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Research Article

HIV-infected patients are defective in their ability to produce interleukin (IL)-12 in vitro in response to pathogenic bacteria and parasites. IL-12 enhances the patient's depressed natural killer cell cytotoxic activity, peripheral blood lymphocyte production of interferon-gamma (IFN-gamma), and proliferative T cell response in vitro to recall antigens, HIV antigens, alloantigens, and mitogens. However, these effects represent short-lived responses and imply the need for chronic IL-12 therapeutic administration in the clinical setting. To identify any long-term effects of IL-12 on T cell differentiation toward Th1 cells, peripheral blood T cells from 10 HIV-infected patients at different stages of disease were cloned by limiting dilution in the presence or absence of IL-12 and tested for cytokine production in response to stimulation with anti-CD3 antibodies and phorbol diesters IL-12 present during the first 2 wk of clonal expansion determined a stable severalfold enhancement on the ability of both CD4+ and CD8+ clones to produce IFN-gamma. Because priming for high IFN-gamma production is probably the most important mechanism by which IL-12 induces generation of efficient T helper type 1 (Th1) cells, these results suggest the possibility that IL-12 treatment in vivo of HIV-infected patients may stimulate a protective Th1 response against opportunistic pathogens and possibly HIV itself.

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Priming for High Interferon-γ Production Induced by Interleukin-12 in Both CD4+ and CD8+ T Cell Clones from HIV-infected Patients

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Abstract

HIV-infected patients are defective in their ability to produce interleukin (IL)-12 in vitro in response to pathogenic bacteria and parasites. IL-12 enhances the patient's depressed natural killer cell cytotoxic activity, peripheral blood lymphocyte production of interferon-γ (IFN-γ), and proliferative T cell response in vitro to recall antigens, HIV antigens, alloantigens, and mitogens. However, these effects represent short-lived responses and imply the need for chronic IL-12 therapeutic administration in the clinical setting. To identify any long-term effects of IL-12 on T cell differentiation toward Th1 cells, peripheral blood T cells from 10 HIV-infected patients at different stages of disease were cloned by limiting dilution in the presence or absence of IL-12 and tested for cytokine production in response to stimulation with anti-CD3 antibodies and phorbol diesters. IL-12 present during the first 2 wk of clonal expansion determined a stable severalfold enhancement in the ability of both CD4+ and CD8+ clones to produce IFN-γ. Because priming for high IFN-γ production is probably the most important mechanism by which IL-12 induces generation of efficient T helper type 1 (Th1) cells, these results suggest the possibility that IL-12 treatment in vivo of HIV-infected patients may stimulate a protective Th1 response against opportunistic pathogens and possibly HIV itself. (*J. Clin. Invest.* 1995. 96:1677–1682.) Key words: interleukin-12 • interferon type II • HIV infections • CD4-positive T lymphocytes • CD8-positive T lymphocytes

Introduction

The progression of human immunodeficiency virus (HIV) infection is associated with a progressive loss in the ability of patients' T cells to proliferate in response to HIV antigens, recall antigens, alloantigens, and mitogens (1, 2). The ability of the patients' T cells to mediate an efficient cell-mediated immune response to HIV has been postulated to correlate with

protection from infection in seronegative high-risk individuals or with long-term survival in HIV-infected patients (1, 3). One hypothesis to explain the decreased cell-mediated response in HIV-infected patients is that during disease progression, CD4+ T helper cells type 2 (Th2)[1] producing cytokines such as interleukin-4 (IL-4), IL-5, and IL-10 and favoring humoral immune responses predominate over Th1 cells, which produce IL-2 and interferon-γ (IFN-γ) and favor cell-mediated immune responses and phagocytic cell activation (3–6). The deficient Th1 response in HIV-infected patients could also account for the susceptibility of these patients to opportunistic infections, which in most cases are represented by intracellular pathogens against which cell-mediated responses and phagocytic cell activation are most effective (5). Although the hypothesis of a Th2 predominance in HIV-infected patients has been challenged on the basis of increased expression of Th1-cytokine mRNA expression in peripheral blood and lymph nodes (7, 8), several investigators have confirmed an increased production of IL-4 in CD4+ cells (9, 10), suggesting an increase in Th0 cells which produce both IL-4 and IFN-γ and, at least in some patients, an increased proportion of cells producing Th2 cytokines within the CD8+ cell subset (11–13).

