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M Clerici, ... , T A Wynn, G M Shearer

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Research Article

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Changes in Interleukin-2 and Interleukin-4 Production in Asymptomatic, Human Immunodeficiency Virus-seropositive Individuals

Mario Clerici,* Frances T. Hakim,* David J. Venzon,[‡] Stephen Blatt,[§]

Craig W. Hendrix,[§] Thomas A. Wynn,¹ and Gene M. Shearer*

*Experimental Immunology Branch and, [‡]Biostatistics and Data Management Section, National Cancer Institute; ¹Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; and [§]HIV Unit, Wilford Hall Medical Center, Lackland Air Force Base, Texas 78236

Abstract

Infection with HIV results in an incremental loss of T helper cell (TH) function, which can occur years before CD4 cell numbers are critically reduced and AIDS is diagnosed. All TH function is not affected, however, because B cell activation and hypergammaglobulinemia are also characteristic of this period. Recently, in a murine model of AIDS an early loss in production of the CD4 cytokines IL-2 and IFN- γ was correlated with an increase in the B cell stimulatory cytokines IL-4, IL-5, and IL-10. We therefore assessed the production of IL-4 generated by PBL from HIV-seropositive (HIV⁺) individuals who did not have AIDS, yet who exhibited different TH functional categories based on their IL-2 production profiles. We observed that the decreases in recall antigen-stimulated IL-2 production were accompanied by an increase in IL-4 production. The loss of recall antigen-stimulated responses in HIV⁺ individuals could be reversed in vitro by anti-IL-4 antibody. Our results suggest that the TH functions assessed by IL-4 production replace the normally dominant TH function of antigen-stimulated IL-2 production in the progression toward AIDS, and raise the possibility of cytokine cross-regulation in AIDS therapy. (*J. Clin. Invest.* 1993. 91:759–765.) Key words: interleukin-2 • interleukin-4 • interleukin-10 • human immunodeficiency virus infection • T lymphocytes

Introduction

It is well established that defects in T helper (TH)¹ immune function can be detected in HIV seropositive (HIV⁺) patients long before a decline in the number of CD4⁺ T lymphocytes is evident (1–4). In particular, we have previously demonstrated that asymptomatic, HIV⁺ individuals can be divided into four distinct subgroups, based on in vitro TH function of their PBL, as assessed by IL-2 production and proliferation in response to: recall antigens such as tetanus toxoid and influenza A virus (FLU); irradiated, HLA-disparate PBL (ALLO); and PHA (4). Individuals whose PBL generated positive responses to all

three stimuli were designated +/+/+; those who responded to ALLO and PHA, but not to FLU were -/+/+; and those who responded only to PHA were -/-/+ (4). This pattern of TH function has been verified using more than 1,000 HIV⁺ individuals studied over a 5-yr period. Several hypotheses have been advanced to explain this early event in HIV-induced immune dysfunction. These include: (a) a selective HIV-induced depletion of memory T lymphocytes (5, 6); (b) loss in function of antigen presenting cells (7–10); (c) activation of a subpopulation of suppressive cells (11–13); (d) generation of soluble suppressive factors (13–16), including HIV products (17–21), and TGF- β (22); and (e) the generation of autoantibodies which exhibit an inhibitory effect on TH lymphocytes (23–25). Recent evidence demonstrating the independent and reciprocal production of TH1 and TH2 cytokines by clones of murine (26–32) and human (32–39) CD4⁺ cells led us to reexamine the above categories of HIV-induced TH dysregulation for production of the TH2 cytokine IL-4. In the present study we have tested whether the loss of IL-2 production characteristic of HIV infection is associated with augmented production of IL-4. Our results support the hypothesis that the earlier stages of HIV infection are dominated by production of IL-4, with concomitant inhibition of IL-2 cytokines (40). This cytokine profile has been postulated to indicate a switch from a “TH-1-like” to a “TH-2-like” cytokine pattern.

Methods

Patients and clinical evaluation. HIV⁺ patients were obtained from Wilford Hall United States Air Force (USAF) Medical Center, Lackland Air Force Base, TX. Individuals were diagnosed as being HIV infected if they had anti-HIV antibodies demonstrated on two specimens tested by the HIV enzyme immunoassay (Abbott Laboratories, Irving, TX) and confirmed by Western blot analysis (Roche Biomedical Laboratories, Burlington, NC). Western blots were considered positive if they showed at least two of the following three bands reactive: p24, gp41, and gp120 or gp160. Patients were classified according to Walter Reed staging system (41). The HIV-seronegative (HIV⁻) control donors were normal healthy volunteers. Lymphocyte counts and T cell subsets were determined using laser-based flow cytometry (Epics Profile; Coulter Electronics, Inc., Hialeah, FL), and OKT4A (anti-CD4) and OKT8 (anti-CD8) monoclonal antibodies (Orthodiagnosics Systems, Raritan, NJ). All patient and control studies were approved by the Institutional Review Boards of both the National Cancer Institute and the U. S. Air Force.

Processing of blood. Whole blood from HIV⁺ individuals was drawn in tubes containing heparin (Vacutainer; Becton-Dickinson & Co., Rutherford, NJ) and shipped from Lackland, TX to Bethesda, MD, overnight at ambient temperature. Whole blood from HIV⁻ individuals was collected in the same fashion and allowed to sit overnight at ambient temperatures. PBL were separated on lymphocyte separation medium (LSM; Organon Teknika Corp., Durham, NC), washed twice

Address reprint requests to Dr. Gene M. Shearer, Experimental Immunology Branch, National Cancer Institute, Building 10, Room 4B-17, National Institutes of Health, Bethesda, MD 20892.

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1. Abbreviations used in this paper: ALLO, irradiated, HLA-disparate PBL; CTLL, cytotoxic T lymphocyte line; HIV⁺, HIV⁻, HIV seropositive, seronegative; TH, T helper.

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in PBS, and resuspended at 3×10^6 /ml in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 0.5% penicillin, 0.5% streptomycin, 1% glutamine, and 10 mM Hepes. Viable cells counts were determined by trypan blue exclusion.

