

Is CD4⁺ cell depletion due to rapid elimination by HIV and failure of the immune system to replace these cells at the required rate? Increasing evidence suggests that this is not the case, and that infection-induced immune activation drives both viral replication and CD4⁺ cell depletion.

CD4⁺ T-cell depletion in HIV infection: Are we closer to understanding the cause?

ZVI GROSSMAN^{1,2},
MARTIN MEIER-SCHELLERSHEIM²,
ANA E. SOUSA³,
RUI M.M. VICTORINO³ & WILLIAM E. PAUL²

The cause of the progressive depletion of CD4⁺ T cells in HIV-infected people is one of the most fundamental and controversial issues in AIDS research. HIV infects and kills CD4⁺ T cells. The infection results in high T-cell activation and turnover. An immediately intuitive assumption is that HIV-mediated destruction of CD4⁺ cells directly reduces the number of these cells and that the high turnover rates of T cells and the slow progression to AIDS reflect a long, but eventually lost struggle of the immune system to replace killed cells in its effort to maintain T-cell homeostasis¹⁻⁴.

However, HIV mainly infects *activated* CD4⁺ cells, and activated cells normally follow different dynamics than cells that belong to resting populations whose numbers are controlled by homeostatic mechanisms (Fig. 1a). Upon activation, T cells undergo several rapid rounds of division, and then they stop dividing and most die. Some of the activated cells escape this activation-induced cell death (AICD) and (re-)enter the population of resting memory cells. An alternative explanation for the high turnover rates of T cells in HIV infection may be that a large number of cells stimulated by antigen and/or inflammatory molecules rapidly replicate and subsequently die, as opposed to the immune system responding to high rates of virus-mediated death with high rates of homeostatic proliferation.

Conflicting interpretations of *in vivo* DNA-labeling studies

Recent studies have used DNA labeling to investigate the turnover dynamics of T cells in HIV-infected patients and uninfected individuals^{2,5,6}. In such studies, the DNA precursors administered are incorporated during the S-phase of the cell cycle. Bromodeoxyuridine (BrdU) or newly synthesized nucleotides generated from deuterium (²H)-labeled precursors (such as deuterated glucose) have both been used to tag DNA synthesized during the administration period of the labeling substance. During and after the administration, the proportion of labeled cells or of labeled DNA is measured. The time-dependence of the fraction of labeled cells or DNA can then be analyzed by fitting a mathematical model to the data. Whether this procedure allows one to draw conclusions regarding proliferation and death rates and the composition of the cell populations obviously depends on the degree to which the model's variables and parameters faithfully represent the real biological entities and their interrelationships.

Mohri *et al.*² observed that the fraction of CD4⁺ and CD8⁺ cells whose DNA becomes labeled in the course of a three-week administration of BrdU is greater in SIV-infected macaques than in uninfected animals. After BrdU administration ceased, the proportion of labeled cells rapidly dimin-

ished in both CD4⁺ and CD8⁺ cell populations. The authors excluded major division-induced dilution of the BrdU tag based on direct examination of label intensities. The rapid decline in label

detection then indicates that labeled cells die more frequently than they divide, as balanced division and death would imply that on average one labeled cell dies for every new one that is produced by a dividing labeled cell. The authors assumed that the imbalance of division and death was characteristic of the general population of turning-over cells. In order to reproduce the quasi-steady state of the total cell numbers during the asymptomatic phase of the infection, Mohri *et al.* had to postulate a massive supply of T cells from a source (either the thymus or the population of resting T cells) to compensate for the excessive cell death. The accelerated death of both CD4⁺ and CD8⁺ cells was attributed to virus-mediated destruction.

