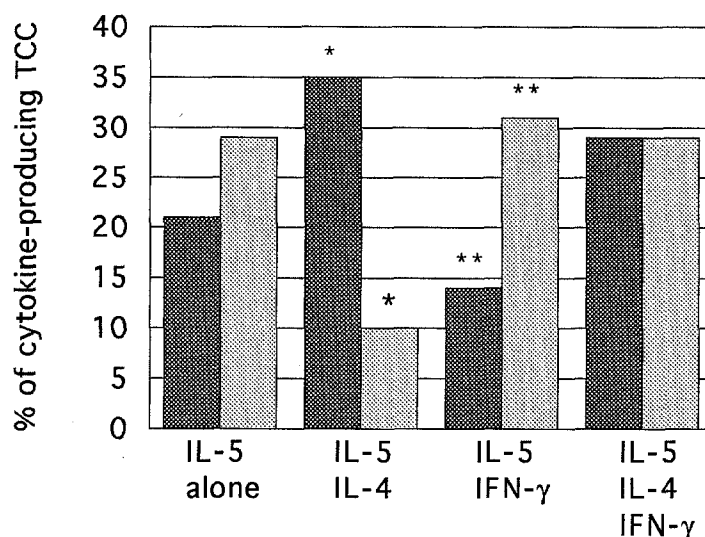


FIG. 1. Distribution of IL-5-producing TCCs obtained from the lesional skin of patients with AD. The percentages of IL-5-producing TCCs from patients with AD (■) and from control subjects (□) were subdivided according to their patterns of cytokine production. The amounts of cytokines were assessed as described in Methods; the cutoff limits are the mean values of irradiated stimulated feeder cells + 3 SDs. * and ** $p < 0.0001$.

viruses.¹⁶ Conversely, T_{H2} -like cells support a very strong humoral (non-phagocyte-dependent) response with the production of IgE isotype and the recruitment of circulating basophils and eosinophils.¹⁶ The existence of other phenotypes showing the secretion of both T_{H1} - and T_{H2} -type cytokines (named T_{H0}) has also been reported.¹⁴

One of the purposes of this study was to establish the role of T-cell subsets infiltrating the skin of patients with AD in determining the elevated serum IgE levels.^{17,18} If such a correlation does exist, persons affected by AD who show high IgE serum levels would have in their skin proportionally more IL-4-producing (or T_{H2}) T lymphocytes than those with low IgE serum levels. Furthermore, because more than 80% of patients with AD have elevated levels of IgE antibodies specific for environmental allergens and immediate-type skin reactivity to them (mainly *Dp*), aeroallergens passing directly through the skin may be involved in the pathogenesis of AD lesions.¹⁹ Thus another aim of this study was to define the frequency and cytokine profile of T cells specific for two very common aeroallergens (i.e., *Dp* and Lol p 1), in the lesional skin of patients with AD.

We have found that in patients with AD, the proportions of IL-4 and IFN- γ -producing TCCs were significantly increased and decreased, respectively, in comparison with those of control TCCs. Accordingly, when T-helper subsets were considered on the basis of their IL-4 and IFN- γ produc-

tion profile, T_{H1} - and T_{H2} -like TCCs were significantly decreased and increased, respectively. This suggests that T cells infiltrating lesional skin in AD mainly belong to the T_{H2} subset. The proportions of T_{H2} cells, however, had large individual variation, ranging from 11% to 70% of all tested TCCs. Of note is that a few or no T_{H2} cells were found in TCCs from nonatopic control subjects, whereas relatively high (15% and 27%) percentages of T_{H2} cells were found among TCCs from atopic patients with respiratory symptoms but no AD. This finding strongly suggests that the prevalence of T_{H2} -type cells in the skin may be a characteristic of atopic subjects rather than limited to those with AD. Even though reduced in comparison with TCCs from control subjects, high proportions of TCCs generated from the skin of patients with AD produced IFN- γ as well. Because IFN- γ has been shown to induce immunopathologic changes similar to those observed in several inflammatory skin diseases,²⁰ including contact sensitivity (which is frequently observed in patients with AD), it is possible that this cytokine also plays a role in the pathogenesis of AD lesions. Indeed, van der Heijden et al.²¹ showed that virtually all skin-derived TCCs (both allergen-specific and nonspecific clones) produced IFN- γ . Similarly, Sager et al.¹⁸ found that the majority of TCCs derived from positive patch test sites of patients with AD secreted IFN- γ with or without IL-4, even though a small proportion of allergen-specific TCCs dis-