IL-2 production and proliferation. The ability of PBL to produce antigen-induced IL-2 or to proliferate was determined by culturing the PBL at 37°C in a moist, 7% CO₂ atmosphere. PBL were either unstimulated, or were stimulated with: FLU (A/Bangkok RX73 H3N2) (1:500 concentration); 5,000 rad ALLO PBL from unrelated HIV-donors; or PHA diluted 1:100 (Gibco Laboratories). For IL-2 production, 3×10^5 PBL were cultured in 96-well, flat-bottom culture plates (Costar Corp., Cambridge, MA) for 7 d in the presence of 2 µg/ml of the human anti-IL-2 receptor antibody anti-Tac, to prevent IL-2 consumption by the stimulated cells (4). For IL-2 production, culture supernatants were frozen and stored at -20°C until assayed for IL-2 content. The IL-2 assays consisted of culturing 8×10^3 of the IL-2-dependent cytotoxic T lymphocyte line (CTLL) cell line per well in 96-well, flat-bottom microtiter plates, in the presence of four twofold dilutions of unstimulated or antigen-stimulated culture supernatants, as previously described (4). 24 h later, the cultures were pulsed with 1 µCi of [³H]thymidine, and harvested after 18 h using a 96-well cell harvester (Tomtec Inc., Orange, CT). ³H determinations were made using a spectrometer (Betaplate; Pharmacia LKB Biotechnology, Piscataway, NJ). In the assays in which inhibition of FLU stimulated proliferation was reversed by anti IL-4 antibody, PBL were stimulated for 5 d with FLU in the absence, or in the presence of 5 µg/ml of anti-IL-4 (BL-4P) (Genzyme Corp., Boston, MA); anti TGF-β (R&D Systems, Minneapolis, MN); or anti-IFN-γ (gift from Dr. Alan Sher, Laboratory of Parasitology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) antibodies.

IL-4 production. The ability of PBL to produce mitogen-stimulated IL-4 was determined by culturing 3×10^6 /well PBL in 24-well plates (Linbro; Flow Laboratories, Inc., McLean, VA) at 37°C in a moist, 7% CO₂ atmosphere. PBL were either unstimulated or were stimulated with PHA diluted 1:100 (Gibco Laboratories). The culture media consisted of RPMI 1640 (Gibco Laboratories). Supernatants were harvested after 80 h of culture, because kinetic studies indicated that 80 h was the optimal time for assessing lymphokines detected by CT.h4S cells (see Fig. 1 A). The CT.h4S cell line was generously provided to us by Dr. William E. Paul and Ms. Carol Kinzer (Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) (42). The murine CT.4S line that detects murine IL-4 was transfected with the human IL-4 receptor gene by Drs. William E. Paul and M. Patricia Beckmann (Immunex, Inc., Seattle, WA). We determined the IL-4 specificity of this CT.h4S cell line by adding a polyclonal rabbit anti-human IL-4 (BL-4P) (Genzyme Corp.) which completely blocked supernatant-induced stimulation of the CT.h4S cells (see Fig. 1 B). Supernatants were frozen and stored at -20°C until assay for IL-4 content. The IL-4 assays consisted of culturing 5×10^3 of the IL-4-dependent CT. h4S cell line per well in 96-well, round-bottom microtiter plates, in the presence of four twofold dilutions of unstimulated or mitogen-stimulated culture supernatants. 48 h later, the cultures were pulsed with 1 µCi of [³H]thymidine, and harvested after 18 h with a 96-well cell harvester (Tomtec Inc.). ³H determinations were performed by using a spectrometer Beta-plate; Pharmacia LKB Biotechnology). For each supernatant dilution, the "background" values of unstimulated PBL, which never exceeded 1,000 cpm, have been subtracted from the data of the stimulated cultures. Results are presented in relative units of IL-2. The units were calculated as a constant times the reciprocal supernatant dilution corresponding to half-maximal CTLL proliferation. This dilution was computed by extrapolation of the line generated by linear regression analysis of the cpm for CTLL proliferation as a function of the supernatant dilution, using off plateau values.

Detection of IL-4 mRNA. RNA from PBL of HIV⁺ individuals either unstimulated or stimulated with PHA for 8 h was isolated by treatment of the cells with an RNA solvent (RNAzol; Tel-Test, Inc., Friendswood, TX). IL-4 transcript levels were assessed using a modi-

fied quantitative reverse transcriptase PCR technique previously described (43, 44). Briefly, 1 µg of total RNA was reverse transcribed using Moloney leukemia virus reverse transcriptase (Gibco, BRL, Gaithersburg, MD) in a 25-µl reaction. The reaction mixture was then diluted 1:8, and 10 µl of diluted product was used for specific amplification of IL-4 mRNA using Taq DNA Polymerase (Promega Corp., Madison, WI). The PCR product was separated on a 1% agarose gel and transferred to a membrane (Hybond N⁺; ECL Amersham International, Amersham, UK) using standard blotting techniques. Southern transfers were subsequently probed with internal cytokine-specific oligonucleotides, and visualized using the chemiluminescent detection system (ECL; Amersham International). Autoradiographs were scanned with a scanner (600 ZS; Microtek, Torrance, CA) calibrated with a densitometer step tablet (Kodak, Rochester, NY). The amount of PCR product was determined by comparison of signal density to that of standard curves generated from simultaneously amplified step-wise dilutions of reverse transcriptase product from samples with high amounts of IL-4 mRNA. Results were normalized for the relative quantity of total mRNA used in the reverse transcriptase reaction through parallel amplification and analysis of transcripts from the housekeeping gene hypoxanthine-guanine phosphoribosyl transferase. PCR reactions were strictly defined for the IL-4 primer pair such that a log-linear relationship was obtained between the amount of initial cytokine transcript and the signal density in the detection system. The PCR primer pairs were chosen to span at least one intron. Nucleotide sequences for sense and antisense primers, and probes, respectively, were as follows: hypoxanthine-guanine phosphoribosyl transferase, CGAGATGTGATGAAGGAGATGG, GGATTATACTGCCTGACCAAGG, and GCTGACCTGCTGGATTACAT; IL-4, ACTTTGAACAGCCTCACAGAG, GATCGTCTTTAGCCTTCC, and CATGAG-AAGGACTCGCT.

