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Augmented HIV-specific Interferon-gamma Responses, but Impaired Lymphoproliferation During Interruption of Antiretroviral Treatment Initiated in Primary HIV Infection

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Abstract

Background—Antiretroviral therapy (ART) introduced during primary HIV infection followed by treatment interruption (TI) is postulated to enhance virologic control through induction of HIV-specific CD4⁺ T-cells, which foster virus-specific CD8⁺ T-cells that suppress virus replication. This hypothesis was evaluated in 21 subjects enrolled in ACTG 709, a substudy of ACTG 371, which prospectively evaluated subjects who received ≥ 1 year of ART initiated in acute or recent HIV infection followed by TI.

Methods—Lymphoproliferation was assessed by [methyl-³H]thymidine incorporation and HIV-specific CD8⁺ T-cell interferon-gamma responses by enzyme-linked immunospot-forming (ELISPOT) assays. Virologic success was defined as sustained viral load $< 5,000$ copies/mL for 24 weeks after TI.

Results—HIV-specific lymphoproliferative responses were detected at least once in 5 (24%) of 21 subjects, were generally transient, and were unrelated to HIV-specific interferon-gamma responses ($p > 0.4$). HIV-specific CD8⁺ interferon-gamma responses increased after 48 weeks of ART ($p = 0.03$), but failed to predict virologic success ($p = 0.18$). Compared to seronegative subjects, lymphoproliferation to *Candida*, cytomegalovirus, and alloantigens was similar in HIV-infected subjects during ART, but lower during TI ($p \leq 0.04$).

Conclusion—HIV-specific CD8⁺ T-cell interferon-gamma responses expand during ART following primary HIV infection, but are not related to HIV-specific lymphoproliferative responses nor virologic success. Impaired non-HIV antigen-specific lymphoproliferation associated with TI suggests this strategy could be deleterious.

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Introduction

Primary HIV infection is often associated with a flu-like illness and high-titer viremia that gradually declines over a period of weeks to months to a quasi-steady state known as the virus set-point¹. Severity of clinical symptoms^{2,3} and virus set-point following primary infection¹ are predictive of rates of disease progression suggesting that early virus-host interactions may determine disease outcome. Rosenberg *et al.*⁴ reported that antiretroviral therapy (ART) initiated in primary HIV infection followed by treatment interruption (TI) resulted in viral loads under 5,000 copies/mL for sustained periods of time in 7 of 8 subjects, a significantly larger proportion than was observed to have virus set-points below 5,000 copies/mL in a cohort of untreated seroconverters. Individuals treated during primary HIV infection had substantially more vigorous virus-specific CD4+ T-cell responses than individuals with chronic treated or untreated HIV infection^{4,5}, and those who maintained low viral loads during TI experienced expansions in HIV-specific CD8+ T-cell interferon-gamma responses⁴. In light of these data and the known helper functions provided by CD4+ T-cells to cytotoxic CD8+ T-cells⁶, it was hypothesized that the mechanism underlying reduced viremia following treatment of acute HIV infection was the induction and preservation of HIV-specific CD4+ T-cell responses, which fostered robust anti-viral CD8+ T-cell responses.

AIDS Clinical Trials Group (ACTG) 371 was implemented to test the efficacy of ART followed by TI in acute and recent HIV infection and results of this study have been published elsewhere⁷. ACTG 709 was an immunology substudy of ACTG 371 that was designed to evaluate the impact of early ART and TI on HIV-specific CD4+ and CD8+ T-cell responses. The primary hypotheses underlying ACTG 709 were that 1) early treatment of HIV infection results in robust HIV-specific lymphoproliferative responses; 2) these responses are associated with vigorous HIV-specific CD8+ T-cell interferon-gamma responses; and 3) HIV-specific CD8+ T-cell responses correlate with low-level viremia during TI. Secondary analyses examined the impact of this strategy on lymphoproliferative responses to non-HIV microbial antigens and alloantigens.

