

# Cellular restoration in HIV infected persons treated with abacavir and a protease inhibitor: age inversely predicts naive CD4 cell count increase

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**Objective:** To characterize early and later indices of cellular restoration among HIV-1 infected persons treated with abacavir and one protease inhibitor and to identify predictors of CD4 cell increases.

**Methods:** Flow-cytometric analyses of lymphocyte phenotypes among 71 antiretroviral treatment naive adults in a 48 week treatment trial.

**Results:** During the first 4 weeks of therapy, increases in naive and memory CD4 cells and in B cells were seen; naive CD8 cells increased while CD8 cells remained stable as memory CD8 cells decreased. During the second phase total CD4 and naive CD4 and CD8 cells increased while total CD8 and memory CD8 cells decreased. The numbers of CD4 cells that expressed CD28 increased from a median of  $308 \times 10^6/l$  at baseline to  $477 \times 10^6/l$  at week 48. Higher baseline plasma HIV-1 RNA levels predicted the magnitude of early CD4 ( $r = 0.35$ ;  $P = 0.01$ ), memory CD4 ( $r = 0.38$ ;  $P = 0.001$ ) and CD28 CD4 cell ( $r = 0.29$ ;  $P = 0.01$ ) restoration but was not related to second phase changes. Younger age predicted a greater second phase (but not first phase) increase in naive CD4 cells ( $r = -0.31$ ;  $P = 0.03$ ).

**Conclusions:** Higher baseline levels of HIV-1 replication determine the magnitude of first phase CD4 cell increases after suppression of HIV-1 replication. Second phase (primarily naive) CD4 cell increases are not related to HIV-1 replication but are inversely relate to age suggesting that thymic potential is a major determinant of long term cellular restoration in HIV-1 infected persons receiving antiretroviral therapy.

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## Introduction

The recent ability to suppress HIV-1 replication after combination antiretroviral therapies has provided both

laboratory [1–3] and clinical [4–6] evidence of substantial immune restoration in persons with HIV-1 related immune deficiency. Phenotypic monitoring of circulating lymphocyte populations in persons with

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moderately advanced HIV-1 infection has revealed a two phase restoration [2,3,7]. The first phase – occurring within the first 4–8 weeks of therapy – has been associated with rapid increase in the numbers CD4 T cells, CD8 T cells, B cells but not cells of the natural killer cell phenotype. A second phase of cellular restoration has been characterized primarily by an increase in circulating naive CD4 and CD8 T cells, and a decrease in circulating CD8 memory cells. The first phase increases have been attributed largely to redistribution of cells from lymphoid tissue to the circulation [8] and the second phase cellular changes have been thought to represent an increase in thymic production of naive T cells [9,10] and a concurrent disappearance of memory CD8 T cells that may in part represent death of HIV-1-reactive cells as HIV-1 antigens are cleared [11].

CNA-2004 was initiated to test the activities of abacavir when combined with five different inhibitors of HIV-1 protease. The documented high level *in vivo* activities of protease inhibitors and the nucleoside inhibitor abacavir and the relative simplicity of the regimens provided the rationale for testing the two drug regimens for the treatment of HIV-1 infection. The predictors and correlates of first phase and second phase lymphocyte restoration were analyzed in HIV-1 infected persons who were naive to antiretroviral therapies. These studies were focused on CD4 cell responses – in particular the changes in total CD4 cells, naive CD4 cells (as defined by expression of CD45RA and CD62L), memory CD4 T cells (as defined by CD45RO expression and the absence of CD45RA expression) and CD4 cells expressing CD28, a coreceptor for T cell activation. This additional marker of CD4 cell restoration was chosen for study as CD4 cells of HIV-1 infected persons frequently lack expression of this marker [12] and CD28 expression on CD4 cells tends to predict *in vivo* immune function as reflected in the ability to respond to immunization with recall and presumed neo-antigens [13].

## Methods

### Subject population and study design

This study (Glaxo-Wellcome CNA-2004) was conducted at eight study sites in the USA. All study sites had investigational review board approval to perform the study and all subjects gave written informed consent prior to participation. Antiretroviral therapy naive HIV-1 seropositive male and female subjects were eligible to participate in the study if they were aged 16 years or over with CD4 lymphocyte counts  $\geq 100 \times 10^6$  cells/l and plasma HIV-1 RNA of  $\geq 5000$  copies/ml within 14 days of study entry.

