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

Mario Clerici,* Naomi I. Stocks,* Robert A. Zajac,‡ R. Neal Boswell,‡ Daniel R. Lucey,‡ Charles S. Via,* and Gene M. Shearer*
**Experimental Immunology Branch, Division of Cancer Biology and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; and ‡HIV Unit/SGHMMM, Lackland Air Force Base, Texas 78236*

Abstract

We have tested the T helper cell (T_H) potential of asymptomatic, HIV seropositive (HIV$^+$) patients, using an in vitro assay for IL-2 production. Peripheral blood leukocytes (PBL) from 74 HIV$^+$ patients and 70 HIV$^-$ control donors were tested for T_H function when stimulated with influenza A virus (FLU), tetanus toxoid (TET), HLA alloantigens (ALLO), or PHA. Of the HIV$^+$ patients, four different response patterns were observed: (*a*) patients who responded to all four stimuli (16%); (*b*) patients who were selectively unresponsive to FLU and TET, but responded to ALLO and PHA (54%); (*c*) patients who were unresponsive to FLU, TET, or ALLO, but responsive to PHA (16%); and (*d*) patients who failed to respond to any of these stimuli (14%). Our results indicate a time-dependent progression from a stage responsive to all four stimuli to a stage unresponsive to any of the stimuli tested, progressing in the order outlined above.

The earliest T_H defect is the loss of responses to FLU and TET, indicating a selective defect in CD4$^+$ MHC self-restricted T_H function. The later loss of ALLO and PHA IL-2 responses suggests more severe T_H dysfunction involving both CD4$^+$ and CD8$^+$ T cells. None of these patterns of T_H unresponsiveness in asymptomatic HIV$^+$ individuals were correlated with CD4$^+$ cell numbers nor with Walter Reed staging criteria. This study indicates that the in vitro T_H assay used can detect multiple stages of immune dysregulation early in the course of HIV infection and raises the possibility that staging of HIV$^+$ patients should include in vitro T_H functional analyses of the type described here.

Introduction

It is well established that the T lymphocyte-mediated immune defects in patients with AIDS are associated with a severe reduction in the number of CD4$^+$ T lymphocytes (1–5). Furthermore, it is considered that the loss of CD4$^+$ T cells is the result of infection with HIV, although the mechanism(s) by which HIV depletes CD4$^+$ T cells has not been determined. However, before a severe depletion of CD4$^+$ cells, a long period without apparent syndrome progression can ensue between the time of infection and development of AIDS symptoms (6–10). This period is characterized by near normal numbers of CD4$^+$ cells, absence of symptoms, positive delayed skin reactions, and positive in vitro proliferative responses to antigenic and mitogenic stimuli, and can be followed by a progressive and sometimes rapid deterioration resulting in symptomatic AIDS.

Early detection of HIV-induced, immune-related changes is important for identification and understanding of the initial events leading to HIV-associated immune dysregulation. Furthermore, detection of early changes in immune parameters would be useful for timing of therapeutic intervention as well as for monitoring immunologic changes that might result from therapy. Early indicators of progression toward AIDS include reduced numbers of CD4$^+$ cells, a decline of antibodies specific for HIV viral proteins, and an appearance of viral antigens (1–10). In a previous study, we observed that PBL from some otherwise asymptomatic HIV seropositive (HIV$^+$) individuals exhibited a defect in cytotoxic T lymphocyte (CTL)[1] responses to influenza A virus, but not to HLA alloantigens (11). These results can be explained by a defect in CD4$^+$ T helper cell (T_H) function, even though CD4$^+$ cell numbers were not critically reduced in these patients.

In the present report we have assessed T_H function by using an in vitro assay for IL-2 production. PBL from Walter Reed Stage 1 (WR 1) and 2 (WR 2) patients were stimulated with influenza virus (FLU), tetanus toxoid (TET), alloantigens (ALLO), and PHA. This panel of stimuli was selected for study because T_H responses to FLU and TET have been recently shown to be MHC self-restricted and require CD4$^+$ T_H and autologous antigen-presenting cells (APC). In contrast, the T_H responses to ALLO and PHA can utilize both CD4$^+$ and CD8$^+$ T cells (11–13).[2] Using this approach, we have identified three categories of T_H dysfunction among HIV$^+$ patients whose clinical stages were WR 1 or WR 2. These findings may be valuable as a prognostic indicator for progression toward symptomatic AIDS.

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

Address reprint requests to Dr. Shearer, Experimental Immunology Branch, National Cancer Institute, Building 10, Room 4B17, National Institutes of Health, Bethesda, MD 20892.

The present address of Dr. Via is Rheumatology Division, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201.

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1. *Abbreviations used in this paper:* ALLO, alloantigens; CTL, cytotoxic T lymphocyte; FLU, influenza virus; LSM, lymphocyte separation medium; TET, tetanus toxoid; T_H, T helper cell; WR 1 and WR 2, Walter Reed stages 1 and 2.

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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 the Walter Reed staging system (14). The HIV^- control donors were USAF personnel of the same sex and with the same age range as the HIV^+ donors.

