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CD8 T cell proliferative capacity is compromised in Primary HIV-1 infection

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Abstract

Understanding the correlates of immunity that control HIV-1 infection is imperative to our understanding of HIV-1 disease and vaccine development. HIV-1 specific cytotoxic T lymphocytes (CTL) are fundamental to the control of viremia, however which T cell repertoire components enact this control remains unclear. We hypothesize that polyfunctional HIV-1-specific CD8 T cells capable of viral control are present in most patients early in infection and these cells are distinguished by their ability to secrete IL-2 and proliferate. We examined HIV-1-specific CD8 T cell proliferation and cytokine secretion in primary HIV-1 infection (PHI) using known HIV-1 CTL epitopes to exclude CD4 bystander effect. We found that only a subset of PHI patients demonstrated "CD4 independent" CD8 proliferation *ex vivo*. The remainder of the patients lacked HIV-1-specific CD8 T cells with proliferative capacity, even after the addition of exogenous IL-2. Amongst the proliferators, IL-2 production from the total HIV specific CD8 T cell population correlated with proliferation. Surprisingly though, we did not routinely detect both IL-2 secretion and proliferative capacity from the same antigen specific CD8 T cells. Thus there are distinct and heterogeneous populations of CD8 T cells- phenotypically characterized by either proliferation, or IL-2 secretion, and few with dual capacity. Generation of these responses may be an important measure of HIV-1 control but are not universal following PHI. Furthermore, the heterogeneity of this population suggests that a simple measure of an effective vaccine response remains elusive.

Introduction

Primary HIV infection (PHI) is associated with a burst of viremia, massive systemic dissemination and generalized immune activation¹⁻⁴. While the impact on absolute CD4 T cell count in the plasma may be minimal and transient, the impact on gut CD4 T cells and other arms of the immune system is profound and possibly irreversible⁵⁻⁷. Despite multiple lines of evidence demonstrating that cytotoxic T lymphocytes (CTL) play an important role

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in viremic control during HIV-1 infection, we still do not have a complete understanding of protective correlates of immunity or deleterious consequences of generating this response in the setting of acute viremia and immune activation^{8,9}. CTL have numerous effector functions, most notably polyfunctional cytokine secretion and proliferative capacity, which have been demonstrated in long term non-progressors, those patients able to control virus without antiretroviral therapy¹⁰⁻¹³. While these types of responses appear to be deficient in patients with a relative lack of viral control, it remains unclear if polyfunctional and proliferative CTL actually help contain virus or are simply the result of successful viral inhibition by other mechanisms.

Several groups have demonstrated a progressive functional loss of CTL with disease progression. HIV-1-specific CTL lose proliferative capacity before non-HIV-specific CTL, and this correlates with increasing viral load and loss of absolute CD4 T cells¹⁴⁻¹⁶. Cytokine secretion is also compromised in progressive disease with loss of IL-2, TNF- and eventually IFN-^{17,18}. It remains unclear whether all HIV-1 patients generate polyfunctional CTL with proliferative capacity that is lost with progressive disease, or if only a subset of patients are capable of generating these responses. Furthermore, several laboratories have suggested that IL-2 secretion, from either dual IFN- /IL-2-secreting CD8 T cells or CD4 T cells, is necessary to support CD8 T cell proliferative capacity but whether this occurs at the outset during PHI has not been demonstrated^{18,19}.

Because all patients demonstrate some level of viremic control in PHI and likely have sufficient CD4 T cell help at the time these cells are generated, we hypothesized that polyfunctional HIV-1-specific CD8 T cells capable of viral control are present in most patients in PHI and have a phenotype of IL-2 secretion and proliferative capacity. Here we demonstrate that HIV-1-specific CTL with proliferative capacity are not uniformly observed in PHI. In addition, the capacity to secrete cytokines after specific HIV-1 peptide stimulation is characterized by significant inter- and intra-patient variability. These defects are only partly explained by the lack of IL-2 producing HIV-1-specific CD4 or CD8 T cells in early infection and do not obviously correlate with polyfunctional T cell responses. This work adds to our understanding of correlates of immunity to HIV infection and challenges the paradigm of using surrogate responses such as cytokine secretion to measure vaccine efficacy.

