Increased levels of serum IgE and eosinophilia have been described in human immunodeficiency virus (HIV) infection, almost exclusively in patients with CD4+ cell count <200 cells/µl. IgE production is regulated by CD4+ T helper type 2 (Th-2) lymphocytes, producing interleukin 4 (IL-4) and expressing a ligand for the B cell-specific CD40 molecule (CD40 ligand [L]). A shift to a Th-2-like pattern of cytokine secretion has been postulated to be associated with progression toward acquired immunodeficiency syndrome (AIDS). We studied three AIDS patients with very high levels of IgE and almost complete depletion of CD4+ lymphocytes, suggesting that IgE synthesis could not be driven by CD4+ cells. IgE in vitro synthesis by cells from such patients was, however, inhibited by anti-IL-4. We show that both CD8+ T cell lines and the majority of CD8+ T cell clones derived from these patients produce IL-4, IL-5, and IL-6 in half of the cases together with interferon γ (IFN-γ). 44% of CD8+ T cell clones expressed a CD40L, and the supernatants of the clones were capable of inducing IgE synthesis by normal B cells costimulated with anti-CD40. CD8+ T cells in these patients therefore functionally mimic Th-2 type cells and may account for hyper-IgE and eosinophilia in the absence of CD4+ cells. The presence of such CD8+ cells may also provide a source of IL-4 directing the development of predominant Th-2 responses in HIV infection.
both IL-4 and IL-5 by CD8+ T cells from these subjects, as well as the ability to express a CD40 ligand (CD40L), and to stimulate IgE synthesis by normal B lymphocytes, indicate that these cells can functionally mimic CD4+ Th-2 cells and account for the clinical features presented by AIDS patients with hyper-IgE.

Materials and Methods

Patients. We studied three male patients (age 32-59 yr) affected by AIDS, with very high levels of IgE (serum levels were 12,000, 2,600, and 3,400 kU/L, respectively), eosinophilia (900 ± 150 cell/mm3), chronic diffuse pruritic dermatitis, repeated Staphylococcal abscesses, and Candida infections. These patients had CD4+ cell counts of 0.4, 5, and 0.3% of peripheral blood lymphocytes (PBL), respectively, corresponding to absolute numbers of 1,38, and 4 CD4+ cells/μL. Parasites were absent, both in skin and stools, and no other known cause of eosinophilia was found.

Flow Cytometric Analysis. For double fluorescence analysis cells were stained with the following mAbs, PE- or FITC-conjugated: anti-CD3 (Leu 4), anti-CD4 (Leu 2a, all from Becton Dickinson Immunocytometry Systems, Mountain View, CA). A chimeric molecule generated by fusing the extracellular domain of CD40 to the heavy chain of IgG1 (a kind gift of I. Stamenkovic, Harvard University, Cambridge, MA), was used for the determination of CD40L expression on the surface of T cells, and revealed by indirect immunofluorescence with a FITC-conjugated F(ab)2 goat antiserum to human IgG (Serotec, Oxford, UK). Normal polyclonal human IgG were used as control. Flow cytometric analysis was performed on a Cytoron (Ortho Diagnostic Systems, Raritan, NJ) after electronic gating on lymphocytes.

Mononuclear Cell Cultures. Lymphoprep (Nycomed, Oslo, Norway)-separated PBMC were suspended in RPMI 1640 (GIBCO BRL, Gaithersburg, MD) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO BRL), 2 mM t-glutamine (GIBCO BRL) and 10% FCS (Hyclone, Logan, UT) (complete medium) at 106 cells/ml. Duplicate cultures of 1 ml were set up for 10 d, with or without the following: cycloheximide (100 μg/ml; Sigma Chemical Co., St. Louis, MO), and goat neutralizing anti-IL-4 (R&D Systems, Inc., Minneapolis, MN), or normal goat serum as control, at different concentrations (1-100 μg/ml). Supernatants were then collected and stored in aliquots at -80°C for up 3 wk until analysis for IgE determinations.

