

Th2-like CD8⁺ T Cells Showing B Cell Helper Function and Reduced Cytolytic Activity in Human Immunodeficiency Virus Type 1 Infection

By Enrico Maggi, Maria Grazia Giudizi, Roberta Biagiotti, Francesco Annunziato, Roberto Manetti, Marie-Pierre Piccinni, Paola Parronchi, Salvatore Sampognaro, Loretta Giannarini, Giuliano Zuccati,* and Sergio Romagnani

From the Division of Clinical Immunology and Allergy, and the *Division of Dermatology, University of Florence, 50134 Florence, Italy

Summary

We analyzed at clonal level the functional profile of circulating or skin-infiltrating T lymphocytes from two individuals infected with the human immunodeficiency virus type 1 (HIV-1), suffering from a Job's-like syndrome (eczematous dermatitis, recurrent skin and sinopulmonary infections, and hypergammaglobulinemia E) and showing virtually no circulating CD4⁺ T cells. Most of the CD3⁺ T cell clones generated from both patients were CD4⁻ CD8⁺ TCR $\alpha\beta$ ⁺. The others were CD4⁻ CD8⁻ TCR $\alpha\beta$ ⁺ which exhibited reduced mRNA expression for the CD8 molecule or no mRNA expression for either CD4 or CD8 molecules. The great majority of both CD4⁻ CD8⁺ and CD4⁻ CD8⁻ did not produce interferon (IFN) γ and exhibited reduced cytolytic activity. Rather, most of them produced large amounts of both interleukin (IL) 4 and IL-5 and provided B cell helper function for IgE synthesis. These data suggest that a switch of cytolytic CD8⁺ T cells showing a Th1-like cytokine secretion profile to cells that make Th2-type cytokines, exhibit reduced cytolytic potential, and provide B cell helper function can occur in the course of HIV-1 infection. These cells may contribute to the reduced defense against viral infections and intracellular parasites and account for the elevated IgE serum levels, eosinophilia, and the allergic-like clinical manifestations seen in a proportion of HIV-1-infected individuals.

CD8⁺ T cells are a major defense against viral infections and intracellular parasites, as their production of IFN- γ and TNF- β , as well as their cytolytic activity, are key elements in the immune response to these pathogens (1, 2). Recently, however, it was shown that the cytokine profile of CD8⁺ T cell clones derived from patients with lepromatous leprosy was characterized by high levels of IL-4. These clones were similar to murine and human CD4⁺ Th2 cells and were designated type 2 CD8⁺ cells (3). Furthermore, it has been reported that murine CD8⁺ T cells can be primed in vitro to produce IL-4 (4), and when activated in vitro in the presence of IL-4, can switch development to a CD8⁻ CD4⁻ Th2-like phenotype that is not cytolytic and that does not produce IFN- γ (5).

In this report we have examined the functional profile of circulating and skin-infiltrating T lymphocytes from two HIV-infected individuals suffering from a Job's-like syndrome (association of eczematous dermatitis, otitis, and sinopulmonary infections with recurrent skin lesions and hypergammaglobulinemia E) (6-10), showing very low numbers of CD4⁺ circulating T lymphocytes (<50/ μ l of blood). Anal-

ysis at clonal level revealed that in both patients, virtually all CD8⁺ circulating T lymphocytes had switched in vivo from the production of Th1-type cytokine IFN- γ to the production of Th2-type cytokines, IL-4, IL-5, and IL-10. Moreover, high proportions of Th2-type CD4⁻ CD8⁻ TCR $\alpha\beta$ ⁺ cells that expressed reduced mRNA encoding for the CD8 molecule or no mRNA encoding for either CD4 or CD8 molecules were found. Both CD4⁻ CD8⁺ and CD4⁻ CD8⁻ Th2-like clones showed poor cytolytic activity and provided B cell helper function for IgE synthesis.

Materials and Methods

Patients. The study was performed in four HIV-1-infected patients and two HIV-1-seronegative healthy volunteers. According to the Centers for Disease Control (Atlanta, GA) criteria (11), two of the patients were classified in the Group IV Subgroup C-1 because they had suffered from *Pneumocystis carinii* and *Toxoplasma gondii* infection, respectively: one in the Group IV Subgroup D (Kaposi's sarcoma), and one in the Group IV Subgroup C-2 (oral leukoplakia and candidiasis). Two of the four HIV-1-infected patients also had a Job's-like syndrome (recurrent skin lesions caused by *Staph-*

