High-level HIV-1 viremia suppresses viral antigen-specific CD4$^+$ T cell proliferation

Andrew C. McNeil, W. Lesley Shupert, Christiana A. Iyasere, Claire W. Hallahan, JoAnn Mican, Richard T. Davey, Jr., and Mark Connors*

Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

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In chronic viral infections of humans and experimental animals, virus-specific CD4$^+$ T cell function is believed to be critical for induction and maintenance of host immunity that mediates effective restriction of viral replication. Because *in vitro* proliferation of HIV-specific memory CD4$^+$ T cells is only rarely demonstrable in HIV-infected individuals, it is presumed that HIV-specific CD4$^+$ T cells are killed upon encountering the virus, and maintenance of CD4$^+$ T cell responses in some patients causes the restriction of virus replication. In this study, proliferative responses were absent in patients with poorly restricted virus replication although HIV-specific CD4$^+$ T cells capable of producing IFN-γ were detected. In a separate cohort, interruption of antiretroviral therapy resulted in the rapid and complete abrogation of virus-specific proliferation although HIV-1-specific CD4$^+$ T cells were present. HIV-specific proliferation returned when therapy was resumed and virus replication was controlled. Further, HIV-specific CD4$^+$ T cells of viremic patients could be induced to proliferate in response to HIV antigens when costimulation was provided by anti-CD28 antibody *in vitro*. Thus, HIV-1-specific CD4$^+$ T cells persist but remain poorly responsive (produce IFN-γ but do not proliferate) in viremic patients. Unrestricted virus replication causes diminished proliferation of virus-specific CD4$^+$ T cells. Suppression of proliferation of HIV-specific CD4$^+$ T cells in the context of high levels of antigen may be a mechanism by which HIV or other persistently replicating viruses limit the precursor frequency of virus-specific CD4$^+$ T cells and disrupt the development of effective virus-specific immune responses.

In a number of animal models of viral pathogenesis, virus-specific CD4$^+$ T cells are critical in induction or maintenance of an effective CD8$^+$ cytotoxic T lymphocyte response that mediates restriction of virus replication (1–3). Acute or chronic virus infections in humans or experimental animals typically result in induction of CD4$^+$ T cell responses that can be demonstrated by proliferation to virus antigens *in vitro* long after control of infection due to persistence of memory CD4$^+$ T cells. Unlike most other viral infections in humans, HIV-1 infection also is characterized by the early disappearance of CD4$^+$ T cell-mediated proliferative responses to HIV antigens, a response that typically remains absent in the untreated individual (4–7). Because HIV infects CD4$^+$ T cells, it is believed that the early loss of these proliferative responses may be the result of deletion of HIV-specific cells early in infection when they encounter the virus (4).

However, loss of HIV-specific CD4$^+$ T cell responses during infection is not universally the case (4–6, 8–14). A relatively rare subgroup of patients (<0.8% of the HIV-infected population) maintain <50 copies of virus RNA per ml of plasma without antiretroviral therapy despite 15 or more years of infection. In these long-term nonprogressors (LTNP) that restrict virus replication in the absence of antiretroviral therapy, and some patients treated early in HIV infection, virus-specific proliferative responses are maintained (4–6, 8, 9, 15). Because most previous studies did not examine both the frequency and proliferation of antigen-specific cells, it remains unclear whether the absence of proliferative responses are actually caused by the deletion of antigen-specific CD4$^+$ T cells *in vivo* or dysfunction of these cells. More importantly, most previous studies are correlative and have not addressed the cause-and-effect relationships between CD4$^+$ T cell responses and restriction of virus replication.

In this study, the frequency and function of virus-specific CD4$^+$ T cells were examined, using cytomegalovirus (CMV) and five different HIV antigen preparations in standard and flow cytometry-based assays, in a unique cohort of LTNP, patients with progressive disease, and a separate cohort studied during an interruption of therapy. Although HIV-specific CD4$^+$ T cells persisted in each of these cohorts, viral antigen-specific proliferation was rapidly abrogated in the longitudinal cohort during an interruption of therapy. Thus diminished proliferation of antigen-specific CD4$^+$ T cells was a consequence of high levels of viral replication. These data may suggest a mechanism by which in the context of high levels of virus replication, HIV or other persistently replicating viruses may act to limit the virus-specific immune response of the host.

Methods

Study Population. HIV-1 infection in study participants was documented by HIV-1/2 immunoassay. All subjects signed informed consent approved by the National Institute of Allergy and Infectious Diseases investigational review board. Each of these patients was not receiving antiretroviral therapy during the study period. In the subset of patients that have appeared in previous publications (9, 16) the corresponding numbers are used to permit cross-referencing. Patients 3, 8, 9, 10, and 13 in the therapy interruption cohort (17) were reassigned 203, 208, 209, 210, and 213, respectively in this study.

