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Getting to the HAART of T cell dynamics

Detailed analysis of the dramatic T cell changes accompanying HIV disease point to homeostatic dysregulation as a primary cause of immunodeficiency (pages 208–214 and 215–221).

MARIO ROEDERER

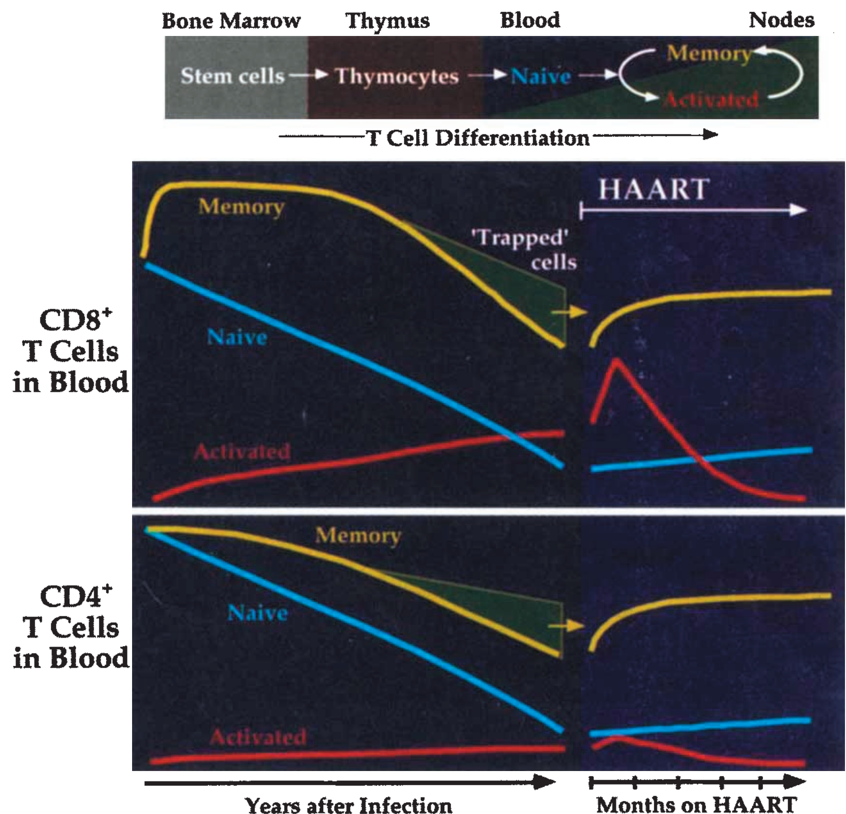
THE DISCOVERY (nearly fifteen years ago) that CD4 is the primary receptor that HIV uses to enter T cells provided a neat solution to the question of why CD4⁺ T cells are progressively lost during HIV disease. According to this theory, HIV infects CD4⁺ T cells, then lyses them during the productive phase of the viral life cycle. A direct corollary of this hypothesis is that removal of virus from the host should restore CD4⁺ T cells, leading to immunological recovery of the infected person. In early 1995, two *Nature* papers[1,2] purported to show exactly this result. Effective anti-retroviral therapy caused immediate and large increases in the numbers of CD4⁺ T cells—putatively, by reducing viral-induced cytolysis while maintaining high levels of CD4⁺ T cell proliferation. These reports received enormous publicity in the popular press, with vivid portrayals of a 'massive immunological war' in which billions of CD4⁺ T cells were produced and destroyed daily.

However, there has been considerable debate about this simple hypothesis. The *Nature* papers ignited a heated controversy that resulted in publication of several well-designed and informative studies, which raised serious doubts about this 'war'. In this issue of *Nature Medicine*, reports by Pakker *et al.*[3] and Gorochov *et al.*[4] provide the final nails in the coffin for models of T cell dynamics in which a major reason for changes in T cell numbers is the death of HIV-infected cells. The papers present extensive data on the remodeling of the T cell compartment in HIV-infected individuals after treatment with Highly Active Anti-Retroviral Therapy (HAART, a combination of three antiviral drugs including at least one protease inhibitor).

Through the early stages of HIV disease, numbers of CD4⁺ T cells decline whereas the total CD8⁺ T cell compartment expands (see figure). However, the recent application of flow cytometric techniques that accurately identify subsets of T cells, showed that this increase in the CD8⁺ T cell compartment is comprised entirely of memory and activated T cells[5]. In fact, within an individual, naive CD8⁺ T cells decline at the same rate as naive CD4⁺ T cells[6]. Because CD8⁺ T cells cannot be infected by HIV, and naive CD4⁺ T cells are relatively resistant to productive HIV infection[7–9], these declines cannot be directly attributed to HIV-mediated cytolysis. In the later stages of disease, both memory CD8⁺ and memory CD4⁺ T cells decline at similar rates[6].