Lymphocyte counts and T cell subsets were determined using laser-based flow cytometry (Coulter Epics Profile; Coulter Electronics, Inc., Hialeah, FL) and OKT4A (anti-CD4) and OKT8 (anti-CD8) monoclonal antibodies (Orthodiagnostics Systems, Raritan, NJ).

Skin testing for recall antigens was performed using intradermal injections of 0.02 ml of PPD (5TU) (Connaught Laboratories, Ontario, Canada), *Candida albicans* ($^1/_{500}$) (Hollister-Stier, Spokane, WA), Tricophyton ($^1/_{500}$) (Hollister-Stier, Spokane, WA), tetanus toxoid ($^1/_5$), and mumps (full strength) (both from Connaught Laboratories).

In vitro tests for T_H function. Whole blood from HIV^- and HIV^+ individuals was drawn in Vacutainer tubes containing preservative-free heparin (Becton-Dickinson & Co., Rutherford, NJ) and shipped from Lackland, TX to Bethesda, MD overnight at ambient temperature. PBL were separated on lymphocyte separation medium (LSM; Organon Teknika Corp., Durham, NC), washed twice in PBS, and resuspended at 3×10^6/ml in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 0.5% penicillin and 1% glutamine. The number of viable cells was determined by trypan blue exclusion and hemacytometer. For IL-2 production, 1 ml of PBL was added per well to 24-well flat-bottom Linbro tissue culture plates (Flow Laboratories, Inc., McLean, VA). For the proliferative assay, 0.1 ml of PBL was added per well, in triplicate wells, to 96-well flat-bottom tissue culture plates (Costar, Cambridge, MA). The PBL were cultured without stimulation or were stimulated with (*a*) influenza A/Bangkok RX73 (at a final dilution of 1:1,000) as previously described (11); (*b*) tetanus toxoid (at a final dilution of 40 lf/ml) (Massachusetts Department of Health, Boston, MA); (*c*) a pool of irradiated (5,000 rad) PBL from two or more unrelated HIV^- donors (2×10^6/well for IL-2 production and 2×10^5/well for proliferation); and (*d*) PHA (Gibco Laboratories) diluted 1:200. Pooled AB^+ plasma was added to each well (final dilution 1:20). Supernatants of stimulated and unstimulated cultures were harvested 7 d later and frozen at −20°C. For studies of IL-2 production, the anti-IL-2 receptor antibody, monoclonal anti-TAC (a kind gift from Dr. T. A. Waldmann, Metabolism Branch, NCI, NIH, Bethesda, MD), was added at the initiation of culture at a final concentration of 10 μg/ml, in order to block IL-2 consumption (15). The supernatant IL-2 activity was assessed as the ability to stimulate the proliferation of the IL-2-dependent cell line, CTLL. Assay cultures consisted of 8×10^3 CTLL/well and five successive twofold dilutions of supernatant. 24 h later, the cultures were pulsed with 1 μCi of [^{3}H]thymidine (ICN Radiochemicals, Irving, CA) and harvested 18 h later. Results are expressed as mean counts per minute for three replicate wells for a given supernatant dilution. Standard errors were always < 10% of the mean values. For each IL-2 assay, a standard titration curve was determined using recombinant IL-2. However, we were not able to accurately calculate IL-2 units because many of the IL-2 titration curves generated by antigenic stimulation of PBL from immune deficient HIV^+ donors were flat and were not parallel to the standard curves nor the curves generated by PBL from the HIV^- controls. Therefore, in Figs. 1, 3, and 4, we show the entire titration curves.

For the proliferative assay, cultures were pulsed with 1 μCi of [^{3}H]thymidine 6 d after sensitization and harvested 18 h later. For both assays, thymidine incorporation was determined using a model LS1801 β-spectrometer (Beckman Instruments, Fullerton, CA).

Determination of responsive and unresponsive patients. For both the IL-2 and proliferative assays, patients were defined as responsive to a given antigen if the mean cpm of their stimulated cultures was > 3 SD above the mean unstimulated cpm of the HIV^- control donors. The cutoff value for IL-2 production was 7,300 cpm and was derived from a mean of 70 HIV^- donors. Supernatant dilutions of both 1:2 and 1:4 were used for determination of responsiveness for the IL-2 assay. The cutoff value for proliferation was 3,138 cpm and was derived from a mean of 58 HIV^- donors.

Statistical analysis of data. Row (R) × column (C) contingency tables were set up as shown in Tables I and II for testing possible correlations of the four different T_H functional categories with $CD4^+$ cell numbers or WR staging (16). The sum of the χ^2 value was calculated by the sums of the equation $\chi^2 = (f - F)^2/F$, where f is the observed frequency and F is the expected frequency. The degrees of freedom for this analysis are: df = (R − 1)(C − 1). Student's t tests were performed for the comparisons of two independent samples of unequal size as described by Snedecor and Cochran (16), and P values were determined.