Proliferation Assays. Standard proliferation assays were performed as described (4, 8). Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood or apheresis donor packs by sodium diatrizoate density centrifugation (Organon Teknika–Cappel). Cryopreserved samples maintained at −140°C for less than 1 year were thawed for use in proliferation assays. Cells were incubated (100,000 cells/well) in triplicate in the presence of each of the following antigens: 10 μg/ml HIV-1$_{IIIB}$ p24, HIV-1$_{IIIB}$ gp160 (Advanced Biotechnologies, Columbia, MD), HIV$_{SF\text{-}2}$p24, HIV$_{SF\text{-}2}$p17, or HIV$_{SF\text{-}2}$p55 (Austral Biological); 1/100 dilution of CMV lysate, CMV control lysate (BioWhittaker); 2 μg/ml phytohemagglutinin (PHA) (Sigma); or medium. On day 3, cells incubated with PHA were pulsed for 6 h with 1 μCi per well of [^{3}H]thymidine and harvested. Wells containing all other proteins or controls were similarly pulsed and harvested on day 5. Results shown are representative of assays performed in triplicate on 2–4 occasions from a single leukapheresis.

Abbreviations: LTNP, long-term nonprogressors; CMV, cytomegalovirus; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; CFSE, carboxyfluorescein diacetate-succinimidyl ester.

*To whom reprint requests should be addressed at: National Institutes of Health, Building 10, Room 11B-09, 10 Center Drive, MSC 1876, Bethesda, MD 20892. E-mail: mconnors@nih.gov.

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Flow Cytometry. Freshly isolated PBMC were aliquoted at 2–5 million per 1 ml in stimulation tubes (Sarstedt) and rested overnight. Anti-CD28 antibody (5 μg/ml, PharMingen) and individual HIV antigens (10 μg/ml) were then added to each tube. Staphylococcal enterotoxin B (10 μg/ml final concentration; Toxin Technology, Sarasota, FL) was used as a positive control. Incubation, fixation, permeabilization, and staining were performed as described (9). All cells were simultaneously stained with PerCP-anti-CD3, allophycocyanin–anti-CD4, phycoerythrin–anti-IL2 (Becton-Dickinson), and FITC-anti-IFN-γ (PharMingen) antibodies. A total of 50,000 $CD3^+$ $CD4^+$ cell events were collected per sample. IFN-γ^+ virus-specific cell frequencies are reported after subtraction of medium and isotype controls as described (18). For comparison with previous studies, where noted, a correction for the frequency of memory cells was performed (18). We used 5-(and-6)-carboxyfluorescein diacetate-succinimidyl ester (CFSE; Molecular Probes) according to the manufacturer's protocols. The CCR7 antibody (BLR2 clone 3D12) was a generous gift of Martin Lipp (Max-Delbruck-Center for Molecular Medicine, Berlin). In separate experiments 5×10^8 PBMC were incubated for 14 h with either CMV or p24 antigen or medium alone, and IFN-γ secreting cells were isolated with magnetic beads (Miltenyi Biotec, Auburn, CA) using an AutoMACS separator (Miltenyi Biotec) on deplete-sensitive mode. Depletion of IFN-γ secreting cells (>99.8% depletion) was confirmed on day 0 by flow cytometry. Data were collected on a FACSCalibur dual-laser cytometer (Becton-Dickinson) and analyzed by using CELLQUEST software (Becton-Dickinson) and FLOWJO software (TreeStar, San Carlos, CA).

Statistical Analysis. $CD4^+$ T cell counts or plasma viral RNA levels were compared by using the Wilcoxon two-sample test. A Wilcoxon two-sample test with a Bonferroni adjustment of P values for multiple testing was used to compare the frequency of p55, p24, or CMV-specific IFN-γ^+ cells in groups A and B. Within the longitudinal cohort, $CD4^+$ T cell counts or PHA mean responses were compared by an ANOVA with Tukey's multiple comparison test. Survival of $CD4^+$ T cells in culture was compared by using the Student's t test. The effect of addition of anti-CD28 was tested with the Wilcoxon signed rank test with a Bonferroni adjustment to P values for multiple testing.