Subjects received open label therapies: abacavir 300 mg twice daily plus one of the assigned protease inhibitors: (i) indinavir 800 mg three times daily; (ii) saquinavir 1200 mg three times daily; (iii) ritonavir 600 mg twice daily; (iv) nelfinavir 750 mg three times daily; or (v) amprenavir 1200 mg twice daily. Subjects continued their randomized therapy for 48 weeks unless they met protocol-defined virologic switch criteria, defined as failure at week 8 to achieve  $\geq 1.0$  log<sub>10</sub> copies/ml reduction from baseline in plasma HIV-1 RNA, or failure at week 16 or thereafter to achieve a plasma HIV-1 RNA level at or below the limit of detection (400 copies/ml). Subjects who met the switch criteria were eligible for one of three options: (i) to continue randomized therapy; (ii) to discontinue the assigned protease inhibitor and continue to receive abacavir in combination with any other antiretroviral therapy prescribed; or (iii) to discontinue all study medication and withdraw from the study.

Blood samples were collected prior to dosing, at pre-entry (screen), day 0, day 3/4, day 7, day 10/11 and weeks 2, 3, 4, 16, 32, and 48. Plasma HIV-1 RNA was measured using a quantitative HIV-1 RNA PCR assay (Amplicor HIV-1 MONITOR, Roche Molecular Systems, Branchburg, New Jersey, USA) with a sensitivity of 400 copies/ml (standard assay); virologic data are reported separately (D. McMahon, M. Lederman, D. Haas, R. Haubrich, J. Stanford, E. Cooney, J. Horton, D. Kelleher, L. Ross, A. Cutrell, D. Lee, W. Spreen and J. Mellors, unpublished data).

Lymphocyte subset analyses were performed at pre-entry (screen), day 0, day 3/4, day 7, day 10/11 and weeks 2, 3, 4, 16, 32, and 48. Selected lymphocyte surface antigens were measured on whole blood using a validated four-color flow cytometric methodology using a FACSCalibur instrument (Becton Dickinson, San Jose, California, USA). Patient blood samples were drawn in EDTA-containing vacutainer tubes and were transported to a central clinical laboratory for analysis; sample stability was confirmed for up to 48 h following sample collection [14]. Fluorescein isothiocyanate (FITC)/phycoerythrin/peridinin chlorophyll-a protein/allophycocyanin labeled antibody combinations were prepared in a single lot under good manufacturing practice conditions to monitor CD25, CD28, CD38/HLA DR, CD45RA/CD45RO, CD45RA/CD62L and CD95 expression on both CD3/CD4 and CD3/CD8 lymphocytes (Pharmingen, San Diego California, USA). Absolute CD3/CD4 and CD3/CD8 cell counts were monitored using True Count beads.

### Statistical analysis

Results from all five study regimens were pooled for analysis. All patients remaining on study contributed data for these analyses. First phase changes were estimated for each subject by fitting a regression line

through all data points available from baseline through week 4. Second phase and total changes were estimated by subtracting week 4 or baseline values from week 48 values, respectively.

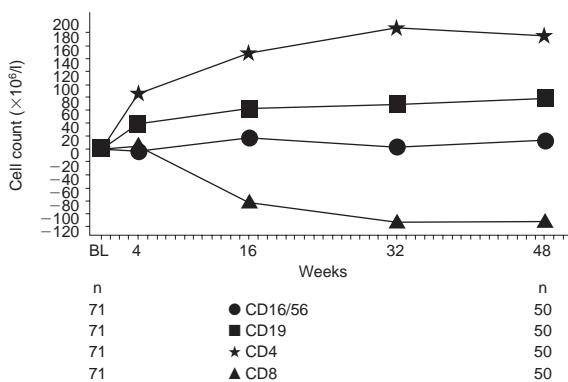
Associations between variables were explored by calculating Spearman correlation coefficients for pairs of continuous variables with corresponding *P* values. A Wilcoxon rank sum test was used for calculating relationships between sex and variables of interest. No adjustment was made for multiple comparisons so all results are considered descriptive.