Qualitatively similar kinetic patterns of labeling and delabeling have now been reported by the same group in a study in which deuterated glucose was administered for seven days to HIV-infected and uninfected individuals⁵. As before, the authors assumed that the per-cell proliferation and per-cell death rates of labeled cells were constant throughout the observation time, both during and after the labeling period. Mass spectrometric measurement of the [²H]DNA content in the cell population, as it was used in this study, methodically excludes any influence of dilution, because without fresh precursor, cell division does not change the ²H content in the population's DNA. The decline of the label then directly reflects cell death. Again, the authors could not fit their data to their model with balanced division and death and had to postulate infusion from a source. Moreover, the analysis suggested to the authors that in HIV-infected people, CD4⁺ cells, but not CD8⁺ cells, were destroyed more rapidly than in uninfected individuals, and that production increased to keep up with the rate of loss. They concluded that CD4 depletion is primarily a consequence of increased cellular destruction, not decreased cellular production.

Different findings were reported by Kovacs *et al.*⁶ who measured and analyzed the delabeling kinetics following BrdU pulse-labeling *in vivo*. Using a more phenomenological modeling approach, they did not find significant differences between CD4⁺ and CD8⁺ cells. Moreover, they showed that although the fraction of dividing cells in patients on highly active anti-retroviral therapy (HAART) was much lower than in untreated patients, the decay rates were similar. These findings imply that the death of cells that had divided was largely independent of HIV. The authors attributed the enhanced turnover in CD4⁺ and CD8⁺ cells to immune activation.

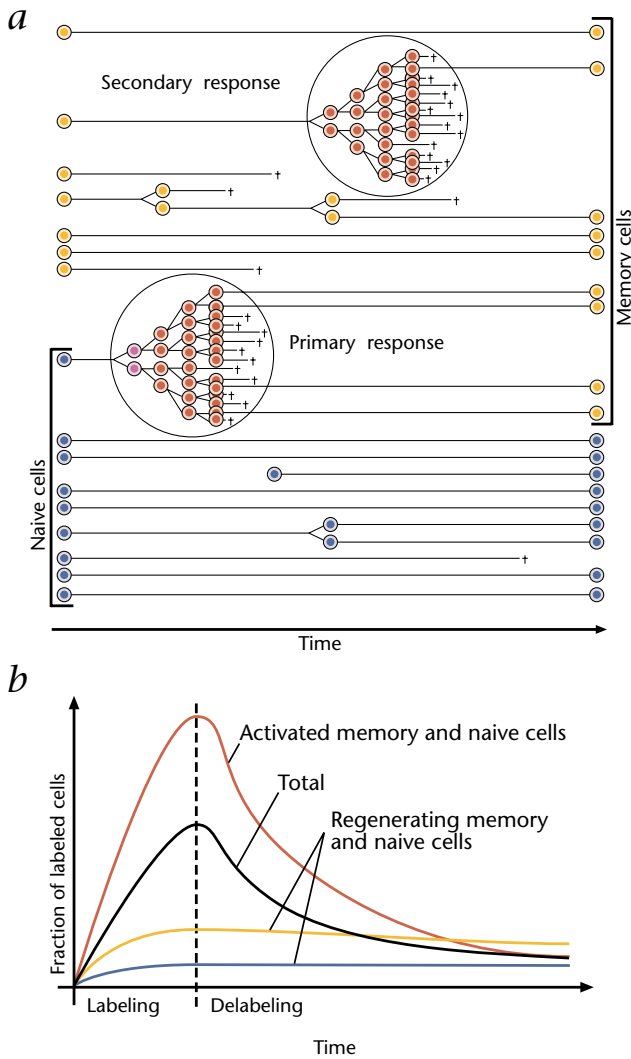


Fig. 1 Regenerative proliferation and transient clonal expansion contribute to T cell turnover. **a**, Cell division, differentiation and death of T cells. Naive cells (blue) and memory cells (yellow) stochastically divide and die (†). Naive cells also receive immigrants from the thymus. Antigen encounter triggers proliferation bursts (red) by specific naive or memory cells. Naive cells undergoing such a response may pass through a transition stage (purple). Most of the cells generated in proliferation bursts die but the fraction surviving is greater among naive as opposed to memory cells. Memory cells are replenished by cells surviving antigen-driven proliferation bursts. **b**, A generic labeling experiment reflecting these events. The fraction of labeled regenerating cells (blue for naive, yellow for memory) rises during the labeling period and after labeling ceases slowly falls. For BrdU labeling this fall is due to dilution (neglecting thymic input). For deuterated glucose, the fall is due to cell death. Among cells undergoing antigen-driven proliferation bursts (red), the labeled fraction rises rapidly during the labeling period and falls rapidly in the post-labeling period. The total fraction of labeled cells (black) is a weighted average of these fractions. It shows a peak and a biphasic decay.

lead to acquisition of label mainly by cells that are in the rapid expansion stage of their response. In the post-labeling period, these labeled cells are more likely to die than divide.