Results

HIV-Specific $CD4^+$ T Cells Persist but Proliferation Is Absent in Patients with High-Level Viremia. Proliferative responses and frequencies of cytokine producing HIV-specific $CD4^+$ T cells were measured in a cohort of 26 HIV-1-infected patients not receiving antiretroviral therapy. To better define the magnitude of significant proliferative responses that occur during HIV-1 infection, we included a unique cohort of LTNP patients that typically maintain <50 copies of HIV RNA/ml plasma in this group. *In vitro* proliferative responses to a panel of purified HIV antigens that included p55, two preparations of p24, p17, and gp160 were measured. Data are expressed as net cpm (NCPM) as stimulation indices do not permit the magnitude of the response to be evaluated and tended to be quite variable. Only patients with nonprogressive infection and very low levels of plasma virus RNA were found to have proliferative responses significantly greater than 18 uninfected controls (>1,000 NCPM; Fig. 5*A*, which is published as supporting information on the PNAS web site, www.pnas.org). The responses to p55, p24, or p17 were highly dichotomous across patients in that responses were either present to most HIV antigens or completely absent. In only three patients were significant responses to gp160 detected (range 1,536 to 13,730 NCPM; not shown). To further analyze the $CD4^+$ T cell responses of patients with proliferative responses, patients were separated into those with (group A; $n = 16$) and without (group B; $n = 10$) a proliferative response to p24 antigen (Table 1). There was no significant difference in the absolute $CD4^+$ T cell counts between the two groups (Fig. 5*B*; $P > 0.5$). The median plasma viral RNA for group A was 50 copies/ml and for group B was 22,923, a highly significant difference (Fig. 5*C*; $P < 0.001$). Thus proliferative responses to HIV antigens were confined to those patients with very low levels of plasma viral RNA that make up a very select subgroup of LTNP.

The distributions of IFN-γ^+ p55-specific cell frequencies were not different (group A median 0.13% vs. group B median 0.04%; $P > 0.5$) although the distributions were different for p24 (median 0.13% vs. 0.02% in group B; $P = 0.04$) (Fig. 1 *A* and *B*). For reference to previous studies (18), comparisons also were made after a correction for the frequency of memory $CD4^+$ T cells (those outside the naive $CD95^{dim}$, $CD27^+$, $CD45RO^-$ subset) but were little changed for p55 (0.17% in group A vs. 0.05% in group B; $P > 0.5$). After correcting for the frequency of memory $CD4^+$ T cells the distributions of p24-specific cells were no longer significantly different (0.18% in group A vs. 0.03% in group B; $P = 0.24$). The frequencies of p24 or p55-specific IL-2^+ cells were at or below the assay detection limit. The distributions of frequencies of CMV-specific $CD4^+$ T cells were not significantly different between groups A and B [median 2.7% vs. 1.4%; $P > 0.5$ (memory median 3.63% vs. 4.77%; $P > 0.5$)]. The frequency of cells that produced IL-2 in response to CMV antigen was modestly reduced in group B (group A median 1.01% vs. group B 0.46%; $P = 0.03$). Despite similar frequencies of CMV-specific IFN-γ^+ $CD4^+$ T cells in the two groups, the proliferative response to CMV was diminished but readily detectable in group B (median 32,037 cpm group A vs. 5,828 cpm group B; $P = 0.004$). Taken together these data are consistent with modestly diminished, but readily demonstrable, responses to recall antigens early in HIV infection previously described (19, 20), but a much more profound effect in responses to HIV antigens. However, consistent with one recent report (21), the absence of HIV-specific responses in viremic patients was not caused by the absence of HIV-specific $CD4^+$ T cells to each of the antigens measured.

High Levels of Virus Replication Causes Diminished Proliferation of HIV-Specific $CD4^+$ T Cells. Although absent proliferative responses to HIV antigens were associated with very high levels of plasma viral RNA, the causal role of these responses in the restriction of virus replication remained unclear. Because HIV-1 can affect the frequency and function of $CD4^+$ T cells, causality becomes circular. It is possible that HIV-1-specific $CD4^+$ T cell responses *in vivo*, or by proxy those detected *in vitro*, cause the restriction of virus replication. Alternatively, viremia might have an effect on $CD4^+$ T cell proliferative responses. To address these questions, we selected a subset of eight patients from a recently described cohort of 27 patients receiving antiretroviral therapy that maintained plasma viral RNA <50 copies/ml for >1 year (17), which had demonstrable proliferative responses to HIV antigens. The presence of a proliferative response to HIV antigens was not predictive of restriction of virus replication in that these patients experienced a rapid rebound and peak of plasma viremia within $\approx$4 weeks of therapy interruption similar to other cohort patients. The responses to PHA were unchanged over this time (Fig. 2*A*; $P = 0.2$). Despite significant p24-specific proliferation before therapy interruption, the increase in plasma virus was associated with a disappearance of this proliferative response (duration of interruption 35–111 days; median 45 days). This response was regained, generally at a lower level, only after antiretroviral therapy was resumed. Fig. 2*B* shows the proliferative responses in comparison to the frequency of IFN-γ^+ cells during therapy interruption in a subset of patients from whom flow cytometry data were obtained. Similar to responses previously described for viruses that typically do not chronically replicate in humans (22), proliferative responses to HIV antigens were present, yet HIV-specific $CD4^+$ T cells were either very low or not detected before therapy interruptions. During antiretroviral therapy interruption and viremia, there was a transient increase in the frequencies of IFN-γ^+ antigen-specific cells