Methods

Study Subjects

HIV-infected subjects were individuals enrolled into ACTG 371⁷, a prospective trial of ART initiated in acute or recent HIV infection, who agreed to participate in a substudy (ACTG 709). This required donation of additional blood at study entry, 48 weeks after initiation of therapy, 4 weeks after treatment interruptions, and 24 weeks after treatment interruptions. Acute infection was defined as having a plasma HIV RNA concentration more than 2000 copies/mL within 14 days of study entry and either a negative ELISA or a positive ELISA but a negative or indeterminate western blot (CDC/ASTPHLD criteria) or a positive ELISA and western blot in conjunction with either a negative ELISA or a plasma HIV RNA concentration less than 2000 copies/mL in the 30 days prior to entry. Recent infection was defined as a positive ELISA and western blot within the 14 days prior to entry, but a negative ELISA or plasma HIV RNA concentration less than 2000 copies/mL within the 31–90 days before entry or a positive ELISA and western blot and a nonreactive detuned ELISA in patients with more than 200 CD4+ cells/ μ L all within the 21 days before study entry. If plasma HIV RNA was below 50 copies/mL and CD4+ T-cells $>200/\text{mm}^3$ after 52 weeks of treatment, ART was interrupted. If viremia rebounded $>50,000$ copies/mL on two consecutive measurements or above 5,000 copies/mL on three consecutive measurements, the cycle of treatment and TI was repeated. The primary endpoint of virologic success was defined as achieving a viral load less than 5,000 copies/mL at 24 weeks following either the first or second TI. Thirty-three (83%) of a proposed total of 40 subjects were enrolled in

ACTG 709, and 21 (64%) of these individuals underwent TI and are included in this analysis. Seronegative control subjects were laboratory workers tested annually for HIV antibodies. The study was performed in accordance with federal human experimentation guidelines; all subjects provided informed consent in accordance with local institutional review boards.

Peripheral Blood Specimens

Peripheral blood specimens were collected in sodium heparin-containing tubes (BD Vacutainer, San Diego, CA) and shipped overnight to the University of Colorado AIDS Clinical Trials Group Immunology Laboratory. Specimens obtained at the University of Colorado were held overnight at room temperature, to maintain comparable handling conditions⁸. PBMC were isolated by ficoll-hypaque density centrifugation and used immediately in lymphocyte proliferation assays or cryopreserved for future studies.

Lymphocyte Proliferation Assays

Lymphocyte proliferation was assessed by [methyl-³H]thymidine incorporation using four replicate wells as previously described⁹ using the following antigens: *Candida albicans* (20 µg/mL, Greer Laboratories, Lenoir, NC), tetanus toxoid, (0.5 Lfu/mL, Connaught Laboratories, Willowdale, Ontario), HIV-1 p24 or control antigen (1 µg/mL, Protein Sciences, Meriden, CT), HIV-1 env2–3 SF2 or control antigen, (0.5 µg/mL, Chiron Corporation, Emeryville, CA), *Mycobacterium avium complex* (MAC) antigen (0.5 µg/mL Statens Serum Institut, Copenhagen, Denmark), cytomegalovirus or control antigen (1/50 dilution, provided by Dr. Adriana Weinberg, University of Colorado Denver)¹⁰, and alloantigens consisting of pooled PBMC from 15 unrelated donors that had been previously gamma-irradiated, cryopreserved, and stored in liquid nitrogen. PHA (5 µg/mL, Sigma, St. Louis, MO) was included as a positive control. Stimulation index (SI) was calculated as median counts per minute (cpm) in the stimulated wells divided by median cpm in relevant control wells. If the observed SI was less than 1, then a value of 1 was assigned.