Staphylococcus aureus, repeated episodes of otitis and/or sinusitis, oral candidiasis, and staphylococcal subcutaneous "cold abscesses"). Both patients had high serum IgE levels (7,700 and 3,100 U/ml, respectively) and hypereosinophilia (absolute eosinophil counts 900 and 1,500 cells/ μ l, respectively). They were anamnesticly free of histories of allergic diseases or atopic dermatitis and never had documented staphylococcal infections before their seroconversion to HIV-1. None of them had detectable parasitic infestations. The other two HIV-1-infected patients, as well as the two HIV-1-seronegative healthy individuals, had normal serum IgE levels (<100 U/ml) and normal values of circulating eosinophils (<150 cells/ μ l). Immunophenotyping of PBMC by flow cytometry revealed very low levels of CD4⁺ T cells (<50/ μ l) in all four HIV-1-infected patients but normal values in the two HIV-1-seronegative subjects (1,200 and 1,150 cells/ μ l, respectively). Absolute values of CD8⁺ T cells were within normal range (350–600 cells/ μ l) in both HIV-1-infected patients and healthy subjects. All patients and controls gave informed consent for the studies.

Reagents. PHA was purchased from GIBCO BRL (Gaithersburg, MD) and PMA from Sigma Chemical Co. (St. Louis, MO). Recombinant IL-2 was a kind gift of Eurocetus (Milan, Italy). Recombinant IL-5 and -10 were purchased from Amersham International (Amersham, Bucks, UK) and Genzyme Corp. (Cambridge, MA), respectively. Anti-CD3, -CD4, -CD8, and - α β TCR mAb were purchased from Becton Dickinson & Co. (Mountain View, CA).

T Cell Cloning System. PBMC were obtained from the four HIV-1-infected patients and the two HIV-seronegative healthy volunteers by the Ficoll-Hypaque gradient centrifugation technique, and T cells cloned according to a previously described technique that allows the clonal expansion of virtually every T cell regardless of its antigen specificity (12, 13). Briefly, PBMC were seeded under limiting dilution conditions (0.3 cells/well) in round-bottomed microwells containing 10⁵ irradiated allogeneic spleen cells (as feeder cells) and PHA (1% vol/vol) in a final volume of 0.2 ml

RPMI 1640 medium supplemented with 2 mM L-glutamine, 2 \times 10⁻⁵ M 2-ME (complete medium) containing human recombinant IL-2 (20 U/ml) and 10% FCS (Hyclone Laboratories, Logan, UT). Growing microcultures were then supplemented at weekly intervals with IL-2 (20 U/ml) and 10⁵ irradiated feeder cells.

In two HIV-infected patients (one without and one with Job-like syndrome) T cell clones were also derived from skin biopsy specimens. To this end, biopsy specimens were cultured in complete medium, supplemented with IL-2 (50 U/ml) three times a week, and cultures continued for an additional 9 d. Tissue specimens were then disrupted and viable T blasts resuspended in IL-2-supplemented medium for an additional 3 d. To generate T cell clones, T blasts were seeded under limiting conditions with PHA, feeder cells, and IL-2, as described above.

Immunophenotyping of T Cell Clones. Cell surface marker analysis of T cell clones was performed on a Cytoron Absolute cytofluorimeter (Ortho Pharmaceuticals, Raritan, NJ) by using fluoresceinated or phycoerythrinated anti-CD3, -CD4, -CD8, or - α β TCR, as described (14).

T cell clones lacking both CD4 and CD8 on their surface were analyzed for the expression of CD4 and CD8 mRNA by PCR technology, as described (15). To this end, total cellular RNA was prepared from cells by a single step guanidium isothiocyanate-phenol-chloroform extraction method. The first strand cDNA was synthesized using 4 μ g total RNA, reverse transcriptase (Moloney murine leukemia virus [M-MLV]; GIBCO BRL) and oligodT primer (Pharmacia, Uppsala, Sweden) in a final volume of 40 μ l at 37°C for 1 h. The products thus obtained were denatured and 5 μ l were utilized in the amplification reaction with specific primers for CD4 and CD8 molecules (Clontech Laboratories, Palo Alto, CA). The PCR amplification was carried out with 2.5 Taq polymerase (Perkin Elmer Cetus, Norwalk, CT) and 500 pmol of each primer, and consisted of 30 cycles followed by a 15-min final extension at 72°C. The amplified products were run on a 1.8% agarose gel, as described (15).

