

## ABNORMAL CLONES OF T CELLS PRODUCING INTERLEUKIN-5 IN IDIOPATHIC EOSINOPHILIA

HANS-UWE SIMON, M.D., SABINE GISELA PLÖTZ, M.D., REINHARD DUMMER, M.D., AND KURT BLASER, PH.D.

### ABSTRACT

**Background** The cause of persistent eosinophilia and the hypereosinophilic syndrome is unknown. Recent work suggests that in some patients with the hypereosinophilic syndrome, a clone of abnormal T cells produces large amounts of interleukin-5, a cytokine required for the growth and differentiation of eosinophils. We examined T-cell surface markers, rearranged T-cell-receptor genes, and in vitro production of cytokines by T cells from patients with idiopathic eosinophilia.

**Methods** The expression of surface molecules on T cells was measured by flow cytometry. Cytokine expression was measured by enzyme-linked immunosorbent assay, flow cytometry, and immunohistochemical analysis. To identify dominant (clonal) rearrangements of the T-cell receptor within the lymphocyte population, Southern blot analysis ( $\beta$  chain) and the polymerase chain reaction ( $\gamma$  chain) were performed according to standard protocols.

**Results** Among 60 patients with idiopathic eosinophilia, 16 had circulating T cells with an aberrant immunophenotype. In each of these patients, the abnormal immunophenotype was unique. Evidence of clonal rearrangements of the T-cell receptor was obtained in 8 of the 16 patients. In most instances, the abnormal T cells expressed large amounts of surface proteins associated with T-cell activation (the  $\alpha$  chain of the interleukin-2 receptor and the HLA-DR antigen). Moreover, the aberrant T cells produced large amounts of interleukin-5 in vitro.

**Conclusions** Clonal populations of abnormal T cells producing interleukin-5 occur in some patients with idiopathic eosinophilia. (N Engl J Med 1999;341:1112-20.)

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THERE is increasing evidence that the secondary eosinophilia present in patients with allergic and parasitic diseases is due to the production of cytokines by activated T cells.<sup>1-3</sup> Previous studies have found that in some cases of the hypereosinophilic syndrome (which is characterized by the presence of more than 1500 eosinophils per cubic millimeter for longer than six months; the absence of evidence of parasitic infections, allergic diseases, or other disorders associated with eosinophilia; and the infiltration of tissues by eosinophils),<sup>4</sup> there is clonal expansion of CD3-CD4+ or CD3+CD4-CD8- T cells.<sup>5-7</sup> The CD3+CD4-CD8- immunophenotype is typical of immature T cells, whereas the CD3-CD4+ immu-

nophenotype is abnormal because the CD3 molecule normally occurs on all CD4+ T cells. Furthermore, these T cells were shown to produce large amounts of interleukin-5,<sup>5-7</sup> a cytokine required for the differentiation of eosinophils.<sup>3</sup> Such cases were believed to represent a premalignant lymphoproliferative disorder, but it is unclear how often abnormal T cells occur in persistent eosinophilia.

We assessed 60 patients with idiopathic eosinophilia. Among this group, we identified 16 patients who had T cells with an abnormal immunophenotype. At the time of the initial blood analysis, 15 of these 16 patients had no evidence of a malignant lymphoproliferative disorder. The immunophenotypically abnormal T cells in the blood of these patients produced large amounts of interleukin-5.

### METHODS

#### Patients

A total of 59 adults and 1 child with idiopathic eosinophilia were evaluated during the period from June 1993 to December 1998. Immunologic analysis of blood leukocytes revealed a subgroup of 16 patients in whom T cells with an aberrant immunophenotype were detected.

#### Immunofluorescence Analysis

Peripheral-blood T lymphocytes were analyzed by standard flow-cytometric techniques. Initial screening for T cells with an aberrant immunophenotype was performed with a panel of monoclonal antibodies against the lineage-associated T-cell markers CD2, CD3, CD4, CD5, CD6, CD7, and CD8 in two-color immunofluorescence analysis (all antibodies were obtained from Coulter, Hialeah, Fla.).

The aberrant expression of at least one T-cell surface molecule led to further characterization of these cells. For this purpose, we used the monoclonal antibody that detected decreased or increased staining of the surface marker to identify the abnormal T cells. This antibody was used in combination with fluorescein- or phycoerythrin-conjugated monoclonal antibodies against activation-associated molecules (CD25, HLA-DR, and CD95) and fluorescein-conjugated monoclonal antibodies against several  $V\beta$  families of the T-cell receptor. CD25 is the  $\alpha$  chain of the interleukin-2 receptor, HLA-DR is a family of class II HLA molecules, and CD95 (also called the Fas receptor or APO-1) is a receptor that induces programmed cell death (apoptosis) in activated T cells.

In additional experiments, staining for interleukin-5 in aberrant and CD25+ circulating T cells was performed with two-color fluorescence analysis,<sup>8</sup> in which fluorescein- and phycoerythrin-

From the Swiss Institute of Allergy and Asthma Research, University of Zurich, Davos, Switzerland (H.-U.S., K.B.); the Research Center for Environment and Health, Technical University of Munich, Munich, Germany (S.G.P.); and the Department of Dermatology, University of Zurich, Zurich, Switzerland (R.D.). Address reprint requests to Dr. Simon at the Swiss Institute of Allergy and Asthma Research, University of Zurich, Obere Strasse 22, CH-7270 Davos, Switzerland, or at hus@siaf.unizh.ch.

