T cell turnover and thymic function in HIV-1 infection
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Citation for published version (APA):
Hazenberg, M. D. (2002). T cell turnover and thymic function in HIV-1 infection
Discordant responses during antiretroviral therapy: role for immune activation and T cell redistribution rather than true CD4 T cell loss

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Chapter 4

Abstract

We studied T cell dynamics in four patients who initially responded well to highly active antiretroviral therapy (HAART) but subsequently experienced virological failure. Maintenance of peripheral blood CD4+ T cell counts was associated with low levels of immune activation. Low reactivity to rebounding virus may preserve normal T lymphocyte distribution over blood and tissues and be associated with stable peripheral blood T cell numbers in virological failures to HAART.
Discordant responses during HAART

A proportion of HIV-1 infected individuals that receive highly active anti-retroviral therapy (HAART) fails to completely suppress the virus, or goes through one or more periods of virus relapse after initial adequate control. Virus rebound is usually associated with a decline in peripheral blood CD4+ T cell numbers, however, a subset of patients was found to maintain peripheral blood CD4+ T cell counts or even experienced an increase in CD4+ T cell numbers in the blood, despite high levels of plasma HIV-1 RNA (1-4). This may be attributable to protease inhibitors (PIs), that were reported to reduce susceptibility of CD4+ T cells to apoptosis (5,6) or that may interfere with cell cycle progression and thereby enhance T cell survival (7,8). Alternatively, PI and / or reverse transcriptase (RT) -resistant virus strains may be less cytopathic to mature or immature (intrathymic) CD4+ T cells (9,10).

Alternative mechanisms remain to be explored, such as the role of immune activation and T cell redistribution. Here, we studied the association between plasma HIV-1 RNA levels, naive, memory and effector CD4+ and CD8+ peripheral blood T cell numbers and T cell activation longitudinally in patients who initially adequately suppressed the virus, but subsequently experienced virological failure despite continued HAART. We selected participants of the Amsterdam Cohort Studies on HIV-1 Infection and AIDS of whom sequential cryopreserved peripheral blood mononuclear cells (PBMC) were available before and during HAART, and who initially experienced sustained virus suppression (plasma HIV-1 RNA less than 400 copies/ml for more than six months) but subsequently developed virological failure (plasma HIV-1 RNA ≥ 1 log increase) for at least six months. Only four patients were identified that complied with these strict criteria as in most virological failures treatment is adjusted within six months and the increase in plasma HIV-1 RNA less pronounced. Selected individuals were male and median age at initiation of this study was 31.5 years (range: 22.8 - 46 years). All patients were HIV-1 seropositive when they entered the cohort; follow up was between 40 and 70 months. Details of the HAART regimens are indicated in Figure 1. T cell division was assessed by measuring the expression of Ki67 (Immunotech, Marseille, France), a protein that is expressed exclusively by cells that are in cell cycle, and naive (CD27+ CD45RO-), CD27+ memory (CD27+ CD45RO+), CD27+ memory (CD27+ CD45RO+), and effector (CD27+ CD45RO+). CD4+ and CD8+ T cells were defined as described previously (11). Plasma HIV RNA was assessed using Roche Amplicor Monitor Standard Assay or Ultra Monitor Assay (Roche Diagnostics, Branchburg, NJ), NucliSens HIV-1 QT assay and NASBA HIV-1 RNA QT (Organon Teknika, Boxtel, The Netherlands), or Quantiplex HIV-1 RNA 3.0 bDNA monitor assay (Chiron Corp., Emeryville, CA).