The dichotomy between Th1 and Th2 responses has been shown to be determined in part by the balance between the production of IL-4, which appears early during an immune response and favors Th2 responses, and IL-12, which favors Th1 responses (11, 14–16). The effect of IL-12 on Th1 cell generation appears to be mediated through an irreversible priming of both CD4+ and CD8+ T cells for high IFN-γ production (11), while negatively affecting the generation of IL-4 producing cells, both in vivo and in vitro, through still unclear selective mechanisms, possibly depending in part on IFN-γ production (11, 14, 15, 17, 18).

The ability of peripheral blood mononuclear cells (PBMC) from HIV-infected patients to produce IL-12 in vitro in response to *Staphylococcus aureus* (19) or to the opportunistic pathogen *Toxoplasma gondii* (20) is profoundly impaired even in patients who are asymptomatic and have a nearly normal number of peripheral blood CD4+ cells. This finding contrasts with the normal or increased production of other phagocytic cell-derived cytokines, such as TNF-α, IL-1β, and IL-6 observed in HIV-infected patients compared with seronegative donors (19) and is reminiscent of the reported deficiency in the production of IFN-α (21). The deficient production of IL-12 in response to infectious pathogens is compatible at least with a suboptimal Th1 response, if not necessarily with a predominant Th2 re-

1. *Abbreviations used in this paper:* CDC, Centers for Disease Control; Th0, Th1, Th2, T helper cells types 0, 1, and 2, respectively.

Table I. Clinical Stage and T Cell Counts in HIV-infected Patients in This Study

Patient	CDC stage*	CD4$^+$ cells	CD8$^+$ cells	CD4/CD8 ratio
			cells/mm^3	
1	A1	630	640	1.02
2	A2	260	510	0.51
3	C3	130	400	0.32
4	B2	430	1250	0.34
5	B3	110	930	0.12
6	A1	1510	1240	1.2
7	C3	30	520	0.06
8	A3	150	1000	0.15
9	B3	140	810	0.17
10	A1	580	1040	0.55

* Centers for Disease Control clinical stage.

sponse, and could have pathogenetic significance in the susceptibility of HIV-infected patients to opportunistic pathogens.

In vitro, IL-12 increases the cytotoxic activity of patients' natural killer cells to levels comparable with those of healthy donors and, alone or in combination with IL-2, induces IFN-γ production from patients' peripheral blood lymphocytes (PBL) (22). IL-12 in vitro also partially overcomes the inability of patients' T cells to proliferate in response to HIV antigens, recall antigens, and alloantigens (23, 24) and prevents antigen-induced apoptosis of patients' T cells (25).

In this study, we asked whether IL-12, in addition to transiently improving acute T cell responses, might also affect the differentiation of Th1 cells, which are important in maintaining immune competence in the patients and which, unlike Th2 cells, do not readily support HIV replication (9). Our data indicate that IL-12 induces an irreversible priming for high IFN-γ production in both CD4$^+$ and CD8$^+$ T cell clones derived from HIV-infected patients, which likely represents the most important mechanism by which IL-12 induces Th1 cell differentiation.

Methods

Patient population. Study subjects were recruited from the Immunodeficiency Program of the Hospital of the University of Pennsylvania. CD4$^+$ lymphocyte counts were obtained on the same day that blood was obtained for use in this study. Patients were categorized according to Centers for Disease Control (CDC) criteria on the basis of their CD4$^+$ lymphocyte and HIV-related symptomatology and complications (26). This study was approved by the Institutional Review Board of the University of Pennsylvania.