Methods

Subjects and HLA typing

Blood was collected from HIV infected volunteers (n= 13) at the University of Alabama at Birmingham (UAB) after obtaining written, informed consent, as approved by the Institutional IRB. HLA typing was performed by the tissue typing center at UAB Hospital (Micro SSP HLA Typing System). Volunteers were identified early in infection as part of the National Institutes of Health (NIH) - sponsored Acute Infection and Early Disease Research Program (AIEDRP)²⁰.

Quantification of HIV-1 RNA in plasma and absolute CD4 T cell count

Plasma HIV-1 RNA levels (copies/mL) were measured using Amplicor Ultra Sensitive HIV-1 Monitor assay (version 1.5; Roche Diagnostics Systems). Plasma CD4 T cell counts (cells/mm³) were determined as described previously^{21,22}.

Antigens

PBMC were stimulated using (1) optimized HLA-restricted HIV-1 CD8 T cell epitopes (9-11mers) (10 µM), as described in the LANL HIV Immunology Database, (2) CEF pool

(10 μ M) and (3) HIV-1 pools (Gag, Pol, Nef, Env, Accessory) composed of overlapping peptides, 15-20mers, representing the entirety of HIV-1 (2 μ g/mL) (NIH AIDS Research and Reference Reagent Program), or (4) staphylococcal enterotoxin B (SEB (1 μ g/mL) (positive control).

Proliferation Assay

Cryopreserved PBMC were thawed, stained, and visualized with Trypan Blue to assess viability. They were labeled with 1.25 μ M CFSE (5-(and-6)-carboxyfluorescein diacetate-succinimidyl ester) (Molecular Probes, Eugene, OR) for 4 minutes at room temperature. After washing in PBS, 1×10^6 PBMC were cultured in complete RPMI with 10% AB serum in 48 well plates at 37°C and 5% CO₂. Where stated, PBMC were depleted of CD4 T cells using Dynal beads (Invitrogen). Depletion was determined to be > 96% by flow cytometry. IL-2 was added to cultures at 50 IU/mL where noted.

PBMC or CD4 T cell depleted populations were stimulated with appropriate antigens. We performed studies using 3, 5, or 7 days for the CFSE proliferation assay. We chose a 7 day assay as we found this to be optimal in our studies. After 7 days, the amount of CFSE per cell was determined by flow cytometry. The criterion for a positive proliferative response was determined to be twice that of media alone (background).

Intracellular cytokine staining assay (ICCS)

Standard intracellular cytokine staining was performed as described^{23, 24}. 1×10^6 PBMC were plated in 48 well plates and stimulated with appropriate antigens for one hour followed by incubation with monensin and brefeldin A for 4 more hours.

Flow cytometry

For proliferation and ICCS assays, surface staining was performed with anti-CD3 (APC or Pacific Blue), anti-CD4 (PE), and anti-CD8 (PerCP or PerCP Cy5.5). Cytokine secretion was detected with anti-IFN- γ (Alexa 700), anti-IL-2 (FITC), and TNF- α (PE Cy7). A standard lymphocyte gate was selected from forward scatter versus side scatter plots. Next, CD3+ T cells were selected. A minimum of 5×10^5 CD3+ events were collected. From these populations proliferation by CFSE was determined. For ICCS, CD3+CD8+ or CD3+CD4+ T cells were gated for cytokine analysis (IFN- γ vs IL-2; IFN- γ vs TNF- α). Events were collected using an LSRII flow cytometer (Becton Dickinson) and the data analyzed using FlowJo software (v8.1), as described²⁴. T cells were analyzed for polyfunctional cytokine responses using PESTLE (version 1.4) and SPICE (version 4.1) software (courtesy of Mario Roederer, Vaccine Research Center, Bethesda, MD). After the gates for each of the three single functions were created, we used the Boolean gate platform to create the 8 possible response patterns. After background correction (media only), the percentage of CD8 T cells producing one to three functions were quantified.

Statistics

Using Prism software, statistical analysis included nonparametric Mann-Whitney U test to compare differences between categorical variables. Spearman rank was used as the nonparametric test to compare the relationship between continuous variables. *P* values less than 0.05 were considered significant.