Enzyme Linked Immuno-Spot (ELISPOT) Assays for HIV-specific Interferon-Gamma Responses

Cryopreserved PBMC were thawed, rested overnight, and seeded onto nitrocellulose HA plates (Millipore, Burlington, MA) coated with 10 µg/mL anti-IFN-γ (Mabtech, Stockholm, Sweden) at a concentration of 100,000 cells/well in AIM-V media (Life Technologies, Grand Island, NY) supplemented with 10% human AB serum (Gemini Bio-Products, Calabasas, California). HIV-1 pooled peptides (AIDS Research and Reference Reagent Program, Bethesda, MD) were added to duplicate wells at a final concentration of 2 µg/mL: MN Env (15-mer peptides), HXB2 Gag (15-mer peptides), Clade B Consensus Nef (15-mer peptides), HXB2 Pol (20-mer peptides) and Consensus B Rev (15-mer peptides). A positive control well treated with PHA (20 µg/mL) was included in each assay. Plates were incubated at 37°C for 48 hours, stained with 2 µg/mL biotinylated 7-b6-1 (Mabtech) and spots were developed using Vectastain kit (Vector Laboratories, Burlingame, CA) and 3-amino-9-ethylcarbazole (AEC, Sigma). Spot forming cells (SFC) per well were determined by visual inspection and manual counting. HIV-specific interferon-gamma responses were calculated by subtracting the mean SFC of unstimulated wells from the mean SFC in peptide-stimulated wells. Total HIV-specific interferon-gamma responses were determined by summing individual antigen-specific responses. Results were scaled by multiplying by 10 and expressed as SFC per 10⁶ PBMC; a positive response was defined as ≥20 SFC/10⁶ PBMC and also at least two-fold above levels of SFC in unstimulated wells. CD8 depletion was performed using CD8 Dynabeads, (Invitrogen Dynal As, Oslo) and antibody staining confirmed depletion of >95% of CD8+ cells in all cases.

Statistical Analysis

Immunologic measurements are summarized using medians and rank-based correlations. Groups are compared using the Wilcoxon rank-sum and Fisher's exact tests. Lymphoproliferative responses at each timepoint ($\log_{10}$ SI) are compared to responses in seronegative subjects using generalized estimating equations to account for repeated measurements in seronegative subjects. Changes in proportions are compared using McNemar's test. No adjustments are made for multiple comparisons.

Results

Clinical characteristics and outcomes of the 21 study subjects enrolled in ACTG 709 who underwent TI were similar to those of the 73 subjects enrolled in the parent study who interrupted therapy⁷. The majority (95%) were males, the median age was 36 years at enrollment, and most were white (86%). Median baseline CD4+ T-cell count was 599 cells/mm³ and median baseline HIV RNA concentration was 79,500 copies/mL. Eight (38%) subjects were acutely and 13 (62%) were recently infected. Nine (43%) met criteria for virologic success during the first TI and one additional subject achieved virologic success during a second TI.

Lymphocyte Proliferative Responses

Lymphocyte proliferative responses to microbial antigens and alloantigens are shown in Figure 1. Five (24%) HIV-infected subjects had detectable HIV-specific lymphoproliferative responses, defined as a SI $\geq$ 3 at any time point to HIV env or gag antigen, and no HIV-specific lymphoproliferative responses were detected in control subjects. Previous studies have demonstrated that the lymphoproliferative responses induced by these antigens are primarily mediated by CD4+ T cells^{11, 12}. HIV-specific lymphoproliferative responses were low, with a maximum SI of 14. Only one subject responded to both HIV antigens. HIV-specific responses were transient in all but one subject who had responses to the same antigen twice. One subject developed new HIV-specific lymphoproliferative responses during the first and another during the second TI. Four (80%) of five subjects with HIV-specific lymphoproliferative responses achieved virologic success after 24 weeks of TI, compared to 6 (38%) of 16 without HIV-specific lymphoproliferative responses ($p=0.15$). When analysis was restricted to lymphoproliferative responses detected prior to TI ($n=3$), 100% achieved virologic success compared to 7 (39%) of 18 subjects who did not harbor HIV-specific lymphoproliferative responses ($p=0.09$). Two (25%) subjects with acute infection and three (23%; $p=1.0$) with recent infection had HIV-specific lymphoproliferative responses. Neither baseline CD4+ T-cells ($p=0.35$) nor baseline viral load ($p=0.13$) significantly correlated with proliferative responses to HIV, although median baseline viral load tended to be lower in those with HIV-specific lymphoproliferative responses (52,400 copies/mL) compared to those without lymphoproliferative responses (148,200 copies/mL).