The induction of IgE synthesis by normal B cells from three healthy controls was tested by adding anti-CD40 (mAbs 89, 0.5 μg/ml, a kind gift of Dr. J. Blanchereau, Schering-Plough, Dardilly, France) with or without IL-4 100 U/ml, or 1:10 dilution of supernatants from six CD8+ T cell clones from patient 1. B lymphocytes were obtained from PBMC by immunomagnetic selection with anti-CD3-coated beads (Dynal, Oslo, Norway). 2 × 108 negatively selected cells were cultured for 7 d and supernatants collected for IgE determination.

Immunomagnetic depletion of CD8+ PBMC was performed by using anti-CD8-depleted immunobeads (Dynal, Oslo, Norway). After depletion, CD8+ cells were always <1%. CD8-depleted PBMC were cultured for 7 d at 0.5 × 106 cells/ml and supernatants were tested for IgE synthesis as described.

Generation of T Cell Lines and Clones. Patients' PBMC were stimulated with PHA-P (1 μg/ml; Wellcome, Beckenham, UK) in 24-well flat-bottomed plates (Falcon, Oxnard, CA). CD8-depleted PBMC were cultured for 7 d at 0.5 × 106 cells/ml and supernatants were tested for IgE synthesis as described.

Results and Discussion

Phenotypes of Cells Ex Vivo and in IL-2-dependent T Cell Clones. The dramatic decrease of CD4+ cells in vivo was
accompanied by a relative increase of CD8+ T lymphocytes (58 ± 14%), whose absolute count was only slightly raised, due to the low number of total CD3+ T lymphocytes (average mean, 569 cells/μl). T cell lines were grown from PBMC of the patients, and maintained in rIL-2, after initial stimulation with PHA. Cloning of cells from two lines (patients 1 and 2) was then performed, and growing T cell clones were analyzed 2 mo after seeding. All three cell lines, 24/24 clones from patient 1 and 26/28 from patient 2 were CD3+CD8- and CD3+CD4+CD8-. The almost complete depletion of circulating CD4+ cells was then reproduced also at clonal level in these patients.

Spontaneous In Vitro Synthesis of IgE by PBMC of AIDS Patients. PBMC from the three patients were cultured for spontaneous IgE production for 10 d, and in all instances, net IgE synthesis in supernatants was present at levels ranging from 10.3 to 144.6 ng/ml (Fig. 1, solid bars). No spontaneous net IgE production was detected in cultures from HIV noninfected nonatopic subjects (data not shown). The levels of IgE found in cultures from HIV+ cases with hyper-IgE were similar to those obtained in cultures of PBMC from patients affected by the primary hyper-IgE syndrome (15), a primary immunodeficiency disease in which clinical features are closely related to those observed in our HIV+ cases (19), and where an imbalance between IL-4 and IFN-γ production has been reported (15, 19, 20). These two cytokines are known to exert opposing effects on IgE (21), and their balance ought to be critical in hyper-IgE states.

We therefore asked whether IL-4 was playing a role in the in vitro spontaneous synthesis of IgE occurring in patients severely depleted of CD4+ cells, the principal producers of IL-4 in immune responses. Addition of increasing amounts of neutralizing antibody to IL-4 inhibited IgE production in vitro in a dose-dependent fashion in the range 1-100 ng/ml (Fig. 1, empty bars), indicating that presence of IL-4 was essential for de novo synthesis of IgE in vitro. A control nonimmunized goat serum had no effect upon levels of IgE detected in the culture supernatants (data not shown).

CD4+ cells were severely reduced in these patients, and CD8+ cells accounted for 93-99% of all T lymphocytes. We observed that depletion of these CD8+ cells led to a 6-10-fold reduction of IgE synthesis (Fig. 2). We therefore postulated that these cells might be an alternative source of IL-4 in our cases, and might be actively promoting IgE production through IL-4.