Reagents. Phytohemagglutinin (PHA) was purchased from Grand Island Biological Co. (Grand Island, NY). 12-0-tetradecanoyl-phorbol-13-acetate (TPA) was from Sigma Chemical Co. (St. Louis, MO). Anti-CD3, anti-CD4, and anti-CD8 monoclonal antibodies (mAb) were produced from hybrid cell lines obtained from American Type Culture Collection (ATCC, Rockville, MD). Human recombinant IL-2 (rIL-2, 10^7 U/mg) was a generous gift of Dr. T. Taguchi (Osaka University, Osaka, Japan). Human rIL-12 (Chinese hamster ovary cell–derived) was provided by Dr. Stan Wolf (Genetics Institute Inc., Cambridge, MA).

Generation of CD4$^+$ and CD8$^+$ T cell clones. PBMC were obtained from 4 healthy donors and 10 HIV-infected patients (described in Table I) after Ficoll-Hypaque gradient centrifugation. T cell clones were gen-

erated from PBMC by limiting dilution (11, 27). 0.5 PBMC/well were seeded in 96-well round-bottom tissue culture plates in 200 μl of RPMI-1640 medium with 10% FCS in the presence of 5 μg/ml PHA, γ-irradiated (50 Gy) allogeneic PBMC (2.5 × 10^4 cell/ml) and RPMI-8866 B lymphoblastoid cells (10^4 cells/well) in the presence of IL-12 (2.5 ng/ml), neutralizing anti–IL-12 mAb C8.6 (10 μg/ml, sufficient to completely neutralize > 1 μg/ml of IL-12, a level much higher than that possibly produced by the feeder cells) (28), or medium. After 3 d, IL-2 (50 U/ml, final concentration) was added to all cultures by replenishing half of the medium. After 1 wk, irradiated PBMC (2.5 × 10^4/well) and RPMI-8866 (10^4/well) as feeder cells were added again; cells were maintained in culture with IL-2 and fed and split as required. Cloning efficiency was calculated for either CD4$^+$ or CD8$^+$ by Poisson distribution as described previously (11, 27, 29), considering as positive wells only those in which a sufficient T cell number was achieved to allow determination of both surface phenotype and cytokine profile. The proportion of CD4$^+$ and CD8$^+$ T cells in the plated PBMC was taken into account in calculating the number of cells from either subset plated per well.

Surface phenotype of T cells. The phenotype of PBMC and T cell clones was examined by immunofluorescence (flow cytometry) using FITC-conjugated anti-CD3, anti-CD4, and anti-CD8 mAbs as described (11).

Induction of cytokine production. T cell clones were resuspended to a concentration of 2 × 10^6/ml in medium and stimulated for 24 h with both anti-CD3 mAb (OKT3, ascites 1:10,000) and TPA (10 ng/ml). The cell-free culture supernatants were collected and stored in aliquots at −20°C until tested for cytokine content.

Quantitation of IFN-γ and IL-4. IFN-γ was quantitated in T cell clone supernatants by radioimmunoassay (RIA) using mAbs B133.1 and B133.5 (30); IL-4 was quantitated by RIA using antibody 4F2 and 5A4 (31), kindly provided by Dr. Lucien Aarden (Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands).

Results and Discussion

T cell clones were generated from peripheral blood of 10 HIV-infected patients (described in Table I) and 4 HIV-negative healthy controls by limiting dilution to allow the clonal expansion of a large proportion of peripheral blood T cells (11, 27). The clones were generated in the presence or absence of IL-12 and in the presence of anti–IL-12 mAb. Consistent with previous studies (9), the clonal efficiency of CD4$^+$ T lymphocytes from HIV-infected patients (37.4±18.9%, mean±SE) was lower than that of the corresponding CD8$^+$ cells (59.8±22.0%) and that observed with total T cells from healthy donors (63.6±23.4%) (Table II). The continuous presence of IL-12 during culture did not improve the clonal efficiency, and in early cloning experiments actually decreased it (not shown). Thus, with some donors, IL-12 was added only in the first 2 wk of cultures, yielding a clonal efficiency similar to that observed in cultures without IL-12. This inhibitory effect of IL-12 in these cultures is possibly explained by previous results showing that high concentrations of IL-12 may decrease proliferation of certain T cell subsets, including CD8$^+$ T cells, induced by high concentrations of IL-2, although IL-12 synergizes with low concentrations of IL-2 (32, 33). The in vivo effect of IL-12 therapy on T cell counts of HIV-infected patients will obviously need to be closely monitored in the clinical trials.