Results

Patterns of T_H responses in WR 1 and WR 2 patients. PBL from 70 HIV^- control donors and 74 HIV^+ WR 1 and 2 patients were tested for in vitro IL-2 production after stimulation with FLU, TET, ALLO, or PHA. The complete IL-2 titration curves for each of these stimuli are presented in Fig. 1 for two HIV controls (Fig. 1, *A* and *F*), four WR 1 patients (Fig. 1, *B–E*), and four WR 2 patients (Fig. 1, *G–J*). The data on these patients were selected from the group of 74 to illustrate four different patterns of IL-2 responsiveness. The first pattern, observed in both WR 1 and WR 2 patients (Fig. 1, *B* and *G*), is characterized by positive IL-2 responses to all four stimuli. In the second pattern, PBL from WR 1 and WR 2 patients (Fig. 1, *C* and *H*) generated near normal IL-2 responses to ALLO and PHA, but failed to produce IL-2 in response to FLU and TET. In the third pattern, PBL from the WR 1 and WR 2 patients (Fig. 1, *D* and *I*) responded to PHA but failed to respond to FLU, TET, and ALLO. Finally, PBL from the WR 1 and WR 2 patients (Fig. 1, *E* and *J*) failed to respond to any of these stimuli. These results indicate that four different states of T_H function can be identified within WR 1 and WR 2 patients. Furthermore, the failure of WR 1 and WR 2 patients to respond to any combination of these stimuli did not appear to be correlated with $CD4^+$ cell numbers (see below).

Using the criteria described in Methods, we determined the number of individuals in the group of 74 patients and 70 controls who responded to FLU, TET, ALLO, or PHA by IL-2 production. We also tested 49 of these same patients and 58 of the controls for proliferative responses to the same stimuli. The numbers and percentages of patients and controls who were unresponsive to each of the stimuli by either of the two T_H tests are presented in Table I. 62 of the 74 HIV^+ donors (84%) failed to respond to FLU or TET by the IL-2 test. A much lower proportion of these donors was unresponsive to ALLO (30%), and an even lower proportion was unresponsive to PHA (14%). In contrast, only one HIV^- control donor was unresponsive to FLU and TET, and none of the controls was unresponsive to ALLO or PHA. There was an exact concordance among individuals for both IL-2 and proliferative responses to FLU and TET. Again, similar to the IL-2 data, fewer patients failed to respond to ALLO than to FLU or TET, and even fewer did not respond to PHA. The percentage of HIV^- controls that was unresponsive to FLU and TET was 1% as measured by IL-2 production and 3–5% by proliferation. All of the controls responded to ALLO and PHA by both assays. For convenience, we shall refer to those patients who re-