## Results

Eighty-two patients enrolled in the trial. There were 66 men and 16 women. Their median age was 36 years (range 18–65 years). The median  $\log_{10}$  plasma HIV-1 RNA was 4.78 and the median baseline CD4 cell count was  $349 \times 10^6$  cells/l. Of these patients, 74 received antiretroviral therapy. Baseline immunologic data are available for 71 patients and with gradual attrition, 55 patients completed the 48 weeks of study (eight were lost to follow-up, six had adverse events, two had inadequate suppression of HIV-1 replication, and one each withdrew consent, had a protocol violation or left for unspecified reasons). Seven patients who completed 48 weeks of study continued abacavir but switched to non-randomized treatment because of adverse events or incomplete suppression of HIV-1 replication. Complete immunologic data were available for 50 patients and these data were used for the present report. By reporting median changes in phenotypic indices of cellular restoration, all useful data were included.

### Cellular restoration

Dual antiretroviral therapy with abacavir plus a protease inhibitor in treatment naive HIV-1 infected patients resulted in dramatic changes in circulating lymphocyte populations (Fig. 1). Circulating CD4 lymphocytes in-

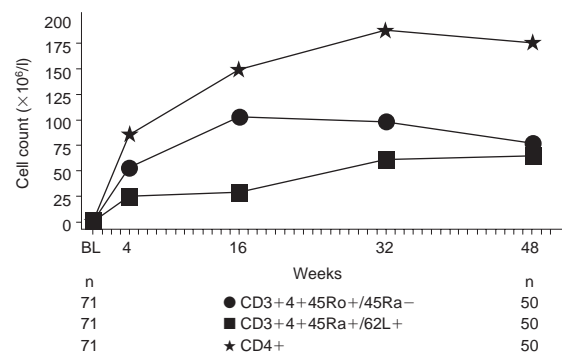


**Fig. 1.** Changes in CD4, CD8, CD16/CD56 and CD19 positive cells. Data represent median changes from baseline.

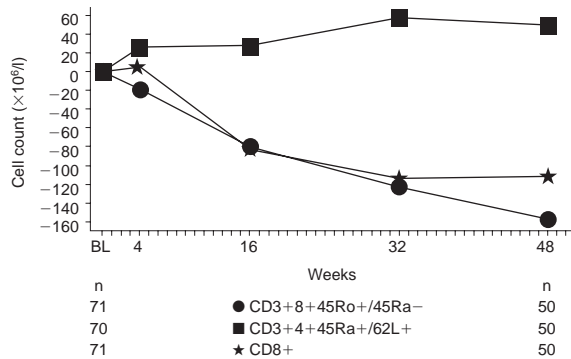
creased by a median of  $86 \times 10^6$  cells/l within 4 weeks and by  $187 \times 10^6$  cells/l by 48 weeks. In contrast, the median number of circulating CD8 cells remained stable for the first 4 weeks after treatment initiation and then fell progressively with a median  $113 \times 10^6$  cells/l decrease by week 32 and a  $100 \times 10^6$  cells/l decrease by week 48. Circulating B lymphocytes as identified by expression of CD19, manifested a rapid initial increase of  $39 \times 10^6$  cells/l by week 4 that remained stable throughout the remainder of the study period. There was no appreciable change in the numbers of circulating natural killer cells as defined by expression of CD16 or CD56.

During the first 4 weeks after treatment initiation, there was an acute increase in the numbers of memory (CD45RO+/CD45RA-) CD4 cells and in the numbers of naive (CD45RA/CD62L) CD4 cells (Fig. 2). Naive CD4 cells increased by a median of  $24 \times 10^6$  cells/l, memory cells by a median of  $53 \times 10^6$  cells/l and total CD4 cells increased by a median of  $86 \times 10^6$  cells/l over this time period. Over the second phase of the study (from week 4 to week 48) naive CD4 cells increased by an additional  $32 \times 10^6$  cells/l, memory CD4 cells by  $9 \times 10^6$  cells/l and total CD4 cells increased by  $79 \times 10^6$  cells/l. It should be noted that total lymphocyte counts may not be reflected in the sums of phenotypically defined naive and memory subpopulations; moreover the median changes are also not necessarily additive.