TABLE IV. T-helper cell subsets of CD4⁺ TCCs derived from the skin of patients with AD and control subjects

	No. of TCCs tested	No. of T-helper subsets (%)		
		T _{H1} -like	T _{H0} -like	T _{H2} -like
Patients with AD				
AD1	10	0 (0)	3 (30)	7 (70)
AD2	42	5 (12)	16 (38)	21 (50)
AD3	27	14 (52)	10 (37)	3 (11)
AD4	9	4 (44)	2 (22)	3 (33)
AD5	38	14 (37)	19 (50)	5 (13)
AD6	62	22 (35)	25 (40)	15 (24)
AD7	52	14 (27)	15 (29)	23 (44)
AD8	17	7 (41)	3 (18)	7 (41)
All TCCs	257	80 (31)*	93 (36)†	84 (33)‡
Control subjects				
NAD1	43	30 (70)	13 (30)	0 (0)
NAD2	35	17 (49)	18 (51)	0 (0)
HD	42	23 (55)	14 (33)	5 (12)
AR1	33	24 (73)	4 (12)	5 (15)
AR2	22	12 (55)	4 (18)	6 (27)
All TCCs	175	106 (61)*	53 (30)†	16 (9)‡

T-helper subsets were defined according to IFN- γ and IL-4 production (IL-4 alone: T_{H2}-like cells; IFN- γ alone: T_{H1}-like cells; both IL-4 and IFN- γ : T_{H0}-like cells) under PMA plus anti-CD3 mAb stimulation as described in Table III.

NAD, Nonatopic dermatologic disorder; HD, healthy donor.

* $p < 0.0001$.

† $p = \text{NS}$.

‡ $p < 0.0001$.

TABLE V. Specificity of CD4⁺ TCCs derived from the lesional skin of patients with AD and control subjects

	No. of TCC tested	No. of TCCs specific for* (%)		
		<i>Dp</i>	Lol p 1	SK
Patients with AD				
AD2	55	3 (5)	1 (2)	NT
AD3	18	1 (6)	NT	NT
AD5	36	1 (3)	0 (0)	NT
AD6	66	3 (5)	3 (5)	1 (2)
AD7	71	1 (1)	2 (3)	4 (6)
All TCCs	246	9 (4)	6 (2)	5 (2)
Control subjects				
HD	53	2 (4)	0 (0)	0 (0)
AR1	52	0 (0)	1 (2)	0 (0)
All TCCs	105	2 (2)	1 (1)	0 (0)

SK, Streptokinase; NT, not tested; HD, healthy donor.

*Specific proliferative response of T blasts was assessed in the presence of streptokinase or allergens as described in Methods. When the stimulation index was greater than 5, responses were regarded as positive.

played a T_{H1}-like profile of cytokine secretion. More recently, it has been observed that the clinical improvement of skin symptoms in patients with AD after successful therapy was associated with the downregulation of IFN- γ messenger RNA

signals in skin lesions, whereas the expression of IL-4 mRNA remained essentially unchanged.²² On the other hand, the finding that a proportion of TCCs from skin lesions of patients with AD is specific for bacterial antigens suggests that the high

TABLE VI. Cytokine pattern of allergen-specific TCCs derived from lesional skin of patients with AD and control subjects