Table 1. Clinical data

Patient group	Patient	Year of diagnosis	$CD4^+$ T cells, cells/μl	Plasma HIV RNA, copies/ml	Therapy
Group A	7	1985	277	<50	—
	13	1986	1,016	<50	—
	8	1985	664	<50	—
	17	1985	1,073	<50	—
	9	1997	1,079	<50	—
	25	1986	1,028	<50	—
	3	1985	915	<50	—
	4	1985	1,063	<50	—
	6	1986	760	<50	—
	10	1996	602	<50	—
	5	1985	1,105	<50	—
	12	1986	500	<50	—
	31	1992	823	<50	—
	32	1990	422	<50	—
	24	1994	947	481	—
	21	1985	557	2,367	—
Group B	30	1994	1,136	<50	—
	11	1989	1,830	124	—
	16	1994	409	529	—
	1	1985	693	3,996	—
	20	1985	1,311	4,825	—
	19	1984	785	7,774	—
	28	1984	350	35,035	—
	29	1999	488	36,475	—
	14	1998	410	53,158	—
	26	1991	413	132,884	—
Therapy interruption	203	1988	863	<50	D4T/3TC/IND
	219	1986	1,059	<50	D4T/DDI/NVP/3TC/IND/HDU
	220	1996	934	<50	3TC/D4T/IND
	208	1994	641	<50	ZDV/3TC/IND
	209	1995	1,138	<50	D4T/3TC/SQV/NFV
	221	1988	884	<50	D4T/3TC/IND
	210	1987	1,138	<50	ZDV/3TC/IND
	213	1992	659	<50	NVP/3TC/IND

Mean $CD4^+$ T cell counts of uninfected individuals are 912 $\pm$ 24.08 and 528 $\pm$ 20.8 cells/mm^3, respectively in this laboratory. Determination of plasma virus was performed by using bDNA assay (Chiron) with a 50 copies/ml plasma sensitivity. Patient numbers correspond to those that appear in previous publications (see *Methods*).

that then diminished when therapy was resumed. Thus although HIV-specific $CD4^+$ T cells were increased in the peripheral blood during viremia, proliferative responses were absent. This result is consistent with the cross-sectional data and suggests that absence of a proliferative response to HIV antigens is not necessarily a cause, but rather a consequence of high levels of virus replication.

Absence of Proliferative Responses Is Not Caused by Death of Virus-Specific Cells *in Vitro* or Detection of Different Cell Subsets. It remained possible that although antigen-specific cells were detected, proliferation of these cells was not detected in viremic patients because of virus-mediated killing of $CD4^+$ T cells during the 5-day assay for proliferation or that these assays detect different cell subsets. When cells are labeled on day 0 with CFSE, proliferation of cells may be tracked by the decrease in CFSE fluorescence of individual cells with each cell division. In several experiments, a dose of the antiretrovirals zidovudine, didanosine, or indinavir alone or in combination that inhibited HIV replication but did not inhibit antigen-specific proliferation was not found. However, in many cases viral RNA in supernatants of untreated cell cultures from viremic patients remained below 50 copies/ml on day 5. More importantly similar numbers of $CD4^+$ T cells were detected in culture on day 5 between the two groups within the cross-sectional cohort (mean group A 38.5% vs. group B 38.4%; $P > 0.5$), indicating there was no large depletion of $CD4^+$ T cells by HIV in these cultures. In cell cultures from group A patients that were sampled daily, IFN-γ producing cells became $CD4^{bright}$, increased in frequency by day 5 and were almost entirely confined to the subset of cells that proliferated in response to antigen (Fig. 6 *A* and *B*, which is published as supporting information on the PNAS web site). Cells that proliferated over 5 days were within the $CCR7^-$ subset consistent with IFN-γ-producing cells (23). In addition, IFN-γ-producing cells were isolated after stimulation and labeled with CFSE, and proliferation was assessed on day 5 (Fig. 3). Although proliferating CMV-specific cells were divided between IFN-γ- and IFN-γ^+ subsets, p24-specific cells were almost completely confined to the IFN-γ-secreting subset.