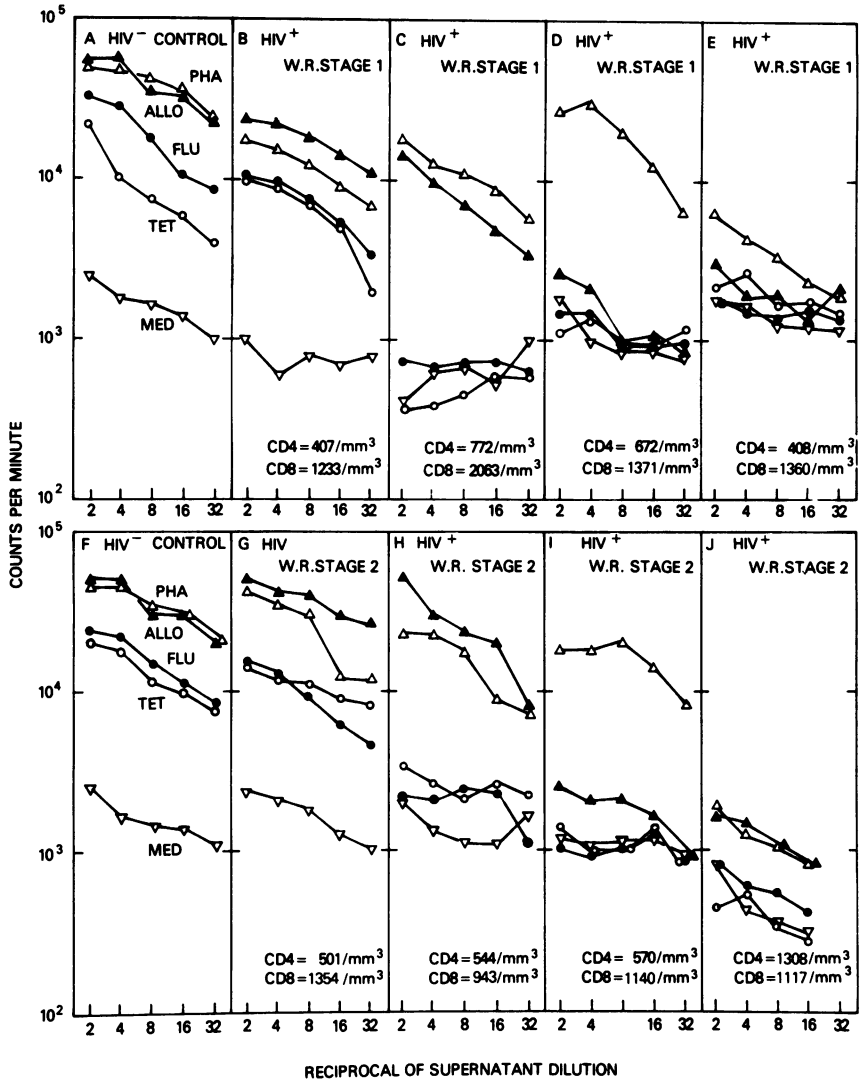

Figure 1. IL-2 production by PBL from two HIV⁻ control donors (*A* and *F*), four WR 1 HIV⁺ individuals (*B–E*), and four WR 2 HIV⁺ individuals (*G–J*). The PBL from these donors were unstimulated (MED, ▽) or were stimulated with FLU (●), TET (○), ALLO (▲), or PHA (△). The titration curves represent the dilutions of culture supernatant used to stimulate the CTLL.

sponded to all four stimuli as +/+/+ (FLU and TET/ALLO/PHA); to those who failed to respond to FLU and TET, but responded to ALLO and PHA as −/+/+; to those who failed to respond to FLU, TET, and ALLO, but responded to PHA as −/−/+; and to those who did not respond to any of the stimuli as −/−/−. It should be noted that no patients were found who (*a*) responded to FLU or TET but not to ALLO or PHA; or (*b*) did not respond to PHA but were responsive to any of the other three stimuli. These results are compatible with a pattern of sequential progression from +/+/+ to −/+/+, to −/−/+, and finally to −/−/−.

Fig. 2 lists the frequencies of these four categories of T_H responsiveness, and within each category, quantitatively summarizes the IL-2 results obtained from each stimulant by plotting individual data points and mean values. The data shown are for a supernatant dilution of 1:4; similar results were obtained at dilutions of 1:2 and 1:8. Among the 40 patients who were selectively unresponsive to FLU and TET (−/+/+), IL-2 production levels to FLU and TET were significantly below those of the HIV⁻ controls (compare Fig. 2 *C* with *A*, and *H* with 2F; $P < 0.01$ for FLU and $P < 0.01$ for TET), and also significantly below those of the responsive HIV⁺ patients (compare Fig. 2 *C* with *B*, and *H* with *G*; $P < 0.05$ for FLU and $P < 0.05$ for TET). IL-2 production did not differ significantly among these three groups for responses to ALLO and PHA (Fig. 2, *K–M* and *P–R*). The 12 patients who responded only to

Table I. Fraction and Percentage of Unresponsive Patients to FLU, TET, ALLO, and PHA

	Tested by IL-2 production				Tested by proliferation			
Donor status	FLU	TET	ALLO	PHA	FLU	TET	ALLO	PHA
HIV⁺ patients	62/74	62/74	22/74	10/74	39/49	39/49	11/49	2/49
	84%	84%	30%	14%	80%	80%	22%	4%
HIV⁻ controls	1/70	1/70	0/70	0/67	3/58	2/57	0/58	0/56
	1%	1%	0%	0%	5%	3%	0%	0%

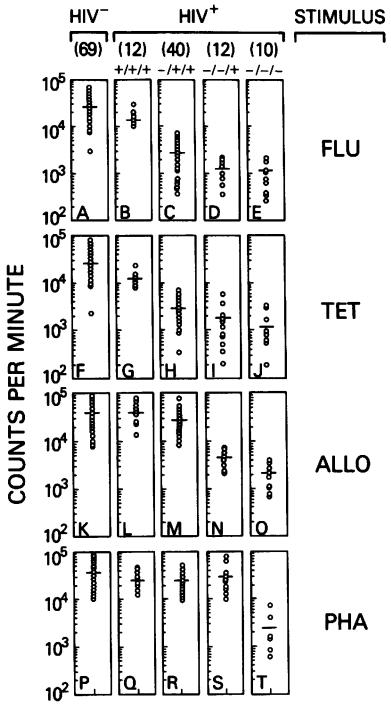

Figure 2. Mean and individual values for IL-2 production (detected at a supernatant dilution of 1:4) by PBL from HIV⁻ (*A, F, K, P*) and HIV⁺ (all other panels) individuals to: FLU (*A–E*); TET (*F–J*); ALLO (*K–O*); or PHA (*P–T*). +/+/+ indicates individuals whose PBL generated normal IL-2 responses to all four stimuli; −/+/+ indicates individuals whose PBL were selectively deficient in IL-2 responses to FLU and TET; −/−/+ indicates individuals whose PBL were deficient in IL-2 responses to FLU, TET, and ALLO, but not to PHA; −/−/− indicates individuals whose PBL were deficient in response to all four stimuli. Number of donors in each category is shown in ( ) in donor status column. In some of the panels (for example, 69 HIV⁻ and 40 −/+/+ donors), overlapping values did not permit showing of all points.

