The impact of HIV on naïve T-cell homeostasis

Correlational data and mathematical modeling suggest that increased division rate of naïve T cells, not reduced thymic output, lowers the frequency of original thymic emigration among these cells in HIV infected individuals. But are there alternate explanations for these data? (pages 1036–1042)

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DURING UNTREATED HIV infection, naïve CD4$^+$ T cells, and eventually CD8$^+$ cells, are depleted more rapidly than memory cells. An impaired supply of T cells from a functional thymus has been proposed as a possible mechanism for this depletion. Recent thymic emigrants (RTE) carry a traceable molecular marker, a T cell receptor excision circle (TREC). TREC content in a cell population is affected both by changes in thymic output and by the division history of the cells[1]. This is because TRECs do not replicate during mitosis. Indeed, memory T-cell populations contain 10 to 100-fold fewer TRECs than naïve populations. This has been taken into account in evaluating TREC data[1]. However, it has been believed that proliferative dilution of TRECs among naïve cells was slow and most importantly, constant. In this issue, Hazenberg *et al*[2]. present data to suggest that this assumption may be wrong.

Hazenberg *et al.* analyzed the relative impact of changes in thymic output and in naïve T-cell division rate on TREC content[2]. From equations describing how numbers of naïve T cells (N), and of TRECs (T) change with time, the steady-state TREC content, *T/N*, was derived. It is written as $c/(1+D)$, where c is *T/N* in pure RTE and D a dilution factor. D is essentially $N\alpha/\sigma$ where α is the per-cell division rate and σ is the immigration rate of RTE. Thus, dilution depends on the ratio between the production rate of non-TREC containing cells through cell division and the thymic output of TREC containing cells.

This analysis has important implications for naïve T-cell homeostasis. In the absence of cell division, TREC content does not change even when σ does. Because naïve T-cell TRECs decline as people age, division must contribute to maintenance of the population. Furthermore, N is not just passively adjusted to a gradually declining thymic output, because then N would remain proportional to σ and T/N would not change. This argues for active homeostasis, whereby division rate/cell increases and/or death rate diminishes as N decreases. The authors' simulations favor increased division rate, in agreement with recent experiments showing accelerated naïve T-cell division in lymphopenic mice[3].

TREC content of blood cells is reduced within a few months after infection[1]. Hazenberg *et al.* show that such change cannot result from a sharp decline in thymic output, even if such decline did occur, because of the low death rate of naïve cells[2]. By contrast, a several-fold increase in division rate of naïve cells can lead to a rapid fall in TREC content. If such an increase occurs, the reductions in TREC content during HIV infection cannot be taken as evidence for diminished thymic function.

Naïve T cells express both CD27 and the RA isoform of CD45, while most memory cells are believed to express the RO isoform. Hazenberg *et al.* measured TREC content of CD45RA$^+$CD4$^+$ and CD45RA$^+$CD8$^+$ cells in HIV-infected individuals and controls and also the frequency of naïve (CD45RO$^-$ CD27$^+$) cells that expressed Ki67, a cell-cycle marker[2]. They report reduced TREC content in the RA$^+$ populations from infected individuals, more pronounced in the CD8$^+$ subset. The reduction correlated with an increase in the proportion of Ki67$^+$ T cells in the corresponding naïve populations. Relying both on these correlations and on their general analysis, they suggest that the division rate of naïve T cells is substantially increased in HIV infected individuals, leading to the dilution of TRECs.

The authors interpret their elevated Ki67 naïve frequencies to reflect a general cytokine induced increase in naïve cell proliferation[2]. This may not be the case, as increased Ki67 expression may reflect proliferation by cells entering an activated state and thus not be an indication of overall naïve cell turnover. This is an equally plausible interpretation, considering that there are two modes of lymphocyte proliferation—regenerative and transitional. Regenerative division and death of naïve and memory cells are slow and stochastic, and daughter cells maintain phenotype, viability and proliferative capacity. In contrast, antigen and inflammatory signals can induce activation bursts involving rapid proliferation over a period of days or weeks. This is associated with maturation/differentiation, leading subsequently to death of most activated cells within a comparable period (except for a minority that are added to the memory pool). Stimulated cells that fail to become fully activated may become reversibly refractory to activation, or 'anergic'. Local, recurrent, asynchronized activation bursts may contribute steadily to the overall turnover[4] (Fig.1).

Recent findings based on DNA-labeling experiments, where the fraction of labeled cells was monitored during labeling and 'delabeling' periods, suggest that a substantial antigen-induced transitional proliferation occurs during HIV infection[5,6]. Theoretical analysis predicts a pronounced peak and biphasic decay kinetics[5] (Fig.1, insert), and this prediction is

