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# Rapid CD4$^+$ T-cell turnover in HIV-1 infection: a paradigm revisited

**Katja C. Wolthers, Hanneke Schuitemaker and Frank Miedema**

*Although progression to AIDS has generally been believed to be related to exhaustion of the capacity for CD4$^+$ T-cell renewal due to persistently enhanced CD4$^+$ T-cell turnover, this view is now increasingly being challenged. Here, Katja Wolthers, Hanneke Schuitemaker and Frank Miedema discuss these new experimental findings and propose alternative explanations for CD4$^+$ T-cell depletion in human immunodeficiency virus 1 infection.*

Over the past ten years, many pieces of information have gradually changed the picture of human immunodeficiency virus 1 (HIV-1) pathogenesis. Originally, it was believed that the virus was dormant during clinical latency, despite the well-established effects on immune function and immune activation that occur even before CD4$^+$ T cells are depleted[1]. With the development of polymerase chain reaction (PCR)-based amplification techniques and the discovery of high amounts of virus in the lymphoid tissues, it was realized that there might be more virus in the body than was previously thought[2–6]. However, the first insight into the magnitude of viral replication dynamics was provided by studies from Ho *et al.*[7] and Wei *et al.*[8] showing the daily virus production to be in the order of $10^{10}$. In addition, virus half-life was determined to be 6 h in a follow-up study by Perelson and colleagues[9].

Viral load correlates with CD4$^+$ T-cell decline and disease progression[10,11]; however, the basis for this decline, one of the main features of HIV-1 infection, is still not completely understood. The rate of CD4$^+$ T-cell decline is slow and relatively constant in asymptomatic HIV-infected individuals, with a more rapid decline related to the emergence of syncytium-inducing (SI) variants[12,13]. High virus replication may have considerable impact on CD4$^+$ T-cell turnover. In the seminal studies from Ho *et al.*[7] and Wei *et al.*[8], CD4$^+$ T-cell dynamics were calculated from changes in CD4$^+$ T-cell counts after therapy with potent antiretroviral drugs. A steep increase in CD4$^+$ T-cell counts was observed in many patients in the first 30 days following start of treatment. It was assumed that these were newly produced CD4$^+$ T cells that were now spared from destruction by HIV-1 because of the interference with virion maturation by the antiretroviral treatment. By assuming a pre-treatment steady state of CD4$^+$ T-cell counts, analogous to the viral kinetics, and assuming the blood lymphocyte pool to be about 2% of the total population, it was calculated that, before treatment, $2 \times 10^9$ CD4$^+$ T cells were produced and destroyed every day. Thus, CD4$^+$ T-cell production was believed to be highly stressed in many patients, even up to 78-fold. It was argued that this high production rate of CD4$^+$ T cells by the hematopoietic system was not infinite and would eventually be exhausted, causing rapid depletion of CD4$^+$ T cells and progression to AIDS. Thus, the high rate of destruction of CD4$^+$ T cells was the principal driving force of HIV-1 pathogenesis. For this, the metaphor of a sink was introduced[7], with the tap (CD4$^+$ T-cell renewal) and drain (CD4$^+$ T-cell destruction) equally wide open, eventually exhausting the flow of the tap.

These conclusions have been further underscored by the finding that the half-life of productively HIV-infected CD4$^+$ T cells is in the order of two days[9], which is compatible with a high turnover rate of CD4$^+$ T cells in HIV-1 infection. However, this view of high CD4$^+$ T-cell turnover leading to exhaustion is challenged by results from studies on (1) CD4$^+$ T-cell telomere length, (2) quantification in lymphoid tissues of productively HIV-infected cells and (3) human CD4$^+$ T-cell repopulation kinetics. Here, these findings are discussed in relation to T-cell turnover and CD4$^+$ T-cell depletion in HIV-1 infection.



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## T-cell turnover measured by the loss of telomere length

If $CD4^+$ T cells have a rapid turnover and therefore a high replication rate during HIV-1 infection, this could be reflected in accelerated shortening of $CD4^+$ T-cell telomere restriction fragment (TRF) length. Telomeres are the extreme ends of chromosomes and comprise TTAGGG repeats covering approximately 10 kb in length in humans[14–17]. Several findings have led to the suggestion that telomere length can be used as a measure of replicative history of cells[18,19] and may show accelerated ageing or increased cell replication rates. First, telomeres of somatic cells shorten with age (about 30–50 bp loss per year) and after culture *in vitro*[19–21]. Second, telomere length is predictive for replicative capacity in cultured lymphocytes and fibroblasts[18]. Third, telomere length is maintained in continuously dividing immortalized cells *in vitro*, as well as in tumor cells and germline cells, by the enzyme telomerase; these cell types show high telomerase activity while somatic cells show little or no telomerase activity[22–25].