PHA by IL-2 production (−/−/+) exhibited a significant reduction in mean counts per minute for ALLO when compared with the ALLO response of −/+/+ or +/+/+ patients or the HIV⁻ donors (compare Fig. 2 *N* with *K–M; P* < 0.05). The mean counts per minute for response to PHA, however, was similar among these groups. Finally, the 10 patients who were unresponsive to any of these four stimuli (−/−/−) exhibited a significant reduction in counts per minute for IL-2 production to PHA compared with the groups of patients who responded to PHA (compare Fig. 2 *E* with *P–S; P* < 0.05). The results of Fig. 2 verify that these 74 WR 1 and WR 2 patients can be divided into four functional categories, based on T_H responses to recall antigens, alloantigens, and a T cell mitogen.

Most of the patients in the study were also tested for delayed skin reactions to a panel of antigens including *Candida albicans,* tetanus toxoid, mumps, tricophyton, and purified protein derivative (PPD). Of the 63 WR 1 and 2 patients who were tested for both in vitro IL-2 production and for in vivo skin reactions to TET, 51 were unresponsive to TET by IL-2 production, whereas only 8 were unresponsive to TET by skin test (data not shown).

Lack of correlation between T_H function and either CD4⁺ cell number or WR 1 and WR 2. Contingency tables were prepared in which the numbers of patients in each functional category are shown for three ranges of CD4⁺ cell numbers (Table II) and for WR 1 and WR 2 patients (Table III). Statistical analysis of the data in the R × C contingency comparison of Table II indicates that there was no correlation between any of the four T_H response patterns (by IL-2 or proliferation) and the CD4⁺ cell numbers. A similar analysis of the frequency data for the four T_H categories by either the IL-2 production or proliferation assays for T_H function indicates no correlation of function with WR 1 or WR 2 (Table III).

Table II. Lack of Correlation between T_H Function and CD4⁺ Cell Number

Functional category*	Range of CD4⁺ cell numbers (/mm³) 400–600	600–800	>800	Total	Statistics
By IL-2 production					
+/+/+	6	2	4	12	
−/+/+	14	16	10	40	
−/−/+	4	5	3	12	
−/−/−	7	2	1	10	$\chi^2 = 4.60$
Total	31	25	18	74	$P > 0.50$
By proliferation					
+/+/+	3	2	5	10	
−/+/+	11	13	4	28	
−/−/+	3	4	2	9	
−/−/−	2	0	0	2	$\chi^2 = 7.73$
Total	19	19	11	49	$P > 0.25$

* +/+/+ indicates patients who responded to FLU, TET, ALLO, and PHA; −/+/+ indicates patients who responded to ALLO and PHA, but not to FLU and TET; −/−/+ indicates patients who responded to PHA, but not to FLU, TET, or ALLO; −/−/− indicates patients who did not respond to any of the four stimuli.

Time-dependent changes in T_H function. The data illustrated in Figs. 1 and 2 suggest a progression from the functional stage in which the patient is responsive to all four stimuli (+/+/+), through a selective loss in T_H function to recall antigens such as FLU and TET (−/+/+), followed by loss of response to ALLO (−/−/+), and finally to a totally unresponsive state (−/−/−). However, these data, as well as the data collected on most of our 74 patients, represent only one point in

Table III. Lack of Correlation between T_H Function and Walter Reed Stage (WR)

Functional category*	WR 1	2	Total	Statistics
By IL-2 production				
+/+/+	6	6	12	
−/+/+	22	18	40	
−/−/+	6	6	12	
−/−/−	8	2	10	$\chi^2 = 1.71$
Total	42	32	74	$P > 0.50$
By proliferation				
+/+/+	3	7	10	
−/+/+	15	13	28	
−/−/+	5	4	9	
−/−/−	2	0	2	$\chi^2 = 3.84$
Total	25	24	49	$P > 0.25$

* +/+/+ indicates patients who responded to FLU, TET, ALLO, and PHA; −/+/+ indicates patients who responded to ALLO and PHA, but not to FLU and TET; −/−/+ indicates patients who responded to PHA, but not to FLU, TET, or ALLO; −/−/− indicates patients who did not respond to any of the four stimuli.

time for each individual's progression toward symptomatic AIDS. As part of a longitudinal study of T_H function, we have collected and cryopreserved serial blood samples from a limited number of asymptomatic, HIV^+ individuals for the past 5 yr. If the patterns of unresponsiveness shown in Fig. 1 are sequential and represent a progressive loss of T_H function, then it should be possible to detect a sequential loss of T_H function with time in a given patient.