**TABLE 1.** CLINICAL CHARACTERISTICS OF 16 PATIENTS WITH AN ABERRANT T-CELL IMMUNOPHENOTYPE.

PATIENT No.	SEX/ AGE (YR)	DURATION OF EOSINO-PHILIA	TISSUES WITH INCREASED EOSINOPHILS*	CUTANEOUS PRESENTATION	DAILY PREDNISONE DOSE†	IgE‡
		yr			mg	kU/liter
1	F/47	5	Bone marrow, skin, lungs	Erythroderma	20	12,050
2	M/65	20	Bone marrow, liver, spleen, intestine	No skin symptoms	50	12
3	F/43	7	Bone marrow, skin	Urticarial plaques	20	738
4	M/71	1	Skin	Erythroderma	0	1,645
5	M/54	0.2	Bone marrow, skin, spleen	Reddish papules	0	87
6	M/40	1	Bone marrow, skin	Erythroderma	15	32
7	M/70	5	Bone marrow, skin	Reddish papules	6	56
8	M/55	4	Bone marrow, spleen, lungs	No skin symptoms	<1 (inhaled)	<2
9	F/88	13	Bone marrow, skin	Poikiloderma	20	5
10	M/60	2	Bone marrow, skin	Erythroderma	20	416
11	M/63	8	Bone marrow, skin	Urticarial plaques	60	36
12	F/76	7	Bone marrow, skin	Erythroderma	30	232
13	M/87	1	Skin	Reddish, partly confluent papules	0	115
14	M/55	3	Bone marrow, skin	Erythroderma	50	1,147
15	F/59	5	Skin	Erythroderma	0	200
16	M/5	3	Bone marrow	Erythroderma	0	156

\*The presence of eosinophils was shown by routine histologic examination of biopsy specimens. In all bone marrow–biopsy specimens, increased numbers of mature eosinophils were seen, suggesting increased generation of eosinophils.

†In many cases, prednisone was given to control eosinophilic inflammation, which was mostly evident on the skin but was also present, at least in some patients, on other organs. The indicated daily dose of prednisone was given at the time the immunologic data were obtained.

‡Although some of the patients had elevated serum IgE levels, only Patients 1 and 14 had increased specific IgE levels against common aeroallergens, and only Patient 1 had an IgE-mediated allergic disease (allergic asthma). Patients 2 through 16 did not have evidence of IgE-mediated allergic disease. Normal serum IgE levels are below 130 kU per liter. To convert values for IgE to micrograms per liter, multiply by 2.4.

conjugated anti-CD95 monoclonal antibodies were used (Immunotech, Marseille, France). Surface expression of the  $\gamma\delta$  chains of the T-cell receptor was detected with the following fluorescein-conjugated monoclonal antibodies: anti-V $\beta$ 2 and anti-V $\beta$ 19 (Immunotech) and anti-V $\beta$ 5a, anti-V $\beta$ 5b, anti-V $\beta$ 5c, anti-V $\beta$ 6.7, anti-V $\beta$ 8, and anti-V $\beta$ 12 (T Cell Diagnostics, Cambridge, Mass.). Monoclonal antibodies against the  $\alpha/\beta$  T-cell receptor and the  $\gamma/\delta$  T-cell receptor (TCR- $\delta$ 1) were also used (T Cell Diagnostics), as were R-phycoerythrin–conjugated monoclonal antibodies against interleukin-5 (PharMingen, San Diego, Calif.).

To quantify the expression of lineage-associated surface markers, the mean channel fluorescence levels for each marker on the aberrant T cells and the remaining normal T cells in each patient were compared, and the ratios were calculated as the mean channel fluorescence levels for aberrant T cells divided by the mean channel fluorescence levels for normal T cells. A value of 1.0 indicates normal expression of the marker, a value less than 1.0 indicates decreased expression, and a value greater than 1.0 indicates increased expression.

To quantify the expression of activation-associated surface markers on aberrant T cells, we identified the cells with a monoclonal antibody against a lineage-associated molecule, as described above. The mean channel fluorescence level for each activation-associated

marker was compared with that for the isotype-matched control monoclonal antibody for each patient, and the ratios were calculated as the mean channel fluorescence level for an activation-associated molecule divided by the mean channel fluorescence level for control antibody staining. A value of 1.0 indicates no expression, and a value greater than 1.0 indicates expression. Since increased T-cell activation has been reported in patients with asthma,<sup>9</sup> we determined the relative expression of CD25, HLA-DR, and CD95 in peripheral-blood CD4+ T cells from 10 patients with bronchial asthma for comparison with the patients with eosinophilia.