TCC No.	Specificity	Cytokine production (ng/ml)		
		IL-4	IL-5	IFN- γ
Patients with AD				
AD2.2	<i>Dp</i>	4.0	1.0	18.7
AD2.51	<i>Dp</i>	1.8	3.5	<0.1
AD2.84	<i>Dp</i>	1.1	14.0	<0.1
AD3.26	<i>Dp</i>	0.5	7.0	5.3
AD5.22	<i>Dp</i>	1.2	<0.2	1.7
AD6.7	<i>Dp</i>	7.4	28.0	0.2
AD6.16	<i>Dp</i>	1.6	3.2	6.1
AD6.8	Lol p 1	4.0	8.0	<0.1
AD6.20	Lol p 1	1.4	24.0	4.2
AD6.88	Lol p 1	<0.1	2.8	0.3
AD7.25	<i>Dp</i>	0.1	5.0	0.4
AD7.13	Lol p 1	1.1	14.5	14.0
AD7.70	Lol p 1	4.9	2.0	20.5
Control subjects				
HD.28	<i>Dp</i>	10.7	6.4	14.6
HD.57	<i>Dp</i>	<0.1	<0.2	4.0
HD.63	<i>Dp</i>	4.7	3.0	<0.1
AR1.65	Lol p 1	<0.1	3.2	0.7

HD, Healthy donor.

Allergen specificity of TCCs was evaluated as described in Table V and in Methods. The amounts of IL-4, IL-5, and IFN- γ secreted by allergen-specific TCCs were assessed in supernatants (PMA plus anti-CD3 mAb stimulation) by calibrated ELISA, bioassay, and radioimmunoassay as described in Methods. The mean values of duplicate samples are reported. The cutoff limits for the three cytokines (mean values of irradiated stimulated feeder cells + 3 SDs) were >0.2, >0.4, and >0.4 ng/ml, respectively.

but variable IFN- γ production may reflect an antibacterial response, probably caused by a superinfection with staphylococci or streptococci.

When the production of other cytokines characterizing the T_{H1} or T_{H2} phenotype was analyzed, no difference in the proportion of IL-2- and IL-5-producing TCCs was found between patients with AD and control subjects. It is well known that human T_{H2} cells derived from peripheral blood mononuclear cells produce IL-4 and IL-5,¹⁰ and it has been suggested that the regulation of IL-4 and IL-5 genes, clustered on chromosome 5, may be linked.²³ However, recent reports indicate that this concept may be simplified.^{24, 25} In our study, more than 60% of IL-5-producing TCCs derived from the skin of control subjects did not produce IL-4, whereas in patients with AD IL-5 production was preferentially associated with T_{H2} - but not with T_{H1} -like cells. This suggests that in the lesional skin of patients with AD there is a higher number of "classical" T_{H2} cells contemporarily able to induce and maintain inflammation, based on the ongoing recruitment of mast cells (IL-4) and eosinophils (IL-5) and on several activities of their secreted proteins. Furthermore, of note is that the great

majority of allergen-specific TCCs in the skin produced both IL-4 and IL-5 (associated or not associated with IFN- γ). In any case, the dissociation in the secretion of IL-4 and IL-5 by TCCs generated from the skin remains to be elucidated because this phenomenon may be associated with certain pathophysiologic conditions, such as chronic inflammation in AD.²⁵

