Reconstitution of naive T cells during antiretroviral treatment of HIV-infected adults is dependent on age

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Objective: To determine the influence of age on the regeneration rate of naive and memory T cells in the blood of 45 adults on highly active antiretroviral therapy (HAART).

Methods: The age of the patients ranged from 25 to 57 years. Naive cells were defined as CD45RA+CD27+. Cells negative for CD45RA and/or CD27 were considered memory type cells.

Results: The recovery rates of naive CD4 and CD8 T cells were similar, were negatively correlated with age and were decreasing 5% and 3.6% per year, respectively. In a multivariate regression analysis, only age was significantly correlated with the naive T cell recovery rates. The recovery rate of memory T cells showed no relation to age. The average regeneration rate of naive CD4 T cells during HAART, i.e., 0.34×10^6 cells/l per day, is not lower than regeneration rates in HIV-negative adults following cytotoxic chemotherapy or CD4 monoclonal antibody therapy.

Conclusion: These observations suggest that the thymus contributes considerably to the regeneration of naive T cells in adults on HAART, and that the impact of HIV infection on naive T cell production is small, or rapidly reversible.

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Introduction

Several papers attribute the regeneration of the naive T cells during highly active antiretroviral therapy (HAART) to thymus-dependent production [1–8]. Because of thymic involution with increasing age [9,10], one line of evidence in the literature is that the regeneration rates of total T cell numbers [6,7] and of naive T cell numbers [5] would diminish with age in human adults on HAART. The present study confirms the latter result [5] by finding similar correlations and extends the evidence by fitting the data to the classical exponential function describing the loss of functional thymus tissue in human adults [9].

Methods

The recovery of naive and memory T cells was analysed in all patients from the previously described CHEESE study cohort [11] who had a sustained plasma HIV RNA response to < 50 copies/ml ($n = 45$) until week 48 of the study. Briefly, this is a randomized study comparing antiviral efficacy of zidovudine plus lamivudine plus saquinavir versus zidovudine plus lamivudine plus indinavir. During 48 weeks of treatment, the virological response and the CD4 cell count response did not differ between the two treatment arms. Of the selected patients, 23 were from the indinavir arm and 22 from the saquinavir arm. At baseline, the 45 patients

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from the CHEESE study included in this analysis had CD4 cell counts ($P = 0.7$) and HIV RNA plasma levels ($P = 0.22$) that did not differ from those of the patients excluded from this analysis.

Blood samples were obtained at week −2, at week 0 and every 4 weeks to week 24 and then every 8 weeks from week 24 to week 48 of treatment. Plasma HIV RNA levels were measured using an investigational version of the ultrasensitive quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay (Amplicor HIV-1 Monitor, Roche Diagnostic Systems, Branchburg, New Jersey, USA). The lower limit of detection was 50 copies/ml. Lymphocyte immunophenotyping was performed as previously described [11]. T cells expressing both CD45RA and CD27 were regarded as naive cells. Cells that lacked CD45RA and/or CD27 were designated memory cells [12,13].

Results

Because immune recovery during HAART is dependent on virus suppression [14], the reconstitution of T cells during 48 weeks of therapy was investigated in a group of patients who all achieved plasma HIV RNA levels < 50 copies/ml (Fig. 1). The mean age of the patients at baseline was 37.2 years (range, 25–57 years). The median plasma HIV RNA level at baseline was 72 000 copies/ml. The patients achieved plasma HIV RNA levels < 50 copies/ml within a median period of 16 weeks (range, 4–44). For each patient, the recovery rate of naive and memory T cells in the blood during the initial 48 weeks of HAART was estimated by linear regression. The mean (±SEM) recovery rates of naive CD4 and CD8 T cells were 0.34 ± 0.04 and $0.36 \pm 0.04 \times 10^6$ cells/l per day, respectively. The recovery rates of naive CD4 and naive CD8 T cells were not significantly different (paired samples t test, $P = 0.5$). The recovery of memory CD4 T cells was biphasic, with a rapid recovery rate in the first 4 weeks of therapy ($1.46 \pm 0.4 \times 10^6$ cells/l per day) and a slow recovery rate between week 4 and week 48 ($0.17 \pm 0.06 \times 10^6$ cells/l per day), consistent with previous findings [15]. No differences of recovery rates were observed between the two treatment groups (data not shown).