If HIV-specific cells persist but proliferation is suppressed in the presence of high levels of antigen, it remained possible that cells could be restimulated to proliferate *in vitro*. Antigen-specific proliferation might be restored by provision of a second signal in the form of anti-CD28 or IL-2. The effect of supplemental IL-2 could not be evaluated because a dose was not found (0.02–20 units/ml) that increased the percent of $CD4^+$ T cells that proliferated to HIV antigen above control cultures. However, when anti-CD28 antibody was added to the cultures in the presence of p24, an increase in the percentage of $CD4^+$ T cells that proliferated was observed in patients within group B (Fig. 4 *A* and *B*; $P = 0.03$) and in patients

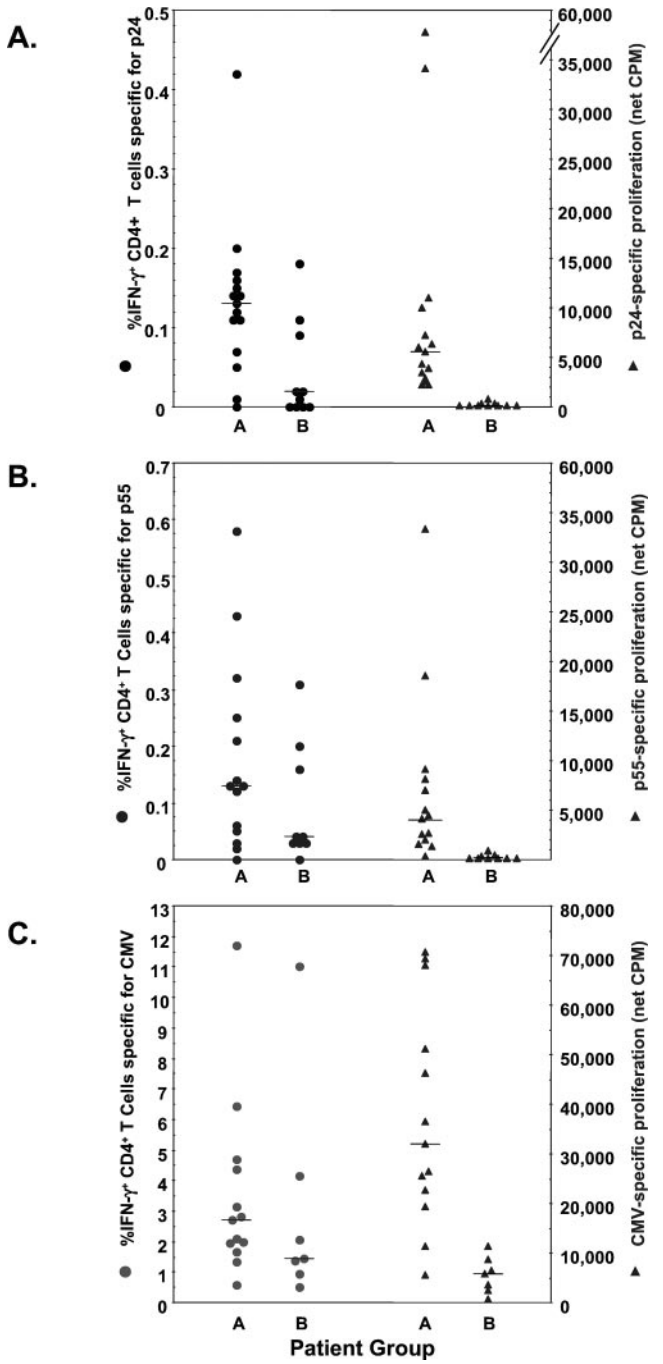

Fig. 1. Detection of HIV-1 antigen-specific $CD4^+$ T cells in patients lacking proliferative responses. The frequency of $CD4^+$ T cells that produce IFN-γ (○) or proliferative responses in PBMC (▲) in response to HIV-1 p24 (*A*), HIV-1 p55 (*B*), or CMV lysate (*C*). Only CMV seropositive patients ($n = 20$) are shown in *C*. Bars represent median values.