Table 1. Surface Phenotype of T Cell Clones Derived from HIV-1-infected Patients with Job's-like Syndrome*

Source of T cells	Absolute numbers of circulating T cells (\times 10 ⁹ /liter)		No. and phenotype of clones obtained		
	CD4 ⁺ CD8 ⁻	CD8 ⁺ CD4 ⁻	CD4 ⁺ CD8 ⁻	CD4 ⁻ CD8 ⁺	CD4 ⁻ CD8 ⁻
HIV-1-seronegative					
Healthy	1.2	0.7	58	32	0
Healthy	1.1	0.5	68	36	0
HIV-1-seropositive					
Group IV-C1	<0.1	0.6	2	22	0
Group IV-D	<0.1	0.4	1	27	1
Skin biopsy			3	35	0
Group IV-C1 and Job's-like syndrome	<0.1	0.7	3	37	18
Group IV-C-2 and Job's-like syndrome	<0.1	0.3	0	40	11
Skin biopsy			1	25	5

* Analysis of the surface phenotype in both fresh circulating T cells and T cell clones was performed on a cytofluorimeter with fluoresceinated anti-CD4 and phycoerythrinated anti-CD8 mAb.

Assessment of Functional Activities of T Cell Clones. The cytokine secretion profile of T cell clones was evaluated by stimulating 10^6 T cell blasts from each clone in 1 ml complete medium with PMA plus anti-CD3 Ab, as reported elsewhere (14–16). After 24 h, culture supernatants were collected and stored at -70°C until used. The quantitative determination of IFN- γ and IL-4 was performed by a commercial RIA (Centocor Inc., Malvern, PA) and a commercial ELISA (British Biotechnology, Oxon, UK), respectively. IL-5 and -10 were quantified by in-house-made capture ELISAs using anti-IL-5 or -IL-10 mAb bound to microwell plates and biotinylated anti-IL-5 or -IL-10 mAbs (Pharmingen, San Diego, CA) as revealing antibodies, respectively. T cell clone supernatants showing IFN- γ , IL-4, -5, or -10 levels 5 SD over the mean levels in control supernatants derived from irradiated feeder cells alone were regarded as positive.

Cytolytic activity of T cell clones was assessed as reported elsewhere (14, 16). Briefly, T cell blasts of each clone were washed three times, counted, resuspended in complete medium, and assayed for cytolytic activity against murine ^{51}Cr P815 mastocytoma cells in the presence of PHA (1% vol/vol) (lectin-dependent assay) or anti-CD3 mAb (100 $\mu\text{g}/\text{ml}$) (redirected system) with an E/T ratio of 1:1. After 4 h at 37°C , 0.1 ml supernatant was removed for measurement of ^{51}Cr release (14, 16).

B cell helper activity for IgE synthesis by T cell clones was evaluated as detailed elsewhere (14). Briefly, 10^5 T cell blasts from each clone were stimulated for 6 h with anti-CD3 mAb and then cocultured for 7 d with 10^5 purified allogeneic B cells. The quantitative determination of IgE in the supernatant was performed by an appropriate RIA (14).

Results

T Cell Clones Generated from HIV-1-infected Subjects with Job's-like Syndrome Are $\text{CD4}^- \text{CD8}^+$ or $\text{CD4}^- \text{CD8}^-$. All clones derived from both HIV-1-infected patients and HIV-1-seronegative individuals were $\text{CD3}^+ \text{TCR}\alpha\beta^+$. As expected, the majority of CD3^+ T cell clones derived from the two HIV-1-seronegative individuals were CD4^+ , the remaining being CD8^+ with a $\text{CD4}/\text{CD8}$ ratio (1.7–1.9) comparable with that found between the two main T cell subsets in freshly prepared blood suspensions (1.7–2.2) (Table 1). This suggests that because of the high efficiency of the cloning technique (>70%), no significant loss of one or another subset had occurred during the cloning procedure. In



Figure 1. Immunophenotyping of T cell clones derived from the peripheral blood of a HIV-infected patient with Job's-like syndrome. (Left) Surface CD4 and CD8 expression determined in six clones (A–F) with flow cytometry after staining with fluorescein-conjugated anti-CD4 and PE-labeled anti-CD8 mAbs. Clones A and B are $\text{CD4}^+ \text{CD8}^+$, whereas the other four clones are $\text{CD4}^- \text{CD8}^-$. (Right) PCR analysis on the same clones (A–F) and on a $\text{CD4}^+ \text{CD8}^-$ clone (G) of mRNA for CD4 (primer 1: nucleotides 1009–1032, sense strand; primer 2: nucleotides 1419–1446, antisense strand; 379 bp) and CD8 (primer 1: nucleotides 776–804, sense strand; primer 2: nucleotides 1126–1154, antisense strand; 438 bp).

contrast, <10% of CD3⁺ T cell clones derived from the four HIV-1-infected patients (showing numbers of circulating CD4⁺ T cells <20/ μ l) were CD4⁺. The great majority of clones were CD8⁺, but in the two HIV-infected patients with the Job's-like syndrome, noticeable proportions of them expressed neither the CD4 nor the CD8 phenotype. Similarly, virtually all clones derived from the skin biopsy specimen of one of the two patients with Job's-like syndrome displayed the CD8⁺ CD4⁻ or the CD8⁻ CD4⁻ phenotype. In contrast, in the other two HIV-1-infected patients, virtually all clones obtained from either blood or skin were CD8⁺ (Table 1).