Results

Conditions for detecting IL-4 production. We have studied the kinetics of production of IL-4 by PBL from six HIV⁺, -/+ individuals and, as shown in Fig. 1 A, maximum production of IL-4 occurred between 48 and 96 h. Therefore, we have used 80 h as the time for detecting IL-4 in this study. To determine whether the CT.h4S cell line detects only IL-4 in our culture supernatants, we stimulated PBL from HIV⁺, -/+ individuals with PHA and attempted to block the stimulatory effect of the supernatants with the anti-IL-4 antibody BL-4P (Genzyme Corp.). As shown in Fig. 1 B, 5 µg/ml of BL-4P added during the assay phase, inhibited the proliferation of the CT.h4S cell line in two independent experiments involving PBL from three different HIV⁺, -/+ individuals. Thus the CT.h4S cell line detects only IL-4 in our cultures of PBL from HIV⁺, -/+ patients.

TH subsets that produce IL-2 and IL-4. To determine whether CD4⁺ or CD8⁺ T cells produce IL-4, we depleted PBL from HIV⁺, -/+ individuals of one or the other of these two T cell subsets by panning on antibody-coated culture plates, and stimulated the cells with PHA. The results of a representative experiment are shown in Fig. 2. In this experiment, IL-2 production was assessed on CTLL at 7 d in the presence of anti-Tac (optimal conditions) (4), and IL-4 was assessed on the CT.h4S cell line at 80 h. Panel A illustrates PHA-stimulated IL-2 production, and demonstrates that unseparated PBL, as well as PBL depleted of either CD4⁺ or CD8⁺ cells produced IL-2. In contrast, (panel B), PHA-stimulated unseparated PBL and CD8⁺-depleted, but not CD4⁺-depleted, PBL produced IL-4. These results demonstrate that PHA stimulation can be used to assess CD4-mediated IL-4

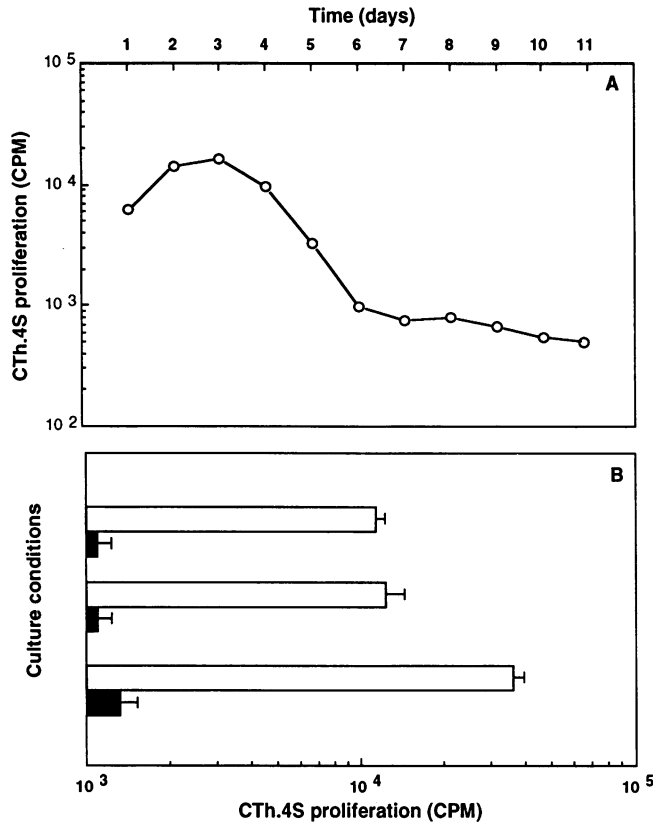


Figure 1. (A) Kinetics of IL-4 production by PHA-stimulated PBL from an HIV⁺, -/+/+ individual assayed on the CT.h4S cell line. Similar results were obtained by PHA-stimulated PBL from five more HIV⁺, -/+/+ individuals. (B) Inhibition of cytokine production by PHA-stimulated PBL from three HIV⁺, -/+/+ individuals to support the proliferation of the CT.h4S cell line in presence of the anti-IL4 antibody BL-4P. (■), no antibody added; (□) 5 μg/ml BL-4P added.

production in whole PBL, but should not be used as an indicator of IL-2 generation by CD4⁺ cells because PHA-stimulated CD8⁺ cells also produce IL-2.

We have compared FLU-stimulated IL-2 (FLU-IL-2) production with PHA-stimulated IL-4 (PHA-IL-4) production and the reasons for such a comparison are listed below. First,

we can stimulate IL-2 production with FLU and other recall antigens, but are unable to detect FLU-stimulated IL-4 production using PBL from either HIV⁻ or HIV⁺ individuals (data not shown). This is consistent with other studies in which IL-4 was not detected by stimulation with recall antigens (45). Second, we can generate PHA stimulated-IL-2 production using unfractionated PBL or PBL depleted of either CD4⁺ or CD8⁺, with PBL from +/+/+ HIV⁺ individuals or HIV-donors (see Fig. 2). Third, proliferation of the CT.h4S cell line was obtained (in the same culture supernatants) using PHA-stimulated unfractionated PBL or PBL depleted of CD8⁺ cells, but not PBL depleted of CD4⁺ cells (see Fig. 2). These findings demonstrate that both CD4⁺ and CD8⁺ cells produce IL-2 when stimulated with PHA, whereas only CD4⁺ cells produce IL-4 when stimulated with PHA. Fourth, FLU-IL-2 production requires previous exposure to influenza antigens, and is mediated by self-restricted CD4⁺ but not by CD8⁺ cells (46). Thus, it is likely that FLU-IL-2 production is mediated by CD4⁺ memory cells. In contrast, PHA-IL-2 production can be mediated by either CD4⁺ or CD8⁺ cells, and may be produced by naive cells.