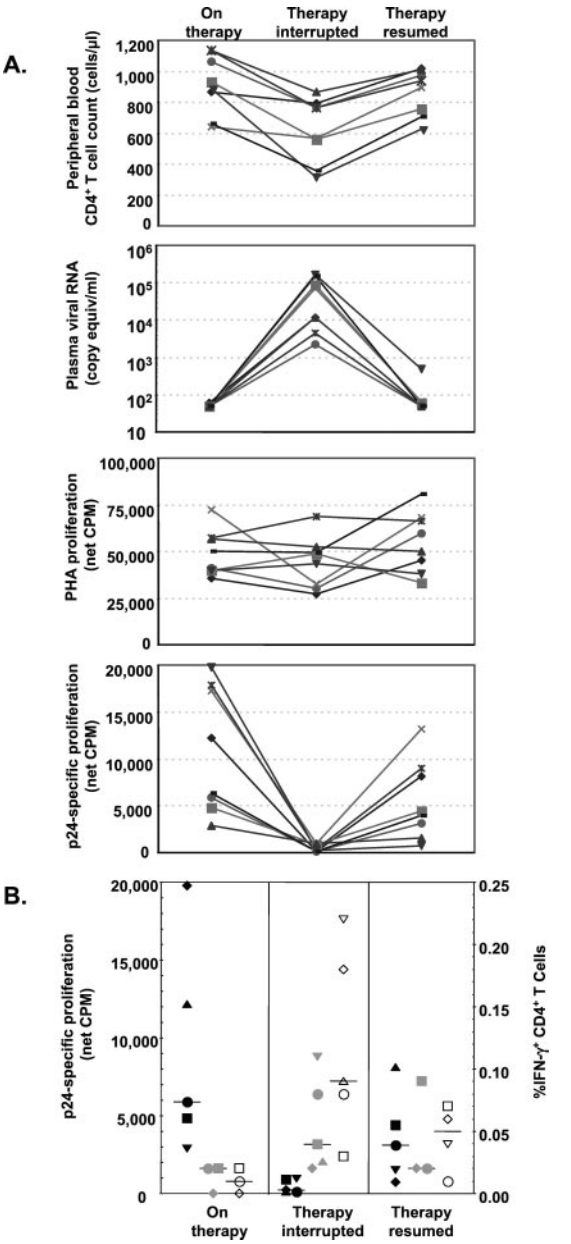

Fig. 2. HIV-specific proliferative responses are suppressed during high-level viremia. (*A*) Changes in peripheral blood $CD4^+$ T cell count, plasma viral RNA, and proliferation in response to PHA or HIV-1 p24 before, during, and after interruption of therapy. The therapy interruption time point shown is just before resumption of therapy (35–111 days; median 45 days). (*B*) The frequency of $CD4^+$ T cells in the peripheral blood that produce IFN-γ in response to HIV-1 p24 (gray symbols) or p55 (open symbols). A subset of patients ($n = 5$) with significant proliferative responses on whom flow cytometry on freshly isolated PBMC could be obtained is shown. HIV-1 p24-specific proliferation (closed symbols) is plotted for comparison.

during treatment interruptions (Fig. 4*C*; $P = 0.03$). Anti-CD28 antibody did not increase the number of proliferating cells in medium, human T cell leukemia virus antigen, or uninfected control cultures, indicating these responses were p24-specific and depended on the presence of antigen-specific cells. It remained possible that p24-specific cells do not persist *in vitro* but were "rescued" from apoptosis by the addition of anti-CD28. However, apoptosis of p24-specific cells would likely occur at frequencies too low to be measured above background by direct assays for apoptosis in 5-day PBMC cultures. To address this possibility, parallel cultures were preincubated in the presence of p24 for 36 h to permit cells primed for apoptosis to die, then supplemental p24 or p24 with anti-CD28 was added. The preincubation with p24 did not diminish the numbers of $CFSE^{low}$ $CD4^+$ T cells, rather the additional preincubation had the effect of increasing the number of these cells (Fig. 4*B*). These results suggest that diminished proliferation detected *in vitro* in viremic patients is not caused by apoptosis of antigen-specific $CD4^+$ T-cells. Because addition of soluble anti-

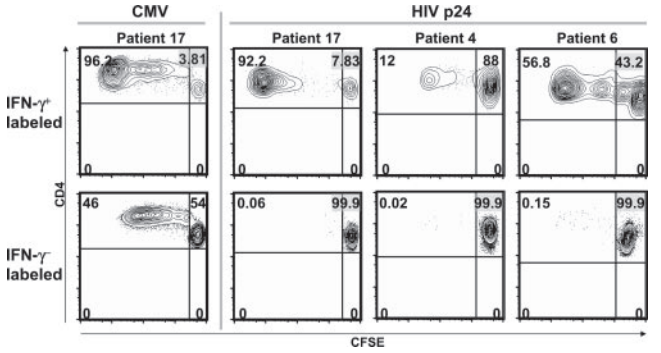

Fig. 3. IFN-γ^+ CD4$^+$ T cells in PBMC proliferate in response to antigen. PBMC were stimulated with the indicated antigen for 14 h, and IFN-γ-secreting cells were isolated. Either IFN-γ^+ or IFN-γ^- cells were labeled with CFSE and recombined with the corresponding unlabeled fraction (see *Methods*) and incubated for 5 days.