Because thymic function diminishes with increasing age, the influence of age on the rate of naive T cell regeneration was examined (Fig. 2). The recovery rates of the naive CD4 and CD8 T cells correlated negatively with the age of the patients ($r = -0.41$, $P = 0.005$ and $r = -0.47$, $P = 0.001$, respectively), and were correlated with one another ($r = 0.7$, $P < 0.001$; not shown). The decrease of the recovery rates with increasing age fitted best to an exponential function with estimated decrease of the recovery rate of 5% per year for the naive CD4 T cells and 3.6% per year for naive CD8 T cells. The recovery rate of memory CD4 T cells was not correlated with age ($r = -0.07$, $P = 0.6$), nor was the daily decrease of memory CD8 T cells ($r = 0.1$, $P = 0.2$). Therefore, the results indicate that the age of patients is an important determinant for the naive T cell regeneration rate.

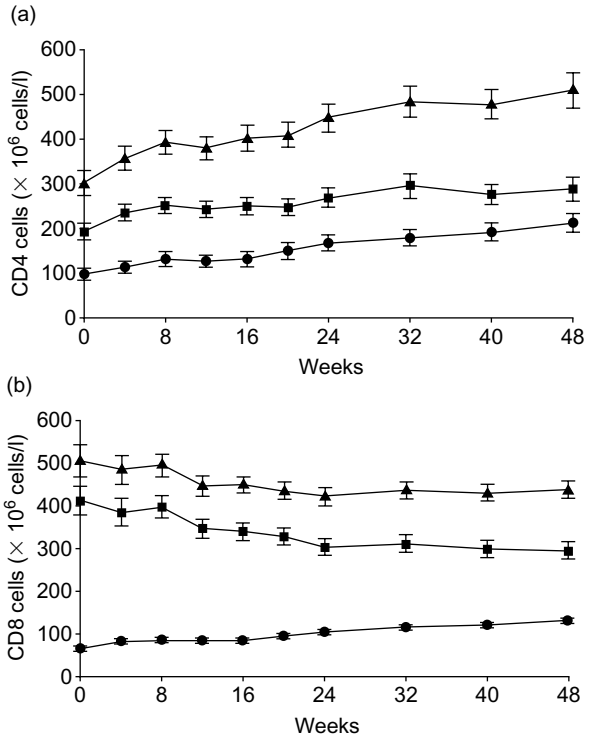

Fig. 1. Reconstitution of absolute number of circulating (a) CD4 and (b) CD8 T cells during 48 weeks of highly active antiretroviral therapy: total count (triangles), memory cells (squares) and naive cells (circles). Bars indicate standard error of the mean.

To investigate whether the regeneration rate of naive T cells depends on the number of pre-existent naive T cells, the relation between pretreatment counts of naive CD4 and CD8 T cells and the recovery rates of the respective T cell subsets was determined. No significant correlations were observed between naive T cell baseline counts and recovery rates ($r = 0.2$, $P = 0.2$, and $r = -0.1$, $P = 0.4$, respectively). This seems in contrast with previous studies [1,16] reporting a positive correlation between baseline naive CD4 T cell counts and the increase of naive CD4 T cells. However, such positive correlations may be spurious (false positive) as a result of a regression-to-the-mean effect, because the naive baseline T cell count (N_0) appears in both correlated variables [i.e., in N_0 itself, and in the increase ($N_t - N_0$) of naive T cells with time t].

