TREC assays are used to detect recent thymic emigrants and quantitate thymic output. However, the longevity of naive T cells combined with T cell division suggest TREC data should be interpreted with caution.

Thymic output: a bad TREC record

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Thymic function in healthy and diseased individuals has received considerable attention over the years, but until recently only indirect estimates of thymic output could be obtained. Thymic function has been measured with computed tomography scans of thymic volume and/or phenotyping of naive T cells in the circulation. In HIV-1–infected individuals thymic volume may relate to thymic output, but it could also represent infiltration of thymic tissue by mature T cells[1]. With flow cytometric analysis for CD45RA and CD62L or CD27 expression, naive T cells can be enumerated in blood and lymphoid tissues. However, because naive T cells are long-lived[2,3], the number of circulating naive T cells represents a composite of production, death, memory cell generation and naive T cell homing to lymphoid tissues, rather than a measure of real-time thymic function.

The recent introduction of the T cell receptor (TCR) excision circle (TREC) assay seemed to enable direct detection of recent thymic emigrants and therefore the quantification of thymic output. High TREC levels were detected in peripheral blood mononuclear cells, lymphocytes or purified T cell fractions during childhood, but declined with increasing age[4]. Surprisingly, TRECs were also readily detectable in elderly people; this was interpreted to reflect continuous thymic output of TREC$^+$ naive T cells even in old age[4]. In HIV-1–infected individuals, TREC levels were significantly lower compared to healthy age-matched individuals and correlated positively with naive T cell numbers. This suggested that HIV-1 was interfering with thymic function. The fact that TRECs were restored in these patients by antiretroviral treatment suggested improved thymic output[4]. Finally, in cancer patients who received stem cell transplantation, TRECs increased rapidly during early T cell reconstitution, often to supranormal levels, which was thought to reflect thymic rebound[5]. The publication of these data led to the general acceptance that TRECs in the peripheral T cell pool are a direct marker for thymic output and that thymic output can be increased following naive T cell depletion.

Indeed, detection of TRECs in T cells does provide evidence for their thymic origin. TRECs are a product of TCR gene rearrangement during intrathymic T cell maturation. Most groups have analyzed V_α signal joint (Sj) TRECs, which are excised late during thymic T cell development as the gene encoding TCRα is rearranged. Shortly after, mature T cells migrate from the thymus into the circulation and about 70% of these contain a Sj TREC. Theoretically, functional TREC$^+$ T cells could also be produced at extrathymic sites, such as the gut. However, TREC levels in those tissues are very low, and in congenitally athymic patients, such as patients with complete DiGeorge Syndrome, no TRECs are detectable[4]. Only after restoring naive T cell production in two DiGeorge patients by transplantation of cultured postnatal thymic tissue were TRECs more abundant[6]. Similarly, in a group of severe combined immunodeficiency patients, TRECs became detectable after hematopoietic stem cell transplantation[7].

Despite the fact that TREC$^+$ naive T cells originate from the thymus, many erroneous conclusions have been drawn from the assumption that TREC data is a measure of ongoing thymic output. Here we discuss two major biological parameters that complicate the interpretation of TREC data as a measure of thymic function: longevity of naive T cells and TREC dilution by division.

Longevity of naive T cells

Although TRECs may serve as a marker for thymic descent, in many instances TREC levels—measured as the number of TRECs per μl of blood (absolute TREC number) or per cell (TREC content)—do not reflect actual thymic output. One important caveat in the interpretation of TREC data is the longevity of naive T cells. Estimating that a healthy adult has a steady state of 10^{11} naive T cells, and a thymic output of 10^7–10^8 naive T cells per day, naive T cells have a lifespan of 1,000–10,000 days[2,3]. Consistently, adult thymectomy does not lead to a rapid decline in naive T cell numbers. Similar data have been reported for juvenile rhesus macaques, where thymectomy does not accelerate age-related naive T cell decline (S.T. Arron, A. Gettie, J. Blanchard, D. Ho & L. Zhang: Impact of thymectomy on the peripheral T-cell pool in rhesus macaques before and after infection with SIV. Ninth Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 2002). Although TREC decline was faster in these animals compared to sham-thymectomized animals, TRECs were easily detectable nine months after thymectomy. In a group of patients thymectomized three to thirty-nine years prior to analysis, TRECs were also clearly present[4]. Thus, TREC-containing T cells need not be recently produced by the thymus. As a consequence, the mere detection of TRECs in healthy adults or in elderly individuals should not be taken as evidence for ongoing thymic naive T cell production.