CD28 antibody increases infection of CD4$^+$ T cells *in vitro* (16, 24), this result also further confirms that diminished proliferation is not caused by virus-mediated killing. Taken together, these results suggest that virus-specific cells that persist in the peripheral blood of these patients persist *in vitro* and can be restimulated to proliferate in an antigen-specific manner.

Discussion

These results may provide some insight into the causal relationships between HIV-specific CD4$^+$ T cell responses and restriction of virus replication. Previous associations (4–6, 8, 21, 25) between virus-specific proliferative responses and restriction of viral replication were corroborated in the present study by using a larger cohort of LTNP that typically maintain <50 copies of viral RNA per ml of plasma. Based on these previous correlative data it is suspected that loss of a CD4$^+$ T cell proliferative response to HIV-1 antigens, as a proxy for T cell help, causes the loss of restriction of virus replication. However, the results of the present study suggest that this relationship is considerably more complex. Viremia had a very dramatic effect on HIV-specific proliferation detected *in vitro*. These responses were rapidly abrogated in each of eight patients during viremia when therapy was interrupted. The lack of HIV-specific proliferation persisted throughout the viremic period and was restored only when viremia was controlled by antiretroviral therapy. This effect did not appear to be caused by the loss of antigen-specific cells in the peripheral blood. Conversely, although the patients in the longitudinal cohort maintained HIV-specific proliferative responses, restriction of virus replication was ultimately lost (17). This observation is offered with the caveat that this is a relatively small number of patients and needs to be corroborated in other cohorts or possibly with further interruptions of therapy. Nonetheless, the finding of proliferative responses *in vitro* was not predictive of long-term restriction of virus replication in these patients. Taken together these results are consistent with the idea that the loss of HIV-specific proliferative responses is a consequence of poorly restricted virus replication.

Diminished proliferation in response to HIV antigens during viremia shares some similarities with other virus infections. Because proliferative responses to viral antigens are typically found after acute or chronic virus infections of humans and experimental animals it has been suggested that the absence of these responses in most patients is unique to HIV-1. However, these responses occur during the recovery phase after virus replication has been restricted by the host immune response. In most untreated individuals HIV-1 causes persistent viremia. There are several virus infections for which humans typically develop successful immune responses but *in vitro* proliferative responses to viral antigens are

