

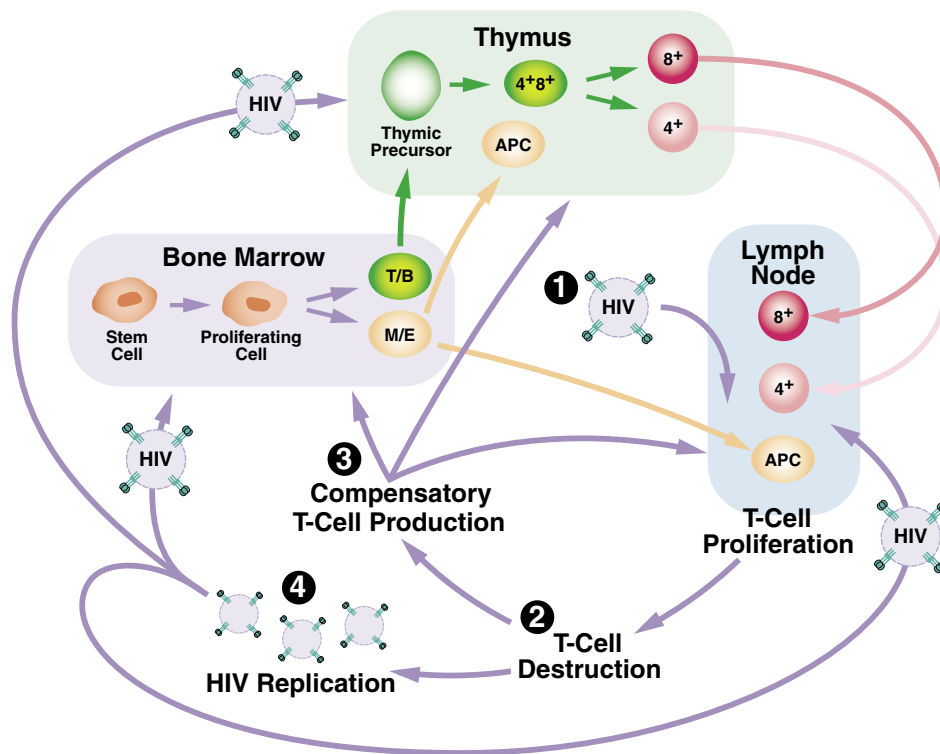
T-Cell Depletion in HIV Disease

The Gladstone Connection

HIV infection leads to a depletion of disease-fighting CD4+ T cells and to the acquired immune deficiency syndrome, or AIDS. Yet, despite years of intensive study, a central question remains: what causes the loss of these vital cells? An answer to this question would facilitate efforts to prevent T-cell destruction in newly infected patients. Reciprocally, the answer might provide clues about reconstituting the immune system of patients with more advanced disease. Research in the laboratory of Joseph M. (“Mike”) McCune at the Gladstone Institute of Virology and Immunology has focused on this question, providing new insights about the disease-causing properties of HIV and about how to reverse them.

The Flow Chart of Destruction and Production

HIV causes T-cell depletion either by increasing the rate of destruction of pre-formed cells (“accelerated destruction”) or by inhibiting the production of new cells (“regenerative failure”). Good evidence exists that each might occur in a



Accelerated T-cell destruction during HIV infection leads to impaired production. The figure depicts a model of disease progression and CD4+ T-cell destruction in three organ systems that produce blood and immune cells. The bone marrow contains stem cells that generate immune (T/B) and blood (M/E) cells. The thymus supports the differentiation of T cells from thymic precursors and selects mature CD3+CD4+ (4+) and CD3+CD8+ (8+) thymocytes, relying in part on antigen-presenting cells (APCs). In the peripheral lymphoid