In a study by Effros *et al.*[26], a substantially decreased TRF length in the expanded population of $CD8^+CD28^-$ T cells from HIV-infected individuals was reported. They concluded that the shorter mean TRF length in the $CD8^+CD28^-$ population was due to additional cell division, and that replicative senescence could contribute to exhaustion of the T-cell response in HIV-1 infection. The change in TRF length of $CD4^+$ and $CD8^+$ T cells from HIV-infected patients has since been compared with that of healthy controls over a follow-up time of 3–9 years[27]. These data confirmed the previous findings by showing an accelerated shortening of telomeres in the total $CD8^+$ T-cell population from most HIV-infected individuals. However, comparison of $CD4^+$ and $CD8^+$ TRF length revealed reduced $CD8^+$ TRF length but normal $CD4^+$ TRF length. Furthermore, analysis of sequential lymphocyte samples showed no accelerated loss of TRF length in $CD4^+$ T cells during the period of HIV-1 infection prior to AIDS diagnosis. Other groups have also not observed any shortening of TRF length of $CD4^+$ T cells from HIV-infected individuals[26,28]. Moreover, in a recent study with HIV-discordant pairs of monozygotic twins, normal or increased $CD4^+$ T-cell TRF length was found while $CD8^+$ T-cell TRF length was decreased in the HIV-positive individual compared with the HIV-negative twin[29]. Thus, $CD4^+$ T-cell TRF length is not decreased in HIV-1 infection. Is this in contradiction with a rapid turnover of $CD4^+$ T cells in HIV-1 infection?

## Alternative explanations

There are several reasons why some caution in the analysis of such data may be appropriate. First, increased telomerase expression might compensate for telomere shortening in $CD4^+$ T cells that are rapidly dividing. However, stable TRF length in $CD4^+$ T cells from HIV-infected individuals does not appear to be associated with high telomerase activity[27]. Furthermore, in HIV-discordant twins, no difference in telomerase activity could be detected in freshly isolated or *in vitro*-stimulated $CD4^+$ T cells from the HIV-infected twin compared with the HIV-negative twin[29]. Thus, potential loss of $CD4^+$ T-cell TRF length with high cell replication is not masked by elevated telomerase activity in these cells.

Second, it has been argued that accelerated telomere loss in $CD4^+$ T cells can only be measured if the cells that have undergone extensive cell division are present in the actual $CD4^+$ T-cell population. In HIV-1 infection, the half-life of productively infected $CD4^+$ T cells, mainly memory cells[30], is in the order of two days[9] and thus these cells are rapidly lost from the population. However, in normal ageing, cell division and cell loss occur at a rate such that homeostasis is maintained, and the TRF length of the total population shortens. This process of ageing is shown in Fig. 1, where three cell compartments are indicated. Naive and memory $CD4^+$ T-cell TRF length shows a remarkably stable difference over time, although both subsets lose TRF length with ageing, as shown by Weng and colleagues[31]. Progenitor cells presumably have higher TRF length than do naive cells but also lose TRF length during ageing[32]. If the memory and naive compartment are somehow linked, a major destruction of $CD4^+$ memory T cells will lead to compensation through enhanced division proximal in the $CD4^+$ life cycle, resulting in a major flux of naive and memory cells; this has also been suggested by the 'tap-and-drain model' proposed by Ho and colleagues[7]. Thus, even if memory cells are lost from the population by killing, and relatively more cells with longer telomeres remain, the remaining population will eventually lose TRF length because cell renewal continues. Analogous to Fig. 1, this 'accelerated ageing' results in an accelerated loss of TRF length throughout the whole $CD4^+$ T-cell population. Thus, according to this model, exhaustion of the renewal capacity is not likely to occur without loss of TRF length in the remaining population.