To establish the nature of CD4⁻ CD8⁻ clones derived from the two HIV-1-seropositive subjects suffering from the Job's-like syndrome, the expression of mRNA for both CD4 and CD8 molecules was evaluated by amplification with PCR. None of the CD4⁻ CD8⁻ clones expressed mRNA for the CD4 molecule. The great majority of them showed a reduced mRNA expression for the CD8 molecule and a minority apparently did not show mRNA expression for either the CD4 or the CD8 molecule (Fig. 1).

CD4⁻ CD8⁺ and CD4⁻ CD8⁻ T Cell Clones Show a Th2-like Cytokine Profile. All CD4⁻ clones from both HIV-1-infected patients and HIV-1-seronegative healthy subjects were then assessed for their cytokine secretion profile after 24-h stimulation with PMA plus anti-CD3 mAb. Clones producing IFN- γ , but no IL-4 or IL-5, were classified as Th1-type, those

producing IL-4 and IL-5, but no IFN- γ were classified as Th2-type, and those showing a mixed cytokine secretion profile were classified as Th0-type (17). The results of these experiments are summarized in Tables 2 and 3. As expected, virtually all CD4⁻ CD8⁺ clones derived from HIV-1-seronegative healthy individuals produced IFN- γ , but no IL-4, -5, or -10 (Th1-type). Similarly, the great majority of CD4⁻ CD8⁺ clones from the two HIV-infected patients who did not suffer from the Job's-like syndrome exhibited a Th1-type profile. In contrast, virtually all CD4⁻ CD8⁺ and CD4⁻ CD8⁻ clones derived from the two HIV-1-infected patients with Job's-like syndrome produced IL-4, -5, and -10, thus exhibiting an opposite (Th2-type) profile. Similarly, the great majority of both CD4⁻ CD8⁺ and CD4⁻ CD8⁻ clones derived from the skin of the patient with Job's-like syndrome were Th2. In contrast, the majority of CD4⁻ CD8⁺ clones derived from the skin of the patient with Kaposi's sarcoma were Th1. However, even in the skin of this patient, high proportions of clones producing IL-4 in addition to IFN- γ (Th0) or IL-4 alone (Th2) were found (Table 2).

CD4⁻ CD8⁺ and CD4⁻ CD8⁻ T Cell Clones Exhibit Reduced Cytolytic Activity and Provide B Cell Helper Function. Cytolytic activity and B cell helper function for IgE synthesis

Table 2. Cytokine Secretion Profile of CD4⁻ T Cell Clones Derived from HIV-1-infected Patients with a Job's-like Syndrome*

Diagnosis	CD8 ⁺			CD8 ⁻		
	Th1	Th0	Th2	Th1	Th0	Th2
HIV-1-seronegative						
Healthy	28	4	0	0	0	0
Healthy	31	5	0	0	0	0
HIV-1-seropositive						
Group IV-C1	18	3	1	0	0	0
Group IV-D	23	2	2	0	1	0
Skin biopsy	16	14	5	0	0	0
Group IV-C1 and Job's-like-syndrome	2	6	29	0	2	16
Group IV-C2 and Job's-like syndrome	6	10	24	1	4	6
Skin biopsy	0	2	23	0	0	5

* T cell clones were stimulated for 24 h with PMA plus anti-CD3 mAb and indicated cytokines measured in their supernatants. T cell clones producing IFN- γ but neither IL-4 nor IL-5 were defined as Th1; T cell clones producing IL-4 and/or IL-5 but not IFN- γ were defined as Th2; T cell clones producing IFN- γ and IL-4 or IL-5 or all three cytokines were defined as Th0.