organs, immune responses by T and B cells are coordinated by APCs. Many of the cells represented here express the cell-surface receptors that form the HIV receptor complex (i.e., CD4 and chemokine coreceptors). HIV infection influences the structure and function of these organs in many different ways. HIV is probably concentrated in draining lymph nodes (1) where it attracts T cells and induces vigorous T-cell proliferation. Such immune activation is associated with accelerated destruction of T cells (2). To compensate

for this loss, “sensor cells” in lymphoid organs may release signal molecules (e.g., interleukin-7) to increase T-cell production at multiple sites (3). These signals may in turn accelerate viral replication and result in the emergence of highly cytopathic variants, leading to the destruction of key progenitor cells in the bone marrow, thymus, or peripheral lymphoid organs (4). Impaired production of new cells from these organs results in the collapse of the immune system and conversion of a high-turnover state to a low-turnover state.

Causes of T-Cell Depletion

Destruction of mature CD4+ T cells

Direct destruction
of infected cells by virus

Indirect destruction
of uninfected cells

Destruction by HIV-specific
cytolytic T cells
or natural killer cells

Autoimmune reactions,
either cellular or humoral

Incorporation into giant cells
by neighboring infected cells

Impaired T-cell production

Direct destruction of blood-forming
progenitor cells or their supporting
environments

Opportunistic infections
of bone marrow

Infiltrating malignancies

Toxic effects of drugs

Deficiencies of vitamins
and other essential factors

given patient, either simultaneously or in sequence. The crux of the issue has been how to measure the relative contributions of accelerated destruction and regenerative failure over time.

To address this question, it is important to remember that the blood system is continuously renewed. Long-lived blood-forming stem cells reside in the bone marrow (or, during fetal life, in the liver), giving rise to short-lived cells that rapidly divide and mature into each of the important blood cell types found in the circulation, including oxygen-carrying red blood cells, bacteria-fighting neutrophils, and lymphocytes, which provide protection against a multitude of infectious agents. T cells, an important subpopulation of lymphocytes, require a period of development in the thymus to fully mature. Most active in early life, the thymus generates a cadre of cells that seed and populate the peripheral lymphoid organs of the body, warding off invading microorganisms throughout life.

Perturbation in the Flow

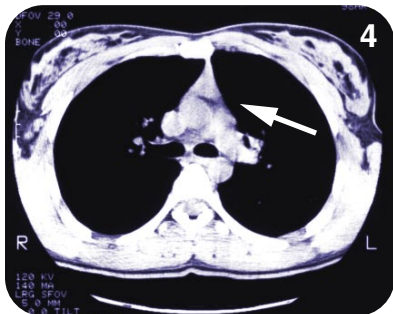
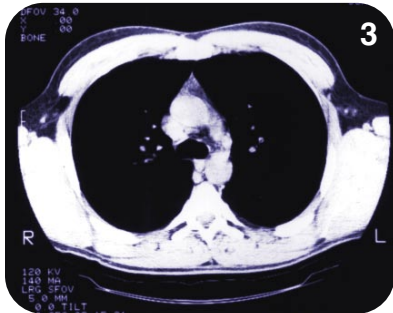
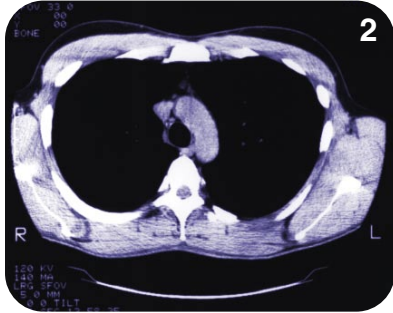
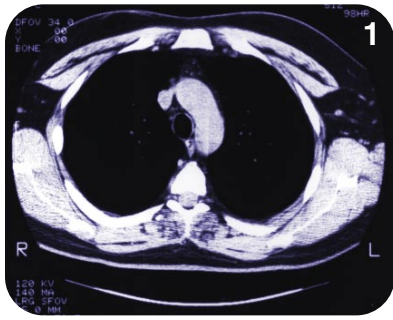
In HIV infection, the immune system itself is attacked. Mature T cells are killed, leading to a clarion call to the bone marrow and thymus to produce replacement T cells. If the rate of production offsets the losses, all is well. However, if the bone marrow and thymus are infected and thereby compromised, their regenerative capacity may be diminished and the floor will fall out. T-cell depletion ensues.