Results

HIV-1-specific CD8 T cell proliferative capacity in PHI

Despite extensive analysis of CD8 T cell proliferation in chronic infection, evaluation of this parameter has been limited in PHI. We therefore examined CD8 T cells for proliferative capacity in patients identified and administered antiretroviral therapy (ART) within 4 months of seroconversion. Thirteen subjects were analyzed for HIV-1-specific CD8 T cell proliferative responses. A representative CFSE proliferation plot is shown (Figure 1). We identified six patients with HIV-1-specific CD8 T cell proliferation (proliferators- defined as CD8 T cell proliferation to at least one tested optimized CTL epitope) and seven without HIV-1-specific CD8 T cell proliferation (non-proliferators- lack of proliferative response to any of the tested optimized CTL epitopes). Clinical parameters, including CD4 count and viral load (at entry and assay date), and demographic characteristics were not statistically different between the groups (Table 1). Two of six proliferators had protective HLA alleles (B*27, B*51). Despite this, most proliferative responses observed were not restricted by these protective alleles (Figures 1, 2A, and 2B).

Of the cohort, approximately 75% (10 of 13 subjects) had detectable proliferative responses to non-HIV antigens (CEF). CEF proliferation magnitude was similar between HIV-1 proliferators and non-proliferators (data not shown). Of those with CEF proliferative responses, most (6 of 10 subjects) had HIV-1-specific CD8 T proliferative responses. Across the cohort, we tested a total of 24 HLA-1 restricted HIV-1 epitopes chosen for their ability to elicit strong IFN- γ ELISpot responses (> 200 SFC/ 10^6 cells)²² and 17 of 24 epitopes elicited HIV-1-specific HIV proliferative responses in one or more of the six “proliferators”.

We next wanted to rescue HIV-specific responses by adding exogenous IL-2. To do this, we further examined 5 patients lacking an HIV-1-specific CD8 T cell proliferative response to at least one tested epitope. In each case, the subjects demonstrated either proliferation to CEF or at least one other HIV-1 CD8 T cell epitope. We were only able to recover one of eight absent proliferative responses, in this case to a non-optimized 20-mer that elicited CD8 but not CD4 T cell responses (Table 2). Thus, even in the earliest stages of HIV-1 infection, CTL proliferation is compromised in many patients as evidenced by IFN- γ secreting CD8 T cells lacking proliferative capacity.

CD4 independent CD8 T cell proliferation

To better understand components necessary to generate an optimal HIV-1-specific CD8 T cell response with proliferative capacity, we sought to determine the functional contributions from CD8 and CD4 T cells. Previous studies suggested that the production of cytokines by proliferating CD8 T cells, specifically IL-2, supports proliferative capacity to HIV antigens¹⁹. Because the addition of exogenous IL-2 did not consistently recover lost proliferative responses, we reasoned that some CD8 T cell proliferative responses in acute disease were primed in the setting of optimal CD4 T cell help and maintained despite the loss of ongoing CD4 help. Furthermore, the majority of the proliferative responses that we detected were to optimized 9-11 amino acid epitopes, expected to elicit CD8 but not CD4 T cell responses. To prove that CD8 T cell proliferation can occur independently after *in vivo* priming, we chose two patients (based on sample availability) from our acute cohort and antigenically stimulated PBMC in the presence (Figure 1, 2B) or absence (Figure 2A, 2B) of CD4 T cells. Even in the absence of CD4 T cells or exogenous IL-2, CD8 T cells were able to maintain proliferative capacity to optimized epitopes. Furthermore, the magnitude of proliferation in response to several epitopes was comparable, regardless of CD4 T cell presence (Figure 2B). We did observe significant CD8 down-regulation on T cells with

relatively little evidence of proliferation from this population except to SEB (Figure 2A). These CD8 low T cells did not co-stain with CD4.

Antigen specific cytokine secretion from CD4 and CD8 T cells in PHI

Several studies have indicated that cytokine secretion, including IFN- γ and IL-2 is associated with viral control^{11, 19, 21, 24}. These functions, especially IL-2, are lost in chronic progressive disease but are present in controllers and some patients treated during PHI²⁴. Furthermore, these cytokines may define a CTL phenotype, established early in infection, capable of viral control. Since neither exogenous IL-2 nor co-culture of CD4 T cells is necessary to elicit HIV specific CD8 T cell proliferation, we sought to determine if functional characteristics of CD8 T cells predict and/or supports their own proliferation. To determine if IL-2 secreted from the CD8 T cells supports proliferation as previously reported¹⁹, we performed ICCS after peptide stimulation, with optimized CD8 T cell epitopes on two subjects demonstrating CD4-independent CD8 T cell proliferation (Figure 2B). We found no evidence of dual secretors (IFN- γ /IL-2), when CD8 T cells were stimulated with the optimized epitope only, despite detectable dual secretors from the same sample when stimulated with staphylococcal enterotoxin B (SEB) (Figure 2C).