The results of this study also showed that there is no significant correlation between the amounts of serum IgE antibodies in each patient and the cytokine profile of skin-infiltrating T cells. Likewise, no relationship was found between the proportions of IL-4-producing TCCs or the mean concentrations of IL-4 produced by TCCs or the percentages of T_{H0} or T_{H2} cells and the serum IgE levels in each patient. Accordingly, no inverse correlation was shown between the serum IgE levels and the proportions of IFN- γ -producing TCCs, or the amounts of IFN- γ produced or the percentages of T_{H1} cells in these subjects. Thus even though the number of patients examined is limited, our findings strongly argue against the simplistic hypothesis of an association between the pattern of cytokine secretion by skin-derived T

cells and serum IgE concentrations in patients with AD. Probably, the levels of IgE antibody in the serum reflect the ability of T cells from draining or other regional lymph nodes rather than that of skin-infiltrating T cells to produce IL-4 (and not IFN- γ). However, because we did not measure IL-13, another T-cell-derived cytokine that is known to be able to induce isotype switching to IgE synthesis,²⁶ in this study, we cannot rule out its role in determining IgE levels in patients with AD.

The role of aeroallergens in the pathogenesis of skin lesions in patients with AD has been recently hypothesized.²⁷ Ramb-Lindhauer et al.¹⁷ have reported that 15% of phytohemagglutinin-induced TCCs derived from epidermal lesional skin of patients with AD proliferate in response to pollen extracts. Furthermore, high proportions (10% and 47%, respectively) of *Dp*-specific TCCs were seen in T cells derived from lesional skin of two *Dp*-sensitive patients with AD with very high serum IgE levels.²¹ Because the proportions of *Dp*-specific TCCs obtained from the peripheral blood of the same patients were lower, the authors favored the conclusion that an accumulation or expansion of allergen-specific T cells occurred in the skin.²¹ In the study by Sager et al.¹⁸ the frequencies of *Dp*-specific T cells derived from positive patch test sites of two patients with AD ranged from 1% to 5%, whereas in the blood the proportion of *Dp*-specific T cells was lower than that in the skin by one order of magnitude. The results of this study of *Dp*- and Lol p 1-specific TCCs partially confirm this latter report,¹⁸ but our values appear to be lower than those reported by the other two authors.^{17, 21} In our study no correlation was found between the proportions of *Dp*- (or Lol p 1-) specific TCCs (or the amounts of IL-4 produced by them) and the values of *Dp*- (or Lol p 1-) specific IgE serum levels in each patient, thus confirming that elevation in IgE levels was not due to the activity of skin-infiltrating T cells. The finding of a proportion of allergen-specific (*Dp*- and Lol p 1) TCCs in the skin of patients with undetectable levels of specific IgE antibodies was also in agreement with such a conclusion. Even though a high proportion of *Dp*-specific T_{H2}-like TCCs has been described at the level of *Dp*-positive patch test sites, thus showing that allergen can quickly expand locally infiltrating allergen-specific T cells or recruit them from the circulation in patients with AD, caution is needed in interpreting these cells as the cause of AD lesions.¹⁹ Indeed, in atopic subjects without AD, *Dp*-specific T_{H2}-like cells can also be recruited and/or strongly ex-

panded after *Dp* skin testing without any sign of atopic lesions.²⁸ Moreover, the fact that allergen-specific TCCs are found in the skin of both healthy subjects and atopic individuals without AD in proportions similar to those of patients with AD argues against a role of these cells in the pathogenesis of skin lesions. Lastly, the hypothesis that antigens and superantigens (mainly derived from gram-positive bacteria) may play a role in generating skin lesions is indirectly supported by the fact that a number of skin-infiltrating TCCs from patients with AD, but not TCCs from control subjects, appear to be specific for bacterial antigens.

Taken together, the results reported here suggest that AD is associated with preferential activation of a T_{H2}-type response at the skin level,²⁹ even though this phenomenon does not appear to be related to the elevation of IgE levels in the serum. The production of cytokines (mainly IL-4 and IL-5) by these T cells may favor the initiation and the maintenance of inflammation in skin, which in many cases appears very early in life. The nature of the stimuli that activate these T_{H2} cells remains unknown. In any case, our results are not in favor of an exclusive role of aeroallergens in the pathogenesis of skin lesions. They rather suggest that several exogenous stimuli, acting together in a genetically determined host, may result in the preferential T_{H2} response.

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