Table 3. Cytokine Secretion Profile of Some Representative CD4⁻ T Cell Clones from HIV-1-infected Patients with a Job's-like syndrome

T cell clone donor	Cell surface phenotype	Cytokine produced*			
		IFN- γ	IL-4	IL-5	IL-10
<i>ng/ml</i>					
HIV-1-seronegative					
	CD8 ⁺	15.5	<0.1	<0.5	<0.5
	CD8 ⁺	2.5	<0.1	<0.5	<0.5
	CD8 ⁺	9.4	<0.1	<0.5	0.7
	CD8 ⁺	7.0	<0.1	<0.5	<0.5
	CD8 ⁺	13.1	<0.1	<0.5	<0.5
HIV-1-seropositive with Job's-like syndrome					
	CD8 ⁺	<0.5	4.7	9.0	0.7
	CD8 ⁺	<0.5	4.6	9.2	3.4
	CD8 ⁺	<0.5	2.3	3.3	<0.5
	CD8 ⁺	<0.5	4.7	1.9	1.3
	CD8 ⁺	<0.5	1.8	1.5	0.7
	CD8 ⁻	<0.5	2.0	1.7	0.6
	CD8 ⁻	<0.5	1.4	9.2	3.2
	CD8 ⁻	<0.5	4.5	8.5	0.9

* 10⁶ T cell blasts from each clone were stimulated for 24 h with PMA plus anti-CD3 mAb and indicated cytokines measured in supernatants by appropriate RIA and ELISA, as reported in Materials and Methods.

were assessed in 18 randomly selected CD4⁻ CD8⁺ clones from the four HIV-1-infected patients and in eight of the two HIV-1-seronegative healthy controls. The great majority of CD4⁻ CD8⁺ clones from the two HIV-1-infected patients with the Job's-like syndrome, showing the Th2-type cytokine secretion profile, exhibited significantly reduced ($p < 0.01$) lectin-dependent or redirected cytolytic activity in comparison with CD4⁻ CD8⁺ clones from both HIV-1-seronegative individuals and HIV-1-seropositive patients without the Job's-like syndrome and showing a Th1-like phenotype. Furthermore, after stimulation with anti-CD3 mAb, they all provided helper function for IgE synthesis in normal B cells, whereas none of the Th1-type CD8⁺ clones derived from the other patients or controls did. Table 4 shows the results obtained with 14 representative clones.

Table 4. Cytolytic Activity and B Cell Helper Function for IgE Synthesis of Some Representative CD4⁻ T Cell Clones from the HIV-1-infected Patients with a Job's-like Syndrome

T cell clone donor	Cell surface and cytokine secretion profile*	Cytolytic activity (percent ⁵¹ Cr release ± SE)†	Helper Function for IgE synthesis‡
HIV-1-seronegative			ng/ml
	CD8 ⁺ Th1	60 ± 5	<0.1
	CD8 ⁺ Th1	55 ± 4	<0.1
	CD8 ⁺ Th1	65 ± 3	<0.1
	CD8 ⁺ Th1	85 ± 7	<0.1
	CD8 ⁺ Th1	48 ± 5	<0.1
	CD8 ⁺ Th1	75 ± 6	<0.1
HIV-1-seropositive with Job's-like syndrome			
	CD8 ⁺ Th2	37 ± 4	7.2
	CD8 ⁺ Th2	34 ± 3	3.5
	CD8 ⁺ Th2	25 ± 4	5.3
	CD8 ⁺ Th2	45 ± 4	2.1
	CD8 ⁺ Th2	42 ± 3	6.3
	CD8 ⁻ Th2	22 ± 2	4.8
	CD8 ⁻ Th2	23 ± 3	7.2
	CD8 ⁻ Th2	17 ± 2	3.1

* Cell surface phenotype was evaluated as reported in the legend of Table 1. Cytokine secretion profile was defined as reported in the legend to Table 1.

† 5×10^3 effector cells were incubated in triplicate with 5×10^3 ⁵¹Cr-labeled P815 mastocytoma cells for 4 h at 37°C in the presence of PHA (1% vol/vol). The ⁵¹Cr release was calculated as described (14).

‡ 10^5 T cell blasts were activated for 6 h with anti-CD3 mAb and incubated for 10 d with 10^5 purified allogeneic B cells. IgE protein was measured in the supernatant by RIA. IgE protein in supernatants of B cells alone was consistently 0.1 ng/ml.

Discussion

Until recently, the CD8⁺ T cell has been seen as an effector cell playing a fundamental role in protection against infection by viruses and intracellular parasites (1, 2). Indeed, their key functions of cytolytic activity and production of IFN- γ and TNF- β were considered their main activities (1, 2). It would be very serious for the host if the CD8⁺ T cells started to produce Th2-type cytokines. This is well demonstrated by the observation that *Schistosoma mansoni* infection, which induces production of Th2 cytokines, was found to impair the CD8 T cell cytolytic activity of the host against *Vaccinia* virus and so, delay clearance (18). Moreover, subsets of CD8⁺ T cells producing Th2-type cytokines have been isolated from patients with the highly aggressive (lepromatous) form of *Mycobacterium leprae* infection (3).