In order to get a more comprehensive assessment of CD4 and CD8 T cell elicited cytokines generated from HIV, we stimulated PBMC with the entirety of HIV using peptide pools (15-20mers) encompassing Gag, Pol, Env, Nef, and accessory proteins and determined the cytokine secretion of IFN- γ , IL-2, and TNF- α from CD4 T cells and CD8 T cells (Figure 3A and B). Cumulative cohort data for CD4 (Figure 3C) and CD8 (Figure 3D) T cell responses to peptide pools is shown. We demonstrated Env-dominant IL-2 responses from both CD4 and CD8 T cells, a phenotype previously described²². Again we sought to determine if cytokine production correlated with epitope specific proliferation. Although we demonstrate CD8 T cell proliferative responses to a variety of optimized epitopes, there was no association between the magnitude of the proliferative response to a given epitope and the magnitude of cytokine secretion from the pool containing the same optimized epitope eliciting the proliferative response.

We next examined polyfunctional cytokine secretion from proliferators and non-proliferators. Surprisingly, we found very few triple functioning cells (IFN- γ , IL-2, and TNF- α) at the earliest time points (data not shown). Rather, the distinguishing characteristic between the two groups was the capacity of CD8 (Figure 4B and D), but not CD4 (Figure 4A and C) T cells to secrete IL-2 in response to peptide pools. Approximately 50% of total CD8 T cell cytokine response from proliferators was composed of IL-2 compared with less than 20% in non-proliferators (Figure 4B). Furthermore, the frequency of IL-2 secreting CD8 T cells was greater in proliferators than non-proliferators ($p=0.01$) (Figure 4D). From this, it is apparent that IL-2 secretion from CD4 T cells is not associated with CD8 T cell proliferation as measured by percent of total response (Figure 4A) or frequency (Figure 4C). We also found a significant correlation between the magnitude of IL-2 secreted by CD8 T cells and the total proliferation of all HIV specific CD8 T cells within a patient ($r = 0.6340$; $p < 0.02$). Notably, while total HIV specific CD8 T cell proliferative response within a patient correlated with CD8 T cell IL-2 secretion, it was not generally produced from the same antigen-specific CTL that was capable of proliferating (Figure 2C). In contrast, no correlation was detected between IL-2 secretion from CD4 T cells and the CD8 T cell proliferative response in early infection ($r = .3350$; $p=0.26$). Neither the total IFN- γ secretion from CD4 or CD8 T cells nor the percentage of multifunctional CD4 or CD8 T cells correlated with proliferation (data not shown).

Discussion

Understanding the correlates of immune protection in HIV-1 infection is imperative to our efforts in the development and evaluation of candidate vaccines. Many studies focus on responses generated early in PHI and maintained in elite or viremic controllers. Here we sought to understand the phenotype of HIV-1-specific CD8 T cells in PHI which are capable of proliferation as this along with cytolytic capacity are most predictive of viral control^{12, 13, 18, 25, 26}. Despite testing all patients within a few months of infection, only half of our cohort had preserved HIV-1 specific CD8 T cell proliferative responses. Therefore we postulated that CD8 T cells were able to support their own proliferation as described for HLA-A*2 SL-9 specific CTL²⁷. Upon stimulation with optimized HIV-1 CTL epitopes, cells capable of proliferation did not uniformly have a phenotype of dual cytokine secretion (IFN- γ /IL-2). Instead, we demonstrate that HIV-1 peptide pool stimulated CD8 T cells from proliferators elicit a heterogeneous population with a greater frequency of IL-2-secreting CD8, but not CD4, T cells when compared to non-proliferators.