Patterns of cytokine production in asymptomatic, HIV⁺ individuals, and HIV⁻ controls. We tested IL-2 production in response to FLU, ALLO, and PHA and IL-4 production in response to PHA by PBL from 18 HIV⁻ control donors and 45 HIV⁺ individuals. Among the HIV⁺ donors, 16 were categorized as +/+/, because they responded to FLU, ALLO, and PHA by IL-2 production; 19 as -/+/, because they did not respond to FLU, but did respond to ALLO and PHA; and 10 as -/-/, because these donors only responded to PHA. We observed a complementary pattern of IL-4 production associated with two of the IL-2 functional groups of individuals. The titration curves for responses of PBL from an HIV⁻ control donor and from HIV⁺ individuals representative of the three distinct IL-2 functional patterns described above are shown in Fig. 3. PBL from both HIV⁻ control donors and from HIV⁺ donors categorized as +/+/ produced appreciable IL-2 in response to stimulation with FLU, ALLO, or PHA (Fig. 3, A and B). IL-4 production was low in these individuals, because CT.h4S proliferation was observed only at the 1:2 dilution of supernatant. In marked contrast to these two groups, HIV⁺ individuals who did not generate IL-2 to FLU, but who produced IL-2 in response to ALLO and PHA (-/+/ by our

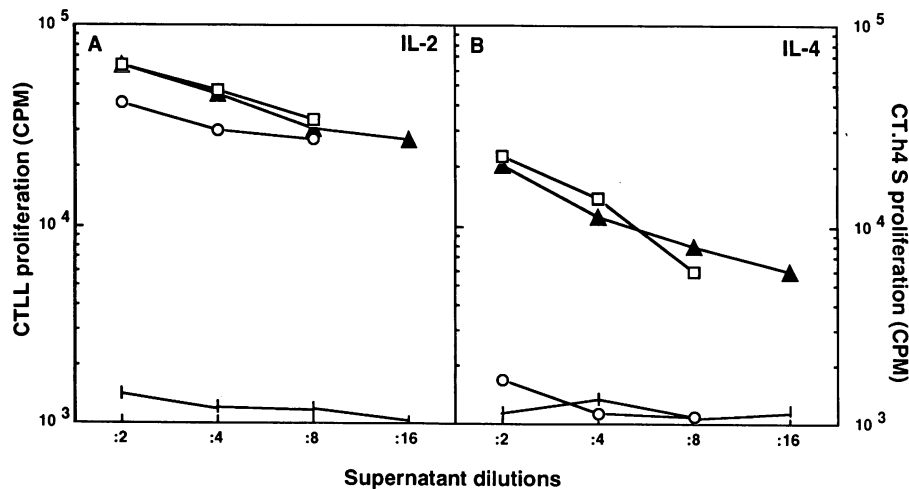


Figure 2. PHA-stimulated production of IL-2 (detected on CTLL) (A), and IL-4 (detected on CT.h4S) (B) by unseparated (▲), CD4-depleted (○), and CD8-depleted (□) PBL from an HIV⁺, -/+/+ individual. The cytokines produced by unstimulated cultures are also shown (+).

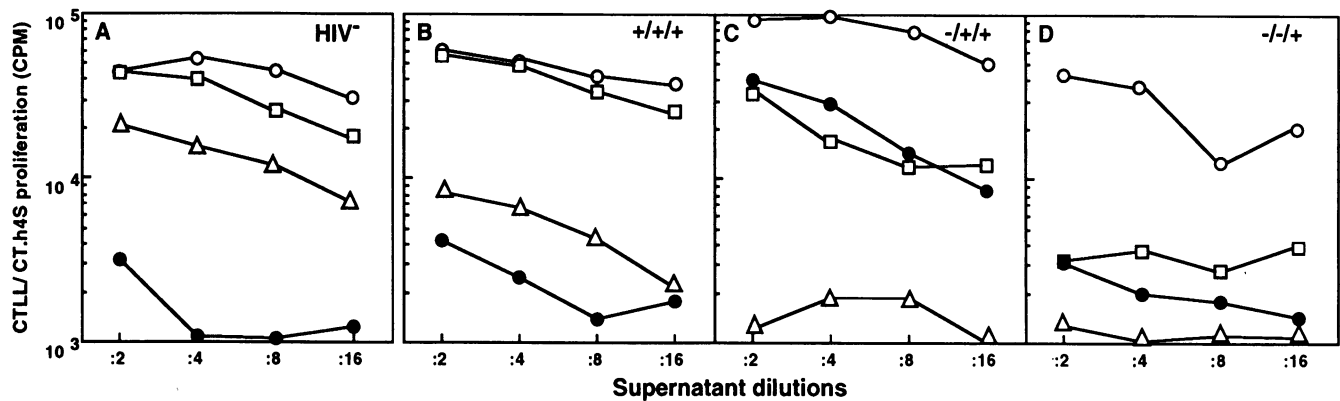


Figure 3. IL-2 (open symbols) and IL-4 (closed symbols) production by PBL stimulated with FLU (Δ), 2×10^6 5,000-rad ALLO (\square), or PHA (\circ , \bullet). (A) PBL from an HIV⁻ donor. (B-D) PBL from three different HIV⁺ individuals whose CD4⁺ cell numbers were, respectively, 574/mm³, 753/mm³, and 437/mm³.

previous designation) (2) generated strong IL-4 responses to PHA (Fig. 3 C). Finally, HIV⁺ donors characterized as $-/-/+$ produced only a marginal PHA-stimulated IL-4 response. These donors produced no detectable IL-2 response to FLU and only a marginal response to ALLO, but still responded strongly to PHA (Fig. 3 D).