After ~ 30 d of culture, cells were tested for production of IFN-γ and IL-4 in response to 24-h TPA and soluble anti-CD3 stimulation (Table II and Fig. 1). The production of IL-4 by clones derived from HIV-infected patients and cultured in IL-2 only or in the presence of anti–IL-12 antibodies was not significantly different from that of clones derived from healthy controls. High levels of average IL-4 production by CD4$^+$

Table II. Cytokine Production from CD4⁺ and CD8⁺ T Cell Clones Generated from PBL of HIV-infected or Healthy Donors in the Presence or Absence of IL-12

Donor	Cloning conditions*	CD4⁺ clones No. (% of efficiency)‡	IFN-γ U/ml ±SE§	IL-4 pg/ml ±SE§	CD8⁺ clones No. (% efficiency)‡	IFN-γ U/ml ±SE§	IL-4 pg/ml ±SE§
HIV⁺							
1	Medium	8 (28%)	324±125	2635±1748	25 (86%)	864±96	29±16
	Anti–IL-12	15 (51%)	323±126	1718±463	23 (79%)	783±98	41±24
	IL-12	0			15 (51%)	1347±112	66±30
2	Medium	9 (31%)	108±66	1971±969	19 (65%)	444±124	20±9
	Anti–IL-12	14 (48%)	113±97	3862±2539	21 (72%)	365±104	43±12
	IL-12	11 (38%)	1332±106	1224±695	18 (63%)	1313±97	32±11
3	Medium	22 (42%)	76±19	788±227	44 (42%)	497±75	37±12
	Anti–IL-12	12 (32%)	65±45	413±122	38 (60%)	323±57	58±20
	IL-12	14 (27%)	626±107	40±23	46 (46%)	450±52	28±12
4	Medium	11 (30%)	441±132	591±218	68 (72%)	532±45	88±29
	Anti–IL-12	9 (24%)	520±126	247±74	30 (33%)	524±72	90±45
	IL-12	7 (19%)	1185±92	334±131	34 (37%)	977±55	11±3
5	Medium	13 (48%)	490±91	67±43	29 (40%)	278±39	12±3
	Anti–IL-12	10 (37%)	153±39	120±62	16 (21%)	178±31	8±2
	IL-12	11 (41%)	944±49	45±20	28 (39%)	808±64	10±3
6	Medium	50 (62%)	258±46	542±124	17 (37%)	240±90	7±3
	Anti–IL-12	67 (81%)	89±32	373±93	23 (50%)	68±46	4±2
	IL-12	15 (19%)	593±110	228±129	10 (22%)	546±182	2±0
7	Medium	0			124 (81%)	288±53	24±6
	Anti–IL-12	2 (15%)	5±0	2±0	136 (88%)	140±31	63±21
	IL-12	0			77 (50%)	987±102	8±8
8	Medium	17 (44%)	452±179	5±1	60 (83%)	722±100	5±2
	Anti–IL-12	14 (32%)	53±25	17±6	53 (74%)	263±52	2±1
	IL-12	12 (30%)	1423±318	37±21	59 (77%)	1018±124	7±2
9	Medium	8 (25%)	2031±258	17±2	39 (67%)	767±154	15±4
	Anti–IL-12	7 (22%)	385±346	49±27	41 (71%)	490±109	67±14
	IL-12	3 (9%)	1268±497	3±1	35 (60%)	1303±179	43±15
10	Medium	18 (64%)	409±141	7±2	9 (25%)	531±106	42±27
	Anti–IL-12	17 (59%)	163±132	15±6	12 (33%)	451±122	2±1
	IL-12	13 (45%)	1267±202	5±2	14 (38%)	1237±186	2±2
HIV⁻							
1	Medium	7 (30%)	472±251	2415±1187	8 (57%)	216±71	572±542
	Anti–IL-12	21 (87%)	215±86	138±38	14 (97%)	199±63	375±207
	IL-12	4 (17%)	1100±46	1849±586	5 (36%)	1134±197	332±271
2	Medium	nd	nd	nd	nd	nd	nd
	Anti–IL-2	22 (*62%)	376±130	568±128	8 (*62%)	283±192	494±254
	IL-12	22 (*83%)	1311±104	502±134	11 (*83%)	1088±178	18±7
3	Medium	28 (*95%)	160±58	265±96	8 (*95%)	312±150	30±24
	Anti–IL-12	26 (*93%)	227±85	638±231	9 (*93%)	256±143	111±102
	IL-12	26 (*87%)	869±105	563±315	7 (*87%)	1350±116	53±31
4	Medium	24 (70%)	292±69	334±91	7 (38%)	188±87	16±9
	Anti–IL-12	17 (50%)	469±158	1243±395	14 (61%)	170±43	56±23
	IL-12	9 (28%)	664±186	618±356	10 (43%)	503±168	45±17