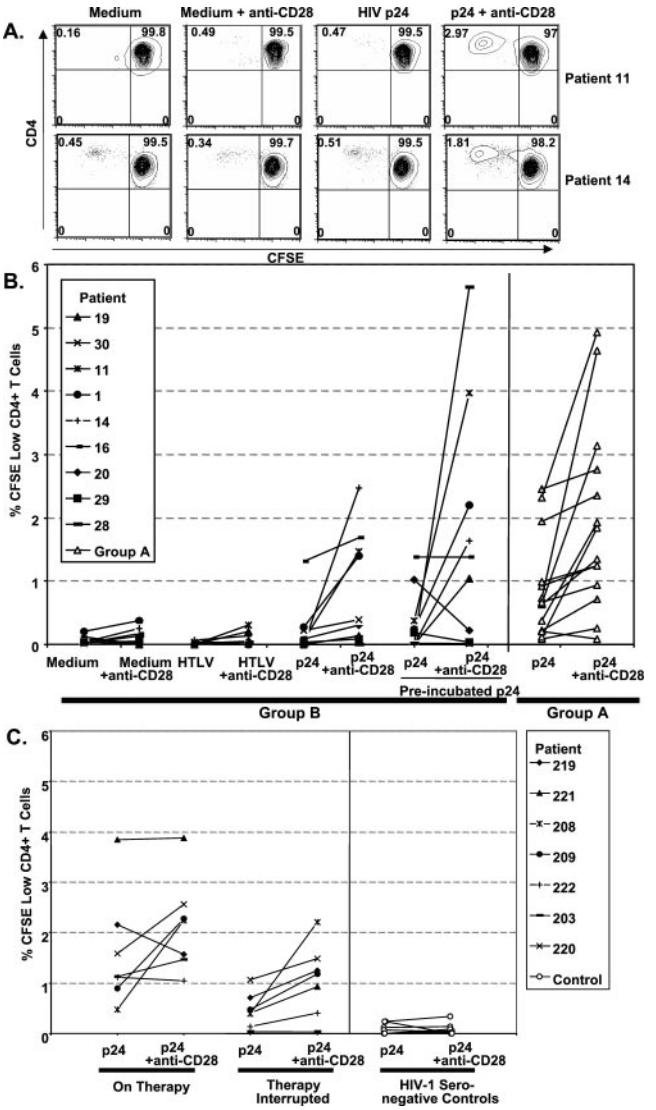

Fig. 4. CD4$^+$ T cells specific for HIV-1 p24 proliferate in viremic patients when costimulation is provided through CD28. (*A*) PBMC labeled with CFSE and incubated for 5 days with or without p24 antigen or anti-CD28 mAb. (*B*) Summary data of the percent CD4$^+$ T cells that are CFSE^{low} after 5 days under the indicated stimulation conditions for nine patients within group B. (*C*) Summary data of the percent proliferating CD4$^+$ T cells of seven patients before interruption of therapy and during viremia when therapy was interrupted. Parallel experiments in 10 HIV seronegative donors are shown for comparison.

absent during viremia. These include measles virus (26), CMV (27), hepatitis B virus (28), and Dengue virus infections (29). The findings in the present study are especially reminiscent of chronic active hepatitis B infection where CD4$^+$ T cell proliferation to hepatitis B antigens appears when virus load is diminished by lamivudine (28). Taken together, the persistence of virus-specific CD4$^+$ T cells, the finding that viremia causes diminished HIV-specific proliferative responses, and the findings above in other virus systems, each call into some question whether the absence of *in vitro* proliferative responses alone should be used as a proxy for absent virus-specific T cell help in HIV infection.

These results also reconcile some recent, seemingly disparate, reports on the HIV-1-specific CD4$^+$ T cell response (4, 18, 25). In one recent study, HIV-specific proliferative responses were inversely correlated with plasma viral RNA (4). However, in a separate report, HIV-1-specific IFN-γ^+ CD4$^+$ T cells were shown

to persist in some patients with progressive disease (18). The results of the present study suggest that this apparent discrepancy is not caused by differences in the sensitivities of the assays, virus-mediated cytopathicity, or apoptotic death of antigen-specific cells, or that these are different cell subsets. Rather, virus-specific cells persist *in vivo*, also persist *in vitro*, yet in viremic individuals *in vitro* proliferation of these cells is suppressed. Thus, HIV-specific $CD4^+$ T cells may not be completely deleted during acute infection, but rather they are detected only in proliferative assays when viremia is controlled by antiretrovirals or the immune response. The precise mechanism(s) by which HIV-1 viremia causes diminished proliferation *in vitro* remain unclear. These changes may potentially be intrinsic to the $CD4^+$ T cell, or the antigen-presenting cell, or operate through suppressive cytokines or immunoregulatory cell subsets. The effect of anti-CD28 and the lack of change in PHA responses during HIV-1 viremia may suggest an effect on antigen-presenting cells, or alternatively, these stimuli simply overcome diminished responsiveness to permit the proliferation of virus-specific $CD4^+$ T cells (30, 31).

Although speculative, it is possible that diminished antigen-specific proliferation of HIV-1-specific $CD4^+$ T cells *in vitro* during viremia may reflect diminished proliferation or function *in vivo*. A progressive decline in precursor frequencies would be consistent with some recent observations in experimental animals under conditions of high levels of antigen. Adoptive transfer of virus-specific $CD4^+$ T cells into mice persistently infected with LCMV results in markedly diminished antigen-specific proliferation of cells isolated from lymphoid tissues although virus-specific cells persist (32). Diminished proliferation or direct infection of HIV-1-specific $CD4^+$ T cells *in vivo* might account for 10- to 20-fold lower frequencies of HIV-specific $CD4^+$ T cells when compared with those specific for CMV, higher frequencies early in infection, and the reconstitution of these responses in only a portion of patients treated with antiretroviral therapy. Although more modest and less consistently observed, effects of viremia on non-HIV-specific responses are also consistent with early decrease in responses to recall antigens (19, 20, 33) and rapid improvement in immune function observed *in vitro* (34, 35) or *in vivo* in some patients given potent antiretroviral therapy before increases in total or naive peripheral blood $CD4^+$ T cells. Finally, these data have some implications for efforts to stimulate HIV-specific immunity through therapy interruptions in infected patients. They suggest that prolonged interruptions of therapy resulting in high-level replication of HIV may not be the best modality for stimulating $CD4^+$ T cell-based cellular immune responses and should be approached with some caution.

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