Analysis of circulating CD8 cell numbers revealed that the decrease in total CD8 cell count was due to a dramatic parallel decrease in the numbers of memory (CD45RO+/CD45RA-) cells (Fig. 3). In contrast, there was a modest  $25 \times 10^6$ /l first phase increase in circulating naive (CD45RA/CD62L) CD8 cells during the first 4 weeks after treatment initiation and a more subtle but persistent increase in these cell numbers resulting in a total  $50 \times 10^6$ /l naive CD8 cell increase by the end of the 48 weeks of study.



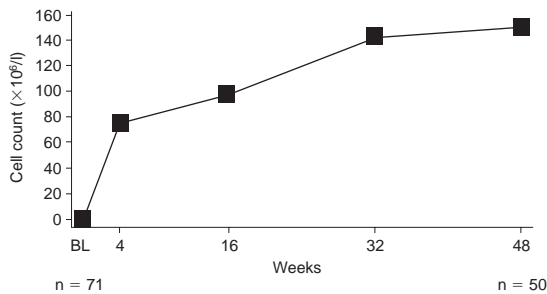
**Fig. 2.** Changes in 'naive' (CD45RA/CD62L) and 'memory' (CD45RO+/CD45RA-) CD4 cells. Data represent median changes from baseline.



**Fig. 3.** Changes in 'naive' (CD45RA/CD62L) and 'memory' (CD45RO+/CD45RA-) CD8 cells. Data represent median changes from baseline.

Treatment was associated with a large decrease in the proportion of circulating CD8 cells that expressed the activation markers CD38 and HLA DR from median of 48% at baseline to 20% at week 48 ( $P < 0.0001$ ). In contrast, the proportion of CD4 cells that expressed both HLA DR and CD38 remained stable with a median of 23% of cells expressing these activation antigens at baseline and 22% at the end of the study.

Treatment was associated with increases in the total numbers of CD4 cells that expressed the coreceptor for T cell activation, CD28 (Fig. 4) with a first phase increase from a median of  $308 \times 10^6$  cells/l at baseline to a median of  $419 \times 10^6$  cells/l at week 4 and a slower second phase increase resulting in a median of  $477 \times 10^6$  cells/l by week 48. There was also a small increase in the percentages of CD4 cells that expressed



**Fig. 4.** Changes in absolute numbers of CD4 cells expressing CD28. Data represent median changes from baseline.

CD28 from a median of 88% at baseline to 89% at 4 weeks to 91% at 48 weeks ( $P = 0.067$  for the baseline-week 48 comparison).

**Correlates and predictors of cellular restoration**

An exploratory analysis was carried out to identify the correlates and predictors of CD4 cell and CD4 cell subset restoration. Correlates and predictors of first phase (defined here as baseline to week 4), second phase (week 4-48) and total (week 0-48) cellular restoration were examined. Baseline values that were used in the analysis included: age, sex, plasma HIV-1 RNA levels and each of the cellular phenotypes that were presented in Figs 1-4 plus the proportion of cells that expressed CD95. Expression of CD95 (Fas) was examined as activation of cells through this receptor can help trigger induction of programmed cell death (apoptosis) [15]. In addition we asked if any early changes in these indices could predict the second phase (week 4-48) and total (week 0-48) changes.

Neither age nor sex predicted the magnitude of first phase CD4 cell restoration (Table 1). Pre-treatment plasma HIV-1 RNA levels were positively correlated with first phase total CD4 cell ( $P < 0.01$ ), memory CD4 cell ( $P < 0.001$ ) and with CD28 CD4 cell ( $P = 0.01$ ) restoration. Neither the frequency of activated (CD38/HLA DR) CD4 cells nor CD8 cells nor the percentage of Fas+ (CD95) CD4 or CD8 cells predicted early cellular restoration. There were no associations between first phase changes in plasma HIV-1 RNA levels or changes in the proportion of activated (CD38/HLA DR) or Fas+ lymphocytes and first phase CD4 cellular restoration (data not shown).