* PBL from HIV-infected (HIV⁺) or healthy (HIV⁻) donors were cloned by limiting dilution in the presence of IL-2 and feeder cells in culture medium (*Medium*) or medium plus 10 µg/ml of neutralizing anti–IL-12 monoclonal antibody C8.6 (*Anti–IL-12*) or 2.5 ng/ml of recombinant human IL-12 (*IL-12*). IL-12 was maintained throughout the culture period for HIV⁺ donors 1, 2, 4, and 5 and HIV⁻ donor 1, whereas it was present only for the first 2 wk for HIV⁺ donors 3, 6, 7, 8, 9, and 10 and HIV⁻ donors 2, 3, and 4. ‡ The indicated number of CD4⁺ and CD8⁺ clones was analyzed for cytokine production. Cloning efficiency was calculated separately for the CD4⁺ and CD8⁺ subsets present in the original PBL preparation, except in the case of PBL preparations from HIV⁻ donors 2 and 3, for which efficiency (indicated by an asterisk) was determined only for total CD3⁺ T cells. § After ~ 30 d of culture, IFN-γ and IL-4 production was evaluated for each clone after stimulation with both anti-CD3 antibodies and TPA in the absence of either IL-12 or IL-2.
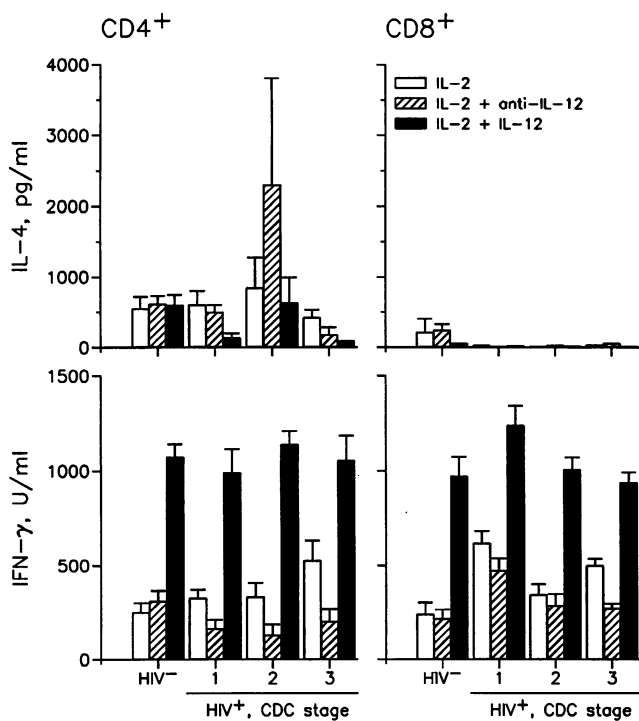
Figure 1. Production of IL-4 and IFN-γ from CD4 $^+$ and CD8 $^+$ clones of HIV-infected and healthy donors generated in the presence or absence of IL-12. PBL from 4 healthy (HIV $^-$) donors and 10 HIV $^+$ donors (3 in CDC stage 1, > 500 CD4 $^+$ cells/mm^3; 2 in CDC stage 2, 200– 500 CD4 $^+$ cells/mm^3; and 5 in CDC stage 3, < 200 CD4 $^+$ cells/mm^3; see Table I) were cloned by limiting dilution in the presence of IL-2 (50 U/ml) and irradiated feeder cells, in culture medium (*open columns*) or medium plus neutralizing anti–IL-12 antibody C8.6 (10 μg/ml, *striped columns*) or human recombinant IL-12 (2.5 ng/ml, *black columns*). IL-12 was present throughout the culture period or, for some donors, only for the first 2 wk, as indicated in the legend to Table II. After ∼ 30 d of culture, the production of IFN-γ and IL-4 by each clone was determined after a 24-h stimulation with both anti-CD3 antibodies and TPA. Results are expressed as mean±SE for all clones obtained from healthy donors or from all HIV $^+$ patients in each stage group.

