A National Tissue Bank to Track HIV Eradication and Immune Reconstitution

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In individuals with human immunodeficiency virus–type 1 (HIV-1) infection, recent antiretroviral drug combinations have proven remarkably successful at suppressing virus production and reducing lymphoid tissue viral reservoirs to levels near or below the limits of detection by existing assays (*1*). With such strong viral suppression, the immune system may regain sufficient lost ground to once again ward off opportunistic pathogens, accounting in part for the recent decline in acquired immunodeficiency–(AIDS)–related deaths in some countries (*2*). Total victory, though, with elimination of the virus and full immune system reconstitution, remains a hoped-for but as-yet-unattained objective. HIV-1 persists in latently infected cells for at least 30 months after initiation of antiviral therapy (*3*), and if such treatment is discontinued, viral load rebounds to pretreatment levels (*4*). HIV-1 may also find refuge in organ sanctuaries; for example, behind the blood-brain barrier where diminished drug concentrations in the central nervous system (CNS) might induce the development of drug-resistant mutants (*5*).

The question of how to attack these documented or putative reservoirs has generated considerable discussion (*6*). One strategy is to stay the course, suppressing active viral replication for the estimated 5 or more years it might take for the latently infected cells to die out. A more activist approach endorses various strategies of immune system stimulation to "flush" hidden HIV-1 out of latently infected cells while under antiretroviral cover to forestall spreading of the roused virus. Either approach poses difficulties. The cost, complexity, and toxicity of current antiretroviral regimens pose vexing issues for waiting strategies, and the prospects for flushing HIV-1 from latently infected cells are uncertain. In either case, both approaches require careful evaluation, like the scope of the problem in the first place, by methods that accurately track infected cells throughout the body. A major issue here is constraints on sampling (Fig. 1). The peripheral bloodstream and cerebrospinal fluid (CSF) reflect to some extent infection in lymphoid tissue (LT) and the CNS, respectively (*1*, *7*). However, assaying these fluids cannot give the same assurance that HIV-1 has been eradicated as an exhaustive survey of organ systems and deep anatomical structures would. Sampling is also an issue in assessing immune reconstitution. Early increases in $CD4^+$ T cells in the blood that are associated with treatment may reflect redistribution from other sites (*8*). The later slow expansion of naïve $CD4^+$ T cells peripherally and in LT is encouraging, but to date the T cell receptor repertoire remains abnormal (*9*). Furthermore, evidence suggests that the pace and extent of immune system renewal vary considerably among treated people. Central questions of how HIV-1 depletes $CD4^+$ T cells and alters T cell homeostasis and about the mechanism and capacity for T cell regeneration in the adult thus remain unanswered. Investigating T cell populations in the gut, lung, and other sites where they may be sequestered, and further examination of the thymus and bone marrow, probably would yield substantial new insights about the human immune system. Once again, such information would help to weigh a waiting strategy against more active interventions such as thymic transplantation or cytokine-induced expansion of T cells.

Are there practical ways to extensively survey organ systems and thus obtain the data needed to guide efforts to eliminate HIV-1 and rehabilitate the immune system? One suggestion, perhaps with Swiftian overtones, is to create a tissue bank of

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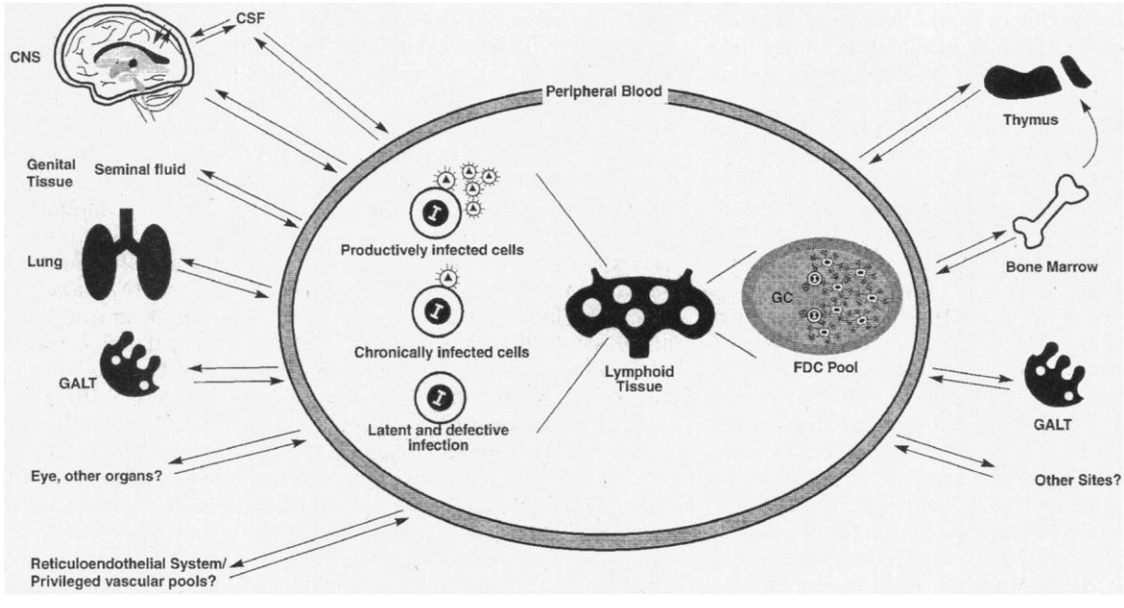