Clinical symptoms of a relative dominance of Th2-mediated responses have been described in HIV-1-infected individuals and include eosinophilia (18), elevation of serum IgE levels (19–21), and allergic manifestations (22). In some HIV-1-infected individuals, the association of eczematous dermatitis, sinopulmonary infections, recurrent skin lesions, hypergammaglobulinemia E, and eosinophilia, has also been reported (8–10), which is reminiscent of the rare primary immunodeficiency disorder, commonly known as Job's, Buckley's, or hyper-IgE syndrome (6, 7). These phenomena are compatible with the recently proposed hypothesis that a shifting of CD4⁺ Th cells from the Th1 to Th2 phenotype occurs in the course of HIV-1 infection and can favor the progression of HIV infection toward the full-blown disease (23).

The finding that the Job's-like syndrome usually develops in the advanced phases of the HIV-1 infection and in patients showing very low numbers of circulating CD4⁺ Th cells (8–10) argues, however, against this simplistic interpretation. Accordingly, in the two patients reported in this study, circulating CD4⁺ T cells were $<50/\mu\text{l}$, suggesting that other cell types would be involved in the production of IL-4 and IL-5 responsible for so elevated serum IgE levels ($>3,000$ IU/ml) and eosinophilia (>500 cells/ μl), respectively, found in the same patients. This view was supported by the results of functional analysis of circulating T cells at clonal level. Indeed, virtually all CD4⁻ CD8⁺ T cell clones derived from both patients showed a clear-cut Th2-type profile of cytokine secretion, whereas the great majority of CD4⁻ CD8⁺ T cell clones obtained from the HIV-1-seronegative healthy individuals, as well as the HIV-1-seropositive patients with similarly reduced numbers of circulating CD4⁺ T cells, but no Job's-like syndrome, had an opposite (Th1-like) profile. Thus, the CD8⁺ Th2-type cells may account for both hyper-IgE (via IL-4 and, possibly, IL-13 production) and hyper-eosinophilia (via IL-5 production) seen in the HIV-1-infected patients with the Job's-like syndrome. It is also noteworthy that these CD8⁺ T cells exhibited markedly reduced cytolytic activity and were unable to produce not only IFN- γ , but also TNF- α and - β (data not shown), whereas a noticeable proportion of them produced IL-10, a powerful inhibitor, together with IL-4, of macrophage inflammatory functions (24, 25). These latter alterations may account for the undue suscepti-

bility to bacterial and fungal infections seen in these patients. In this respect, it is also of note that the majority of T cells present in the skin biopsy specimen of one of the two patients were noncytolytic CD8⁺ clones showing a Th2-like cytokine profile. These cells may be directly involved in the genesis of recurrent skin infections.

Another interesting finding emerging from this study is the demonstration that unusually high numbers of CD4⁻ CD8⁻ T cell clones could be generated from the peripheral blood of both patients. Even these clones, as the CD4⁻ CD8⁺ ones, exhibited a Th2 profile and displayed poor or no cytolytic activity. Analysis of mRNA expression revealed that the majority of them were CD8⁺ T cells that have lost the ability to express the CD8 molecule on their surface, whereas a minority failed to express mRNA for both CD4 and CD8 molecules. These cells strongly resemble CD4⁻ CD8⁻ cells resulting from the switching of CD8⁺ cells incubated in vitro with high IL-4 concentrations (5), suggesting that the CD4⁻ CD8⁻, Th2-like, noncytolytic T cell clones present in HIV-infected patients with the Job's-like syndrome may result from in vivo switching of CD8⁺, Th1-like, cytolytic T cells probably caused by high IL-4 concentrations present in the microenvironment.

The mechanisms accounting for the alterations of CD8⁺ T cells, and in particular the source of IL-4 possibly responsible for their switch to the Th2 profile, are at present unclear. Recently, it has been shown that HIV-1 infection can induce a marked defect in macrophage production of IL-12 (26) and IFN- α (27), which are powerful Th1-inducing agents (28-30). On the other hand, in the murine models, double negative (CD4⁻ CD8⁻ TCR $\alpha\beta$ ⁺) thymocytes have been implicated in providing IL-4 (31), which is critical for the differentiation of naive or memory CD4⁺ T cells into the Th2 subset (16, 32, 33). Further investigation is required to determine whether at least a proportion of the double negative cells here observed represent the equivalent of CD4⁻ CD8⁻ TCR $\alpha\beta$ ⁺ cells described in the mouse (31). The reason why a so dramatic Th1 to Th2 switch occurs only in CD8⁺ T cells from a small proportion of HIV-1-infected patients (for example whether this propensity has some genetic cause) also needs to be elucidated. The understanding of these mechanisms may indeed have wider implications in designing new strategies for the treatment of HIV-1 infection.