The relative lack of proliferative responses in the majority of patients with PHI was unexpected. Many have postulated that CD4 help is necessary in the early priming of CD8 T cells²⁸. During acute infection, there is a transient decline in absolute CD4 T cell number in the systemic circulation but profound early depletion at mucosal sites^{7, 29}. Our cohort was comprised of patients presenting with symptomatic infection and may be skewed towards individuals with very high viral loads and immune activation. It is plausible that proliferating CD8 T cells were primed very early during infection with optimal CD4 help. Subsequently, antigen-specific CD4 T cells may have been deleted or became anergic, leaving behind CD8 T cells that function well for a period of time but rarely persist. In contrast, nonproliferating CD8 T cells initially may have had proliferative potential but eventually became anergic in the highly activated environment of acute HIV infection³⁰. Alternatively, these suboptimal functioning CD8 T cells may have been primed later during infection with little or no CD4 help, making them compromised from the start. Finally, there may be intrinsic differences in the ability of CD8 T cells to proliferate, which are independent of CD4 help^{13, 27}. The fact that we did not observe a correlation between CD8 T cell proliferation and time since infection supports this latter mechanism. Notably, all subjects were treated in this study; thus, early treatment does not explain observed differences in proliferative capacity. Furthermore, while 2 of 13 subjects had protective alleles, only a minority of the observed proliferative responses were to epitopes restricted by these alleles.

In this altered setting of acute HIV infection, it is also possible that seemingly paradoxical immune responses are triggered to dampen the cytokine storm by secreting cytokines such as IL-10, which may have a detrimental response on HIV-1-specific T cell responses. Recent studies demonstrate that blockade of IL-10 in viremic HIV subjects can reverse HIV-1-specific CD8 and CD4 proliferative defects³¹. Furthermore, the function of CD4 T cells could be compromised in settings of such high viremia and may have contributed to development of suboptimal CD8 T cell responses in non-proliferators. This theory is supported by the relative lack of polyfunctional CD4 T cells detected in our cohort, unlike that seen in elite controllers and LTNP.

Controversy exists over the importance of CD8 T cell phenotype and the interplay of IL-2 and proliferation. While studies have implicated IL-2 secretion from CD8 T cells as a signature phenotype of proliferators, our findings suggest it may only be a marker for a broader population of cells with heterogeneous functions. Disparate results have been published regarding the ability to recover defective CD8 T cell proliferation with the addition of exogenous IL-2 or CD4 T cells^{18, 32, 33}. We were unable to recover CD8

proliferative capacity with the addition of IL-2 *ex vivo*, suggesting that CD4 mediated paracrine signaling does not support long term CD8 T cell proliferation. This is supported by recent literature demonstrating that while exogenous IL-2 induces dividing cells to enter more cycles of division, it is unable to rescue cells with defective proliferation³⁴. Clinical trials utilizing the therapeutic administration of IL-2 validate our data as these studies have consistently failed to show a restoration of CD8 T cell responses or control of viremia³⁵. These data suggest that cellular factors other than IL-2 secretion may regulate a CD8 T cells ability to proliferate. Differences in IL-2 receptor expression or signaling may be a better phenotypic representation of HIV-1-specific CD8 T cells capable of proliferation and would explain the differences seen in recent studies and in the present study which indicates compromised proliferative capacity in primary HIV infection³⁶.

In those subjects capable of CD4-independent CD8 T cell proliferation, we could not consistently identify dual (IFN- γ /IL-2) secreting HIV-1-specific CD8 T cells after stimulation with an optimized epitope. This suggests that a simple autocrine signaling pathway supporting CD8 proliferation is also unlikely. It is notable that groups describing dual secreting CD8 T cells with proliferative capacity primarily used peptide pools for stimulation, while our study focused initially on optimized epitopes. Therefore, we now describe a heterogeneous group of CD8 T cells in subjects with proliferative capacity. Within this heterogeneous groups of cells some exhibited IL-2 secretion or proliferative capacity, and few dual secretors with proliferative capacity as described elsewhere¹⁹. This apparent “dichotomous relationship” in IL-2 secretion and effector function was recently highlighted by the observation that perforin secretion and IL-2 secretion do not occur simultaneously^{37, 38}. This supports our observation that the effector function, proliferation, is not always synonymous with IL-2 secretion from the same cell. Furthermore, the detection of IL-2 secreting cells likely represents a cytokine milieu capable of sustaining effector functions rather than defining a cellular phenotype capable of proliferative capacity. Patients with superior viral control likely have this heterogeneous population as described.