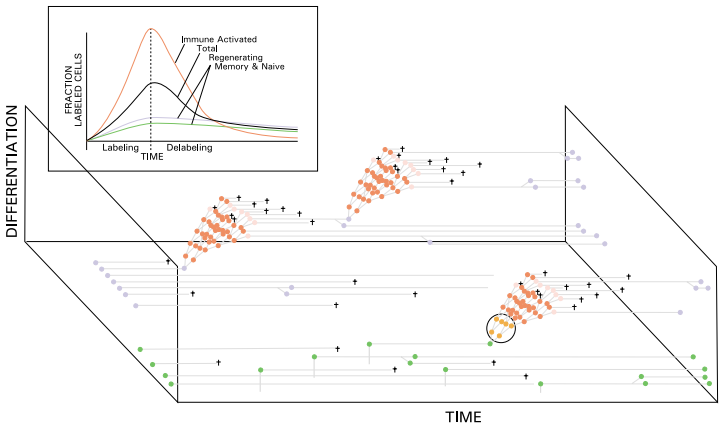

Fig. 1 Regenerative proliferation and transitional expansion contribute to T-cell turnover. The figure provides a small window in time and space into cellular events occurring in a lymphoid tissue. Naïve cells (green) stochastically emerge from the thymus (RTE, vertical lines), divide and die (+). Memory cells (purple) divide and die (+). Antigen triggers proliferation bursts (red; pink for cells that stop dividing). Encircled are cells in transit from naïve to activated phenotype (orange). The *insert* depicts a generic labeling experiment reflecting these events. The fraction of labeled regenerating cells rises during the labeling period and then falls slowly. The fraction of labeled immune-activated cells rises and falls rapidly. The fraction of total cells labeled peaks and then shows bi-phasic decay kinetics.

supported by studies with deuterated 2 deoxyglucose (M. Hellerstein, J. Kovacs, personal communication). Ignoring the transitional component of lymphocyte turnover originally led to confusion and to a failure to predict the bi-phasic kinetics[7]. It was argued that the rapid delabeling reflected generalized, virus-induced killing of $CD4^+$ and $CD8^+$ T cells, requiring a massive compensatory supply from an outside source, possibly the thymus[7]. Proper consideration of chronic, antigen-driven activation is also essential for understanding infection dynamics and the limited impact of HAART[8].

These perspectives suggest possible alternative interpretations for the observed increased expression of Ki67 seen in the naïve population[2]. The transition from naïve to the memory/effector phenotype during antigen-driven bursts is prolonged and involves gradual loss of RA and acquisition of RO[9]. Transitional cells (circled in Fig.1) are likely to express Ki67. Therefore, many $Ki67^+$ naïve cells may in fact be transitional—between the naïve and memory states. Other $Ki67^+$ cells might be anergic[4]; Ki67 may be expressed by non-dividing cells arrested in some phase of the cell cycle[10].

In both cases, the impact of these cells on naïve cell numbers and on TREC dilution would be limited. The percentage of 'naïve' $Ki67^+$ cells may strongly depend on how the naïve cells are defined. Cutoff levels that define a phenotype as RO^- are somewhat arbitrary. Indeed, using strict gating, Douek *et al.* have not observed an increased percentage of $Ki67^+CD4^+$ naïve T cells in individuals with early HIV infection (personal communication). This discrepancy could be due, in part, to a higher mean $CD4^+$ count (600 vs. 400 cells/ml) and to the different criteria used to identify naïve cells in their study.

Hazenberg *et al.*[2] correctly point out that the negative correlation between Ki67 expression and TREC content is further confounded by the finding that RA^+ populations, especially $CD8^+$, contain $CD27^-$memory/effector cells[11]. The fraction of $CD27^-$ T cells in the $CD45RA^+CD4^+$ population is smaller than in the $CD45RA^+CD8^+$ (D. Hamann, personal communication); this could explain the lower TREC content found in the $CD8^+$ subset. Interestingly, it has been proposed that $CD27^-CD45RA^+$ is the phenotype of 'terminally differentiated' effector T cells in acute viral infections[12]. The increased numbers of $CD27^-CD45RA^+$ cells observed during chronic HIV infection is consistent with a hyper-expansion of transitional clones, leading to the continuous production of effector cells.

Hazenberg *et al.* suggest that loss of naïve T cells through activation may account for their gradual depletion[2]. This is consistent with the proposition that many naïve $Ki67^+$ T cells are transitional. But such activation is expected to be largely antigen specific, thus involving only a minority of clones. Alternatively, the transitional component may exert an inhibitory effect on regeneration[13]. In response to such inhibition, the number of regenerating cells would decline to a new homeostatic set point.

This hypothesis[13] explains disease progression not as a result of an 'uncompensated' loss of cells. Rather, set points are pushed down by a growing chronic activation associated with increasing viral load[4,5,14]. Indeed, extending previous studies[15], Hazenberg *et al.* report lower numbers of naïve $CD4^+$ and $CD8^+$ T cells, concomitant with chronic activation, in a group of HIV-negative Ethiopians[2]. These and other hypotheses, including impaired thymic function, remain viable at this time and are not mutually exclusive.

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Lipid biochemistry takes a stand against tuberculosis

Although several excellent drugs are available to treat tuberculosis, their mechanisms are not well understood. Identification of the target of the tuberculosis drug pyrazinamide underscores the role that lipids play in disease pathogenesis, and reveals new avenues for drug design (pages 1043–1047).

PHILIP DRAPER

TUBERCULOSIS IS AN infectious disease caused by *Mycobacterium tuberculosis* that is believed to kill between 2 and 3 million people each year. It commonly affects the lungs, and is fatal in about half of untreated patients. The incidence of tuberculosis has declined dramatically in developed countries due to improved nutrition and housing, and the availability of effective drugs and vaccines. However, it remains as a problem in poorer countries and its overall incidence is increasing worldwide because of the enhanced susceptibility of AIDS patients and the appearance of drug resistant strains.

Tuberculosis requires a long treatment course with a combination of drugs. The time required to treat tuberculosis can be