Fig. 1. Cellular and systemic reservoirs of HIV-1 infection and immune reconstitution. HIV-1 is produced and stored in immune complexes bound to follicular dendritic cells (FDCs) in LT. These tissues and others shown in the figure, such as the CNS, are also sites where virus may persist in long-lived latently or chronically infected cells. Most memory or naïve $CD4^+$ T cells generated by thymopoiesis or other renewal mechanisms reside in LT as well. The correspondence between viral and T cell populations in tissues and the more accessible tissue fluids such as peripheral blood is inexact. A bank of tissues from lymphoid and other organs would provide a better opportunity to address issues of viral eradication and immune reconstitution. GALT, gut-associated lymphoid tissue.

specimens from HIV-1–seropositive individuals who have been treated effectively but suddenly die in vehicle accidents or from other traumas or causes (6). We envision a federal repository of HIV research tissue, funded and organized perhaps by the National Institutes of Health (NIH) and a consortium of governmental and private agencies, located at a single site with a national or international scope for specimen acquisition and distribution. This resource would be advertised to emergency rooms, surgical services, and medical providers likely to encounter victims of trauma or other acute fatal conditions and would function (on a smaller scale) like the United Network for Organ Sharing [UNOS (*10*)] for HIV-infected individuals who are excluded from transplant organ donation. HIV care clinics could offer patients the opportunity to carry cards identifying a willingness to become a "scientific tissue donor" in the event of untimely death. Tissues would be obtained from HIV-1–positive individuals, both treated and untreated controls, by attending physicians who would telephone a 24-hour hotline for emergency consent forms and instructions on harvesting, preserving, and shipping tissues along with blood and CSF, plus a relevant medical history to the repository. Because retention of cellular morphology and small quantities of labile viral nucleic acids is critical, establishing the infrastructure for meticulous tissue preservation by snap freezing and rapid fixation is of paramount importance. A central repository would provide the coordination to ensure uniformity and quality in processing samples. The specimens would then be catalogued, stored, and made available to all qualified investigators via a distribution system maintained by the repository.

The uncertain potential for eradication and immune reconstitution in response to antiretroviral therapy renders even more pressing the need to implement the recommendation of the NIH AIDS Research Program Working Group to develop a central repository of biological specimens (*11*). Based on sudden-accident mortality statistics and national HIV-1 seroprevalence data, we estimate that it might be possible to obtain tissues from 10 to 30 cases for this critical resource over a 2-year period at an estimated cost of $1 million per annum. In our view, the tissue bank proposal is responsive to the urgent need for such a repository. And it has reasonable prospects for success, drawing as it does on the inspiring history, throughout the AIDS pandemic, of the many HIV-1–seropositive individuals who have contributed to the advances in knowledge on which current successes and future hopes are based.

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Toward HIV Eradication or Remission: The Tasks Ahead

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With the advent of combination therapy, it is now possible to achieve durable control of human immunodeficiency virus–type 1 (HIV-1) replication in vivo. This development has led to a substantial decline in AIDS incidence and mortality in the United States in the past 2 years (*1*). The potent antiretroviral agents have also served as a tool to define the kinetics of HIV-1 turnover in infected persons (Fig. 1). When de novo infection is inhibited by drug treatment, cell-free virions are cleared rapidly and productively infected CD4 lymphocytes die after a short life-span (*2*, *3*). Complete elimination of these viral pools could be expected in ~2 months. But slower decay rates have been estimated for additional compartments carrying HIV-1, including infected tissue macrophages, CD4 lymphocytes that harbor infectious genome in a pre-integrated form, and virions trapped on follicular dendritic cells in lymphoid tissues (*4*, *5*). Nevertheless, mathematical projections suggest that these pools could also be eliminated if effective treatment is continued for 2 to 3 years (*4*), thereby raising the possibility of eradication. Recent studies show, however, that infectious HIV-1 persists latently in resting, memory CD4 lymphocytes in a post-integrated form despite 1 to 2 years of combination therapy (6). This latent reservoir of HIV-1, denoted L (Fig. 1), represents the major documented hurdle to virus eradication, although other obstacles such as viral sanctuaries may exist (*7*).

How do such virus-carrying CD4 lymphocytes arise? Do they represent rare survivors of productive infection? Or were they infected at a particular juncture during reversion from an activated state to the resting state? These questions remain unanswered, but it is known that L is small in size, generally ranging from 10^4 to 10^6 cells per host (8) and typically no larger than 10^7 cells (9). Its decay rate, μ (Fig. 1), has not been directly measured with any degree of accuracy, although several studies are now under way. It is known, nonetheless, that memory CD4 lymphocytes have a mean half-life ($t_{1/2}$) of ~3 to 4 months (*10*), which should mirror μ. Although much of the proviral DNA harbored within memory CD4 lymphocytes of infected persons exists as defective forms (9), the decay rate of this DNA may, nevertheless, serve as a surrogate to estimate μ. With this in mind, the decay $t_{1/2}$ of proviral DNA in peripheral blood mononuclear cells of patients receiving effective therapy has been found to be ~3 to 5 months (*4*, *11*), consistent with previous $t_{1/2}$ estimates of memory lymphocytes (*10*). Simple calculations based on these numbers are quite revealing. Approximately 14 to 20 half-lives are required for a pool size of 10^4 to 10^6 to decay to <1. Using 4 months as the $t_{1/2}$, it follows that 5 to 7 years of continuous, completely inhibitory therapy will be necessary to eliminate L. Treatment interruptions that permit HIV-1 replication to resume will rapidly restore the size of L. For larger pool sizes or greater values of $t_{1/2}$, more than 10 years of continuous treatment will be required. A treatment duration this protracted is unacceptable because of the complexity, toxicity, and cost of the current drug regimens, especially when the concept of "maintenance therapy" with a simplified regimen does not seem viable (*12*).

Increasing μ while continuing antiretroviral therapy is a strategy that should be explored. Activation of resting cells results in HIV-1 replication and cell death, whereas the spread of virus remains inhibited by antiretroviral agents. Infectious proviruses are undoubtedly harbored within a diverse

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