This work was supported by grants from the "Istituto Superiore di Sanità" (AIDS Project 1993), the Associazione Italiana Ricerca Cancro, and Consiglio Nazionale Ricerche (Targeted Projects on "Prevention and Control of Disease Factors 94.00637.PF41" and on "Clinical Application of Research in Oncology 93.02233.PF39").

Address correspondence to Dr. S. Romagnani, Istituto di Clinica Medica 3, viale Morgagni 85, 50134 Florence, Italy.

Received for publication 3 February 1994 and in revised form 8 April 1994.

References

1. Kelso, A., A.L. Glasebrook, O. Kanagawa, and K.T. Brunner. 1982. Production of macrophage-activating factor by T lymphocyte clones and correlation with other lymphokine activities. *J. Immunol.* 129:550.
2. Fong, T.A., and T.R. Mosmann. 1990. Alloreactive murine CD8⁺ T cell clones secrete the Th1 pattern of cytokines. *J. Immunol.* 144:1744.
3. Salgame, P., J.S. Abrams, C. Clayberger, H. Goldstein, J. Con-vitt, R.L. Modlin, and B.R. Bloom. 1991. Differing lymphokine profiles of functional subsets of human CD4⁺ and CD8⁺ T cell clones. *Science (Wash. DC)*. 254:279.
4. Seder, R.A., J.-L. Boulay, F. Finkelman, S. Barbiezt, S.Z. Ben-Sasson, G. Le Gros, and W.E. Paul. 1992. CD8⁺ T cells can be primed in vitro to produce IL-4. *J. Immunol.* 148:1652.
5. Erard, F., M.-T. Wild, J.A. Garcia-Sanz, and G. Le Gros. 1993. Switch of CD8 T cells to noncytolytic CD8⁻ CD4⁻ cells that make Th2 cytokines and help B cells. *Science (Wash. DC)*. 260:1802.
6. Davis, S.D., J. Schaller, and R.J. Wedgwood. 1966. Job's syndrome: recurrent "cold" staphylococcal abscesses. *Lancet.* 1:1013.
7. Buckley, R.H., B.B. Wray, and E.Z. Belmaker. 1972. Extreme hypergammaglobulinemia E and undue susceptibility to infection. *Pediatrics.* 49:59.
8. Lyn, R.Y., and J.K. Smith. 1988. Hyper-IgE and human immunodeficiency virus infection. *Ann. Allergy.* 61:629.
9. Raiteri, R., A. Sinicco, P. Gioannini, F. Picciotto, G. Marietti, D. Novero, and M. Pippione. 1993. Job's-like syndrome in HIV-1 infection. *J. Dermatol.* 3:355.
10. Paganelli, R., E. Scala, I.J. Ansotegui, I. Mezzaroma, E. Pinter, P. Ferrara, G.P. D'Offizi, and F. Aiuti. 1993. Hyper-IgE syndrome induced by HIV infection. *Immunodeficiency.* 4:149.
11. Centers for Disease Control and Prevention. 1987. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *Morbid. Mortal. Wkly. Rep.* 35:334.
12. Moretta, A., G. Pantaleo, L. Moretta, J.C. Cerottini, and M.C. Mingari. 1983. Direct demonstration of the clonogenic potential of every human peripheral blood T cell: clonal analysis of HLA-DR expression and cytolytic activity. *J. Exp. Med.* 157:743.
13. Maggi, E., P. Parronchi, D. Macchia, G. Belleli, and S. Romagnani. 1988. High numbers of CD4⁺ T cells showing abnormal recognition of DR antigens in lymphoid organs in