Responses to non-HIV microbial antigens and alloantigens were more robust than those to HIV (Figure 1C–1G). At baseline, HIV-infected subjects had significantly lower lymphocyte proliferative responses to tetanus toxoid, MAC, *Candida*, and CMV antigens compared to 12 seronegative subjects ($p\leq 0.02$). Increases in median lymphocyte proliferative responses to all non-HIV microbial antigens were observed after 48 weeks of ART, but responses to tetanus and MAC antigens remained lower compared to those in seronegative subjects ($p\leq 0.01$). Responses to microbial antigens and alloantigens waned after 4 weeks of TI and all were significantly lower at this time point compared to seronegative controls ($p\leq 0.04$). In the subset of subjects studied at week 24, the majority of whom achieved virologic success, microbial antigen and alloantigen responses were not significantly impaired compared to control subjects. Individuals who reached the study

endpoint of virologic success after 24 weeks of TI tended to have higher lymphocyte proliferative responses to microbial antigens at all time points compared to those who were not successful in reaching the study endpoint (Figure 1); these differences were most pronounced at baseline for CMV ($p=0.001$) and *Candida* ($p=0.07$), and at week 4 of the first TI for MAC ($p=0.015$). There was no significant difference in microbial antigen or alloantigen lymphoproliferative responses at any time point between individuals treated during acute versus recent HIV-1 infection.

HIV-Specific CD8+ T-Cell Responses

HIV-specific interferon-gamma responses are shown in Figure 2. CD8 depletion of PBMC in five subjects demonstrated $\geq 97\%$ reduction in interferon-gamma responses in all instances. At baseline, 6 (32%) of 19 subjects demonstrated virus-specific interferon-gamma responses. Responses were seen to nef (21%), env (16%) and gag (11%); the mean number of HIV antigens recognized by the 6 responders at baseline was 1.7. The proportion of subjects with detectable HIV-specific interferon-gamma responses increased at week 48 to 75% (9/12 subjects; $p=0.03$ compared to 3/12 subjects at baseline). Subjects at week 48 harbored responses to nef (25%), env (42%), gag (58%) and pol (33%); the mean number of HIV antigens recognized by the 9 responders was 2.2. Enrollment during acute versus recent HIV infection was not related to the magnitude or breadth of interferon-gamma responses either at baseline ($p=0.76$ and $p=0.84$, respectively) or week 48 ($p=0.96$ and $p=0.86$, respectively). There was no significant relationship between HIV-specific lymphoproliferative responses and magnitude or breadth of interferon-gamma responses at week 48 ($p=1.0$ and $p=0.68$, respectively). Furthermore, there was no significant relationship between virologic success during TI and interferon-gamma responses at week 48 ($p=0.18$) (Figure 2).

ELISPOT assay results were available on 12 subjects during TI; assays could not be performed in 9 subjects during TI because PBMC were not received in the laboratory, there were insufficient cells, or cells had poor viability. Figure 3 summarizes representative ELISPOT assay results and viral loads from three subjects with virologic success (A–C) and three with virologic failure (D–F). All subjects except A had documented increases in HIV-specific interferon-gamma responses during TI. There was no apparent temporal relationship between increases in interferon-gamma responses and viral load. Indeed, low-level viremia was evident in all subjects with virologic success before increases in HIV-specific interferon-gamma responses occurred, and more than 5-fold increases in interferon-gamma responses were seen in subjects D and E, but did not result in virologic success. The magnitude of interferon-gamma responses during TI varied among subjects, but there was no obvious difference between subjects with virologic success and those with failure.