clones from HIV-infected patients with CD4 $^+$ cell counts 200– 500/mm^3 (Fig. 1) were due mostly to the clones derived from donor 2 (Table II). The IFN-γ production from CD4 $^+$ clones from HIV-infected patients, generated in the presence of anti– IL-12 antibodies, was lower than that from clones generated from healthy donors in the same conditions (*P* < 0.01; Student's *t* test). The fact that this difference was significant in cloning cultures in which endogenous IL-12 was neutralized and that allogeneic irradiated PBMC and RPMI-8866 cells, both of which produce IL-12 (28, 34), were used as feeder cells exclude the possibility that the decreased ability of HIV-infected patients to produce IL-12 (19) during the cloning procedure affected IFN-γ production. However, lack of exposure to IL-12 in vivo might have resulted in a decreased ability of the CD4 $^+$ clones to produce IFN-γ when not exposed to IL-12 during the cloning. These data are consistent with those reported by others (9, 10), although the small number of healthy controls in our experiments, designed to test the cytokine profile of clones from HIV-infected patients, originated in the presence or absence of IL-12, and the use of random cloning rather than the cloning of antigen-specific or memory T cells, as in the above studies,

prevents definitive conclusions on the cytokine profiles of clones from HIV-infected patients versus healthy donors.

The CD4 $^+$ clones generated in the presence of IL-12 from PBL of patients and healthy donors produced significantly higher levels of IFN-γ than the clones generated in the presence of IL-2 only or in the presence of anti–IL-12 antibodies. CD4 $^+$ clones obtained in the presence of IL-12 from HIV-infected patients produced IFN-γ at levels 9.3±7.7-fold higher (mean±SE; range 2.3–26.9-fold) than those from clones derived in the presence of anti–IL-12 antibodies (*P* < 0.001; Student's *t* test). By comparison, clones from healthy donors generated in the presence of IL-12 produced 3.5±1.5-fold (range 1.4–5.1-fold) higher IFN-γ levels (*P* < 0.01) than those generated in the presence of anti–IL-12 antibodies. As graphically shown in Fig. 2 with selected donors, almost every CD4 $^+$ clone generated in the presence of IL-12, from HIV seropositive or negative donors, produced very high levels of IFN-γ, whereas the majority of the clones generated in the presence of anti–IL-12 produced only very low levels of IFN-γ. In some cases, IFN-γ production from clones cultured in the presence of IL-2 and anti–IL-12 was lower than that of clones cultured with IL-2 alone, possibly due to the effect of the IL-12 produced in the cultures by the feeder cells.