We plotted the units of FLU-IL-2 generated as a function of the units of PHA-IL-4 produced for each of the 18 HIV⁻ and 45 HIV⁺ individuals. As shown in Fig. 4, three distinct patterns of cytokine production were observed: (a) defined by IL-2 production as $+/+/+$ HIV⁺, exhibited high IL-2/low IL-4; (b) defined by IL-2 production as $-/+/+$ HIV⁺, exhibited low IL-2/high IL-4; and (c) defined by IL-2 production as

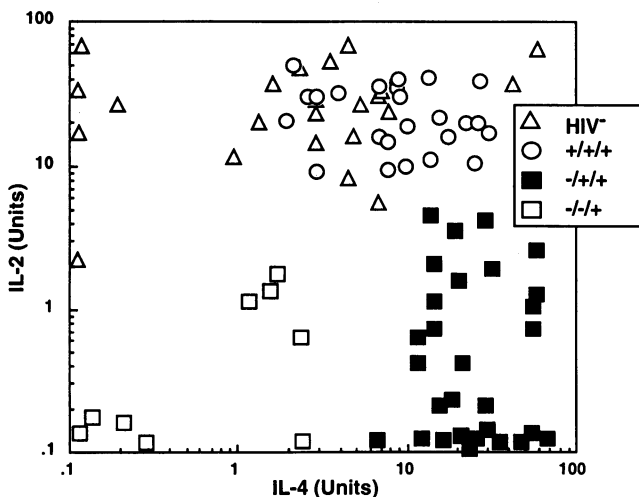


Figure 4. Units of FLU-IL-2 production plotted as a function of units of PHA-IL-4 production by PBL from 18 HIV⁻ individuals (Δ); and 45 HIV⁺ individuals, 16 of whom were $+/+/+$ (\circ), 19 of whom were $-/+/+$ (\blacksquare), and 10 of whom were $-/-/+$ (\square) by IL-2 production. Mean values of each category are indicated by the enlarged symbols. The CD4 counts in the three groups of HIV⁺ individuals were: $+/+/+$ range 232–1,494, median 737/mm³; $-/+/+$ range 72–1,330, median 642/mm³; and $-/-/+$ range 32–1,350, median 416/mm³. There was no statistical correlation between CD4 counts or CD4:CD8 ratios (data not shown) and the IL-2 and IL-4 production profiles.

$-/-/+$ HIV⁺, exhibited low IL-2/low IL-4. Since FLU-IL-2 is involved in categorization of these individuals, differences would be expected between $+/+/+$ individuals and other groups along the IL-2 axis. However, comparison with the PHA-IL-4 indicated that: (a) differed from (b) ($P < 0.00001$ by the Wilcoxon rank sum test); (b) differed from (c) ($P < 0.00001$); and (a) differed from (c) ($P = 0.0012$). The HIV⁻ control and the $+/+/+$ HIV⁺ were also statistically distinguishable ($P = 0.0008$).

Detection of IL-4 mRNA. To provide confirmation that IL-4 production is increased in HIV⁺ individuals, we have performed a preliminary study of the IL-4 mRNA expressed in the PBL of five additional HIV⁺ individuals concurrent with analysis of IL-2 production in our assay. None of the PBL expressed IL-4 mRNA in unstimulated cultures. Upon stimulation with PHA, however, donors 2–5 expressed IL-4 mRNA, whereas donor 1 did not (Fig. 5). Assessment of IL-2 production by the stimulator panel demonstrated that donor 1 was $-/+/+$, donors 2, 3, and 5 were $-/+/+$, and donor 4 was $-/-/+$ by IL-2 production. Thus, the inverse correlation between IL-2 produced in response to FLU and IL-4 produced in response to PHA appeared to be maintained in these studies of IL-4 mRNA levels.

Restoration of antigen-stimulated proliferation in IL-2-deficient patients by stimulation of the PBL in presence of an anti IL-4 antibody. To test whether cytokine cross-regulation could account for the loss of IL-2 production to FLU in $-/+/+$ patients, PBL from six $-/+/+$ HIV⁺ individuals were stimulated with FLU in the presence of antibodies to human IL-4, IFN- γ , and TGF- β . Anti-IFN- γ had no effect on the FLU responses of any of the patients (Fig. 6). Anti-TGF- β restored IL-2 production in only one individual, which may indicate that TGF- β contributes to TH dysregulation in some HIV⁺

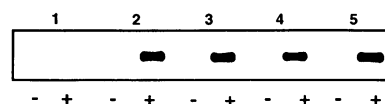


Figure 5. PHA-induced IL-4 mRNA expression by PBL from five HIV⁺ individuals. (–) Without stimulation; (+) 8 h

PHA stimulation. Donor 1 was $-/+/+$, donors 2, 3, and 5 were $-/+/+$, and donor 4 was $-/-/+$ by IL-2 production (4).

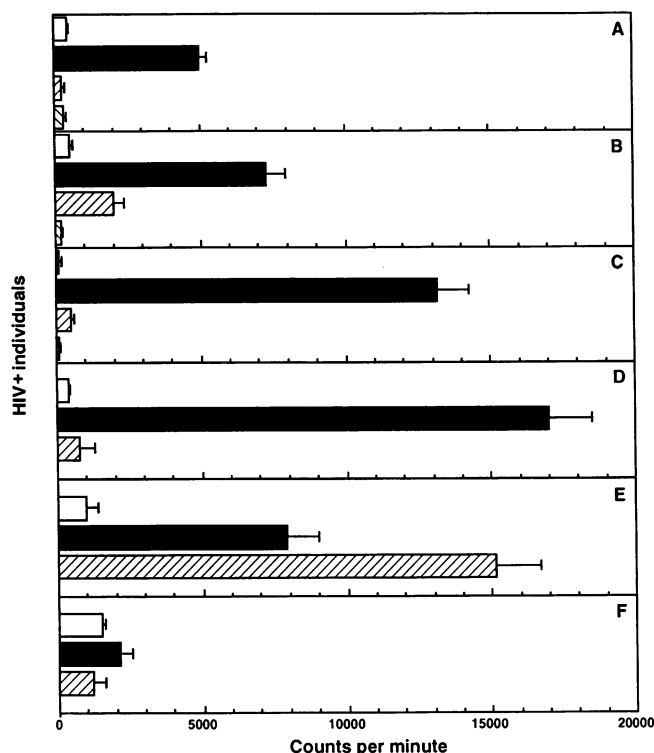


Figure 6. Proliferative TH responses by PBL from six (panels A-F) $-/+/+$ HIV⁺ individuals stimulated with FLU in the absence (\square), or in the presence of 5 $\mu\text{g}/\text{ml}$ of anti-IL-4 (\blacksquare) (BL-4P) (Genzyme Corp.); anti-TGF- β (\square) (R&D Systems); or anti-IFN- γ (\square) (gift from Dr. Alan Sher, Laboratory of Parasitology, NIAID, NIH, Bethesda, MD) antibodies. We observed that anti-IL-4 restored FLU-IL-2 responses in 8:12 individuals tested.

patients and is consistent with a previous report on the immunosuppressive effects of TGF- β (22). In contrast, anti-IL-4 restored FLU-stimulated proliferative responses in PBL from five of these six patients (Fig. 5). These data suggest downregulation of IL-2 production in response to FLU stimulation by IL-4 or the products of IL-4 dependent cells.