This interpretation supports the proposition that the increase in average turnover rates of T cells is a consequence of infection-enhanced immune activation by antigens and inflammation. Statistical analysis of the correlations between immune activation, viral load and CD4 counts during drug-combination treatment (HAART) further supports this proposition⁹. Accordingly, more cells die per unit of time in an HIV-infected individual mainly because more cells have been generated as a result of burst-like activation. If this interpretation is correct, then the vision of resting cells responding to the death of activated cells by being recruited in great numbers into the activated pool⁵ is a reversal of the direction of biological cause and effect.

Importantly, the recent studies clearly revealed a slow-decay phase of labeled cells following the rapid phase^{6,10}, as has been predicted by the immune-activation model⁸. Long-lived labeled cells, both from regenerative proliferation of resting cells and from immune-activated cells that return to the resting state, may account for the slow phase of the biphasic decay (Fig. 1b).

In analyzing their BrdU-labeling results in macaques² and ²H labeling in humans⁵, Mohri *et al.* fitted their data to a model in which labeled activated cells, instead of going through distinct phases of proliferation and subsequent arrest and death (that is, instead of behaving as a cohort), divide and die at constant rates throughout the observation time, both during and after the labeling period. As the kinetics of the labeled T cells was considered representative of the whole population, this required, as we have discussed, that the average death rate of activated T cells be substantially larger than the average proliferation rate, predicting a massive supply of T cells from a source. In contrast, in our interpretation, activated cells selected for observation by short-term labeling do not faithfully represent the total population of activated cells. Whereas the total population maintains a (quasi)-steady state, the selected population does not⁸.

Failure to distinguish between regenerative and burst-like proliferation manifests itself in inconsistencies. According to the analysis presented in the first study, a substantial proportion of the T cells supplied by the source was already labeled in some infected macaques, indicating rapid cellular division

Enhanced T-cell turnover is driven by immune activation

To correctly interpret the labeling results, we need to consider the question of what cellular processes determine the average turnover rate. Lymphocytes manifest two distinct modes of proliferation: regenerative and burst-like⁷ (Fig. 1a). Regenerative division and death of naive and resting memory cells occur at a low rate and are presumably stochastic events. Daughter cells maintain the phenotype of the parents. In contrast, antigens can induce specific resting cells into activation bursts, involving rapid cell proliferation and differentiation into effector cells over a period of days or weeks, and subsequent death of most of the activated cells within a comparable period, except for a minority that become memory cells. These cellular events overlap, but differentiation is accompanied with a gradual cessation of cell division and an increase in the relative probability of death.

In our view, the reported DNA-labeling kinetics^{2,5,6} suggest that activation bursts contribute substantially to T-cell turnover during HIV infection; indeed, they may fully account for the observed increase in average turnover rates. Upon transient administration of precursor, the fraction of BrdU-labeled cells or [²H]DNA in an individual with high levels of immune activation will display a rapid rise, followed by a rapid fall when administration ceases⁸ (Fig. 1b). Burst-like turnover will

within that source (for example the thymus). By contrast, the source was required to supply mainly unlabeled cells in the presence of ^2H , consistent with it being a resting T cell population. Beyond this inconsistency, neither the thymus nor the pool of resting T cells seems capable of continuously supplying new cells at the required rates. In particular, with a fractional input of CD4^+ cells in HIV-infected individuals at a rate of 12% per day⁵, the pool of resting T cells would be depleted within days, not years, unless replenished at a similar rate. However, it is unlikely that the thymus could provide such replenishment, given that thymectomy does not have a discernible effect on the rate of progression to AIDS in SIV-infected macaques¹¹.