Dilution through T cell division

The TREC content of thymocytes isolated from healthy donors is constant with increasing age despite age-related involution of thymic tissue[8]. Thus, diminished thymic output will lower the number of naive T cells produced, but does not change the TREC content of recent thymic emigrants. A simple mathematical model has shown that decreased thymic production cannot solely account for the reduction in the TREC content of peripheral T cells[9]. Peripheral effects like cell division or changes in the cellular lifespans are required to account for changes in the TREC content (**Fig. 1**). Thus, changes in

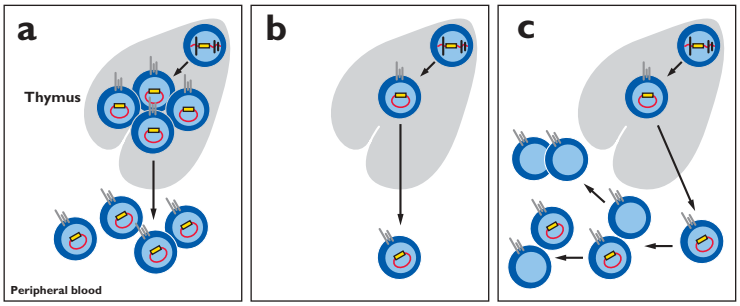

Figure 1. Schematic representation of changes in TREC levels in response to changes in thymic function or T cell division. (**a**) Normal thymic output of TREC$^+$ T cells yields a certain naive TREC content (thymic output = σ and cell division = 0; peripheral T cell pool TREC content = 100%). (**b**) This TREC content does not decline when only the thymic output of naive T cells is lowered (thymic output = σ/4 and cell division = 0; peripheral T cell pool TREC content = 100%). (**c**) Cell division in the peripheral T cell pool reduces the T cell TREC content (thymic output = σ/4 and cell division = α; peripheral T cell pool TREC content <100%).

the TREC content of peripheral T cells need not reflect changes in thymic output.

As TRECs are episomal circles that are not replicated during cell mitosis, they are diluted with each round of cell division. Although the possibility of dilution is frequently mentioned, its implications are only rarely fully acknowledged[10]. TREC levels are often determined with the use of peripheral blood mononuclear cells or purified CD4$^+$ and CD8$^+$ T cells. Interpretation of such TREC data is complicated because of differences in the division frequencies of the naive and memory subpopulations. For instance, increased priming and subsequent clonal expansion of naive T cells is expected to reduce the TREC content of the whole T cell population.

Even the much cleaner TREC data collected from purified naive T cells suffer from biases introduced by naive T cell division (**Fig. 1**). Initially, it was assumed that naive T cell division would be too low to significantly affect the TREC content of naive T cells[4]. Indeed in healthy adults, the proportion of dividing naive T cells is very low. However, in HIV-1–infected individuals and stem cell transplantation recipients, naive T cell division is increased and correlates negatively with the TREC content of naive T cells[11,12]. Some have argued that this is largely due to increased percentages of cells in transition between naive and memory phenotypes, because naive T cell division should always be low and cannot be increased[13]. However, others have shown that depending on the type of stimulus, naive T cells can be induced to divide without phenotype changes. For example, interleukin 7 (IL-7) can induce several rounds of naive T cell division without the acquisition of a memory phenotype[14]. Of note, serum IL-7 is increased during HIV-1 infection[14], which may account for the increased proportion of dividing naive T cells in these patients.

Thymic rebound

In lymphopenic cancer patients receiving stem cell transplantation, immune recovery was characterized by rapid normalization of TREC content, which was interpreted to reflect recovery of thymic function[5]. In some patients TREC contents reached even supranormal levels, suggesting that thymic function may increase to compensate for low naive T cell numbers. This phenomenon was referred to as 'thymic rebound'. However, TREC content is expected to normalize rapidly, because production of only a few TREC$^+$ naive T cells that enter the virtually empty T cell pool is enough to normalize the ratio between TREC$^+$ and TREC$^-$ T cells[12] (**Fig. 2**). Before these recent thymic emigrants divide, the TREC content is indeed expected to be supranormal, even in the complete absence of thymic rebound. Low post-transplantation TREC contents appeared to be associated with increased T cell division rates, leading to dilution of TRECs[12]. Of note, naive T cell numbers remained low even in those patients who were assumed to show thymic rebound based on their transient supranormal TREC levels.