Discussion

In the present study we demonstrate that IL-2 production predominates in the HIV⁻ and the $+/+/+$ HIV⁺ individuals. In $-/+/+$ individuals, such cytokine production is reduced, whereas IL-4 production is elevated. In $-/-/+$ donors, both CD4-mediated IL-2 and IL-4 activity appears to be deficient. We also detected IL-4 message in four HIV⁺ individuals who were functionally deficient by IL-2 production, but not in one individual with normal IL-2 production to FLU, ALLO, and PHA.

We have compared the increase in IL-4 production with a decrease in IL-2 production, because we have previously reported that there is a sequential and progressive loss of TH function assessed by IL-2 production such that the $+/+/+$ state is followed by $-/+/+$, which is followed by $-/-/+$, and finally by $-/-/-$ (4, 47). We have followed the TH function of 80 HIV⁺ individuals by sequential assays of IL-2 production over a 15-mo period. A progressive loss in TH function was

observed in 41:80 (51%) of these HIV⁺ patients. The incidence of spontaneous reversal was $< 7\%$. Our findings are consistent with sequential changes in IL-4 production that are reciprocally correlated with the $+/+/+$ and the $-/+/+$ patterns of IL-2 production reported previously (4).

The mechanism responsible for the switch from a predominance of IL-2 to IL-4 production is not known, but could involve TH1-TH2 cross-regulation by cytokines such as IL-10. IL-10 has been demonstrated to decrease TH1 cytokine production in both murine (28, 29) and human (38, 39) immune systems. Thus, mice infected with the murine retrovirus LP-BM5, which induces immunodeficiency and B cell hyperplasia (48), exhibit increased IL-4 and IL-10 production and decreased IL-2 production with progression of disease (31). An alternate explanation of our data is that HIV might preferentially infect TH that mediate cellular immunity compared with TH that mainly promote humoral immunity. Such a preference for infection of TH1 cells could result from HIV infection of macrophages (49) which might be the antigen-presenting cells for TH1 type responses, in contrast to B cells which could be more efficient antigen-presenting cells for TH2 type responses (50).

An IL-2 $>$ IL-4 pattern might represent an immune-protective phase for HIV infection, because we have observed that $\sim 50\%$ of HIV-exposed, seronegative individuals showed evidence of helper cell priming to HIV *env* (51, 52) and exhibited an IL2 $>$ IL-4 functional profile (Clerici, M., and G. M. Shearer, unpublished observations). These individuals were also virus negative in the blood by PCR assay. Two of the first six individuals followed longitudinally seroconverted and became PCR⁺ at the time of seroconversion (51, 52). One of these two donors lost HIV-specific TH function at the time of PCR and seroconversion, and subsequently developed AIDS. Our results are consistent with a recent work by Yamamura et al. (35), which described in leprosy a TH1 pattern of cytokine production representative of a protective state, and a TH2 pattern associated with progression of disease. Thus, the loss of recall antigen-stimulated IL-2 production with a concomitant increase in IL-4 production is not necessarily unique to HIV infection and may be observed in other conditions that involve chronic stimulation of the immune system.

The present study suggests that cytokine cross-regulation can contribute to the loss of TH function of HIV⁺ individuals before CD4 counts are appreciably reduced. It may be possible to stage HIV⁺ patients by TH function, based on the relative amounts of cytokines that their PBL produce after in vitro stimulation. Studies are planned to test for IL-10 production, a cytokine that could be important in TH cross-regulation during AIDS progression. Our finding that the IL-4 $>$ IL-2 pattern could be reversed by stimulating $-/+/+$ HIV⁺ individuals' PBL in the presence of an anti-IL-4 antibody is consistent with the study in leprosy (35), suggests that cytokine cross-regulation is involved in TH dysregulation in HIV⁺ individuals, and raises the possibility of immunotherapy in HIV disease, based on cytokine cross-regulation.