Immune activation drives T-cell depletion

The concept of burst-like activation of T cells in the context of chronic HIV/SIV infection not only helps in the interpretation of DNA-labeling studies, but may also explain the limited direct impact of virus replication within activated cells and the slow progression of the disease. Activated CD4^+ T cells are the major targets for infection. A fraction of these cells are probably killed by the virus or by cytotoxic T cells (CTLs) that are specific for HIV rather than eliminated through activation-induced cell death. However, it is not clear how this would affect homeostasis—for those cells that die within a short period after activation, it seems irrelevant whether they are killed by virus, CTLs or AICD. Activation events in the presence of virus might result in reduced gain or a small net loss for the resting CD4^+ memory T-cell compartment, which is not measurable in current labeling studies but might be important over a longer period of time.

How are activation, viral replication and CD4 depletion interrelated? Comparison of CD4 depletion and immune activa-

tion within different host–virus systems reveals a consistent relation. Immune activation, CD4^+ T-cell depletion and viremia in untreated HIV-1-infected patients have been compared with those in HAART-failing patients who continue to have increasing CD4^+ cell counts. For any level of viremia, CD4^+ cell turnover rates were higher in untreated patients than in treated patients with drug-resistant virus¹². Furthermore, the natural hosts of SIV, sooty mangabeys and African green monkeys, show no significant increase in immune activation and turnover^{13,14}. Despite high-level virus replication and rapid death of infected cells¹⁵, these animals do not develop progressive depletion of CD4^+ cells. Finally, we have recently observed that for the same level of CD4^+ T-cell depletion, HIV-1- and HIV-2-infected patients exhibited similar elevations in the frequencies of activated and cycling T cells despite large differences in viremia¹⁶. Collectively, these observations indicate that CD4 depletion during the chronic phase of HIV/SIV infection is more directly related to the overall activation and turnover of T cells than to the rate of virus replication. A high turnover rate of infected cells does not necessarily imply a cytopathic virus because AICD alone may account for the short lifespan of infected cells. Thus, the different viruses in the examples above may differ in cytopathicity. But the consistent association of depletion with elevated immune activation suggests that it is the level of activation, not solely differences in cytopathicity, which determines the outcome.

Activation is the machine driving virus production¹⁷. However, once activated, cells are largely expendable, whereas resting cells represent the long-term immunological reservoir under homeostatic control. Accordingly, virus-induced death of activated cells may cause progressive depletion only by impairing the regulation of resting cells. The concept that the im-

Hypothetical mechanisms of activation-driven depletion that do not depend on virus-mediated cell killing

Memory cells are normally activated at a low rate and replaced by new entrants into the memory pool (Fig. 1a). It is generally believed that each cycle of immune activation adds to the number of memory cells. But recent data indicate that, at least under some circumstances, memory cells re-activated by antigen may leave no or little new memory at the end of the response, while activated naive cells are converted into larger numbers of memory cells³¹. Could the net change in memory cells in each activation cycle be negative in the presence of persistent antigen and inflammation? As the size of the naive pool shrinks and its contribution to activation diminishes, might the memory pool slowly exhaust itself?

Notably, HIV mainly targets activated CD4^+ cells and yet the first cells to be depleted are naive CD4^+ and CD8^+ cells. The input of naive T cells from the thymus appears to be substantially reduced in HIV infection^{28,32}, either as a result of virus-induced destruction of thymocytes or because of immune activation-associated inhibition of production. Normally, as the thymic output is reduced with aging, continued slow proliferation of naive cells in the periphery and probably reduced, cell density-controlled cell death help to stabilize the naive T-cell populations at modestly decreased levels^{27,33}. However, if the outflow of naive cells into the memory compartment through immune activation exceeds the maximum replenishment capacities, stability may be impaired^{25,34}. Systemic deple-

tion resulting from such outflow is conceivable only if naive T cells are activated not only specifically but also non-specifically, a phenomenon recently observed during homeostatic proliferation in lymphopenic settings, but which might occur also under other conditions, such as chronic immune activation.