One of the interesting findings of this study was the dominance of IL-2 production from Env specific CD4 and CD8 T cells. While the underlying mechanism of this result is not clear, one possible explanation is that B cells may be playing an important role in antigen presentation³⁹. This may be especially true in the context of HIV-1 infection where monocytes and macrophages are likely functionally skewed early during infection^{40, 41}. Immune globulin present on the surface of B-cells may be taking up Env and efficiently presenting this antigen to CD4 T cells inducing a qualitatively different response than those induced by monocyte or macrophage presentation (i.e. more IL-2). In this scenario Env could be presented by B cells more than other HIV-1 proteins because Env is dominantly present at the viral surface making it more accessible for B cell immunoglobulin interaction.

Numerous studies have examined functionality of CD8 T cells to better understand correlates of immunity. Distinguishing the immunological cause of viral control from the deleterious effects of viremia and immune activation remains a challenge. Studies comparing LTNP and successfully treated patients have sought to determine which immune parameters can be recovered by viral control and thus are unlikely to be the correlate of immune control in EC or LTNP. However, our studies are focused on understanding immune control or compromise occurring during the earliest time points of infection which may determine the level of subsequent viral control. Viral load alone is unlikely to explain differences we observed, because all of our patients had detectable viral load at the time of sampling. Differences in our studies relative to published literature may be explained in part by differences in cohorts, type of antigenic stimulation, and variability in cell analysis. However, our ability to focus on acute/early events of HIV-1-specific T cells stimulated

with optimized epitopes allows this study to generate more precise conclusions about the generation of optimal CD8 T cells during the course of early infection.

While we continue to define the correlates of immune protection, our focus must remain on early and longitudinal studies of the generation and maintenance of protective immune responses. Furthermore, it is essential to continue to delineate those responses that fluctuate with levels of viremia such as polyfunctionality versus those markers that truly identify unique cellular phenotypes predictive of control. Here we demonstrate that not all CD8 T cell responses generated in acute infection have proliferative capacity and that the phenotype of IL-2 secretion, while globally important to support functional CD8 T cells, does not in and of itself define the proliferative phenotype. Rather a more heterogeneous population exists. This is particularly important as we move forward in our evaluations of candidate vaccines, wherein we are searching for those favorable phenotypes both established during acute infection and preserved among elite controllers.

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Figure 1. HIV-1-specific CTL proliferative capacity in PHI

PBMC were labeled with CFSE and stimulated with HIV-1 peptides, Non-HIV-1 peptides (CEF), or SEB for 7 days. CD3+ T cells were analyzed by flow cytometry for proliferation. Proliferation was determined to be 2X unstimulated (media) controls. Strong proliferative responses were seen to all peptides tested in this individual. Peptide key-CEF- (cytomegalovirus, Epstein Barr Virus, Influenza); SEB- (staphylococcal enterotoxin B); A*3 RK9 (RLRPGGKKK); B*35 VY10 (VPLDEDFRKY); A*2 SL9- (SLYNTVATL); IG15- (IPLGDAKL VITTYWG) (B*51); B*35 VY8- VPLRPMTY; B*35 TY9- (TVLDVGDAY).

Figure 2. CD4 Independent CD8 T cell proliferation

A) CD4 Independent CD8 T cell proliferation. PBMC were depleted of CD4 T cells (> 96% purity) prior to labeling with CFSE and peptide stimulation for 7 days. CD3+ T cells were analyzed for proliferation by flow cytometry. Representative proliferative responses after CD4 depletion are shown.

B) CD4 Independent CD8 T cell proliferation. The bar graph demonstrates CD8 T cell proliferation in the presence of CD4 T cells (shaded bars) and the maintenance of CD8 T cell proliferation in the absence of CD4 T cells (open bars) to multiple epitopes in two subjects (PHI 1 and PHI2). Peptide key- see Figure 1 legend and B*27 KK10- (KRWII LGLNK); vif 70 (QVDPDLADQLIHL YY); vpr31 (EAVRHFPRIWLHSLG).