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References

- Giorgi, J. V., J. L. Fahey, D. C. Smith, L. E. Hultin, H. L. Cheng, R. T. Mitsuyasu, and R. Detels. 1987. Early effects of HIV on CD4 lymphocytes in vivo. *J. Immunol.* 138:3725-3730.
- Petersen, J., J. Church, E. Gomperts, and R. Parkman. 1989. Lymphocyte phenotype does not predict immune function in pediatric patients infected with human immunodeficiency virus type 1. *J. Pediatr.* 115:944-948.
- Miedema, F., A. J. Petit, F. G. Terpstra, J. K. Schattenkerk, F. De Wolf, B. J. Al, M. Roos, J. M. Lange, S. A. Danner, J. Goudsmit, et al. 1988. Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. *J. Clin. Invest.* 82:1908-1916.
- Clerici, M., N. I. Stocks, R. A. Zajac, R. N. Boswell, D. R. Lucey, C. S. Via, and G. M. Shearer. 1989. Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. *J. Clin. Invest.* 84:1892-1899.
- Schnittman, S. M., H. C. Lane, J. Greenhouse, and A. S. Fauci. 1990. Preferential infection of CD4+ T memory cells by human immunodeficiency virus type I: evidence for a role in the selective T cell functional defects observed in infected individuals. *Proc. Natl. Acad. Sci. USA.* 87:6058-6063.
- Van Noesel, C. J. M., R. A. Gruters, F. G. Terpstra, and F. Miedema. 1990. Functional and phenotypic evidence for a selective loss of memory T cells in asymptomatic human immunodeficiency virus-infected men. *J. Clin. Invest.* 86:293-300.
- Rich, E. A., Z. Toossi, and H. Fujiwara. 1988. Defective accessory function of monocytes in human immunodeficiency virus related disease syndromes. *J. Lab. Clin. Med.* 112:174-185.
- Petit, A. J., M. Tersmette, F. G. Terpstra, R. E. de Goede, R. A. Van Lier, and F. Miedema. 1988. Decreased accessory cell function by human monocytic cells after infection with HIV. *J. Immunol.* 140:1485-1490.
- Ennen, J., I. Selpp, S. G. Norley, and R. Kurt. 1991. Decreased accessory cell function of macrophages after infection with human immunodeficiency virus type 1 in vivo. *Eur. J. Immunol.* 20:2451-2456.
- Clerici, M., N. I. Stocks, R. A. Zajac, R. N. Boswell, and G. M. Shearer. 1990. Accessory cell function in asymptomatic, human immunodeficiency virus infected patients. *Clin. Immunol. Immunopathol.* 54:168-174.
- Hofmann, B., N. Odum, B. K. Jakobsen, P. Platz, L. P. Ryder, J. O. Nielsen, J. Gerstoft, and A. Svejgaard. 1986. Immunological studies in the acquired immunodeficiency syndrome. II. Active suppression or intrinsic defect investigated by mixing AIDS cells with HLA-DR identical normal cells. *Scand. J. Immunol.* 23:669-675.
- Sadat-Sowti, B., P. Debré, T. Idziorek, J. M. Guillon, F. Hadida, E. Okze-hendler, C. Katlama, C. Maynaud, and B. Autran. 1991. A lectin-binding soluble factor released by CD8+ CD57+ lymphocytes from AIDS patients inhibits T cell cytotoxicity. *Eur. J. Immunol.* 21:737-739.
- Clerici, M., E. Roilides, C. S. Via, P. A. Pizzo, and G. M. Shearer. 1992. A factor from CD8 cells of human immunodeficiency virus (HIV)-infected patients suppresses HLA self-restricted T helper cell responses. *Proc. Natl. Acad. Sci. USA.* 89:8424-8429.
- Cunningham-Rundles, S., M. A. Michelis, and H. Masur. 1983. Serum suppression of lymphocyte activation in vitro in acquired immunodeficiency disease. *J. Clin. Immunol.* 3:156-165.
- Laurence, J., A. Gottlieb, and H. G. Kunkel. 1983. Soluble suppressor factors in patients with acquired immunodeficiency syndrome and its prodrome: elaboration in vitro by T lymphocyte-adherent cell interactions. *J. Clin. Invest.* 72:2072-2079.
- Laurence, J., and L. Mayer. 1984. Immunoregulatory lymphokines of T hybridomas from AIDS patients: constitutive and inducible suppressor factors. *Science (Wash. DC).* 225:66-69.
- Shalaby, M. R., J. F. Krowka, S. E. Gregory, S. E. Hirabayashi, S. M. McCabe, D. S. Kaufman, D. P. Sites, and A. J. Amman. 1987. The effects of human immunodeficiency virus recombinant envelope glycoprotein on immune cell functions in vitro. *Cell. Immunol.* 110:140-152.
- Mann, D. L., F. Lasane, M. Popovic, L. O. Arthur, W. G. Robey, W. A. Blattner, and M. J. Newman. 1987. HTLV-III large envelope protein gp120 suppresses PHA induced lymphocyte blastogenesis. *J. Immunol.* 138:2640-2646.
- Diamond, D. C., B. P. Sleckman, T. Gregory, L. A. Lasky, J. L. Greenstein, and S. J. Burakoff. 1988. Inhibition of CD4+ T cell function by the envelope glycoprotein gp120. *J. Immunol.* 141:3715-3720.
- Weinhold, K. J., H. K. Lyerly, S. D. Stanley, A. A. Austin, T. J. Matthews, and D. P. Bolognesi. 1989. HIV-1 GP120-mediated immune suppression and lymphocyte destruction in the absence of viral infection. *J. Immunol.* 142:3091-3097.
- Viscidi, R. P., K. Mayur, H. M. Lederman, and A. D. Frankel. 1989. Inhibition of antigen-induced lymphocyte proliferation by Tat protein from HIV-1. *Science (Wash. DC).* 246:1606-1609.
- Kekow, J., W. Wachsmann, A. McCutchan, W. L. Gross, M. Zachariah, D. A. Carson, and M. Lotz. 1990. Transforming growth factor- β and noncytopathic mechanisms of immunodeficiency in HIV infection. *Proc. Natl. Acad. Sci. USA.* 87:8321-8326.
- Mittler, R. S., and M. K. Hoffmann. 1989. Synergism between HIV gp120 and gp120 specific antibody in blocking human T cell activation. *Science (Wash. DC).* 245:1380-1383.
- Golding, H., F. A. Robey, F. T. Gates III, W. Linder, P. R. Beining, T. Hoffman, and B. Golding. 1988. Identification of homologous regions in human immunodeficiency virus I gp41 and human MHC class II beta 1 domain. I. Monoclonal antibodies against the gp41-derived peptide and patients' sera react with native HLA class II antigens, suggesting a role for autoimmunity in the pathogenesis of acquired immune deficiency syndrome. *J. Exp. Med.* 167:914-923.
- Golding, H., G. M. Shearer, K. Hillman, P. Lucas, J. Manischewitz, R. A. Zajac, M. Clerici, R. E. Gress, R. N. Boswell, and B. Golding. 1989. Common epitope in human immunodeficiency virus (HIV) I-GP41 and HLA class II elicits immunosuppressive autoantibodies capable of contributing to immune dysfunction in HIV I-infected individuals. *J. Clin. Invest.* 83:1430-1435.
- Mosmann, T. R., and R. I. Coffman. 1987. Two types of mouse T helper cell clone: implication for immune regulation. *Immunol. Today.* 8:223-226.
- Mosmann, T. R., and R. I. Coffman. 1989. TH1 and TH2 cells: different pattern of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7:145-173.
- Fiorentino, D. F., M. W. Bond, and T. R. Mosmann. 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170:2081-2098.
- Fiorentino, D. F., A. Zlotnik, P. Vieira, T. R. Mosmann, M. Hoard, K. W. Moore, and A. O'Garra. 1991. IL-10 acts on the antigen-presenting cells to inhibit cytokine production by Th1 cells. *J. Immunol.* 146:3444-3451.
- Swain, S. L., L. M. Bradley, M. Croft, S. Tonkonogy, G. Atkins, A. D. Weiberg, D. D. Duncan, S. M. Hedrick, R. W. Dutton, and G. Huston. 1991. Helper T cell subsets: phenotype, function and the role of lymphokines in regulating their development. *Immunol. Rev.* 123:115-144.
- Gazzinelli, R. T., M. Makino, S. K. Chattopadhyay, C. M. Snapper, A. Sher, A. W. Hugin, and H. C. Morse III. 1992. CD4+ subsets regulation in viral infection. Preferential activation of Th2 cells during progression of retrovirus-induced immunodeficiency in mice. *J. Immunol.* 148:182-188.
- Scott, P., and S. H. E. Kaufmann. 1991. The role of T-cell subsets and cytokines in the regulation of infection. *Immunol. Today.* 12:346-348.
- Maggi, E., D. Macchia, P. Paronchi, M. Mazzetti, A. Ravina, D. Milo, and S. Romagnani. 1987. Reduced production of interleukin 2 and interferon-gamma and enhanced helper activity for IgG synthesis by cloned CD4+ T cells from patients with AIDS. *Eur. J. Immunol.* 17:1685-1691.
- Gaya, A., O. de la Calle, J. Yague, E. Alsinet, M. D. Fernandez, M. Romero, V. Fabregat, J. Martorell, and J. Vives. 1991. IL-4 inhibits IL-2 synthesis and IL-2-induced up-regulation of IL-2Ra but not IL-2Rb chain in CD4+ human T cells. *J. Immunol.* 146:4209-4214.
- Yamamura, M., K. Uyemura, R. J. Deans, K. Weinberg, T. H. Rea, B. R. Bloom, and R. L. Modlin. 1991. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science (Wash. DC).* 254:277-279.
- Salgame, P., J. S. Abrams, C. Clayberger, H. Goldstein, J. Convit, 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).* 254:279-282.
- Del Prete, G. F., M. De Carli, C. Mastromauro, R. Biagiotti, D. Macchia, P. Falagiani, M. Ricci, and S. Romagnani. 1991. Purified protein derivative of *Mycobacterium tuberculosis* and excretory-secretory antigen(s) of *Toxocara canis* expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. *J. Clin. Invest.* 88:346-350.
- de Waal Malefyt, R., J. Hannen, H. Spits, M. G. Roncarolo, A. te Velde, C. Figdor, K. Johnson, R. Kastelein, H. Yssel, and J. E. de Vries. 1991. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J. Exp. Med.* 174:915-924.
- de Waal Malefyt, R., J. Abrams, B. Bennet, C. G. Figdor, and J. E. de Vries. 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174:1209-1220.
- Shearer, G. M., and M. Clerici. 1992. T helper cell immune dysfunction in asymptomatic, HIV-1 seropositive individuals: the role of TH1-TH2 cross-regulation. *Prog. Chem. Immunol.* 54:21-43.
- Redfield, R. R., D. C. Wright, and E. C. Tramont. 1986. The Walter Reed staging classification for HTLV-III/LAV infection. *N. Engl. J. Med.* 314:131-136.