**Rearrangements of the T-Cell–Receptor Gene**

Southern blot analysis of the gene coding for the  $\beta$  chain of the T-cell receptor was carried out as previously described.<sup>7</sup> To identify clonal rearrangements of the  $\gamma$  chain of the T-cell receptor, we used the polymerase chain reaction (PCR), followed by electrophoresis on a denaturing gel with a formamide–urea gradient.<sup>10</sup> The PCR method incorporated primers for the variable regions 1 to 9, which detect approximately 90 percent of all rearrangements of the T-cell–receptor  $\gamma$  chain. For both Southern blot analysis and the PCR method, DNA preparations from peripheral-blood mononuclear cells were used. Additional DNA

**TABLE 2.** HEMATOLOGIC AND IMMUNOLOGIC FINDINGS IN THE 16 PATIENTS WITH AN ABERRANT T-CELL IMMUNOPHENOTYPE.\*

PATIENT No.	LEUKOCYTES	EOSINOPHILS	LYMPHOCYTES	CD3+CD4+		CD4:CD8 RATIO	CD3-CD16+		CD19+
				cells/mm <sup>3</sup>	percent		percent	percent	
1	11,300	2768	3955	69.8	15.8	4.4	7.6	2.5	
2	10,100	4444	3888	55.0	10.5	5.2	3.0	0.5	
3	8,300	3195	2656	31.9	16.0	2.0	7.3	7.3	
4	9,100	2457	1638	47.1	29.9	1.6	10.0	4.9	
5	13,200	1188	2046	23.7	37.1	0.6	10.3	14.8	
6	12,400	1054	8804	90.0	7.0	12.8	2.0	0.5	
7	8,700	3306	2392	42.2	9.9	4.3	20.6	3.0	
8	7,900	869	2133	11.7	49.3	0.2	12.3	9.2	
9	13,500	5805	810	73.6	11.5	6.4	4.8	3.9	
10	10,600	3180	2438	83.3	8.9	9.4	3.1	1.9	
11	5,100	1326	867	25.0	15.6	1.6	10.9	19.2	
12	10,300	4532	1854	62.0	26.7	2.2	4.2	2.6	
13	10,110	2323	1314	55.1	16.9	3.3	11.5	6.3	
14	10,300	5047	824	59.9	32.9	1.8	2.9	1.9	
15	4,100	1332	738	35.7	7.5	4.7	18.6	11.7	
16	12,000	2760	5700	24.0	29.2	0.8	20.0	17.3	

\*All patients presented with eosinophilia, and Patient 6 presented with an increased lymphocyte count. Lymphocyte subpopulations were identified by two-color immunofluorescence analysis. CD3+CD4+ lymphocytes were considered to be helper T cells (normal range, 26 to 52 percent), CD3+CD8+ lymphocytes were considered suppressor or effector T cells (normal range, 21 to 45 percent), CD3-CD16+ lymphocytes were probably natural killer cells (normal range, 3 to 26 percent), and CD19+ lymphocytes were considered B cells (normal range, 4.5 to 21 percent). The sum of T cells, natural killer cells, and B cells was below 90 percent in many patients, indicating the presence of abnormal T cells, which do not express CD3, CD4, or CD8. For instance, in Patient 3 an additional 33.9 percent of cells were CD3-CD4+ lymphocytes, which do not appear in this table. Another indication of the presence of T cells with an abnormal immunophenotype is a decreased or increased ratio of CD4 cells to CD8 cells (normal range, 1.0 to 3.4), which is apparent in 10 of the 16 patients.

was extracted from purified CD3- T cells with low amounts of CD4 (Patient 7) and biopsy specimens taken from the intestine (Patient 2) and skin (Patients 1, 4, 5, 11, 13, and 15).

#### Expression of Cytokine Genes

Interleukin-5 messenger RNA (mRNA) and protein were measured in peripheral-blood mononuclear cells. Interleukin-5 mRNA was detected by reverse-transcription PCR (RT-PCR),<sup>2,7</sup> and interleukin-5 protein levels were quantified in supernatants from peripheral-blood mononuclear cells by enzyme-linked immunosorbent assay after a 24-hour incubation period without any *in vitro* activation.<sup>7</sup> Levels of interleukin-4 and interferon- $\gamma$  were also measured in these supernatants. Interleukin-5 immunoreactivity was determined in tissue sections by immunohistochemistry with the use of the alkaline phosphatase-anti-alkaline phosphatase technique.<sup>2,11</sup>

## RESULTS

#### Patients

The clinical and hematologic features of the 16 patients we studied are shown in Tables 1 and 2. Only one patient presented with lymphocytosis, and he was subsequently given a diagnosis of the Sézary syndrome. Ten of the patients met the diagnostic criteria for the hypereosinophilic syndrome.<sup>4</sup> The remaining six patients had eosinophilia of unknown

cause, but their conditions did not meet all the criteria for the diagnosis of the hypereosinophilic syndrome. None of the 16 patients had detectable parasitic infection or human immunodeficiency virus infection. Cardiac complications were not observed. Eight patients had elevated serum IgE levels, but only Patient 1 had an IgE-mediated allergic disease (allergic asthma).

Most of the patients were recruited from dermatology clinics. Almost all had pruritic erythroderma, widespread reddish papules, urticarial plaques, or poikiloderma. In most cases, skin biopsies revealed perivascular infiltrations of lymphocytes and eosinophils, with various degrees of epidermal involvement. In 3 of 14 patients, some histologic features of cutaneous T-cell lymphoma were observed.<sup>12</sup> Ten patients were receiving prednisone because of fluctuating symptoms and eosinophilia. The doses of prednisone listed in Table 1 were the amounts given at the time of the immunologic investigations. In all patients, the bone marrow contained numerous mature eosinophils but was otherwise normal. The morphologic features of the peripheral-blood eosinophils were normal in all patients.