IL-12 added during the first 2 wk of clonal expansion of T cells also primed CD8 $^+$ clones for high IFN-γ production (Table II and Figs. 1 and 2). CD8 $^+$ clones from HIV-infected patients and generated in the presence of IL-12 produced on average 3.8±2.2-fold (range 1.4–8.1-fold, *P* < 0.001) higher IFN-γ levels than did CD8 $^+$ clones generated in the presence of anti–IL-12 antibodies compared with a 4.4±1.3-fold increase (*P* < 0.01) observed with clones from healthy donors. The smaller increase observed with CD8 $^+$ than with CD4 $^+$ cells most likely reflects that several of the CD8 $^+$ clones generated in the absence of IL-12 produced high levels of IFN-γ; however, like the CD4 $^+$ clones, virtually all CD8 $^+$ clones generated in the presence of IL-12, from HIV-infected patients or healthy donors, produced high levels of IFN-γ (Fig. 2). Effective priming for high IFN-γ production by IL-12 was observed even in CD8 $^+$ clones from an advanced HIV-infected patient (Fig. 2, *HIV* $^+$ *#7*) with very few circulating CD4 $^+$ cells and from whom almost no CD4 $^+$ clones were obtained (Fig. 2). This patient also represents an example of advanced disease in which, as also reported by others (12), a consistent number of CD8 $^+$ clones with a Th2-like phenotype is observed (Fig. 2).

IL-12 did not significantly affect the production of IL-4 from either CD4 $^+$ or CD8 $^+$ clones, although clones generated in the presence of IL-12 tended toward lower IL-4 production. Thus, unlike IFN-γ production, the presence of IL-12 during clonal expansion of T cells from either HIV-infected or healthy donors was shown to have little effect on IL-4 production, consistent with previous findings (11). It appears that the ability of IL-12 to decrease IL-4 production in polyclonal T cell cultures (14) and in vivo, e.g., in *Leishmania major* infection (17, 18), is due to selective mechanisms that favor either the proliferation of Th1 cells (35, 36) or, possibly through IFN-γ production, the inhibition of Th2 cell growth (37). The ability of IL-12 to prime T cells for high IFN-γ production is most likely the major mechanism by which IL-12 induces generation of Th1 cells in vivo and in vitro. This priming effect has been demonstrated with CD4 $^+$ cells from T cell receptor transgenic mice (38) and with human PBL (11) and cord blood lymphocytes (39). Using cloning conditions in which virtually every T cell from PBL is clonally expanded, we have shown pre-
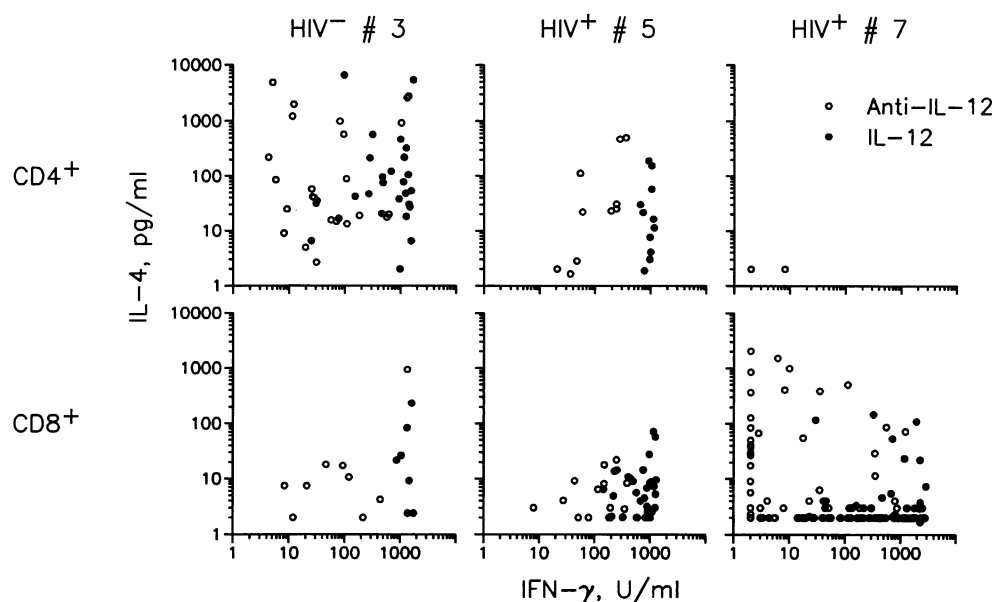
Figure 2. Production of IL-4 and IFN-γ by individual CD4⁺ and CD8⁺ clones of selected healthy (*HIV⁻ # 3*) or HIV-infected (*HIV⁺ # 5 and 7*) donors. Clones were obtained and tested as described in the Fig. 1 legend. O, clones generated in the presence of neutralizing anti–IL-12 antibodies; ●, clones generated in the presence of IL-12. Due to the large number of CD8⁺ clones obtained from donor HIV ⁺ # 7, only a representative randomly selected number of clones is depicted.