Cytokine Production by PBMC, T Cell Lines, and Clones. Supernatants from PHA-stimulated PBMC of the three patients were analyzed by ELISA, and IL-4 was detected at different concentrations (40-1,600 pg/ml). CD8+ T cell lines also produced IL-4 (150-490 pg/ml) upon stimulation by monoclonal anti-CD3 antibody. mRNAs for IL-4, IL-5, and GM-CSF were detected by RT-PCR, as shown in Fig. 3 for the CD8+CD4- T cell line from patient 2. These mRNAs were present both in CD8+ T cells cultured in rIL-2, and after restimulation with anti-CD3; in the latter condition the amount detected seemed to increase. The presence of p55 IL-2 receptor chain RNA showed that the cells were activated in both conditions. IL-5 and GM-CSF are known to promote eosinophil differentiation, growth and activation (7, 22). Thus their production by CD8+ T cells may be related to eosinophilia in these patients. No mRNA for IL-2 and IFN-γ was detected in this cell line, despite maximal stimulation. This cytokine pattern is usually detectable in Th-2 type cells, but it has been reported under certain conditions also in CD8+ lymphocytes (11, 12, 23, 24). Further analysis was made on supernatants of CD8+ T cell clones by ELISA for different cytokines. Data reported in Table 1 demonstrate that a complex mix of cytokine production could be detected in the CD8+ clones from both patient 1 and 2. 67% of the CD8+ clones produced measurable amounts of IL-4, 52% IL-10, and 72% also IFN-γ. IL-5 and IL-6 were consistently detected in all supernatants tested, ranging respectively from 50-2,500 pg/ml and from 18-3,000 pg/ml. Not included in the table, the supernatant of the only CD4+/CD8+ clone from patient 2 contained IFN-γ only, whereas the CD4+ clone from the same patient was a typical Th-2 cell, producing IL-4, IL-5, and IL-10 but no IFN-γ. In 42% of the CD8+ clones we observed both IL-4 and IFN-γ production. This is consistent with previous reports showing that a fraction of alloreactive murine (23) and human
Table 1. Cytokine Production by CD8 + T Cell Clones

| IL-4 | IFN-γ | No. | Percent | Percent IL-10 |
|------|-------|-----|---------|--------------|
| +     | +     | 17  | 42.5    | 76.4         |
| +     | -     | 10  | 25      | 60           |
| -     | +     | 12  | 30      | 16.6         |
| -     | -     | 1   | 2.5     | 0            |

Supernatants of 40 CD8 + T cell clones from patients 1 and 2 were analyzed by ELISA for cytokine content. IL-5 and IL-6 were present in all, with levels ranging from 50 to >2,500 pg/ml, and 18 to >3,000 pg/ml, respectively. Number and percentage of IL-4 and/or IFN-γ producer clones are given. In the last column, the percentage of clones in each row coproducing also IL-10 is indicated.

(24, 25) CD8 + cell clones have the capacity to secrete both cytokines, despite the majority of CD8 + clones produce IL-2 and IFN-γ but no IL-4. Thus, the ability of both T cell lines and about 25% of CD8 + T cell clones to secrete exclusively Th-2 type cytokines after stimulation may be regarded as an abnormal expansion of a CD8 + subset with helper activity. Previous in vivo priming by IL-4 producing Th cells, as demonstrated by in vitro priming of murine CD8 + cells (26) might account for the appearance of this type of cell. In HIV infection a shift to preferential production of IL-4 has been in fact observed (8).