Discussion

ACTG 709 is one of the largest studies to date to evaluate prospectively the impact of ART followed by TI on HIV-specific responses in acutely and recently infected individuals. This study was designed to investigate the findings of Rosenberg et al.⁴, which were subsequently confirmed by Kaufmann et al.¹⁴, that the majority of treated seroconverters with robust HIV-specific lymphoproliferative responses during ART were able to maintain viral loads below 5,000 copies/mL for at least 6 months after one or more TIs. The present study is distinct from these two previous studies in that subjects were not limited to those with robust HIV-specific lymphoproliferative responses, but instead all seroconverters who received early ART and underwent TI were included. Thus, the present study provides information on the impact of this strategy on immune responses in a wider population of seroconverters than those two previous studies. Results of the present study revealed: 1) a minority of subjects treated during primary HIV infection had detectable HIV-specific

lymphoproliferative responses; 2) HIV-specific CD8+ T-cell interferon-gamma responses increased in magnitude and breadth during one year of suppressive ART, but were not related to HIV-specific lymphoproliferative responses; and 3) HIV-specific CD8+ T-cell interferon-gamma responses were not associated with virologic success during TI. Collectively, these data do not support the hypotheses that early treatment of HIV infection frequently results in robust HIV-specific lymphoproliferative responses, that induction of robust HIV-specific lymphoproliferative responses in individuals treated during primary infection augments HIV-specific CD8+ T cell interferon-gamma responses, or that virus-specific CD8+ T-cell interferon-gamma responses mediate viral control during TI.

HIV-specific lymphoproliferative responses were observed in 24% of subjects in the present study and in most instances were transient. The frequency of lymphoproliferative responses in individuals treated during primary infection has varied widely in other studies ranging from 25% to 100% of those treated for one year^{4, 15, 16}. Differences in types and concentrations of HIV antigens used in assays may partially account for these discrepancies. A strength of our study is that seronegative subjects who were studied concurrently demonstrated no evidence of HIV-specific responses, thereby confirming the specificity of the assay. Lymphoproliferative responses in the present study may have been blunted because specimens for immune function assays were shipped overnight or held for 24 hours prior to performance of the assays, procedures that we have previously shown reduce lymphoproliferative responses⁸. The abundance of lymphoproliferative responses to non-HIV antigens, however, indicates that loss of lymphoproliferative responses was relative and not absolute. Importantly, robust lymphoproliferative responses are reduced, but not ablated by shipment of specimens⁸, suggesting that most individuals in our study did not develop robust HIV-specific lymphoproliferative responses despite early initiation of ART.

All three subjects who harbored HIV-specific lymphoproliferative responses prior to TI achieved virologic success, whereas only 39% of the 18 subjects without HIV-specific lymphoproliferative responses maintained low level viremia for at least 6 months during TI. These findings are consistent with previous studies in acute^{4, 15} and chronic HIV infection^{17, 18} that have demonstrated an association between the presence of HIV-specific lymphoproliferative responses, which are largely mediated by CD4+ T cells^{11, 12}, and virologic success upon TI. Initially, many interpreted this as evidence of “immune control” of HIV replication⁴. Nevertheless, multiple lines of evidence including data from this study suggest that HIV-specific CD4+ T-cells do not control viremia. HIV-specific CD4+ T-cells are highly susceptible to HIV infection in vivo¹⁹. Although short bursts of viremia boost virus-specific CD4+ T-cells, the majority of these cells are depleted in association with loss of HIV-specific lymphoproliferative responses during prolonged TI²⁰, consistent with the transient nature of HIV-specific lymphoproliferative responses we observed. Furthermore, induction of HIV-specific lymphoproliferative responses through therapeutic vaccination in a previous study of individuals treated during primary infection failed to reduce viremia during TI²¹. Subjects with HIV-specific lymphoproliferative responses in the present study tended to have lower baseline viral loads than those without HIV-specific responses, and lower baseline viral load was also related to virologic success in the parent study⁷. Similarly, Kaufmann et al.¹⁴ reported that the major predictor of low level viremia during the first treatment interruption was a low viral load prior to initiation of ART. Collectively, these data suggest that preserved HIV-specific lymphoproliferative responses are the consequence of a relatively less virulent HIV infection and not the cause of it. An entry criterion for both Rosenberg’s and Kaufmann’s studies was a robust HIV-specific lymphoproliferative response ($SI \geq 10$) prior to TI⁴. Selection of subjects with less virulent HIV infection may explain why more subjects achieved virologic success in their studies, 88% and 57%, respectively, compared to ACTG 371 (40%)⁷.