To determine whether age is an independent predictor of the naive T cell regeneration rate, a multivariate regression analysis was performed using the regeneration rate of naive CD4 T cells as the dependent variable. Indepen-

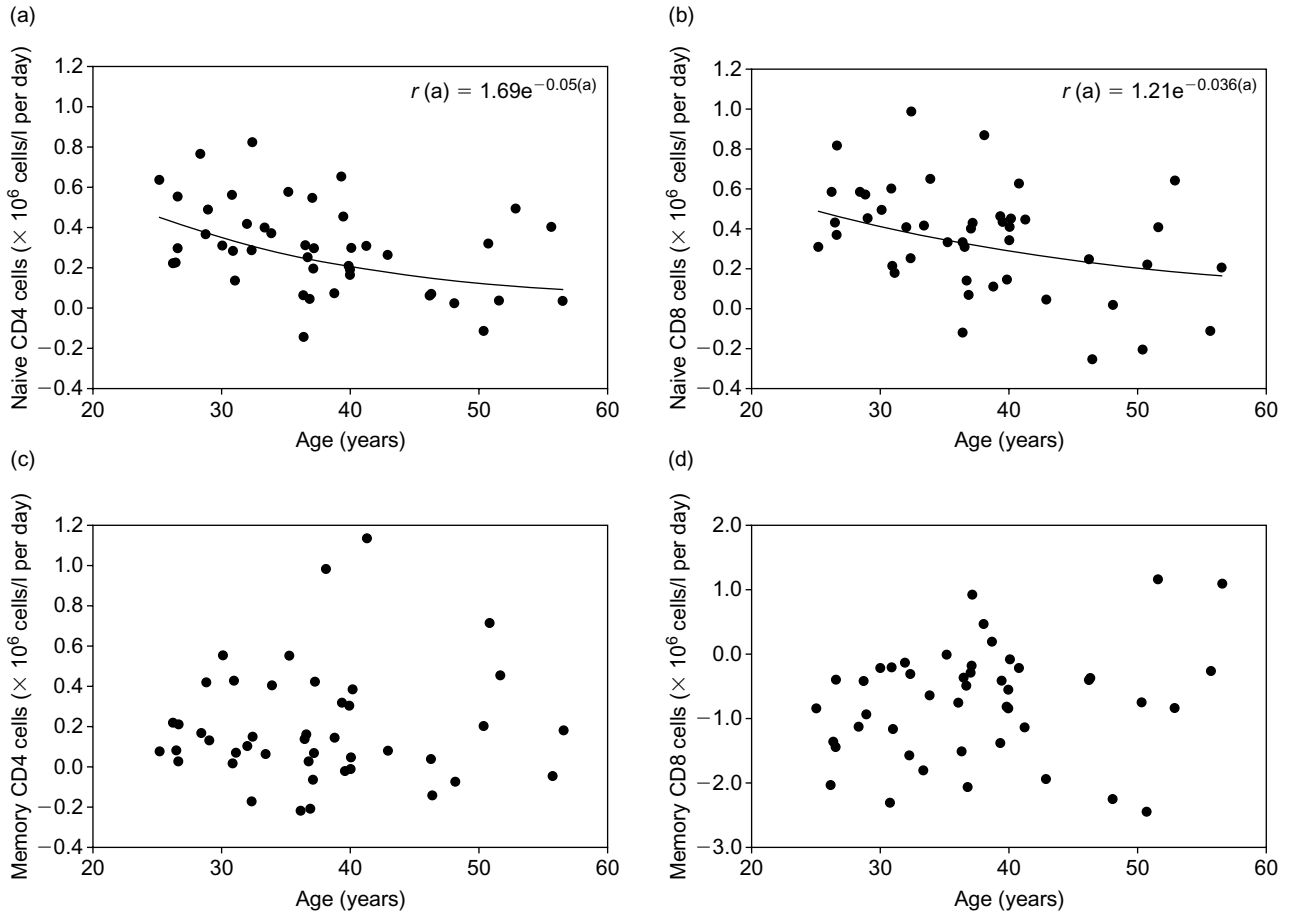

Fig. 2. Age and the recovery rates of T cell subsets: (a) naive CD4 T cells ($r = -0.41$, $P = 0.005$), (b) naive CD8 T cells ($r = -0.47$, $P = 0.001$), (c) memory CD4 T cells ($r = -0.07$, $P = 0.6$) and (d) memory CD8 T cells ($r = 0.1$, $P = 0.2$). The decrease of the recovery rate of naive T cells with increasing age was fitted to an exponential function: $R(a) = p_1 e^{p2(a)}$, where $R(a)$ is the recovery rate and a is the age. For naive CD4 T cells, $p_2 = 0.05 \pm 0.016$, corresponding to a decay rate of 5.0% per year. For naive CD8 T cells, $p_2 = 0.036 \pm 0.016$, corresponding to a decay rate of 3.6% per year.