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TABLE 3. IMMUNOPHENOTYPE OF ABNORMAL T CELLS IN PATIENTS WITH IDIOPATHIC EOSINOPHILIA.\*

PATIENT No.	IMMUNOPHENOTYPE	LINEAGE-ASSOCIATED T-CELL MARKER†							ACTIVATION-ASSOCIATED T-CELL MARKER‡			FREQUENCY  percentage of all lymphocytes	CONFIRMED MONOCLONALITY§
		CD2	CD3	CD4	CD5	CD6	CD7	CD8	CD25	HLA-DR	CD95		
1	CD2 (low), CD3+, CD4+, CD5 (low), CD25+, CD95+	0.38	1.0	1.0	0.24	1.0	1.0	No	32.8	58.2	9.6	23.3	Yes
2¶	CD3+, CD4-, CD8-, CD5-, CD25+, CD95+	ND	1.0	No	No	ND	ND	No	9.1	12.5	9.6	18.0	Yes
3	CD3-, CD4+, CD5 (high), CD25+, CD95+	1.0	No	1.0	4.1	ND	ND	No	8.9	23.5	9.3	33.9	Yes
4	CD3+, CD4+, Vβ5a+, CD25+, CD95+	1.0	1.0	1.0	1.0	1.0	1.0	No	25.3	19.6	5.8	16.0	No
5	CD3+, CD8+, CD6 (high), CD25-, CD95-	1.0	1.0	No	1.0	4.2	1.0	1.0	No	12.0	No	6.5	No
6	CD2-, CD3+, CD4+, CD25+	No	1.0	1.0	ND	ND	ND	No	10.3	19.0	ND	70.0	Yes
7	CD3-, CD4 (low), CD5 (high), CD25+, CD95+	1.0	No	0.69	5.0	1.0	1.0	No	6.9	19.2	7.5	17.5	Yes
8	CD2 (low), CD3 (low), CD8 (high), CD6 (low), CD7 (low), CD25-, CD95-	0.29	0.32	No	No	0.18	0.79	1.55	No	9.8	No	19.7	No
9	CD3+, CD4+, CD7-, Vβ5c+, CD25+, CD95+	1.0	1.0	1.0	1.0	1.0	No	No	7.1	56.8	67.7	14.5	No
10	CD3+, CD4+, CD25+, CD95-	1.0	1.0	1.0	1.0	1.0	1.0	No	9.4	No	No	50.0	No
11¶	CD3+, CD4-, CD8-, CD25-, CD95-	1.0	1.0	No	1.0	1.0	1.0	No	No	No	No	25.0	No
12	CD3 (low), CD4 (low), CD6 (low), CD7-, CD25+, CD95-	1.0	0.32	0.28	1.0	0.35	No	No	17.3	31.1	No	55.8	Yes
13	CD2 (high), CD3+, CD4+, CD6 (low), CD7-, CD25+, CD95+	2.37	1.0	1.0	1.0	0.21	No	No	11.6	17.6	4.2	18.3	No
14	CD3 (low), CD4+, CD5 (low), CD6 (low), CD7-, Vβ6.7+, CD25+, CD95-	1.0	0.17	1.0	0.12	0.08	No	No	7.2	No	No	32.9	Yes
15¶	CD3+, CD4+, CD5 (high), CD7-, CD25+, CD95+	1.0	1.0	1.0	3.6	1.0	No	No	12.9	34.2	4.3	20.0	No
	CD3+, CD4-, CD8-, CD25+, CD95-	1.0	1.0	No	1.0	1.0	1.0	No	18.7	54.9	No	25.0	No
16	CD3+, CD8+, CD5 (low), CD7 (low), CD25-, CD95-	1.0	1.0	No	0.16	1.0	0.81	1.0	No	No	No	22.0	Yes

\*Lineage-associated and activation-associated T-cell surface-molecule expression was measured in peripheral-blood lymphocytes by two-color immunofluorescence analysis.

†The relative expression of the lineage-associated T-cell surface molecules is given in ratios derived from the mean fluorescence levels recorded for the abnormal and the normal T-cell subpopulations, as described in the Methods section. No denotes no expression, and ND not determined.

‡The relative expression of the activation-associated T-cell surface molecules is given in ratios derived from the mean fluorescence levels for the specific surface marker and the isotype-matched control monoclonal antibody, as described in the Methods section. No denotes no expression, and ND not determined. For comparison, we analyzed the expression of the same activation-associated molecules on peripheral-blood CD4+ T cells of 10 patients with bronchial asthma (mean [±SD]: CD25, 1.3±0.1; HLA-DR, 2.8±0.8; and CD95, 2.1±0.7). Thus, in most cases, the T cells with an aberrant immunophenotype represented highly activated T cells.