This scenario forms the working hypothesis underlying many of the studies in the McCune laboratory: that HIV induces accelerated destruction of mature T cells in the periphery, resulting in subclinical disease that erupts into full-blown AIDS and massive T-cell depletion when the production side of the blood-forming system is lost.

To test this hypothesis, Dr. McCune and his colleagues have developed new tools to evaluate human blood formation and its pathology, to monitor human thymic structure and function, and to study the “turnover” of T cells in humans. These tools have allowed them to better visualize the processes of HIV-mediated T-cell depletion in infected patients and have yielded insights into otherwise obscure arenas of human biology.

The SCID-hu Thy/Liv Mouse—A Small-Animal Model for Evaluating Human Hematopoiesis

Since it is difficult to study cells from the bone marrow and thymus of humans, Dr. McCune and his colleagues created a small-animal model of the human blood-forming system. This model is generated by surgically implanting small (1–2 mm³) pieces of human blood-forming organs (including the liver, containing multilineage human blood-forming stem



Images obtained from computed tomography allow for the noninvasive visualization and measurement of the human thymus. The images show a pie-slice view across the chest of different individuals. The backbone is on the bottom. The breastbone is on the top. The dark areas in between represent air-filled lungs surrounding the heart. The thymus, best seen in the bottom figure (arrow), is the triangular white area between the heart and the breastbone. In this individual, it is large and given a score of 4. In the individuals in the films above, it is increasingly smaller and given a smaller score.

cells, and the thymus, containing all of the cues required for T-cell maturation) into the otherwise immunodeficient SCID mouse.

With its human blood-forming system, the SCID-hu Thy/Liv mouse has facilitated the analysis of HIV infection. For example, studies by Dr. Morgan Jenkins and Mary Beth Hanley in the McCune laboratory revealed that HIV infection short-circuits the early stages of blood formation, depleting early progenitor cells that normally move into the thymus. As a result, T-cell production can no longer occur. Other studies have shown that cells within the thymus can also be destroyed. These findings are consistent with the hypothesis that HIV infection causes regenerative failure in infected humans.

Visualizing and Measuring Thymic Function

The thymus rests in one of the most well-protected and sensitive parts of the body: in front of the heart, behind the breastbone, and surrounded by the lungs. Not surprisingly, it is not an easy organ to study. Yet, since HIV depletes T cells in young and older people, it is important to understand whether and how HIV disease affects the thymus. Is it a site for infection? Is it destroyed? Can it regenerate after effective antiretroviral treatment is initiated?

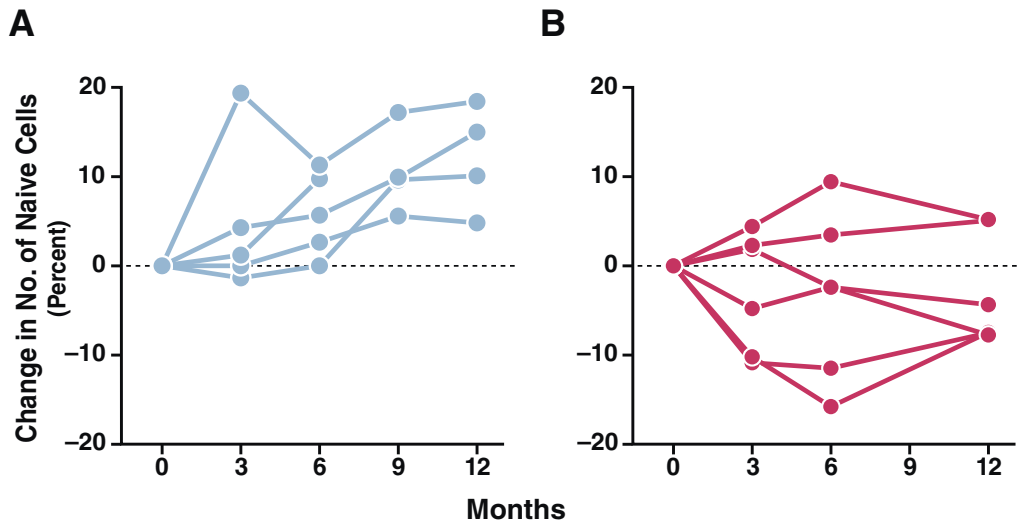
To address these questions, Dr. McCune and his colleagues, including Dr. Michael Gotway of the San Francisco General Hospital Department of Radiology, used computed tomography (CT) to visualize and measure the human thymus. Unexpectedly, they found that the thymus is larger, not smaller, in many adults with HIV disease than in uninfected, age-matched controls.