A more likely possibility is that memory and naive cells have independently regulated homeostasis[33]. The picture now becomes more complex, and additional data regarding TRF length in naive and memory $CD4^+$ T cells from HIV-infected individuals are needed. Recent work shows that no increased loss of TRF length is observed in these cell populations. In addition, these studies suggest that, if the cell production is increased more than twofold, telomere loss is expected, based on a mathematical model of the relationship between telomere length and population dynamics (K.C. Wolthers *et al.*, unpublished). Therefore, normal loss of $CD4^+$ T-cell TRF length during HIV-1 infection probably points towards normal to only slightly increased $CD4^+$ T-cell turnover.

Finally, another explanation for stable TRF length in $CD4^+$ T cells would be that the loss of $CD4^+$ T cells can be compensated for by a small subset of dividing cells such that the overall $CD4^+$ T-cell turnover is not affected. In this case, exhaustion of cell renewal will not occur and $CD4^+$ T-cell decline is not explained. Likewise, if telomeres are maintained in a precursor population by high telomerase activity or an as-yet-unknown alternative mechanism of telomere maintenance, exhaustion of $CD4^+$ T-cell renewal seems unlikely. In these cases, there might be high $CD4^+$ T-cell turnover without loss in TRF length but it is not likely to be the driving force in AIDS pathogenesis.

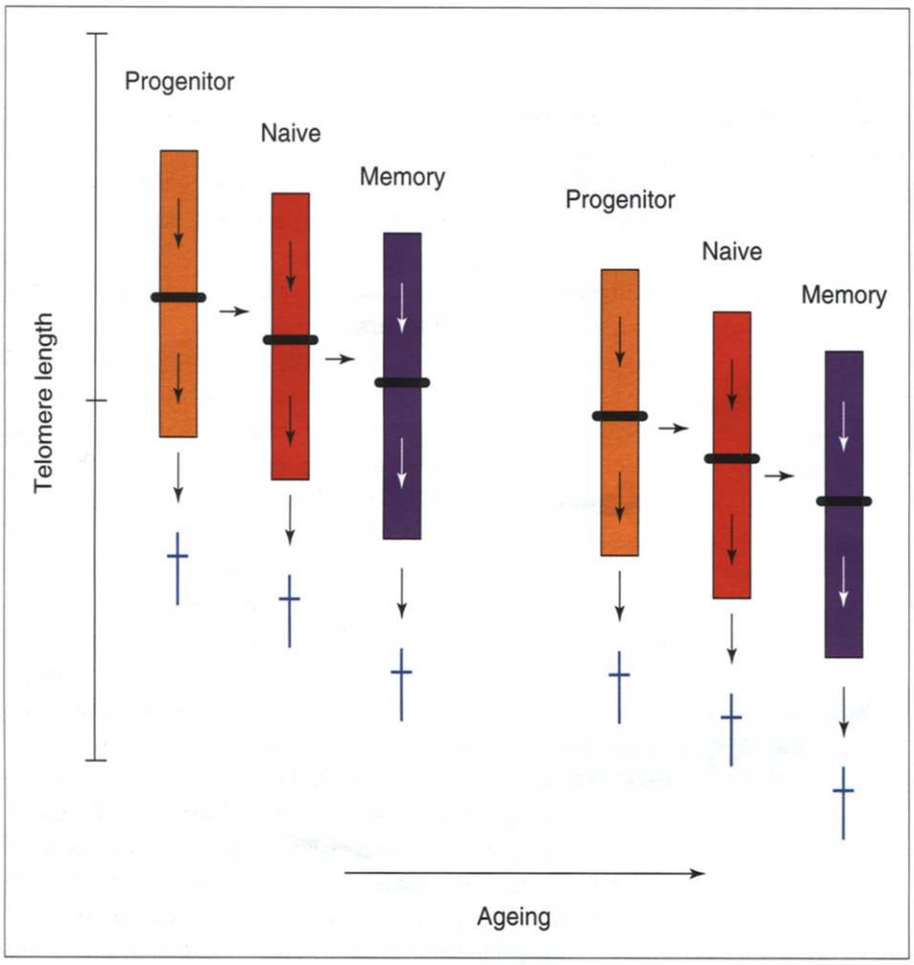


**Fig. 1.** *Diagram showing the postulated development of changes in telomere length during normal ageing where homeostasis is maintained. Rectangles represent a subset of CD4⁺ T cells formed by different generations of cells with different telomere length. Mean telomere lengths are indicated by the black bars. TRF lengths of naive (red rectangles) and memory (purple rectangles) cells differ by 1.4 kb (Ref. 31) and, presumably, progenitor cells (orange rectangles) have higher TRF lengths than do naive cells. Cells lose TRF length because they divide at a certain rate (vertical arrows) and differentiate to the next subset (horizontal arrows). Homeostasis is kept by cell division and cell death (blue crosses). With ageing, all compartments lose TRF length (right panel). If cell loss induced by HIV-1 leads to increased proliferation in all compartments, mean TRF length should decrease at an accelerated rate in all compartments (i.e. 'accelerated ageing' would occur). Abbreviations: HIV-1, human immunodeficiency virus 1; TRF, telomere restriction fragment.*