Baseline predictors of second phase CD4 cellular restorations are shown in Table 2. Age was inversely related to total CD4 cell increases ( $r = -0.26$ ;  $P = 0.07$ ) and this was due largely to an inverse relationship with naive CD4 cell increases ( $r = -0.31$ ;  $P = 0.03$ ) as there was no relationship between age and memory CD4 cell increases. There was a weak inverse relationship between age and increases in CD28 CD4 cells ( $r = -0.23$ ;  $P = 0.11$ ). There was no relationship between sex and any CD4 cellular increases. In contrast with findings for the first phase cellular increases, there

**Table 1.** Baseline predictors of first phase (week 0-4) CD4 cell restoration.

	CD4	Naive CD4	Memory CD4	CD4/CD28
Age (years)	UR	UR	UR	UR
Sex <sup>a</sup>	UR	UR	UR	UR
HIV-1 RNA	$r = 0.35$ $P < 0.01$	UR	$r = 0.38$ $P < 0.001$	$r = 0.29$ $P = 0.01$
CD4/CD38/DR (%)	UR	UR	UR	UR
CD8/CD38/DR (%)	UR	UR	UR	UR
CD4/CD95 (%)	UR	UR	UR	UR
CD8/CD95 (%)	UR	UR	UR	UR

<sup>a</sup>Analyzed using the Wilcoxon rank sum test. UR, Unremarkable ( $-0.2 < r < 0.2$ ;  $P > 0.05$ ).

**Table 2.** Baseline predictors of second phase (week 4–48) CD4 cellular restoration.

	CD4	Naive CD4	Memory CD4	CD4/CD28
Age (years)	$r = -0.26$ $P = 0.07$	$r = -0.31$ $P = 0.03$	UR	$r = -0.23$ $P = 0.11$
Sex <sup>a</sup>	UR	UR	UR	UR
HIV-1 RNA	UR	UR	UR	UR
CD4/CD38/DR (%)	UR	UR	UR	UR
CD8/CD38/DR (%)	UR	UR	UR	UR
CD4/CD95 (%)	UR	UR	UR	UR
CD8/CD95 (%)	UR	UR	UR	UR

<sup>a</sup>Analyzed using the Wilcoxon rank sum test. UR, Unremarkable ( $-0.2 < r < 0.2$ ;  $P > 0.05$ ).

was no relationship between plasma HIV-1 RNA levels and second phase cellular increases. Except for a negative correlation between first phase memory CD4 cell increase and second phase memory CD4 cell increases ( $r = -0.31$ ;  $P = 0.03$ ) there was no relationship between any first phase cellular change and any second phase responses. In addition, neither first phase changes in plasma HIV-1 RNA levels nor activated nor Fas+ CD4 cell nor CD8 cell subsets predicted second phase changes in CD4 cells or CD4 cell subsets (data not shown).

Baseline predictors of 48 week cellular restorations are shown in Table 3. Age was inversely related to 48 week naive CD4 cell increases ( $r = -0.34$ ;  $P = 0.01$ ). Sex was weakly associated with 48 week increases in CD4 cells ( $P = 0.08$ ), memory CD4 cells ( $r = 0.23$ ;  $P = 0.10$ ) and CD28 CD4 cell increases ( $r = 0.23$ ;  $P = 0.12$ ) with women and men experiencing increases of  $80 \times 10^6/1$  CD4 cells,  $53 \times 10^6/1$  and  $88 \times 10^6/1$  memory CD4 cells and  $78 \times 10^6/1$  and  $177 \times 10^6/1$  CD28 CD4 cells respectively. First phase changes in the percentages of activated (CD38/HLA DR) CD4 cells were positively correlated with the 48 week changes in CD4 memory cells ( $r = 0.32$ ;  $P = 0.02$ ); no other first phase changes in cellular phenotype or in plasma HIV-1 RNA level predicted a 48 week CD4 cell change.

## Discussion

Treatment of antiretroviral therapy-naïve patients with a protease inhibitor plus abacavir resulted in sustained increases in circulating CD4 T cells [2,3,16]. A rapid first phase increase of both naive and memory CD4 cells that is largely attributable to redistribution from lymphoid tissue [8,15] was seen within the first few weeks of therapy followed by a slower second phase increase primarily of naive CD4 cells. It is not surprising that both naive and memory cells may be redistributed from lymphoid tissue as the ratio of naive : memory CD4 cells in lymphoid tissue actually exceeds that proportion in peripheral blood [17]. Also observed was a concurrent first phase increase in circulating B lymphocytes while the numbers of circulating cells with natural killer cell phenotype was unaffected as had been reported previously [3]. In contrast with earlier observations among persons with more advanced HIV-1 disease, circulating CD8 cell numbers did not increase significantly during the first phase of cellular restoration and, as had been observed in more advanced disease, the numbers of these cells decreased through the second phase [16]. This decrease was comprised primarily of decreases in memory CD8 cells whereas CD8 cells of a naive phenotype tended to increase progressively throughout the treatment period. Conceivably, CD8 cell trapping in