Tantalizing possibilities are raised by this observation. First, the body may be able to upregulate thymic function and, hence, T-cell production in times of need. Second, although the adult thymus is usually very small (if present at all), it may be “turned on” anew. These possibilities are a focus of research by Dr. Laura Napolitano and her colleagues. CT studies of this type have also made it clear that the thymus is destroyed in later stages of HIV disease, again underscoring the hypothesis of regenerative failure.

Measuring T-Cell Turnover in HIV-Infected Humans

Normally, T cells are produced at a rate that equals their rate of destruction and their number does not change much from day to day. If, however, the rate of production changes and the rate of destruction does not, the total number of T cells in the system will also change. A more complete understanding of this balance sheet would help to explain the mechanisms of T-cell depletion in HIV disease.

To do so, Dr. McCune and Dr. Marc Hellerstein (of UCSF and UC Berkeley) devised a new technique to safely measure T-cell turnover in humans. Using this technique to evaluate patients at various stages of HIV disease and treatment, they found that the rate of production of some cells is increased and that of others is decreased. Importantly, in later stages of HIV disease, long-lived T-progenitor cells are lost and the immune system can no longer regenerate itself.



Growth hormone therapy is associated with an increase in naive CD4+ T cells (A). Naive CD4 T cells increased significantly in the GH cohort in comparison to the pre-GH baseline ($p < 0.05$ at months 6, 9, and 12 versus baseline). (B) No significant changes have occurred in untreated naive CD4 T cells or in treated and untreated CD8 cells (data not shown).

Summary

As questions are answered, new ones take their place. How can we prevent the destruction of these long-lived cells? And how can we restore them? The answers to these and related questions about T-cell production will have implications for the prevention and treatment of HIV disease and for our understanding and treatment of other significant human disease states, such as the immunodeficiency associated with chemotherapy for cancer and bone marrow transplantation. Further work in the McCune laboratory will be aimed at moving these answers toward a theoretical framework with practical applications.

Related Work at Gladstone

Dr. McCune has had productive collaborations with several Gladstone colleagues. Key among these are Drs. Laura Napolitano and Cheryl Stoddart.

Dr. Napolitano's research focuses on immune-based strategies to augment T-cell production and restore the immune system in HIV-infected individuals. With Dr. McCune, she showed that growth hormone (GH) appears to reverse thymic involution and facilitate immune reconstitution in HIV-infected adults. These findings suggest that it may be possible to induce new T-cell production during HIV infection. A larger clinical study of GH therapy is in progress.

Dr. Stoddart's research has concentrated on identifying and evaluating new antiretroviral drugs in the SCID-hu Thy/Liv mouse model developed by Dr. McCune. Recently, she studied a lead drug candidate from the newly discovered class of antiretrovirals termed maturation inhibitors. This small-molecule inhibitor blocks cleavage of p2 from the p25 capsid precursor, resulting in noninfectious virus particles.