viously that high IFN-γ–producing clones are generated as a result of a differentiation effect of IL-12 on each single T cell and not due to a selective effect of IL-12 for growth of IFN-γ–producing clones (11). This priming is long-lasting based on observations that removal of IL-12 from established human T cell clones had no effect on their IFN-γ production (11) and that IL-12 when used as an adjuvant in vaccination in vivo induces a persistent pool of memory Th1-like T cells that responds to secondary antigenic challenge with high IFN-γ production (18, 40). Furthermore, IL-12 was shown to be required in vitro for optimal IFN-γ production and proliferation by Th1 clones (35, 41). However, in mice infected with *T. gondii*, IL-12 production was required only during the first 1 or 2 wk of acute infection to generate a protective Th1 response; maintenance of a Th1 response and high IFN-γ production during a chronic infection was not abolished by treatment with anti–IL-12 antibodies (42). Similarly, we find that the presence of IL-12 for priming of IFN-γ production is required only for the first 1 or 2 wk of clonal expansion, after which the clones from either HIV-infected or healthy donors can be maintained for a further 2 or 3 wk in culture in the absence of IL-12 with no decrease in IFN-γ production ability.

Our results confirm that T cells from HIV-infected patients are responsive to IL-12, consistent with the ability of IL-12 to enhance natural killer cell cytotoxicity (43), to induce IFN-γ production (23, 43), and to enhance antigen-specific in vitro proliferative responses (23, 24) of lymphocytes from HIV-infected patients. However, unlike those effects of IL-12, which represent rapid and short-lived responses, the ability of IL-12 to prime both CD4⁺ and CD8⁺ T cells from patients represents a long-lasting and possibly irreversible response. Because this priming effect of IL-12 has a key and most likely determining role in the ability of IL-12 to induce generation of Th1 cells, these results suggest that IL-12 may be effective in HIV-infected patients in inducing a protective Th1 response against intracellular bacteria and parasites and as an adjuvant in prophylactic vaccination against these pathogens. IL-12 has been proven to be extremely effective in this therapeutic or prophylactic use in experimental animals (17, 18, 40, 44, 45) and several of the pathogens tested, e.g., *T. gondii* and *Mycobacterium avium*,

represent common opportunistic pathogens in AIDS patients. The ability of IL-12 to favor a Th1 response may also increase the resistance of the patients to progression of HIV infection, largely mediated through cellular immunity (46). The concern that IL-12, by activating CD4⁺, may increase HIV replication in vivo is alleviated by the observation that Th1 clones appear to be less permissive than Th2 clones for HIV replication (9), thus raising the possibility that IL-12 in vivo might decrease rather than increase virus replication. Of particular interest is the fact that IL-12 can efficiently prime CD8⁺ cells for high IFN-γ production even in patients with advanced disease and almost no CD4⁺ T cells.

Acknowledgments

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