42. Hu-Li, J., J. Ohara, C. Watson, W. Tsang, and W. E. Paul. 1989. Derivation of a T cell line that is highly responsive to IL-4 and IL-2 (CT.4R) and of an IL-2 unresponsive mutant of that line (CT.4S). *J. Immunol.* 142:800-807.
43. Svetic, A., F. D. Finkleman, Y. J. Jian, C. W. Deffebach, D. E. Scott, K. F. McCarthy, A. D. Steinberg, and W. C. Gause. 1991. Cytokine gene expression after in vivo primary immunization with goat antibody to mouse IgD antibody. *J. Immunol.* 147:2391-2396.
44. Oswald, I. P., T. A. Wynn, A. Sher, and S. L. James. 1992. IL-10 inhibits macrophage microbicidal activity by blocking the endogenous production of TNF- α required as a costimulatory factor for IFN- γ induced activation. *Proc. Natl. Acad. Sci. USA.* 89:8676-8680.
45. Freedman, D. O., A. Lujan-Trangay, C. Steel, C. Gonzales-Peralta, and T. B. Nutman. 1991. Immunoregulation in onchocerciasis. Functional and phenotypic abnormalities of lymphocyte subsets and changes with therapy. *J. Clin. Invest.* 88:231-238.
46. Via, C. S., G. C. Tsokos, N. I. Stocks, M. Clerici, and G. M. Shearer. 1990. Human in vitro allogeneic responses: demonstration of three pathways of T helper cell activation. *J. Immunol.* 144:2524-2530.
47. D. R. Lucey, G. P. Melcher, C. W. Hendrix, R. A. Zajac, D. W. Goetz, C. A. Butzin, M. Clerici, R. D. Warner, S. Abbadessa, K. Hall, et al. 1991. The U.S. Air Force HIV study 1985-1990: immunological analyses, seroconversion and the potential utility of a T-helper functional assay to predict change in CD4+ T-cell counts during early stage HIV infection. *J. Infect. Dis.* 164:631-637.
48. Mosier, D. E., R. A. Yetter, and H. C. Morse III. 1991. Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57BL/6 mice. *J. Exp. Med.* 161:766-784.
49. Gartner, S., P. Markovitz, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTL VIII/LAV infection. *Science (Wash. DC).* 233:215-219.
50. Gajewsky, T. F., M. Pinna, T. Wong, and F. W. Fitch. 1991. Murine TH1 and TH2 clones proliferate optimally in response to distinct antigen-presenting cell population. *J. Immunol.* 146:1750-1758.
51. Clerici, M., J. A. Berzofsky, G. M. Shearer, and C. O. Tackett. 1991. HIV-1 exposure indicated by HIV-specific T helper cell responses before detection of infection by polymerase chain reaction and serum antibodies. *J. Infect. Dis.* 164:178-182.
52. Clerici, M., J. V. Giorgi, C. C. Chou, V. K. Gudeman, J. A. Zack, P. Gupta, H.-N. Ho, P. G. Nishanian, J. A. Berzofsky, and G. M. Shearer. 1992. Cell mediated immune response to human immunodeficiency virus type 1 (HIV-1) in seronegative homosexuals with recent sexual exposure to HIV-1. *J. Infect. Dis.* 165:1012-1019.