§Cells were stained with a panel of anti-Vβ monoclonal antibodies. Binding of specific anti-Vβ monoclonal antibodies was only seen in Patients 4 (Vβ5a), 9 (Vβ5c), and 14 (Vβ6.7), indicating clonal expansion in these cases. To further test whether the abnormal cells represented clonal T cells, we analyzed the rearrangement patterns of the T-cell receptors by Southern blot analysis, polymerase chain reaction, or both using peripheral-blood mononuclear cells or purified aberrant T cells. A confirmation of monoclonality with the use of these techniques was obtained in eight patients only. In addition, clonality studies were performed on skin-biopsy specimens obtained from Patients 1, 4, 5, 11, 13, and 15. All of them were negative.

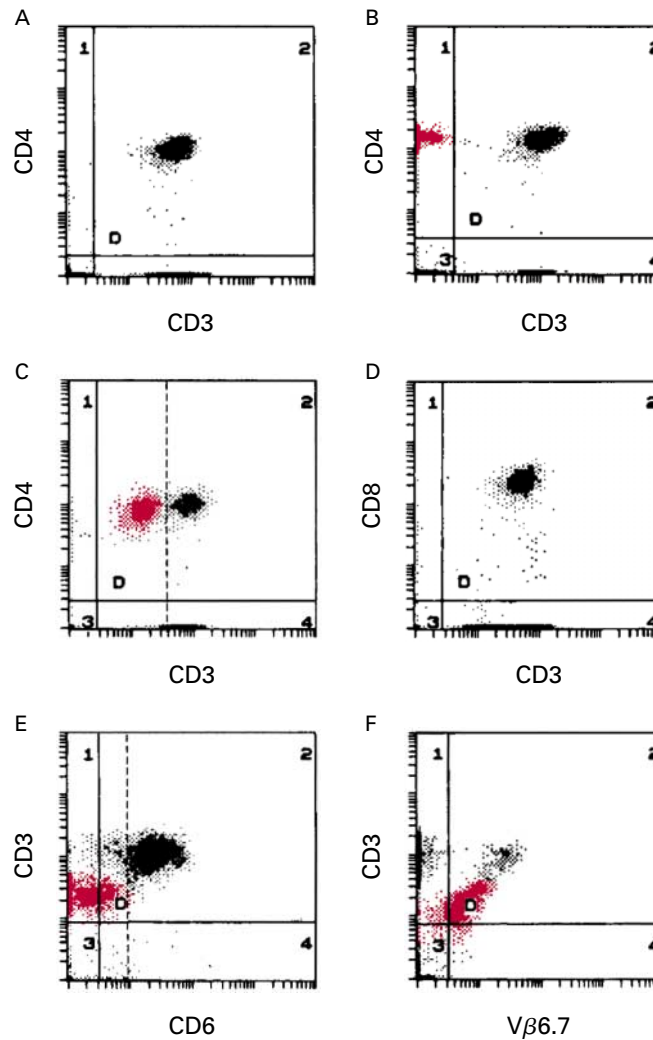
¶The CD3+CD4-CD8- T cells observed in Patients 2, 11, and 15 were positive for α/β T-cell receptors and negative for γ/δ T-cell receptors.

||Patient 15 had two abnormal T-cell subpopulations.

Immunophenotypes of T Cells

Among the 60 patients with idiopathic eosinophilia, 16 had circulating T cells with an abnormal immunophenotype (Table 3). In 10 of these 16 patients, the ratio of CD4 cells to CD8 cells was decreased or increased (Table 2). Direct evidence of the presence of aberrant T cells was obtained by two-

color immunofluorescence assays (Fig. 1). Each patient had a unique subgroup of T cells with an aberrant immunophenotype (Table 3). Nine patients (Patients 1, 4, 6, 9, 10, 12, 13, 14, and 15) had abnormal CD3+CD4+CD8- T cells, three patients (Patients 5, 8, and 16) had abnormal CD3+CD4-CD8+ cells, and three patients (Patients 2, 11, and 15)



**Figure 1.** Identification of T Cells with an Aberrant Immunophenotype in Peripheral Blood by Two-Color Immunofluorescence Assay.

Panel A shows CD3+CD4+ T cells from a normal control subject. A single T-cell subpopulation was observed. Panel B shows CD3+CD4+ T cells from a patient with idiopathic eosinophilia (Patient 3). Approximately 51.5 percent of the CD4+ T cells were CD3- (red). Panel C shows two subpopulations of CD4+ T cells (Patient 14). One had normal CD3 expression, and the other (approximately 55 percent of the CD4+ T cells) expressed a low amount of CD3 (red). The original settings of the analyzer were based on staining of control monoclonal antibody. The dotted line represents the cutoff channel for the separate analysis of the CD4+ cells with low expression of CD3 and normal CD3+CD4+ T cells. The ratio of the CD3 mean channel fluorescence levels of these two CD4+ T subpopulations was 0.17. In contrast with the CD4+ T cells, CD8+ T cells from this patient had normal expression of CD3 (Panel D). The CD4+ T cells with low expression of CD3 had additional immunophenotypic abnormalities. For instance, these T cells also expressed low levels of CD6 (Panel E). The dotted line represents the cutoff for the separate analysis of cells with low expression of CD3 and CD6 and CD3+CD6+ T cells. The ratio of the CD6 mean fluorescence levels of these two T-cell subpopulations was 0.08. Panel F shows that only a small subpopulation of T cells with normal CD3 levels stained with anti-V $\beta$ 6.7 monoclonal antibody (black). In contrast, all T cells with low levels of CD3 expressed a T-cell receptor that belongs to the V $\beta$ 6 family, suggesting clonal expansion (red).