Activated T cells are normally found only in peripheral tissues. Their expansion in the blood of HIV-infected adults is indicative of an active immune response even during the later stages of disease. The increase in the release of these cells into the blood during HIV disease might be caused by, for example, progressive damage to lymph node architecture, or homeostatic mechanisms that attempt to maintain peripheral T cell numbers to compensate for the loss of naive and memory T cells.

Within weeks after initiation of HAART therapy, there are significant increases in the numbers of B cells, and of CD4⁺ and CD8⁺ T cells in the blood. More refined immunophenotyping shows that the T cell increases are restricted to memory



T cell dynamics before and after successful HAART (highly active anti-retroviral therapy). (Top) Naive T cells, matured from precursors in the thymus, represent about 50% of T cells in the blood of healthy adults. Upon exposure to antigen, these cells divide and differentiate into activated effector cells in lymph nodes and other peripheral tissues. After resolution of the response, most activated T cells die by apoptosis, but a few resting memory T cells recirculate through the blood. (Bottom) Both naive CD4⁺ and naive CD8⁺ T cells decline during HIV disease progression, even though both are resistant to productive HIV infection. Memory CD4⁺ (and later, memory CD8⁺) T cells also decline at similar rates. Activated CD8⁺ cells increase throughout disease; activated CD4⁺ cells are typically only found in the late stages of disease. Successful HAART reduces viral burden >100-fold, relieving the immune response and causing many cells trapped in lymph nodes to become redistributed to the blood. Note that activated T cells have a very short lifespan (a few days) compared to memory or naive T cells (which have lifespans of up to many years).

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populations. In contrast, naive T cells and Natural Killer (NK) cells show no increase in blood after therapy. Importantly, the cell types that show HAART-induced increases (B cells and memory T cells) are those that can maintain long-term residence in lymph nodes. Naive T cells and NK cells do not dwell in nodes, and their numbers in blood do not immediately respond to HAART.

As Pakker *et al.*[3] argue in their paper, these data are powerful evidence that the increase in cell numbers observed shortly after initiation of HAART are caused by T cell redistribution and not T cell proliferation. The redistribution hypothesis—summarized by Mosier[10] and Sprent and Tough[11] in response to the two *Nature* papers[1,2]—states that during active viral replication and the concomitant cellular immune response, a large number of B and T cells may be 'trapped' in peripheral sites (for example, by antigen, cytokine, or chemokine signals). After initiation of HAART, when HIV is effectively removed from the system, the immune response begins to resolve, and cells pour out of the inflamed lymph nodes back into the blood.

Pakker and colleagues take this hypothesis one step further, and suggest that the degree of trapping increases as disease progresses (closely following on Mosier's prediction[10] that trapping increases as viral load increases). This simple hypothesis explains why the response to HAART tends to be greater in individuals with lower CD4+ T cell counts—a finding that could not be explained by models of proliferative expansion (in which recovery should be proportional to the number of cells).

Functional recovery of the T cell compartment is only complete when the repertoire of T cell receptors is restored, so that potentially all antigens can be recognized. The decrease in naive and memory T cell populations during disease progression means that the repertoire becomes increasingly restricted, finally resulting in immunodeficiency.

Gorochov and co-workers[4] confirm the findings of others[12] that the T cell receptor repertoire in HIV-infected individuals is significantly perturbed from the normal distribution found in healthy adults. This perturbation is caused by two distinct mechanisms: a loss of unique T cell clones (repertoire restriction), and an expansion of antigen-specific clones causing an over-representation of certain receptor types.

These investigators[4] further show that after successful therapy (that is, viral load reduction in response to HAART), the perturbation in the CD4 (but not CD8) repertoire is reduced. (Connors *et al.*[12] were unable to detect such a normalization in their subjects). The apparent increase in diversity (once virus is removed) most likely reflects the death of activated T cells, which include highly-expanded HIV-specific clones, resulting in a net normalization of the T cell receptor repertoire.

Perhaps the most exciting prospect is that the repertoire is becoming diversified through production of naive T cells. As first shown by Autran and colleagues[13] and confirmed by Pakker *et al.*[3], the number of naive T cells slowly increases over a six month period after initiation of HAART, demonstrating for the first time that this therapy may indeed allow for T cell reconstitution. Notably, this reconstitution occurs only in individuals who show reductions in viral load in response to HAART. It is also likely that failure of HAART, which occurs in many patients over time, will also be accompanied by a re-initiation of cell losses and repertoire restriction.