HIV-1 infection

TREC analyses have led to confusing conclusions, especially in HIV-1 research. Decreased CD4$^+$ T cell TREC content and reduced proportions of naive T cells during HIV-1 infection have been taken to reflect decreased thymic function[4]. However, as noted earlier, the effects of decreased thymic function on naive T cell numbers and TREC content are expected to be very slow because of the longevity of naive T cells[9]. Indeed, after thymectomy of healthy juvenile rhesus macaques, TREC content did not decrease as rapidly as in SIV-infected animals (S.T. Arron, A. Gettie, J. Blanchard, D. Ho & L. Zhang: Impact of thymectomy on the peripheral T-cell pool in rhesus macaques before and after infection with SIV. Ninth Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 2002). Changes in the naive T cell pool during HIV-1 infection are therefore most likely related to increased activation and proliferation of T cells, leading to the dilution of TRECs and to a lower proportion of naive T cells relative to the memory T cell pool. Increased T cell division as the main reason for lower TREC content has been questioned by one group, who reported normal proportions of dividing naive T cells in the presence of decreased TREC content[13]. However, increased cell division does not need to be within the naive T cell pool to have a major impact on TREC levels. Even when patients still have normal proportions of dividing naive T cells, increased activation and expansion of memory T cells will lower TREC numbers when they are measured per μg of CD4$^+$ T cell DNA or per 10^6 CD4$^+$ T cells. Moreover, increased cell division need not be persistent to affect TREC content. Even intermittent periods of T cell activation and expansion will lower the TREC content to an extent that may be detectable for considerable periods of time thereafter. Indeed, when telomere length—a marker for the replicative history of cells—and TREC content of peripheral blood mononuclear cells or purified CD4$^+$ T cells were measured, a significant positive correlation was found[15,16]. Finally, upon treatment of HIV-1–infected individuals with antiretroviral therapy (ART), increases in naive TREC content were reported[4] that correlated with a decline in naive T cell division rates[9]. Recovery of naive CD4$^+$ TREC content during ART therefore seems to involve reduced dilution through rapid normalization of cell division, ongoing (but not necessarily increased) naive T cell production by the thymus and possibly redistribution of TREC$^+$ naive T cells from lymphoid tissues. As it is not possible to distinguish between these phenomena, these data fail to provide evidence for recovery of thymic function in HIV-1–infected patients receiving ART.

Conclusion

We have discussed the dilution effect of cell division and the effect of the longevity of naive T cells on the interpretation of TREC data. For simplicity we have ignored the possibility that diminished thymic output with increasing age would also lower the TREC

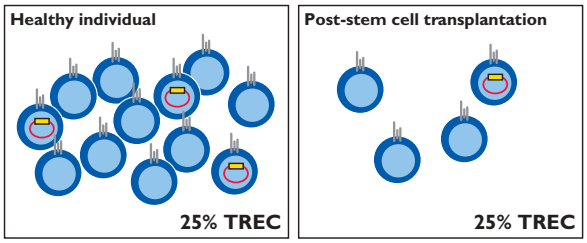

Figure 2. Schematic representation of peripheral blood TREC reconstitution following stem cell transplantation. Because only a few TREC$^+$ naive T cells can normalize TREC content following T cell depletion, measurement of TREC contents in this setting does not allow quantitative estimates of thymic output.

content of naive T cells by increasing the lifespan of naive T cells[9]. In any case, with aging and in clinical conditions such as HIV-1 infection or immune recovery following stem cell transplantation, the number of TRECs and the T cell TREC content are determined both by thymic output and by peripheral events. Although the presence of TRECs in naive T cells is indicative of their thymic descent, TREC-containing naive T cells are not by definition recent thymic emigrants. In addition, as TRECs are passed on to daughter cells upon cell division, even TREC$^+$ naive T cells may have undergone some rounds of proliferation. Estimating thymic function by the TREC assay therefore remains an indirect method that needs to be interpreted with caution.

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1. Haynes, B.F. *et al. J. Clin. Invest.* **103**, 453–460 (1999).
2. Sprent, J. & Tough, D.F. *Science* **265**, 1395–1400 (1994).
3. Clark, D.R., De Boer, R.J., Wolthers, K.C. & Miedema, F. *Adv. Immunol.* **73**, 301–327 (1999).
4. Douek, D.C. *et al. Nature* **396**, 690–695 (1998).
5. Douek, D.C. *et al. Lancet* **355**, 1875–1881 (2000).
6. Markert, M.L. *et al. N. Engl. J. Med.* **341**, 1180–1189 (1999).
7. Patel, D.D. *et al. N. Engl. J. Med.* **342**, 1325–1332 (2000).
8. Jamieson, B.D. *et al. Immunity* **10**, 569–575 (1999).
9. Hazenberg, M.D. *et al. Nature Med.* **6**, 1036–1042 (2000).
10. Zhang, L. *et al. J. Exp. Med.* **190**, 725–732 (1999).
11. Hazenberg, M.D. *et al. Blood* **95**, 249–255 (2000).
12. Hazenberg, M.D. *et al. Blood* **99**, 3449–3453 (2002).
13. Douek, D.C. *et al. J. Immunol.* **167**, 6663–6668 (2001).
14. Unutmaz, D., Pileri, P. & Abrignani, S. *J. Exp. Med.* **180**, 1159–1164 (1994).
15. Richardson, M.W., Scerstiuk, A., Hendel, H., Cheung, T.W., Zagury, J.F. & Rappaport, J. *Biomed. Pharmacother.* **54**, 21–31 (2000).
16. Koetz, K. *et al. Proc. Natl. Acad. Sci USA.* **97**, 9203–9208 (2000).