had abnormal CD3+CD4-CD8- cells (Table 3). CD3-CD4+ T cells were found in two patients (Patients 3 and 7). Patient 15 had two populations of aberrant T cells. In 13 patients, abnormal expression of the lineage-associated T-cell markers CD2, CD5, CD6, and CD7 contributed to the overall aberrant immunophenotype. Loss of expression as well as decreased or increased amounts of surface proteins was found in individual patients (Fig. 1 and Table 3). Excluding Patient 6, who had an absolute lymphocyte count of almost 9000 per cubic millimeter, the proportion of abnormal T cells in the remaining 15 patients ranged from 6.5 percent to 55.8 percent of all lymphocytes. In the patient with lymphocytosis, 70.0 percent of the lymphocytes had an aberrant immunophenotype (CD2-CD3+).

Figure 2 and Table 3 show that T cells with an aberrant immunophenotype also had signs of *in vivo* activation, since they expressed increased levels of CD25 (the  $\alpha$  chain of the interleukin-2 receptor), HLA-DR molecules, or both. The expression of CD25 and HLA-DR by these cells was often extremely high as compared with the peripheral-blood CD4+ T cells of patients with bronchial asthma (Fig. 2B and Table 3). Another abnormality was the absence of CD95, the Fas receptor involved in apoptosis, in the aberrant T cells of 8 of 15 patients. In three patients (Patients 4, 9, and 14), we identified a population of monoclonal T cells by flow cytometry; all the T cells with an aberrant immunophenotype in these patients expressed one particular family of V $\beta$  genes (Fig. 1F and Table 3).

In eight patients, the clonal nature of the abnormal T cells was suggested by the results of analysis of the  $\beta$ - and  $\gamma$ -chain genes of the T-cell receptor by Southern blotting or PCR analysis or both (Table 3). In the remaining eight patients, these molecular studies failed to detect monoclonality. Nevertheless, the aberrant T cells of these patients did not bind eight monoclonal antibodies against different V $\beta$  families, suggesting the possibility of oligoclonal populations.

Patient 9 was studied for more than 13 years. During the first two years after the onset of the disease, as ascertained by the diagnosis of pruritic poikiloderma, the number of eosinophils and the ratio of CD4 T cells to CD8 T cells in peripheral blood were within the normal range. Two years later, the CD4:CD8 ratio was 7.7 and the number of peripheral-blood eosinophils was 3082 per cubic millimeter but remained constant for more than nine years. A PCR-based test for the presence of clonal rearrangements of the  $\gamma$  chain of the T-cell receptor, performed eight years after the initial skin symptoms appeared, was negative. After five more years, a monoclonal T-cell population was detected by flow cytometry.

In Patient 14, therapy with interferon alfa-2b (In-

tron, Essex, Munich, Germany) was associated with the disappearance of the atypical clonal T cells within a few weeks. The patient had no evidence of circulating aberrant T cells and had low numbers of eosinophils for about nine months. Then, because of an exacerbation of skin symptoms, the treatment with interferon alfa-2b was stopped. Circulating T cells with the same aberrant immunophenotype reappeared, and malignant T-cell lymphoma was subsequently diagnosed on the basis of a lymph-node biopsy. The transition to malignant T-cell lymphoma was also observed in Patients 2 and 12 and was associated with a rapid increase in the numbers of leukocytes, lymphocytes, abnormal T cells, and eosinophils in peripheral blood. In these three patients, the lymphoma cells had the same immunophenotype as the clonal subpopulation of T cells in the blood.