- volved by Hodgkin's disease. *Blood*. 71:1503.
14. Del Prete, G.-F., M. De Carli, M. Ricci, and S. Romagnani. 1991. Helper activity for immunoglobulin synthesis of T helper type 1 (Th1) and Th2 human T cell clones: the help of Th1 clones is limited by their cytolytic capacity. *J. Exp. Med.* 174:809.
 15. Del Prete, G.-F., M. De Carli, F. Almerigogna, M.-G. Giudizi, R. Biagiotti, and S. Romagnani. 1993. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J. Immunol.* 150:353.
 16. Maggi, E., P. Parronchi, R. Manetti, C. Simonelli, M.-P. Piccinni, F. Santoni-Rugiu, M. De Carli, M. Ricci, and S. Romagnani. 1992. Reciprocal regulatory role of IFN- γ and IL-4 on the in vitro development of human TH1 and TH2 clones. *J. Immunol.* 148:2142.
 17. Street, N.E., J.H. Schumaker, T.A.T. Fong, H. Bass, F.D. Fiorentino, J.A. Leverah, and T.R. Mosmann. 1990. Heterogeneity of mouse helper T cells: evidence from bulk cultures and limiting dilution cloning for precursors of TH1 and TH2 cells. *J. Immunol.* 144:1629.
 18. Actor, J.K., M. Shirai, M.C. Kullberg, R.M.L. Buller, A. Sher, and J.A. Berzofsky. 1993. Helminth infection results in decreased virus-specific CD8⁺ cytotoxic T-cell and Th1 cytokine responses as well as delayed virus clearance. *Proc. Natl. Acad. Sci. USA.* 90:948.
 19. Fleury-Feith, J., J.T. Ven Nhieu, C. Picard, E. Escudier, and J.F. Bernaudin. 1992. Bronchoalveolar lavage eosinophilia associated with *Pneumocystis carinii* pneumonitis in AIDS patients. *Chest.* 95:1198.
 20. Maggi, E., M. Mazzetti, A. Ravina, C. Simonelli, P. Parronchi, D. Macchia, P. Biswas, M. Di Pietro, and S. Romagnani. 1989. Increased production of IgE protein and IgE antibodies for fungal antigens in patients with acquired immunodeficiency syndrome (AIDS). *Res. Clin. Lab.* 19:45.
 21. Wright, D.N., R.P. Nelson, D.K. Ledford, E.F. Caldes, W.L. Trudeau, and R.F. Lockey. 1990. Serum IgE and human immunodeficiency virus (HIV) infection. *J. Allergy Clin. Immunol.* 85:445.
 22. Butkus Small, C., A. Kaufman, M. Armenaka, and D.L. Rosenstreich. 1993. *J. Infect. Dis.* 167:283.
 23. Clerici, M., and G.M. Shearer. 1993. A TH1 and TH2 switch is a critical step in the etiology of HIV infection. *Immunol. Today.* 14:107.
 24. Powrie, F., S. Menon, and R.L. Coffman. 1993. Interleukin-4 and interleukin-10 synergize to inhibit cell-mediated immunity in vivo. *Eur. J. Immunol.* 23:3043.
 25. Moore, K.W., A. O'Garra, R. de Waal Malefyt, P. Vieira, and T.R. Mosmann. 1993. Interleukin-10. *Annu. Rev. Immunol.* 11:165.
 26. Chehimi, J., S.E. Starr, I. Frank, A. D'Andrea, X. Ma, R.R. MacGregor, J. Sennelier, and G. Trinchieri. 1994. Impaired interleukin 12 production in human immunodeficiency virus-infected patients. *J. Exp. Med.* 179:1361.
 27. Gendelman, H.E., R.M. Friedman, S. Joe, L.M. Baca, J.A. Turpin, G. Dvekster, M.S. Meltzer, and C. Dieffenbach. 1990. A selective defect of interferon α production in human immunodeficiency virus-infected monocytes. *J. Exp. Med.* 172:1433.
 28. Parronchi, P., M. De Carli, R. Manetti, C. Simonelli, S. Sampognaro, M.-P. Piccinni, D. Macchia, E. Maggi, G.F. Del Prete, and S. Romagnani. 1992. IL-4 and IFN (α and γ) exert opposite regulatory effects on the development of cytolytic potential by Th1 and Th2 human T cell clones. *J. Immunol.* 149:2977.
 29. Manetti, R., P. Parronchi, M.-G. Giudizi, M.-P. Piccinni, E. Maggi, G. Trinchieri, and S. Romagnani. 1993. Natural killer stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J. Exp. Med.* 177:1199.
 30. Hsieh, C.-S., S.E. Macatonia, C.S. Tripp, S.F. Wolf, A. O'Garra, and K.M. Murphy. 1993. Development of Th1 CD4⁺ T cells through IL-12 produced by Leisteria-induced macrophages. *Science (Wash. DC).* 260:547.
 31. Zlotnik, A., and A.G.D. Bean. 1993. Production of IL-4 by non-Th2 T-cell subsets: possible role of CD4⁻ CD8⁻ $\alpha\beta$ TCR⁺ and CD4 subset T cells in T helper subset regulation. *Res. Immunol.* 144:606.
 32. Swain, S.L., A.D. Weinberg, M. English, and G. Huston. 1990. IL-4 directs the development of Th2-like helper effectors. *J. Immunol.* 145:3796.
 33. Seder, R.A., W.E. Paul, M.M. Davis, and B.F. de St. Groth. 1992. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4⁺ T cells from T cell receptor transgenic mice. *J. Exp. Med.* 176:1091.