HIV-specific CD8+ T-cell interferon-gamma responses increased in both magnitude and breadth during one year of ART, but were not predictive of virologic success, similar to what has been reported in other studies of TI in primary infection^{5, 14, 15}. Importantly, the present study illustrates that CD8+ interferon-gamma responses can be augmented during TI even in subjects without vigorous HIV-specific lymphoproliferative responses prior to TI. Marked epitope- and allele-specific differences in the ability of HIV-specific CD8+ T cells to neutralize HIV-1 in vitro have been observed despite similar interferon-gamma responses²², suggesting that interferon-gamma responses are not reflective of CD8+ T cell antiretroviral activity. More recently, the interaction between peptide and MHC class I alleles has been linked to durable virologic control in chronic HIV infection²³. Associations between low-level viremia and HIV-specific CD8+ T-cell maturation levels^{24, 25}, cytokine profiles^{26, 27}, and release of a soluble, non-cytotoxic factor²⁸ have been described as well. Whether these characteristics of CD8+ T cells account for the virologic control seen in subjects in the present study, and whether they are modifiable by early initiation of ART are unanswered questions.

If timing of initiation of ART in primary infection is critically related to preservation of lymphoproliferative responses or magnitude of interferon-gamma responses, one would anticipate that subjects treated earliest would have the most vigorous immune responses. In the present study there was no evidence for more robust immune responses to HIV or other antigens in those treated in acute versus recent infection. In the parent study ACTG 371⁷ and in the study by Kaufmann et. al.¹⁴, the timing of initiation of ART was not related to durability of virologic control during TI. Because subjects were not randomized to treatment according to disease stage, it is not possible to derive definitive conclusions from these observations. Nevertheless, these data suggest that there is no dramatic enhancement of antigen-specific lymphocyte proliferative responses or HIV-specific CD8+ T cell responses from early initiation of ART in primary HIV infection.

Impairments in lymphoproliferative responses to all non-HIV microbial antigens were demonstrated at baseline in HIV-infected subjects compared to seronegative subjects. ART initiated during primary infection was associated with normalization of lymphoproliferative responses to the ubiquitous antigens *Candida* and CMV, whereas neither tetanus nor MAC-specific lymphoproliferative responses normalized on ART, consistent with findings in chronic infection^{29, 30}. Importantly, lymphoproliferative responses to all non-HIV microbial antigens and alloantigens worsened during TI and were impaired 4 weeks after TI compared to those in seronegative controls. This is the first study to report the impact of TI on lymphoproliferative responses to multiple non-HIV microbial antigens and alloantigens in individuals treated during acute HIV infection. These findings contrast with those of Rosenberg et. al.⁴ who reported that tetanus-specific lymphoproliferative responses were not altered in the context of TI, and concluded from these limited data that the intervention was safe. Progressive impairments in lymphoproliferation to HIV antigens, recall antigens, alloantigens, and mitogens were the first functional defects described in association with HIV infection³¹. These impairments correlate with CD4+ T-cells, but are also predictive of disease progression independent of viral load and CD4+ T-cell count³². Taken together, these data suggest that TI in individuals treated during early HIV infection results in significant immune impairments.