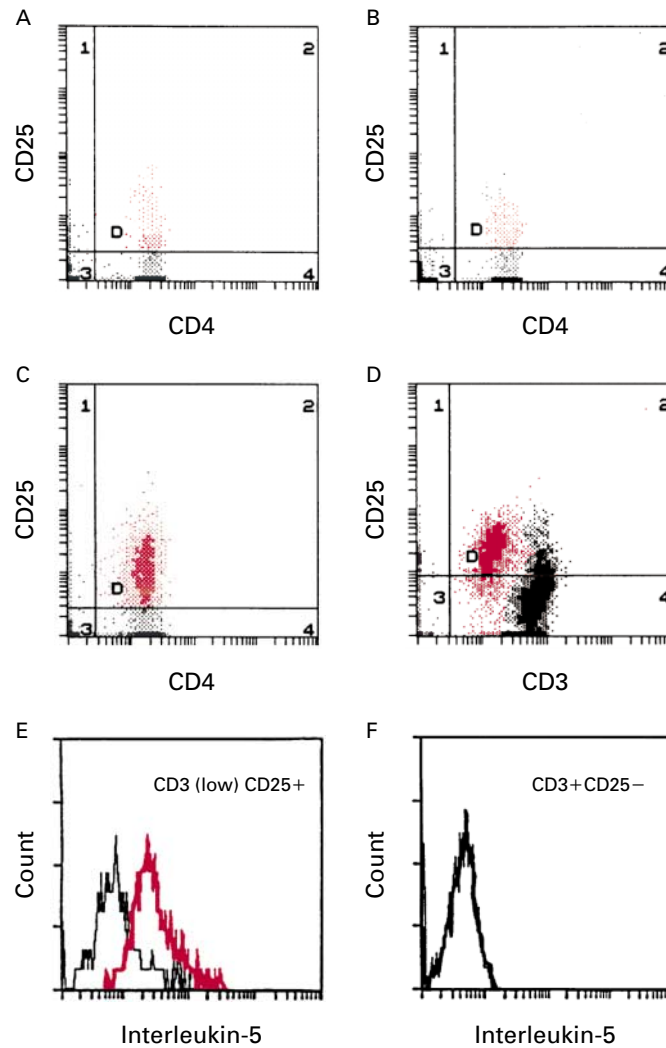
#### Production of Interleukin-5

*In vitro*, unstimulated peripheral-blood mononuclear cells from 12 of 14 patients tested released detectable amounts of interleukin-5 into the supernatant within a 24-hour incubation period (Table 4). In addition, interleukin-5 mRNA was detected in these cells by RT-PCR (data not shown). Interleukin-4, interferon- $\gamma$ , or both were found in the supernatants of cultured peripheral-blood mononuclear cells in six patients (Table 4). Unstimulated peripheral-blood mononuclear cells from control subjects did not release measurable amounts of interleukin-4, interleukin-5, or interferon- $\gamma$  (data not shown). As shown in Figures 2C, 2D, and 2E, monoclonal populations of T cells with high levels of expression of CD25 from Patients 10, 14, and 15 contained high amounts of cytoplasmic interleukin-5, as assessed by flow cytometry. Clonal T cells from Patients 2 and 3 were purified and then cultured. We found high levels of interleukin-5 in supernatants of these cultures (data not shown); T cells with an aberrant immunophenotype were also identified in tissues and found to express interleukin-5 (Fig. 3).

#### DISCUSSION

In this study of 60 patients with idiopathic eosinophilia (median number of eosinophils, 3051 per cubic millimeter), we identified 16 patients with abnormal T cells in peripheral blood. Cultures of the peripheral-blood mononuclear cells of 12 patients revealed detectable amounts of interleukin-5 in the supernatant, and the abnormal T-cell populations of 7 of these patients were directly shown to express high levels of interleukin-5. It is likely that the abnormal T cells producing interleukin-5 caused the eosinophilia in these 16 patients.

The abnormal T cells in the 16 patients were identified by studies of their surface markers. In each case, the constellation of abnormalities of these markers was unique, but in 14 patients the aberrant T cells



**Figure 2.** Analysis of Circulating CD25+ T Cells.

Panel A shows that only a small subpopulation of CD4+ T cells expresses CD25 (red) in normal subjects. Panel B shows a histogram of CD4+CD25+ T cells in a blood sample taken from a patient with bronchial asthma. The percentage of activated T cells (red) in patients with asthma is usually slightly higher than in normal subjects. The mean ( $\pm$ SD) ratio of the mean fluorescence levels for CD25 and an isotype-matched control monoclonal antibody in 10 patients with asthma was  $1.3 \pm 0.1$ . Panel C shows CD4+CD25+ T cells from Patient 14. Staining with CD3 demonstrated that the CD4+ T cells with low amounts of CD3 expressed large amounts of CD25 (Panel D, red). The relative expression of CD25 calculated as a ratio as described above was 7.2. Such highly activated T cells are usually not observed in patients with asthma or atopic dermatitis. The cells were then analyzed by two-color immunofluorescence (Panels E and F). CD25+ T cells with low amounts of CD3 expressed interleukin-5 (Panel E, red). Black lines indicate staining for the control monoclonal antibody. CD3+CD25- T cells did not produce detectable interleukin-5 (Panel F). The line indicating staining for the control monoclonal antibody is superimposed on the line indicating staining for interleukin-5. The relative expression of interleukin-5 in CD25+ T cells with low amounts of CD3 was determined by the ratio of the mean fluorescence levels recorded for monoclonal antibodies against interleukin-5 and the isotype-matched control (1.88). Similar results were obtained for Patients 10 and 15.

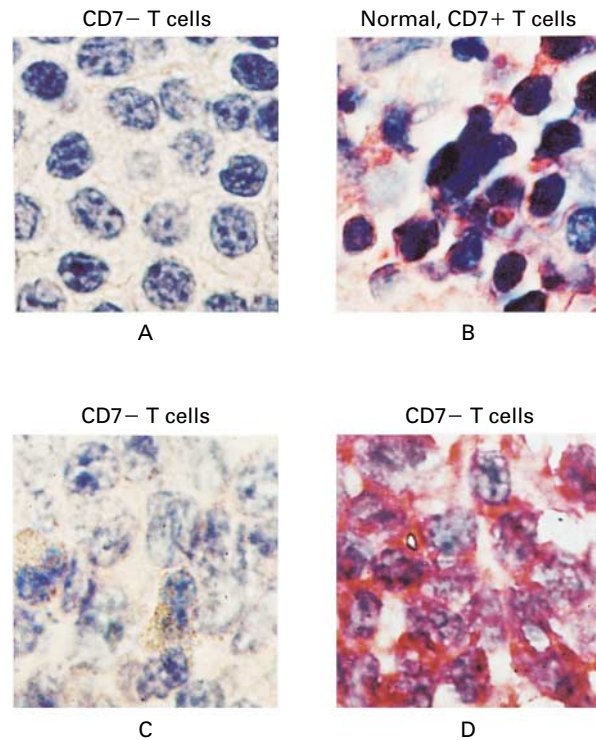
**TABLE 4. CYTOKINE PRODUCTION BY CULTURED PERIPHERAL-BLOOD MONONUCLEAR CELLS.\***