Not only do cell numbers change in the course of HIV infection, but so do the functional characteristics of T cells and other cells. Such changes may be causally related. We have proposed a unifying model in which changes in T-cell counts reflect functional modifications^{7,23}. It is conceivable that products of activation, such as pro-inflammatory factors and other mediators of inflammation, affect the migration patterns, viability and response characteristics of the resting lymphocytes chronically exposed to these factors²³. Part of the depletion of resting CD4^+ and CD8^+ cells from the blood can be explained by redistribution into the lymphoid tissue compartment^{18,34}. Another part is related to the generalized 'energy' of resting T cells in HIV-infected individuals³⁵. A likely explanation for this energy is activation-threshold tuning—a dynamic adaptation to recurrent signals that are not strong enough to induce full activation³⁶. Such adaptation would render lymphocytes less responsive to both antigenic and regenerative signals and might also reduce their viability³⁶. As explained in the text, reduced viability and/or responsiveness of resting T cells to homeostatic signals would result in the down-regulation of steady-state cell numbers but not necessarily in instability.

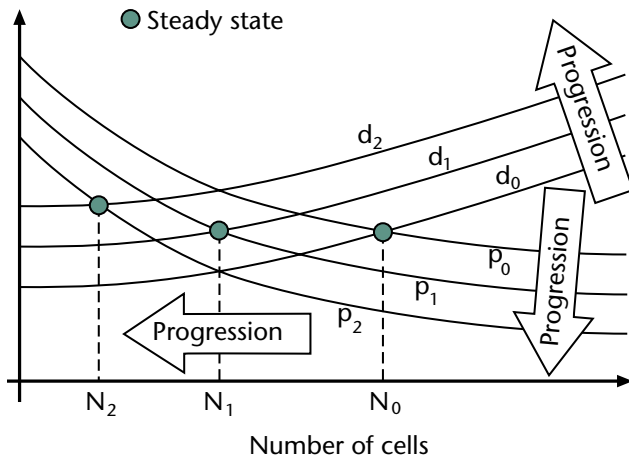


Fig. 2 Progressive depletion of resting T cells is depicted as a shifting steady state. The curve marked p_0 represents a plausible dependence of the (per-cell) production rate of resting T cells (either naive or memory) on the cell number, N , in an uninfected individual. The higher N , the lower is p_0 . d_0 , an increasing function of N , represents normal cell elimination rate as it would change with increasing cell number. The intersection of these two curves, where production and elimination rates are equal, is the normal steady state, with cell number N_0 . After this individual becomes infected, the elimination rate, for any value of N , may be higher than normal (see text), resulting in an upward-shift of the whole d -curve from d_0 to d_1 . In parallel, the p -curve may shift downward (see text). The intersection point shifts as well, from N_0 to a smaller number of cells, N_1 . At a later time, with further increase in the level of immune activation, the production and elimination curves shift further, to p_2 and d_2 , respectively, and the steady-state cell number is consequently reduced from N_1 to N_2 . Note that we have used the terms “production” and “elimination” rather than “proliferation” and “death”. Production also includes inflow from a different compartment (from the thymus in the case of naive T cells; from the naive compartment for memory cells). Similarly, “elimination” of naive cells includes differentiation into the memory compartment.

part of viral replication is largely indirect helps to explain the disjunction between viral replication and CD4⁺ T-cell depletion alluded to above.

How can virus-induced death of activated cells impair the regulation of resting cells? If virus or CTLs eliminate a substantial proportion of activated CD4⁺ cells, too few activated CD4⁺ cells may be converted into resting memory cells to replace those lost by activation. In that case, the pool of CD4⁺ memory T cells might be gradually depleted. An explanation for the slow pace of depletion is that the activation rate of resting cells is low, which is not inconsistent with the relatively high turnover rates of activated cells because the latter may proliferate extensively before undergoing cell death. Radically different circumstances may prevail during acute infection (and in the final stages of the infection^{17,18}), as recently described¹⁹. When macaques are infected with highly virulent SIV, most of the CD4⁺ T cells in their intestinal tissues, which were either already activated or became quickly activated, are rapidly infected and eliminated within two weeks¹⁹. This shows the destructive potential of viral replication but underscores the difference between non-equilibrium and quasi-steady-state dynamics.