Fig. 3 illustrates a longitudinal comparison of FLU-stimulated IL-2 production by PBL from three donors who have been involved in this study for several years. PBL from the donor shown in Fig. 3 *A* were cryopreserved in 1983 and in 1988. This donor remained asymptomatic throughout the 4.5-yr period and had 573/mm^3 of $CD4^+$ cells at the time of the last blood collection. PBL from the donor in Fig. 3 *B* were cryopreserved in 1984 and in 1987. This donor experienced a reduction in $CD4^+$ cells (from 500 to 139/mm^3) during this period, but was not diagnosed with lymphadenopathy or AIDS. PBL from the donor in Fig. 3 *C* were cryopreserved in 1985 and 1988. During this time interval, this donor experienced a reduction in $CD4^+$ cells from 715 to 235/mm^3, but remained otherwise asymptomatic. The two preparations of PBL from each of these three donors were thawed simultaneously and tested for their ability to generate IL-2 in response to stimulation with FLU and TET (donor in Fig. 3 *A*) or with FLU only (donors in Fig. 3, *B* and *C*). These results (Fig. 3) demonstrate that in these three donors there was a time-dependent, selective loss of T_H function to CD4-dependent antigens such as FLU and TET and suggest that a similar pattern of selective loss of T_H response occurred in the other donors who were selectively defective in their responses to FLU and TET. It should be noted that the time-dependent loss of T_H response to FLU and TET in the donor in Fig. 3 *A* was not due to a critically low number of $CD4^+$ cells. Due to limitations on the number of cryopreserved PBL available, it was not possible to test these individuals for T_H function to ALLO or PHA. It should also be noted that PBL from HIV^- donors frozen in 1983 and tested in 1988 still retained a strong T_H function to FLU and TET (data not shown).

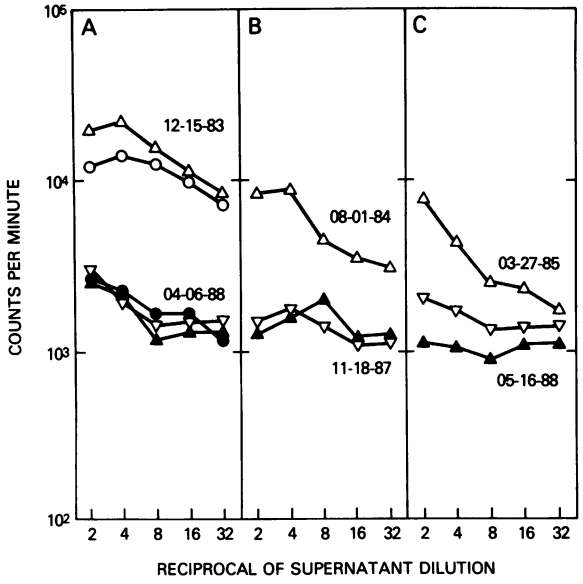

Figure 3. IL-2 production by PBL from three HIV^+ individuals taken at different time intervals during progressive loss of T_H cell function. PBL were unstimulated (▽) (mean value of the two unstimulated cultures), or stimulated with FLU (△, ▲) or TET (○, ●). Open symbols indicate the response from the earlier bleed; closed symbols indicate from the later bleed. The numbers in each panel indicate the two dates that blood was drawn from a donor to be used in the comparative test. Both samples of the PBL from each donor were cryopreserved and tested in the same experiment.

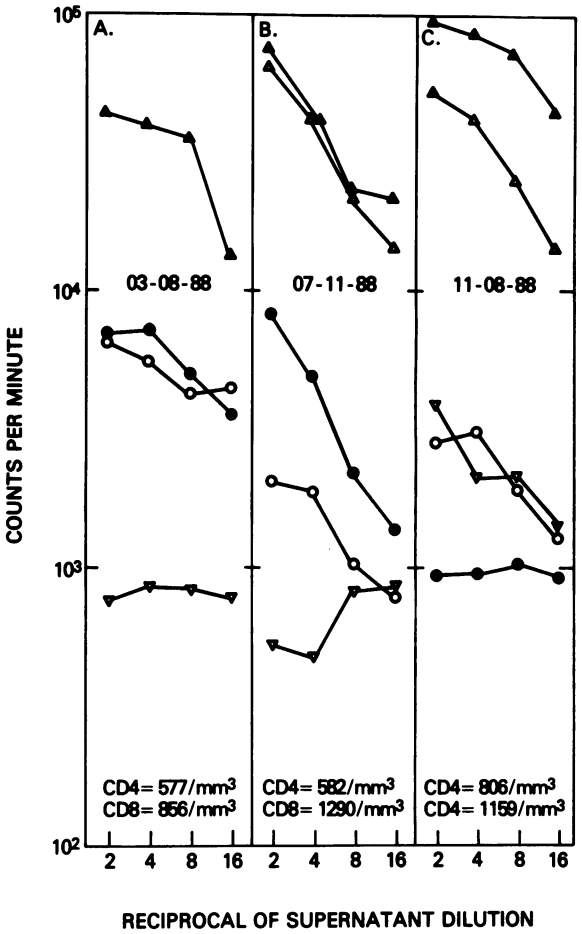

Figure 4. IL-2 production by PBL from a single asymptomatic, HIV^+ individual (WR 1) taken at three 4-mo time intervals. PBL were unstimulated (▽) or stimulated with FLU (●), TET (○), ALLO (▲), or PHA (△). The numbers in each panel indicate the dates that the blood was drawn and tested. $CD4^+$ and $CD8^+$ cell numbers are shown in the lower part of each panel.