Individual data are depicted in Figure 1a-d. All patients initially had high plasma HIV-1 RNA concentrations that rapidly reached less than 400 copies/ml when HAART was initiated. CD4+ T cell numbers increased and Ki67 expression declined in this period. During subsequent virological failure, two patterns could be
distinguished. In patients 6181 and 6156, high plasma HIV-1 RNA levels were associated with increased proportions of Ki67\(^+\) CD4\(^+\) T cells and a decline in CD4\(^+\) T cell numbers (Figure 1a-b), whereas in patients 3558 and 8324, virus rebound was associated with relatively low proportions of Ki67\(^+\) CD4\(^+\) T cells and stable CD4\(^+\) T cell numbers (Figure 1c-d). We plotted the fraction of Ki67\(^+\) CD4\(^+\) T cells measured at three time points (before initiation of HAART, during virus suppression, and during virus rebound) of the four patients and compared these with the proportion of Ki67\(^+\) CD4\(^+\) T cells of a group of untreated HIV-1 infected individuals and a group of healthy lab donors (11) (Figure 1e). The proportions of dividing CD4\(^+\) T cells of patients 3558 and 8324 that were relatively low before initiation of HAART remained low during virologic failure, whereas patients 6156 and 6181 rapidly rebounded to high levels of CD4\(^+\) Ki67 expression. The proportion of Ki67\(^+\) CD4\(^+\) T cells was associated with the proportion of Ki67\(^+\) CD8\(^+\) T cells for each individual at all time points (Figure 1f). In all patients, Ki67 expression of naive, memory and effector T cell subsets followed patterns that were parallel to each other (data not shown).

HIV-1 infection characteristically leads to hyperactivation of the immune system, reflected in upregulated cytokine levels and lymphocyte homing receptors. This has been shown to increase the proportion of T cells migrating to lymphoid tissues (12). In the first weeks following initiation of HAART, when plasma HIV-1 RNA is significantly reduced, immune hyperactivation diminishes and previously sequestered lymphocytes are rapidly released into the circulation leading to a normalised distribution of lymphocytes between blood and tissues (13).

We observed relatively rapid changes in peripheral blood CD4\(^+\) T cell numbers involving both naive and memory T cells following treatment failure which reversed quickly during subsequent successful adjustment of treatment. The decline in peripheral blood CD4\(^+\) T cell numbers in patients that responded to rebounding virus with high proportions of Ki67\(^+\) dividing T cells may be related to increased activation-induced sequestration of T cells in lymphoid tissues. Interestingly, maintenance of peripheral blood CD4\(^+\) T cell numbers despite virological failure was associated with limited immune activation, which may result in low activation-induced T cell sequestration to lymphoid tissues.

Peripheral blood CD4\(^+\) T cell counts are commonly used to evaluate HIV disease progression. It has, however, been shown that in untreated HIV-1 infection the decline in peripheral blood CD4\(^+\) T cell numbers may overestimate true CD4\(^+\) T cell loss, as depletion is less pronounced in lymphoid tissue (14). Our data suggest that this may also be the case during virological failure to HAART. In fact, these data and those from other laboratories (15) indicate that the transient reduction in peripheral blood CD4\(^+\) T cell numbers during structured treatment interruptions (STI) in chronically HIV-1 infected patients may be caused by immune activation and increased tissue sequestration, rather than to a true loss of T cells.
Discordant responses during HAART

Figure 1. (a-d) Plasma HIV-1 RNA, CD4+ T cell numbers and the proportion of Ki67+ CD4+ T cells of four patients during untreated HIV-1 infection, during HAART and during subsequent virological failure. Solid lines: number of CD4+ T cells (per µl blood); dashed lines: plasma HIV-1 RNA (copies per ml); vertical grey bars: Ki67+ CD4+ T cells (%); horizontal grey bars: period during which each patient was treated with HAART. Characters represent drugs: Z zidovudine, L lamivudine, SA saquinavir, N nelfinavir, D didanosine, ST stavudine, I indinavir, R ritonavir. (e) Rebound of immune activation during virological failure to pre-treatment levels. Depicted are the proportions of Ki67+ CD4+ T cells before initiation of HAART (pre-HAART), during successful virus suppression (HAART) and during virus rebound (VF) of patients 6181 (circles), 6156 (squares), 3558 (triangles) and patient 8324 (diamonds). For comparison, median proportions of Ki67+ CD4+ T cells of a group of untreated HIV-1 infected individuals (grey bar, n = 16) and a group of healthy donors (white bar, n = 5) are shown (11). (f) Correlation between activation of CD4+ and CD8+ T cells. For each patient the proportion of Ki67+ CD4+ T cells is plotted against the proportion of Ki67+ CD8+ T cells at all time points. Symbols are similar to those in (a).