C) Induction of cytokines from CD8 T cells. PBMC from PHI 1 & 2 were stimulated with optimized HIV-1 epitopes and SEB. After gating on CD3+CD8+ T cells, the production of IFN- γ , IL-2 and co-expression of both cytokines was determined. A positive response was determined to be 2X media background.

Figure 3. Induction of cytokine secretion from CD4 and CD8 T cells in Acute HIV-1 infection

A/B) PBMC were stimulated with HIV-1 peptide pools encompassing the entirety of HIV-1. Representative Intracellular Cytokine Staining data demonstrates the induction of Gag specific secretion of IFN- γ , IL-2, and TNF- α from CD4 (panel A) and CD8 (panel B) T cells.

C/D) Frequency of cytokine secreting cells from stimulation of CD4 (panel C) or CD8 T cells (panel D). PBMC from PHI subjects were stimulated *in vitro* with HIV-1 peptide pools (20 mers) encompassing the entirety of HIV (Gag, Pol, Env, Nef, and the rest of the accessory proteins). The cumulative responses for all patients are depicted for each cytokine, IFN- γ (black box), IL-2 (white box), and TNF- α (hatched box).

Figure 4. CD8 T cells with preserved proliferative capacity have increased IL-2 secretion from CD8 T cells compared with non-proliferators

A/B) Subjects were divided into 2 groups, proliferators and non-proliferators based on their proliferative capacity to optimized HIV-1 CTL epitopes. For each subject the portion of the total CD4 (panel A) or CD8 (panel B) T cells that secreted either IL-2 or IFN- γ was calculated and summed for the groups (proliferators vs. non-proliferators).

C/D) The frequency of IL-2 secreting CD4 (panel C) or CD8 (panel D) cells are depicted. Each dot represents one patient and peptide pool combination. The bar represents the median.

Table 1

Clinical and Demographic^a characteristics of PHI Cohort^b

Group	Subject	Viral Load at Entry (copies/mL)	Days of Infection	At the time of Assay		
				Viral Load (copies/mL)	CD4 + T cell count (cells/mL)	Days on ART
Proliferators	PHI#1	235,500	77	1,800	479	26
	PHI#2	48,000	59	29,000	549	14
	PHI#3	800,000	67	15,000	663	28
	PHI#4	535,000	80	535,000	534	1
	PHI#5	512,000	50	512,000	456	1
	PHI#6	100,000	98	99,000	864	1
Non-Proliferators	PHI#7	5,000,000	38	16,000	489	13
	PHI#8	1,000,000	38	700,000	567	2
	PHI#9	3,300,000	36	2,000	956	12
	PHI#10	554,000	178	300	685	74
	PHI#11	111,000	135	18,993	286	1
	PHI#12	499,441	88	4,600	551	16
	PHI#13	264,000	70	36,000	379	21

^a 85% W; 92% M; average age 38

^b All volunteers were identified early in infection as part of AIEDRP. All had documentation of negative ELISA within at least 4 months of enrollment and met the ADIERP criteria for recent infection (documented seroconversion within 3 months, a desensitized or detuned ELISA consistent with infection within the prior 120 days, or primary HIV-1 infection as demonstrated by high viral loads in the setting of negative or indeterminate HIV-1 serologic analysis).

Table 2

Effect of IL-2 in recovery of T cell responses to HIV

Subject	Antigen	Frequency	Epitope sequence
PHI#5	media	0.38	
	p33	0.57 ^a	KILEPFRKQNPDIVIYQYMD
	N8	17^b	PQVPLRPMTKAAAVDLSHFL
	SEB	29	
PHI#8	media	0.99	
	Vr 31	0.38 ^a	EAVRHFPRIWLHSLG
	TL9	0.17 ^a	TPQDLNTML
	NN17	0.25 ^a	EVGFPVRPQVPLRPM
	RK10	0.18 ^a	RRTWKSIVK
PHI#10	SEB	48	
	media	0.033	
	vif 54	0.17^c	GKAKGWFYRHHYEST
	CEF	1.16	
PHI#11	Media	0.67	
	TL9	0.26 ^a	TPQDLNTML
	CEF	1.76	
PHI#13	SEB	28	
	media	0.36	
	Nef 18	0.31 ^a	PVRPQVPLRMTYKA
	CEF	4.58	
	SEB	23	

^aNot Recovered^bEnhanced^cRecovered