To obtain a more precise time estimate for development of T_H unresponsiveness, an asymptomatic, HIV^+ donor was studied at 4-mo intervals to detect possible loss of T_H function. Fig. 4 illustrates the titration curves for IL-2 production by PBL stimulated with FLU, TET, ALLO, or PHA. In March 1988, the patient was marginally responsive to FLU and TET, but was strongly responsive to ALLO (Fig. 4 *A*). (PHA was not tested in this experiment.) During the next 8 mo, the patient's FLU and TET responses continued to decline to unstimulated levels, whereas the ALLO and PHA responses remained strong (Fig. 4, *B* and *C*). During this time interval, the patient did not develop any symptoms and $CD4^+$ and $CD8^+$ T cell numbers increased slightly. In all three experiments, PBL from HIV^- control donors generated potent T_H responses to all four stimuli (data not shown). These data demonstrate that loss of T_H function to recall antigens can occur abruptly and is not neces-

sarily associated with symptomatic changes nor with a reduction in $CD4^+$ cell numbers.

To further investigate possible progression in T_H defects, we have studied IL-2 responses of PBL from patients in the more advanced WR 3 to WR 6. If the failure to produce IL-2 in response to ALLO represents T_H dysfunction associated with more advanced stages of AIDS progression, then a larger proportion of WR 3 to WR 6 patients should be unresponsive to FLU or TET, and also to ALLO. The proportions of IL-2 responses by 22 patients are summarized in Table IV and are compared with the proportions of responses by our 74 WR 1 and WR 2 patients using the same two stimuli. The majority of the WR 3 to WR 6 failed to respond to FLU or ALLO (64%) compared with only 30% of the WR 1 and WR 2 patients who failed to respond to both stimuli. The statistical likelihood that these frequency differences between WR 1 and WR 2 and WR 3 to WR 6 patients were due to chance was < 1%. Thus, these results support the hypothesis that the T_H functional phases shown in Figs. 1 and 2 represent a sequential progression of immune dysfunction that is associated with progression toward AIDS.

Discussion

Other studies have reported that the T cell responses to recall antigens may be defective in some HIV^+ patients who do not have AIDS (17–20). Our laboratory (11) has previously demonstrated a selective defect in CTL responses to FLU but not to ALLO in asymptomatic, HIV^+ individuals. Because the deficient CTL response to FLU could be corrected in vitro by recombinant IL-2, the CTL defect appeared to reflect an underlying deficiency in $CD4^+$ T_H function. A similar IL-2-correctable defect in CTL function has been reported in patients with symptomatic AIDS (21). These findings indicate that: (*a*) IL-2 is a helper lymphokine for human CTL generation; and (*b*) this lymphokine is deficient in HIV-infected individuals. The present study measures T_H function directly by assessing IL-2 production and extends these findings by: (*a*) identifying a series of three distinct T_H functional defects in asymptomatic patients; (*b*) demonstrating a time-dependent progressive and selective loss of T_H function in individual asymptomatic patients; and (*c*) attempting to correlate these defects with clinical staging criteria.

The three different patterns of T_H dysfunction identified among these asymptomatic patients were (*a*) a selective loss of T_H function to recall antigens such as FLU and TET, but retention of T_H function upon stimulation to ALLO or PHA (54%); (*b*) an absence of T_H function to the recall antigens and to ALLO, but preserved responses to PHA (16%); and (*c*) a lack of response to all of the stimuli (14%). Furthermore, 16% of these asymptomatic patients were responsive to all of the stimuli. None of the three patterns of T_H dysfunction could be attributed to a critical reduction in $CD4^+$ cell numbers. PBL from many of the same patients who were positive to tetanus by skin test were negative by IL-2 production and by [^{3}H]thymidine incorporation after in vitro stimulation with tetanus. This discrepancy in the in vivo and in vitro immunological tests could be due to one of several possibilities: (*a*) the in vitro tests introduce artifacts that are not observed by in vivo delayed skin reactions; (*b*) the in vitro tests utilize different functional subsets of T lymphocytes and/or APC than the delayed skin reaction; or (*c*) the in vitro IL-2 production and [^{3}H]thymidine incorporation assays are more sensitive and detect immune dysfunction before the delayed skin reaction. We favor the third hypothesis because we have observed a progression in loss of IL-2 production to recall antigens as a function of time, which in WR 1 and WR 2 patients is not correlated with a decline in $CD4^+$ cell numbers. Thus, it is likely that our in vitro data reflect an early stage of $CD4^+$ T_H dysfunction. The observation that none of 22 advanced WR 3 to WR 6 patients responded by IL-2 production to FLU (compared with the 16% of WR 1 and WR 2 patients who responded to the two recall antigens) supports the hypothesis that loss of T_H function to FLU and TET is an early sign of immune dysfunction that is progressive. Furthermore, our observation that a higher percentage (64%) of more advanced patients failed to respond to FLU and ALLO than did WR 1 and WR 2 patients supports our interpretation that the absence of T_H responses to ALLO represents advancing stages of more severe immune dysfunction. Recently, using T lymphocytes and APC from healthy, HIV^- donors, we have demonstrated that T_H responses to FLU and TET require $CD4^+$ T_H cells and autologous APC. In contrast, T_H responses to ALLO can utilize three different T_H-APC pathways: (*a*) $CD4^+$ T_H and autologous APC that process and present alloantigenic HLA determinants in association with self-HLA products; (*b*) $CD4^+$ T_H that recognize ALLO directly on allogeneic APC; and (*c*) $CD8^+$ T cells that recognize ALLO directly on allogeneic APC (13).[2] Likewise, PHA responses can be mediated by both $CD4^+$ and $CD8^+$ T cells (12). Therefore, the selective defect in T_H responses to FLU and TET in 84% of our patients can be attributed to an early immune dysfunction that selectively affects $CD4^+$ T_H and/or autologous APC. $CD8^+$ T cells would be unaffected and could respond to ALLO and PHA.