There are several important lessons to be learned from these experiments. First and foremost, we must always remember that the blood is an imperfect reflection of the immune system. It is not representative of peripheral tissues, and distributions of cell types between blood and those sites change dynamically during disease progression. This should come as no surprise to AIDS researchers, as Rosenberg and colleagues showed us nearly a decade ago that the drop in CD4+ T cell counts in blood is far greater than in the lymph nodes[14]. Furthermore, the end-stage of disease is signaled by an inverted CD4/CD8 ratio in the lymph nodes, an event which happens much later than in the blood[14].

Second, we must recognize that it is critical to measure, in as much detail as possible, the dynamics of *all* of the lymphocyte subsets during disease and therapy. Certainly, had the impact of HAART on the CD8+ T cell compartment been reported in the *Nature* papers[1,2], the immunological interpretations of the results would have been completely different.

Third, it is now apparent that we must pursue therapies aimed at immune reconstitution by targeting mechanisms of T cell production and homeostasis. A combination of HAART, to block viral replication, with a therapy that promotes active production of naive T cells (and a diversification of the T cell repertoire) will be critical for restoring long-term health in HIV-infected individuals.

Finally, the facts (1) that HIV uses CD4 as its primary receptor, and (2) that CD4+ T cell numbers decline during AIDS, are only an unfortunate coincidence that have led us astray from understanding the immunopathogenesis of this disease. HIV leads to the progressive destruction of all T cell subsets, irrespective of CD4 expression. Ultimately, AIDS is a disease of perturbed homeostasis. Only when we understand how the body regulates T cell numbers will we be able to find the mechanism(s) by which HIV destroys the immune system.

1. Ho, D. D. *et al.* Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373**, 123–126 (1995).
2. Wei, X. *et al.* Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* **373**, 117–122 (1995).
3. Pakker, N. G. *et al.* Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: A composite of redistribution and proliferation. *Nature Med.* **4**, 208–214 (1998).
4. Gorochov, G. *et al.* Perturbation of CD4 and CD8 T-cell repertoires during progression to AIDS and regulation of the CD4 repertoire during anti-viral therapy. *Nature Med.* **4**, 215–221 (1998).
5. Roederer, M. *et al.* CD8 naive T cell counts decrease progressively in HIV-infected adults. *J. Clin. Invest.* **95**, 2061–2066 (1995).
6. Roederer, M., De Rosa, S. C., Herzenberg, L. A. & Herzenberg, L. A. Immunology of T cells in AIDS: Dynamics revealed by eight-color flow cytometry. In *Immune Modulating Agents* (ed. Kresina, T.) 209–220 (Marcel Dekker, Inc., New York, 1998).
7. Woods, T. C., Roberts, B. D., Butera, S. T. & Folks, T. M. Loss of inducible virus in CD45RA naive cells after human immunodeficiency virus-1 entry accounts for preferential viral replication in CD45RO memory cells. *Blood* **89**, 1635–1641 (1997).
8. Spina, C. A., Prince, H. E. & Richman, D. D. Preferential replication of HIV-1 in the CD45RO memory cell subset of primary CD4 lymphocytes *in vitro*. *J. Clin. Invest.* **99**, 1774–1785 (1997).
9. Roederer, M., Raju, P. A., Mitra, D. K., Herzenberg, L. A. & Herzenberg, L. A. HIV does not replicate in naive CD4 T cells stimulated with CD3/CD28. *J. Clin. Invest.* **99**, 1555–1564 (1997).
10. Mosier, D. (Letter). *Nature* **375**, 193–194 (1995).
11. Sprent, J. & Tough, D. (Letter). *Nature* **375**, 194 (1995).
12. Connors, M. *et al.* HIV infection induces changes in CD4+ T-cell phenotype and depletions within the CD4+ T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. *Nature Med* **3**, 533–540 (1997).
13. Autran, B. *et al.* Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science* **277**, 112–116 (1997).
14. Rosenberg, Y. J. *et al.* Variation in the CD4+ and CD8+ populations in lymph nodes does not reflect that in the blood during SIVMNE/E11S infection of macaques. *J. Med. Primatol.* **21**, 131–137 (1992).

Beckman Center, B011
Stanford University School of Medicine
Stanford, California 94305–5318, USA
email: Roederer@Stanford.Edu