## New insights with regard to CD4⁺ T-cell turnover in HIV-1 infection

The initial idea of high CD4⁺ T-cell turnover in HIV-1 infection was deduced from the rise of CD4⁺ T cells after 30 days of therapy. Indeed, with high amounts of cytopathic virus produced every day, high CD4⁺ T-cell turnover seems logical and explains the ultimate CD4⁺ T-cell depletion. However, several immunologists have expressed caution regarding the assumptions used for modelling of CD4⁺ T-cell dynamics[34–37]. First, the initial rise in CD4⁺ T cells might not necessarily reflect newly formed CD4⁺ T cells. Also, redistribution or transient peripheral expansion of memory cells might be involved, precluding extrapolation to pre-steady-state kinetics. If the post-treatment repopulation is due to proliferative expansion of cells, then one could assume that HIV-1 interferes with peripheral T-cell proliferation. Antiviral treatment would then lift the obstruction and result in post-treatment expansion. As yet, there is no specific evidence to support this mechanism.

More likely, the rapid post-treatment increase of CD4⁺ T cells in the blood is caused by redistribution of CD4⁺ T cells from tissue to blood, which is due to redistribution of memory cells in the first three weeks[38] (N.G. Pakker *et al.*, unpublished). Changes in distribution of T cells between blood and tissues following resolution of HIV-related chronic T-cell activation are in fact to be expected.

Furthermore, the implicit assumption was made that a turnover of $2 \times 10^9$ CD4⁺ T cells per day is high; however, the normal rate of T-cell turnover in adults is not accurately defined, and the total number of lymphocytes in the body can only be estimated. In humans, the persistence of altered karyotypes after irradiation has been used to calculate estimates of T-cell turnover. T-cell division varied from once every 3.5 years for naive cells to once every 22 weeks for memory cells[39]. Roughly speaking, naive cells divide once every 1000 days and memory cells divide once every 150 days. Thus, per day, cell division occurs in 0.1% of naive cells and 1% of memory cells, that is, $10^8$ dividing naive and $10^9$ dividing memory cells per day. To what extent these figures are representative of normal T-cell turnover in humans remains to be elucidated.

Although an unanticipatedly high number of virions are produced each day, a relatively small number of infected CD4⁺ T cells might actually be required for this, depending on the burst size (the progeny released by one infected cell in its lifetime). Quantitative *in situ* detection of infected cells has demonstrated that maximally $2 \times 10^8$ cells per day are infected[40]. The frequency and total body load of activated CD4⁺ T cells with integrated HIV-1 DNA was estimated to be even lower, in the order of $3 \times 10^7$ cells[41]. This implies that small amounts of cells (<0.1% of the total population) are infected and are able to produce large amounts of virus. However, uninfected CD4⁺ T cells are also lost in HIV-1 infection due to bystander mechanisms[42]. If normal turnover (i.e. production and destruction) is approximately $10^8$–$10^9$ per day, and the additional cell loss in HIV-1 infection is in the same order of magnitude, then only a twofold increase in production can compensate for this extra cell loss. As explained above, this will not be reflected in accelerated loss of TRF length.

Another way to obtain an estimate of turnover rates of T cells is to measure the frequency of cells with a loss-of-function mutation at the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus. In HIV-infected patients with CD4⁺ T-cell counts >300 cells $mm^{-3}$, no increased frequency of HPRT mutants could be observed;

in patients with $CD4^+$ T-cell counts of 100–300 cells $mm^{-3}$, only some of the patients showed increased mutation frequencies[43]. Low TRF length has also been reported in HIV-infected individuals with $CD4^+$ T-cell counts <200 cells $mm^{-3}$ (Ref. 44). This might suggest that, later in HIV-1 infection, when $CD4^+$ T cells have declined in number, production rates are increased. However, in HIV-infected individuals with $CD4^+$ T-cell counts >300 cells $mm^{-3}$, there is no evidence for an increase in T-cell turnover and therefore $CD4^+$ T-cell decline cannot be explained by exhaustion of renewal due to virus-induced cell destruction.