Enthusiasm for TI as a strategy to reduce antiretroviral drug use has waned substantially over the past five years. TI during chronic HIV infection resulted in worse clinical outcomes in subjects enrolled in the SMART study³³. In addition, observational studies suggest that initiation of ART at CD4+ T cell counts above 500 cells/mm³ is associated with better clinical outcomes than when ART is initiated at conventional CD4+ T cell threshold levels³⁴. Data from the present study demonstrating impairments in antigen-specific

lymphoproliferative responses during TI in subjects with primary infection further support the notion that continued ART is beneficial even in early disease. New initiatives to enhance HIV prevention by early treatment of all individuals with ART³⁵ further weigh against strategies that use TI. Recent data from a randomized controlled study of nine months of ART followed by TI versus standard of care treatment for primary HIV-infection suggest that early treatment confers a 4 month delay in initiation of ART³⁶, consistent with observations from Kaufmann et. al's uncontrolled study that virologic control conferred by early ART is not durable¹⁴. If additional data from larger randomized controlled studies confirm this modest benefit, it seems unlikely that this strategy will continue to be pursued. Nevertheless, the improvement in virologic control conferred by ART in primary infection remains unexplained. A better understanding of the mechanisms underlying the transient virologic control conferred by early treatment and TI could nonetheless provide important insight into HIV pathogenesis and potentially lead to development of novel and more durable therapies to treat and prevent HIV infection.

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Figure 1.

Lymphoproliferative responses to microbial antigens and alloantigens in individuals with primary HIV infection before and during ART and TI. PBMC from HIV-infected and seronegative control subjects were stimulated with (A) HIV env antigen, (B) HIV p24 antigen, (C) Tetanus toxoid, (D) MAC antigen, (E) *Candida* antigen (F) CMV antigen, and (G) alloantigens for six days, proliferation was assessed by [methyl-³H]thymidine incorporation, and the stimulation index was determined relative to control wells. Horizontal bars indicate median values. Seronegative subjects had lymphoproliferative assays performed on 4 (range, 1 to 13) different days; each donor's geometric mean SI is plotted. Diamonds indicate subjects with acute HIV infection, octagons indicate subjects with recent

HIV infection, and triangles indicate seronegative subjects. Open symbols indicate HIV-infected subjects who achieved virologic success after one or two TI, and closed symbols indicate HIV-infected subjects who did not achieve virologic success. Asterisks (*) indicate results in seropositive subjects that were statistically different from those in seronegative controls. Horizontal gray dotted lines indicate a SI of 3, the threshold value for a positive response.

Figure 2.

Frequencies of HIV-specific interferon-gamma responses in HIV-infected subjects before and after one year of ART. HIV-specific interferon-gamma responses were determined in ELISPOT assays using overlapping peptides to HIV env, gag, nef, pol and rev. Diamonds indicate subjects with acute HIV infection, and octagons indicate subjects with recent HIV infection. Open symbols indicate HIV-infected subjects who achieved virologic success after one or two TI, and closed symbols indicate subjects who did not achieve virologic success. Horizontal lines indicate median values.

Figure 3.

HIV-specific interferon-gamma responses (triangles) and plasma viral loads (circles) during the study from subjects with virologic success (A–C) and virologic failure (D–F). Periods of TI are indicated in gray. Subject A met the study definition of virologic success during the first TI, but subsequently experienced three consecutive viral loads >5,000 copies/mL requiring reinstitution of ART. He failed to maintain virologic suppression below 5,000 copies/mL during the second TI. Subjects B and C both achieved virologic success during the first TI, and maintained low-level viremia throughout the rest of the study. Subjects D–F failed virologically during both the first and the second TI. Subject F remained off ART after virologic failure during the second TI by his own choice, not in accordance with study

protocol. Subjects A and B were recruited during acute HIV infection, and the other subjects were recruited during recent HIV infection.