dent variables (covariates) in the model were age, baseline CD4 count, baseline viral load, and baseline naive CD4 T cells. Only age had a significant (inverse) correlation with the naive CD4 T cell regeneration rate in this multivariate analysis ($P = 0.015$). The P values of the correlations with the other covariates were larger than 0.21. A similar multivariate analysis for the naive CD8 T cells revealed that age was also significantly (inversely) correlated with their regeneration rate ($P = 0.001$).

Discussion

The negative correlations between naive T cell recovery and age are very similar to correlations reported earlier [5]. Although an influence of age on thymus-independent pathways cannot be excluded, the most plausible explanation for these inverse correlations in both studies is that the thymus-dependent pathway plays an important role in naive T cell regeneration, because thymic function is known to diminish with age [9,10]. A caveat to be considered for both studies is that older patients may also have had the longest duration of HIV infection and hence may have accumulated more damaged thymic tissue. However, both in our study and in the Lederman *et al.* study [5], laboratory markers of duration of infection, baseline CD4 cell count and plasma HIV RNA levels, did not correlate with either age or the recovery rates of naive T cells (not shown). The duration of infection is, therefore, not likely to be a confounding factor.

In another study, it was reported that the number of circulating naive (CD45RA+CD62L+) CD4 T cells increased during HAART in a thymectomized patient [17]. However, this increase was exclusively observed in the first 12 weeks of HAART and the number remained constant thereafter. The initial rise of naive CD4 T cells in the thymectomized patient [17] could, therefore, be explained by a redistribution of naive T cells from (lymphoid) tissue to blood, which typically occurs in the first weeks of HAART [5,15].

We extend the Lederman *et al.* results [5] by showing

that the regeneration rates of naive CD4 and CD8 T cells decrease 5.0% and 3.6% per year for naive CD4 and CD8 T cells, respectively (Fig. 2). Because the volume of the thymic epithelial space in adults above 20 years of age decreases by 4.1% per year [9], this strongly suggests that the recovery of naive T cells in adult patients on HAART is limited by the amount of thymic parenchyma [4]. The fact that we found a positive correlation between the CD4 and CD8 naive T cell recovery rates is also in agreement with a common dependence on thymic production.

The mean rate of increase of the naive CD45RA+ C27+CD4 T cells in this study (i.e., 0.34×10^6 cells/l per day), and that of the CD45RA+CD62L+CD4 in the Lederman *et al*. study [5] (i.e., 0.17×10^6 cells/l per day) is fairly similar. The same is true for naive CD8 T cells, where these numbers were 0.36×10^6 and 0.15×10^6 cells/l per day, respectively. Interestingly, these rates of increase of naive CD4 T cells in our patients are not lower than the regeneration rates observed in HIV-seronegative adults following iatrogenic CD4 T cell depletion by either antineoplastic chemotherapy or treatment for rheumatoid arthritis with CD4 monoclonal antibodies [18–22]. After iatrogenic T cell depletion, the regeneration rates of naive CD4 T cells varied from 0.005 to 1.1×10^6 cells/l per day [22]. Part of the variation is probably a consequence of differences in the phenotypic definition of the naive T cells in these earlier studies. Since we have defined naive T cells as CD45RA+CD27+ cells, we have been more stringent than the previous studies, but nevertheless find estimates that are at least as high as those after iatrogenic T cell depletion. Taken together, our data suggest that the HIV-induced dysfunction of progenitor cells or thymus is either of minor importance or reversed after introduction of HAART.

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