The loss in T_H function in asymptomatic patients is not due to a critical reduction in $CD4^+$ cell numbers and, therefore, must be attributed to some other mechanism(s). $CD4^+$ cells could be infected without being killed and such infected cells would be nonfunctional (22). However, this possibility appears to be unlikely because in situ hybridization studies indicate that the frequency of infected leukocytes is less than $1/10^3$ (23). An alternative possible mechanism is suggested by the recent report that the sera of some AIDS patients (24), as well as some asymptomatic HIV^+ individuals (25), contain an antibody that reacts with a nonpolymorphic component of human MHC class II molecules. These antibodies can block antigen-stimulated proliferative responses of $CD4^+$ T cells from HIV^- individuals (24). Thus, these autoantibodies might block MHC class II self-restricted interactions between $CD4^+$ and APC required for FLU and TET responses. A third possibility is that HIV infection activates a regulatory cell that selec-

*Table IV. Evidence for a Progressive and Sequential Loss of T_H Function in More Advanced Walter Reed Stages (WR)**

T_H response to		Fraction and percentage of patients in	
FLU	ALLO	WR 1 and 2	WR 3–6
+	+	12/74 (16%)	0/22 (0%)
−	+	40/74 (54%)	8/22 (36%)
−	−	22/74 (30%)	14/22 (64%)

* $\chi^2 = 9.82$; $P < 0.07$.

tively suppresses CD4-mediated responses (26). Despite the fact that regulatory cells that selectively suppress $CD4^+$ T_H function have been described in murine models of T_H dysfunction (27, 28), we have not yet detected such a regulatory cell in HIV patients. Fourth, because HIV infects $CD4^+$ monocytes and macrophages (29, 30), and because this early defect is limited to antigens that require $CD4^+$ T_H cells and autologous APC, it is possible that antigen-presenting function rather than T_H function is defective in these patients. However, preliminary studies from our laboratory comparing the T_H and APC functions of cells from individual, asymptomatic patients, before and after the loss of T_H response to FLU and TET, have not shown any defect in APC function (manuscript in preparation). Thus, the mechanism(s) responsible for the early T_H dysfunction in asymptomatic, HIV^+ individuals remains unresolved, but is under investigation.

We have defined responsive and unresponsive patients by the statistical method described in Methods. Stimulation indices of 2.0 or greater have also been used to define responsiveness in other studies (31). We compared both methods in the present study, and found no differences in the percentage of unresponsive donors for the [^{3}H]thymidine assay by either approach. Thus, 80% of the WR 1 and WR 2 patients were unresponsive to FLU and TET, irrespective of which method was used. However, we did find that a lower percentage (57%) of patients were unresponsive to FLU and TET if we used a stimulation index of 2.0 rather than 3 SD above the mean background method (84%). Regardless of which method is used and whether T_H function is measured by IL-2 production or by [^{3}H]thymidine incorporation, a majority of WR 1 and WR 2 patients are unresponsive to recall antigens.

Despite our finding that the WR 1 and WR 2 patients exhibit $CD4^+$ T_H defects, they do not have opportunistic infections. It is possible that the loss of $CD4^+$ T_H function is responsible for a nonprogressive period after HIV infection. For example, the observation that $CD4^+$ T cells must be activated for HIV to be infectious (32, 33) is compatible with the hypothesis that a nonprogressive period could be associated with a condition of $CD4^+$ T_H unresponsiveness. If $CD4^+$ T_H were unresponsive to antigenic stimulation, further progression toward AIDS by more extensive infection of the remaining uninfected $CD4^+$ T cells might be impaired. Thus, this unresponsive condition may indeed be protective and represent a period in which down-regulation of $CD4^+$ T_H function inhibits further spread of HIV infection.

Because none of the four distinct patterns of T_H function identified in HIV^+ asymptomatic individuals were correlated with either $CD4^+$ cell numbers nor with WR staging, we suggest that an in vitro test for T_H function be incorporated into HIV staging criteria. This assay could include IL-2 production to one or more recall antigens to assess $CD4^+$ T_H-self APC interactions and include the IL-2 responses to ALLO and PHA to detect the presence of more severe immune functional defects.

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Note added in proof. Since submission of this manuscript, an additional 206 WR 1 and WR 2 patients have been tested for T helper function for IL-2 production in response to FLU, TET, and ALLO (but not to PHA). 82/206 (40%) responded to all stimuli: 88/206 (43%) failed to respond to FLU and TET, but responded to ALLO; and 36/206 (17%) failed to respond to any of the three stimuli.

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