**Table 3.** Baseline predictors of total (week 0–48) CD4 cellular restoration.

	CD4	Naive CD4	Memory CD4	CD4/CD28
Age (years)	UR	$r = -0.34$ $P = 0.01$	UR	$r = -0.22$ $P = 0.12$
Sex <sup>a</sup>	$P = 0.08$	UR	$P = 0.10$	$P = 0.12$
HIV-1 RNA	$r = 0.26$ $P = 0.07$	UR	$r = 0.26$ $P = 0.07$	UR
CD4/CD38/DR (%)	UR	UR	UR	UR
CD8/CD38/DR (%)	UR	UR	UR	UR
CD4/CD95 (%)	UR	UR	UR	UR
CD8/CD95 (%)	UR	UR	UR	UR

<sup>a</sup>Analyzed using the Wilcoxon rank sum test. UR, Unremarkable ( $-0.2 < r < 0.2$ ;  $P > 0.05$ ).

lymphoid tissues is more pronounced as HIV-1 disease progresses, resulting in a greater magnitude redistribution after viral replication is halted in these subjects. Consistent with this is the observation that circulating CD8 cell numbers tend to be increased in persons with earlier HIV-1 disease yet with more advanced disease, CD8 cell counts tend to fall and this is associated with an accelerated risk of disease progression [18].

Antiretroviral therapy was also associated with an increase in the numbers of CD4 cells expressing CD28, a key co-receptor for T cell activation. In earlier studies, it had been shown that the expression of CD28 on CD4 cells was a predictor of the ability of HIV-1 infected persons to mount an *in vivo* immune response to immunization with both recall and presumed neo- antigens [13]. As CD28 costimulation is required for optimal CD4 T cell responses *in vitro*, increases in the numbers of CD28 co-expressing CD4 cells probably reflects heightened CD4 cell functional capacities as a consequence of suppression of HIV-1 replication.

As had been observed in earlier studies [1–3], decreases in immune activation were seen after suppression of HIV-1 replication with antiviral therapies indicating that in stable and untreated HIV-1 infection, it is HIV-1 replication that largely drives immune activation. Of note, the proportion of CD4 cells that expressed HLA DR+ CD38+ was not significantly affected by treatment. Among a group of 15 healthy control subjects, the mean percentage of CD8 cells that expressed HLA DR and CD38 was 7.4% (range, 2.5–14.4%) and for CD4 cells, the mean percentage of HLA DR and CD38 cells was 10.0% (range, 3.9–15.9%; data not shown). Thus in both CD4 and CD8 cells, levels of immune activation after 48 weeks of therapy remained abnormally elevated. Whether the persistent immune activation seen after suppression of HIV-1 replication [19–21] is a reflection of lower level but persistent HIV-1 replication or continued dysregulated immune activation in HIV-1 disease or a combination of the two phenomena remains to be determined. In this regard, 40 out of 50 patients (80%) who remained on study at 48 weeks and who comprised the population for this report had plasma HIV-1 RNA levels < 50 copies/ml.

When baseline predictors of cellular restoration were explored, it was found that age, baseline plasma HIV-1 RNA levels and to a lesser and non-significant extent, sex, predicted the magnitude of cellular restoration. As we and other investigators have reported earlier, baseline plasma HIV-1 RNA levels were predictive of the magnitude of CD4 cell increases after initiation of suppressive antiretroviral therapies [3,17,22,23]. In this study, we demonstrate that plasma HIV-1 RNA levels