Although the total number of CD8⁺ T cells increases during most of the asymptomatic period of HIV infection, naive and resting memory CD8⁺ cells progressively decline as do naive and memory CD4⁺ cells²⁰. This suggests activation-driven processes of attrition, independent of HIV-induced cell death. Several mechanisms that have been proposed are briefly described in the box (page 321). These include reduced production and increased differentiation of naive T cells, net loss in the number of resting memory T cells during immune activation cycles, and adaptive tuning of cell-activation thresholds.

Each of these mechanisms—as well as the one related to virus-dependent elimination of activated CD4⁺ cells described above—involves a negative effect of chronic activation on the balance between cell production and cell loss within a given compartment of resting T cells. This would result in the down-regulation of steady-state cell numbers but not necessarily in instability (Fig. 2). When the cell number drops—as a result of increased outflow, reduced inflow or infection-related suppression of either cell viability or regenerative capacity—homeostatic mechanisms act to restore or increase these capacities,

temporarily stabilizing the cell population at a new steady state with a lower number of cells.

Mohri *et al.* have rejected suppressive effects of the infection on cell production processes as a possible cause of depletion because they wrongly argued that, to maintain a quasi-steady state, the death rate of T cells would have to decrease, which they considered unlikely in the context of HIV infection⁵. This argument is based on a misconception. Given the focus on resting T-cell homeostasis, we do not exclude regulation by decreasing death rate. But even if this rate is larger than normal, a new steady state of resting T cells may be reached and maintained in the face of infection-related suppression, provided that cell production is sufficiently upregulated by homeostatic mechanisms in response to the reduction in cell number (Fig. 2).

If homeostatic regulation of resting T cells is indeed effective, progressive depletion by any of the attrition mechanisms proposed depends on a progressive increase in the level of immune activation, and the rate of that increase determines the rate of progression. The activation level, in turn, is variably determined, in different host–virus systems, by the viral load. We argue that increased activation in HIV-infected individuals is due to increased antigenic and/or inflammatory stimuli. The question of whether a fixed viral load is sufficient to drive disease progression or if it is the progressive increase in viral load that drives progression has received surprisingly little attention. We think the latter is more likely. Although HIV-RNA levels are relatively stable initially, following the acute, dynamic phase of infection, the important concept of an early-determined viral ‘set point’ does not actually imply that RNA levels remain constant for an extended period after seroconversion²¹.

Increasing viral load and activation result in a progressive down-regulation of resting cell numbers through anergy and ‘attrition’. The effect on total T-cell numbers is complex, because the number of activated cells increases. CD8⁺ cells are known to expand more extensively than CD4⁺ cells during immune responses, and this might account for the overall increase in CD8⁺ T-cell counts during the less-advanced stages of progression, while CD4 counts progressively decline. Additionally, the clonal expansion of activated CD4⁺ cells is curtailed by virus-induced cell death, resulting in a smaller average size of this population.

The intuitively appealing but simplistic vision of an ongoing competition between massive destruction and massive replacement of CD4⁺ cells is still energetically advocated by some. Others envision a classical confrontation between protective immune responses and an evasive virus. But a growing body of evidence and reasoning point to infection-induced immune activation both as the driving force behind virus replication and as a facilitator of CD4⁺ cell depletion^{22–30}. We argue that increased immune activation leads to increased proliferation that is physiologically controlled by increased cell death. Viral replication within activated CD4⁺ cells does not interfere with this balance in the short term. Thus, the increase in CD4⁺ T-cell death does not create a demand for an equally massive homeostatic response. Immune activation maintains viral replication and drives the progression of HIV disease by destabilizing or progressively changing the homeostatic steady states of resting cell populations, naive and memory. Novel approaches are required to quantitatively characterize these effects, with a new focus on resting lymphocytes and their interface with the overtly activated population.

Acknowledgments

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¹Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

²Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

³Institute of Molecular Medicine, Faculty of Medicine of Lisbon, Portugal.