**CD40L Expression by Stimulated CD8 + Cells.** The possibility of a direct induction of IgE synthesis by CD8 + T cell clones was further suggested by the expression of a CD40L on the surface of the cells. This was tested using a fusion product of CD40 with human IgG1 heavy chain, and detected by cytofluorimetry. 22 out of 50 CD8 + T cell clones examined after 72 h of culture with rIL-2 expressed a CD40L (Fig. 4, illustrating two representative positive clones from each patient). Only a small minority of CD8 + T lymphocytes have been reported to express a CD40L after activation (27), whereas nearly half of the CD8 + clones derived from HIV + patients with hyper-IgE had a stable expression of this molecule. In a patient with primary hyper-IgE syndrome, only CD4 +, but no CD8 +, T cell clones expressed the CD40L (data not reported). However, preliminary data from AIDS patients without features of hyper-IgE show that CD40L is expressed by CD8 + clones under the same
The presence of CD8+ lymphocytes expressing a CD40L, and able to produce IL-4, IL-5, and GM-CSF suggests that these cells are directly capable of causing the main features of this HIV-associated syndrome, i.e., hyper-IgE production and eosinophilia.

**Induction of IgE Synthesis by CD8+ T Cell Clones Supernatants.** Since both IL-4 and IFN-γ were produced, alone or in combination, by the majority of T cell clones examined, we tested the effects of the addition of clone supernatants on the in vitro production of IgE by normal B lymphocytes. B cells purified from a normal individual were cultured for 7 d in the presence of anti-CD40, without or with either 100 U/ml rIL-4 (positive control), or 1:10 dilutions of six different CD8+ T cell clones supernatants from patient 1. All six selected clones produced IL-4 (15–60 pg/ml), but five of the six also secreted IFN-γ. In five cases the addition of the supernatants resulted in the stimulation of IgE synthesis above control cultures with anti-CD40 alone (Fig. 5). This shows that CD8+ T cells from HIV+ patients with hyper-IgE have the characteristics of Th-2 type cells, directly inducing IgE synthesis by normal B cells. These cells may be able to drive hyperproduction of IgE, as well as eosinophilia, in patients with almost complete depletion of CD4+ lymphocytes. It is possible that CD8+ cells endowed with Th-2 type competence arise earlier in the course of HIV disease, and may be the source of IL-4 directing CD4+ cells to shift toward a Th-2 phenotype (28). The expansion of this subset of CD8+ cells may account for the clinical aspects manifested by a small proportion of patients with advanced stage of HIV disease. Alternatively these CD8+ cells may appear as a consequence of the prevailing Th-2 type of response in HIV infection (8), and this would be consistent with the presence of increased IgE levels in the late phase of HIV disease, parallel to the decrease of CD4+ cells (2, 3).

Figure 5. In vitro synthesis of IgE by normal B cells induced by anti-CD40 and rIL-4 or CD8+ T cell clones supernatants. B cells were negatively selected from normal PBMC by immunomagnetic depletion with anti-CD3-coated beads. 2 × 10^6 B cells were cultured for 7 d in the presence of anti-CD40 (mAb 89, 0.5 μg/ml), and rIL-4 (100 U/ml), or 1:10 dilutions of supernatants of CD8+ T cell clones nos. 1, 2, 6, 10, 13, and 24 from patient 1. IgE levels below 100 pg/ml are indicated as undetectable.

Table 5. In vitro synthesis of IgE by normal B cells induced by anti-CD40 and rIL-4 or CD8+ T cell clones supernatants. B cells were negatively selected from normal PBMC by immunomagnetic depletion with anti-CD3-coated beads. 2 × 10^6 B cells were cultured for 7 d in the presence of anti-CD40 (mAb 89, 0.5 μg/ml), and rIL-4 (100 U/ml), or 1:10 dilutions of supernatants of CD8+ T cell clones nos. 1, 2, 6, 10, 13, and 24 from patient 1. IgE levels below 100 pg/ml are indicated as undetectable.

| Control | rIL-4 | clone 1 | clone 2 | clone 3 | clone 4 | clone 5 | clone 6 | clone 7 | clone 8 | clone 9 | clone 10 | clone 11 | clone 12 | clone 13 | clone 14 | clone 15 | clone 16 | clone 17 | clone 18 | clone 19 | clone 20 | clone 21 | clone 22 | clone 23 | clone 24 |
|---------|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Undetectable | Undetectable | 100 | 1000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 |

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