PATIENT No.	INTERFERON- $\gamma$	INTERLEUKIN-4	INTERLEUKIN-5
	picograms per milliliter		
1	<20	<20	222
2	<20	<20	15
3	<20	<20	111
4	39	146	46
5	<20	<20	222
6	ND	ND	ND
7	ND	ND	25
8	ND	<20	<5
9	ND	234	54
10	100	<20	62
11	42	<20	118
12	<20	43	<5
13	<20	<20	7
14	<20	87	117
15	ND	ND	ND
16	ND	ND	6

\*Cytokine concentrations were measured by enzyme-linked immunosorbent assay in supernatants from untreated peripheral-blood mononuclear cells (10 million per milliliter) after a 24-hour incubation period (the concentrations of cytokine released by peripheral-blood mononuclear cells from control subjects were below the limits of detection — i.e., <20 pg per milliliter for interferon- $\gamma$  and interleukin-4 and <5 pg per milliliter for interleukin-5). ND denotes not determined.

had the immunophenotype of activated T cells. The abnormal T cells constituted 6.5 percent to 55.8 percent of the circulating lymphocytes, and in eight patients they had the features of a clonal population. Also in eight patients, the aberrant T cells lacked detectable receptors for the Fas ligand (CD95), which is a mediator of apoptosis. It is likely that CD95-mediated apoptosis, which is essential for homeostasis of lymphocytes,<sup>13</sup> does not function in these patients. Treatment with low-dose interferon- $\alpha$ -2b might induce apoptosis in T cells that lack CD95.<sup>14-16</sup>

It was not possible to demonstrate the presence of clonal T cells in eight patients by molecular techniques, but the presence of an aberrant T-cell immunophenotype implied the presence of a clone. These observations concur with previous work in which clonal rearrangements of the variable-region genes of the T-cell receptor were not found in some well-documented cases of T-cell lymphoma.<sup>17,18</sup> Our PCR technique may not have covered all possible clonal  $\gamma$ -chain rearrangements of variable regions 10 and



**Figure 3.** Expression of Interleukin-5 in T Cells with an Aberrant Immunophenotype ( $\times 1000$ ).

The CD7- T cells of Patient 14 were detected in lymph-node tissue (Panel A). In contrast, normal T cells in tonsillar tissue expressed CD7 (Panel B). The CD7- cells demonstrated clear evidence of expression of interleukin-5 (Panel C), but not granulocyte-macrophage colony-stimulating factor (Panel D). Aberrant T cells expressing interleukin-5 were also detected in the skin of Patients 1 and 9.

12. Another possibility is that the limited sensitivity of the technique precludes the demonstration of a monoclonal cell population in all cases.<sup>18</sup> The lack of detectable clonal rearrangements of T-cell receptors does not rule out the possibility of a premalignant or neoplastic clone of T cells. As we observed in Patient 3, the detection of a clonal gene rearrangement was possible only several years after the identification of peripheral-blood T cells with an aberrant immunophenotype.

Eight patients had some clinical features of the Sézary syndrome, including pruritic scaling erythroderma.<sup>19,20</sup> Indeed, Patient 6 was ultimately given a diagnosis of the Sézary syndrome. However, in all other patients, this diagnosis was ruled out. In our study, we did not observe any CD4+CD7-CD25-T cells, which are typical of cells in patients with the Sézary syndrome.<sup>21,22</sup>

Our results indicate that clonal populations of T cells with an abnormal immunophenotype that secrete interleukin-5 are present in some patients with

idiopathic eosinophilia. It is unknown whether these cells arise in reaction to chronic inflammation (e.g., inflammatory skin disease) or represent the precursors of neoplastic T cells that secrete abnormally high amounts of interleukin-5 (thereby causing eosinophilia). Nevertheless, it may not be coincidental that in 4 of the 16 patients we studied, T-cell lymphoma or the Sézary syndrome was ultimately diagnosed. The fact that the lymphoma cells in three patients had the same immunophenotype as the clonal population of T cells in the blood supports the idea that abnormal T cells in patients with persistent eosinophilia can be the precursors of malignant T cells.

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**CORRECTION**

**Abnormal Clones of T Cells Producing Interleukin-5  
in Idiopathic Eosinophilia**

Abnormal Clones of T Cells Producing Interleukin-5 in Idiopathic Eosinophilia . On page 1119, Panels C and D of Figure 3 were transposed. The CD7<sup>+</sup> T cells showed no expression of granulocyte-macrophage colony-stimulating factor (Panel C) but demonstrated clear evidence of expression of interleukin-5 (Panel D). We regret the error.