## $CD4^+$ T-cell depletion through limited renewal?

Why then do $CD4^+$ T cells decline in HIV-1 infection? If it is not excessive loss of cells driving the immune system to exhaustion, then it might be that the immune system is not able to generate sufficient numbers of cells. Two explanations may account for this: (1) active interference with renewal of $CD4^+$ T cells or (2) the immune system is not able to keep up with the chronic loss of $CD4^+$ T cells that takes place every day because of its innate limited regenerative capacity.

Inhibition of the renewal of $CD4^+$ T cells might be due to virus-induced changes in precursor cells or their environment. HIV-1 can act at the level of thymocyte infection and depletion[45]. In the severe combined immunodeficiency mouse grafted with human tissue (SCID-hu model), infection with T-cell-tropic syncytium-inducing (SI)[46] HIV-1 variants induced more rapid and severe thymocyte depletion compared with infection with macrophage-tropic non-SI (NSI) viruses[47,48]. This is fully compatible with $CD4^+$ T-cell depletion in humans infected with these different HIV-1 variants[12]. Recently, it was demonstrated that NSI and SI variants use different co-receptors for cell entry[49–52]. They can both use β-chemokine receptor CCR5 but SI variants are additionally able to use the α-chemokine receptor CXCR4 (Ref. 53). At present, the expression of these chemokine receptors on thymocytes or bone marrow (BM) precursors is unknown. It can be envisaged that SI variants have the capacity to infect and destroy thymocytes or other precursors, thereby impairing the capacity for T-cell renewal. Several reports have shown that hematopoiesis is disturbed in HIV infection at the level of BM precursors, either by infection of $CD34^+$ (immature BM) cells[54,55] or by indirect mechanisms that do not require $CD34^+$ cells to be infected[56,57]. Recently, evidence has been obtained for impaired T-cell regeneration capacity in a fetal thymic organ culture (FTOC) using peripheral blood mononuclear cells from HIV-infected patients[58]; the most affected was the $CD4^+$ T-cell generation. Taken together, these data indicate that HIV-1 can act at the level of T-cell development.

The alternative explanation for the loss of $CD4^+$ T cells might be a limited rate of renewal of the immune system in adults. Several recent studies on the repopulation of $CD4^+$ T cells after anti-HIV therapy and chemotherapy or BM transplantation have provided evidence that repopulation of T cells in adults is slow and that $CD4^+CD45RA^+$ T cells produced *de novo* are the essential component of $CD4^+$ T-cell repopulation[38,59–61]. Although one might argue that hematopoiesis may be damaged under these clinical conditions, recovery is also slow in patients who have undergone T-cell-depletion by $CD4^+$ T-cell-depleting antibodies as therapy for multiple sclerosis[62], indicating that regeneration in general is slow. If the immune system cannot keep up with moderately enhanced but chronic cell loss because it cannot substantially increase the rate of renewal, this will lead to a decline in $CD4^+$ T cells over time. If, in all phases of HIV-1 infection, T-cell renewal is already at its maximum capacity, then the emergence of more-cytopathic SI variants will lead to more-rapid $CD4^+$ T-cell decline.

## Concluding remarks

In summary, new data point towards a different cause of $CD4^+$ T-cell depletion in HIV-1 infection. Exhaustion of renewal driven by huge turnover of $CD4^+$ T cells seems no longer a plausible cause for $CD4^+$ T-cell depletion. Alternatively, $CD4^+$ T-cell depletion might be caused either by interference with renewal due to virus-related damage, or by an intrinsically limited renewal rate of the immune system. As suggested previously[43], the sink empties not because the drain is wide open, but because the tap is damaged, or unable to keep a homeostatic level when the drain is slightly more open.

The mechanism responsible for the insufficient supply of $CD4^+$ T cells may have implications for immunological reconstitution. In the case of a limited renewal rate of the hematopoietic system, complete silencing of viral replication may eventually result in a rebound of the immunological system. If, however, HIV-1 has caused damage to precursors or their environment, suppression of viral replication alone will not be sufficient for immune reconstitution. Furthermore, if immune reconstitution is mainly dependent on $CD45RA^+$ T-cell repopulation, it indicates that restoration of the immunity of patients following antiretroviral therapy may be much slower than has been anticipated thus far.

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