predict the magnitude of first phase increases in total CD4 cells, memory CD4 cells and CD28 CD4 cells but not naive CD4 cells and that there is no relationship between plasma HIV-1 RNA levels and second phase cellular increases. In contrast, Renaud *et al.* had reported a correlation between the change in plasma HIV-1 RNA levels and the magnitude of second phase (month 2–24) CD4 cell restoration [23] and Connick *et al.* found a correlation between the magnitude of first year CD4 cell increases and changes in plasma HIV-1 RNA levels [16]. This is probably a consequence of the incomplete control of HIV-1 replication seen in those studies where predominantly treatment-experienced populations with median baseline plasma HIV-1 RNA levels of > 10<sup>5</sup> and 10<sup>4.9</sup> copies/ml respectively had median decreases of approximately 1.6 and 1.9 log<sub>10</sub> respectively. Thus in those studies, a relationship between virus-induced cell losses and immune homeostasis during the second phase of restoration may underly this relationship. In contrast, 80% of the patients reported in this study had plasma HIV-1 RNA levels < 50 copies/ml at week 48; thus the second phase increases in our study performed among treatment-naive patients is likely less affected by continued detectable HIV-1 replication and its effects on cellular homeostasis. Thus we propose that higher baseline levels of HIV-1 replication induce greater cellular trapping [8] and greater lymphocyte death [15] in lymphoid tissue. Since the bulk of the first year CD4 cell increase is seen during the first phase of cellular restoration, the relationship between HIV-1 replication and cellular increases is largely dependent upon the effect of suppression of HIV-1 replication and cellular redistribution. It is important to note that the magnitude of the progressive increase in naive CD4 cells is not related to plasma HIV-1 RNA levels and this suggests, but does not prove, that the magnitude of thymic output may not be as affected directly by HIV-1 replication as is the magnitude of cellular trapping and redistribution after suppression of HIV-1 replication.

This study is the first to demonstrate that age inversely predicts the magnitude of naive CD4 cell increases after application of suppressive antiviral therapies. This relationship is demonstrable for the second phase of cellular increase, for the total 48 week period of study but not for the first phase. As the greater first phase naive CD4 cell increase (Fig. 2) is probably in part a consequence of redistribution from lymphoid tissue, we suspect that this contribution obscures the relationship between age, thymic output and naive CD4 cell increases. Renaud *et al.* reported a marginally significant inverse relationship between age and early CD4 cell restoration [23]; naive cells were not enumerated in that study that involved as many as 317 patients. Conceivably, younger patients with larger thymuses may produce and trap more naive CD4 cells during

sustained HIV-1 replication and redistribution of these cells during the first phase of cellular restoration may underlie the tendency to greater CD4 cell increases seen in younger patients in that study. In this regard, we have recently reported greater first phase naive CD4 cell restoration among persons with larger thymuses treated with combination antiretroviral therapy [10]. Thymus involution with age is well-recognized [24] and modest immune impairments with age may be attributable at least in part to failure of thymic output [9]. Age is also associated with a poorer survival in persons with HIV-1 infection [25–27] and although this may be related to failure of thymic productivity, this remains to be demonstrated. In this regard older age is associated with a decreased proportion of circulating T cells containing excision circles found as a result of T cell receptor rearrangements during T cell development [28]. Although these analyses must be considered exploratory, age also was inversely correlated with baseline CD8 naive T cell numbers ( $r = -0.25$ ;  $P = 0.04$ ) and the magnitude of the 48 week naive CD8 cell increase ( $r = -0.32$ ;  $P = 0.03$ ) suggesting the importance of age in thymic function and naive cell restoration after suppression of HIV-1 replication.

Women tended to have a diminished CD4 cell response and a diminished memory CD4 cell increase over 48 weeks of therapy although the difference between men and women in the magnitude of these responses was not significant. Nonetheless, additional studies in larger populations are needed to ascertain if sex differences determine the outcome of HIV-1 infection both before and after antiretroviral therapies as some earlier studies have found differences in plasma HIV-1 RNA levels, CD4 cell counts, disease course and durability of viral suppression after administration of HAART among HIV-1-infected women [29–32].

In summary, treatment of HIV-1-infected persons with abacavir and inhibitors of the HIV-1 protease resulted in a pattern of phenotypic cellular restoration that is similar to the pattern observed among persons receiving treatment with three antiretroviral drugs. A biphasic increase in CD4 T cells was observed that comprised all subsets in the first phase and primarily naive cells during the second phase. In addition, there were increases in the numbers of CD4 cells that expressed the co-receptor for T cell activation – CD28. Baseline plasma HIV-1 RNA levels predicted the magnitude of first phase cellular restoration suggesting that the magnitude of HIV-1 replication determines the degree of cellular trapping. Older age predicted a smaller naive cell restoration during the second phase of cellular restoration suggesting that thymic reserve may determine the magnitude of new cell production and restoration after HIV-1 replication is suppressed.

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