AIDS Rev 2002;4:157-164

Immune Restoration after Treatment of HIV-1 Infection with Highly Active Antiretroviral Therapy (HAART)

Hernan Valdez

Center for AIDS Research, Case Western Reserve University and University Hospitals, Cleveland, Ohio, USA

Abstract

The availability of combination antiretroviral therapy (HAART) has been associated with dramatic decreases in HIV-related morbidity and mortality. These clinical benefits are probably mediated by a decrease in HIV-1 replication and an increase in the number and function of peripheral blood CD4+ lymphocytes. Despite many years of maintaining plasma HIV-1 RNA levels below the limits of detection, many patients do not achieve normal CD4+ lymphocyte counts. A larger proportion of patients who delay HAART for longer have incomplete numerical CD4+ restoration compared to patients who start therapy earlier. Even in patients who normalize their CD4+ lymphocytes insert counts, immune function remains impaired among those who delay HAART for longer periods. Whether subclinical immune deficiency will be associated over longer periods of follow-up with adverse clinical outcomes such as an increased number of infections and malignancies remains to be determined. If prolonged subclinical immunodeficiency is associated with adverse outcomes, the use of immune-based therapies may benefits patients while helping us ascertain the residual deficits responsible for incomplete immune restoration.

Key words

Antiretroviral therapy. CD4+ lymphocytes. Lymphocyte function. Immunization. Immune restoration.

Introduction

Infection with HIV-1 results in a progressive loss in the number and function of CD4+ lymphocytes that puts patients at risk of developing opportunistic infections and neoplasms[1-4]. The introduction of potent combination antiretroviral therapy for HIV-1 infection in the mid-1990s resulted in dramatic declines in HIV-1 associated morbidity and mortality[5-8]. Combinations of antiviral medications (highly active antiretroviral therapy or HAART) have become the standard of care for HIV-1 infection[9].

Shortly after the initiation of HAART there is a rapid decline in the incidence of opportunistic infections[5,6]. Nevertheless, despite continued long-term suppression of HIV-1 replication and a slow but continued rise in the number of CD4+ cells, many patients who start HAART during moderately advanced disease do not achieve normal numbers of peripheral blood CD4+ cells[10,11]. In addition, many other lymphocyte subpopulation abnormalities that correlate with immune dysfunction re-

Correspondence to:
Hernan Valdez
Center for AIDS Research
Case Western Reserve University and University Hospitals of Cleveland
2061 Cornell Road, Room 401B
Cleveland, OH 44106 USA
Phone: 216-844-2057 - Fax: 216-844-5523
E-mail: valdez.hernan@clevelandactu.org

main[11,12]. It is unknown whether these persistent abnormalities will resolve with continued suppression of HIV-1 replication. More importantly, whether this subclinical immune deficiency will translate into adverse clinical outcomes remains unknown. In this regard, patients with hematological malignancies who experience incomplete immune restoration after chemotherapy remain at heightened risk for acquiring opportunistic infections[13,14].

This review will describe the numerical and functional immune restoration seen in patients who achieve successful long-term control of HIV-1 replication. We will show that immune restoration is incomplete and that the proportion of patients who achieve incomplete immune restoration is larger the longer therapy is delayed. We propose that by conducting well-designed clinical trials aimed at normalizing immune function in patients who exhibit incomplete restoration we hold the promise of offering clinical benefit to these patients while we learn about the dysfunctions responsible for this incomplete restoration. Also, we will be able to explore new ways of testing the utility of immune-based therapies.

Mechanisms of Peripheral Blood CD4+ Cell Recovery

First Phase Rise in CD4+ Lymphocytes. Shortly after HAART initiation there is a rapid rise in the number of peripheral blood CD4+ lymphocytes. This rise occurs during the first twelve weeks after HAART initiation and represent between 50 and 75% of the CD4+ cell rise an average patient will experience during the first year of therapy[15]. Initial redistribution of lymphocytes from lymph nodes into circulation appears to be responsible for this initial rise; lymphocyte redistribution is supported by mathematical models[16] and by the observation that only lymphocyte subpopulations present in lymph nodes (T-cells and B-cells, but not NK cells) increase in peripheral blood during this first phase[17]. During untreated HIV-infection there is an increase in the expression of proinflammatory cytokines in lymph nodes[18] that results in increased expression of adhesion molecules[19] and "trapping" of lymphocytes in lymph nodes. Many of these lymphocytes will probably die due to activation-induced cell death[20]. A few weeks after starting HAART the expression of proinflammatory cytokines, adhesion molecules, and the proportion of apoptotic lymphocytes in lymph nodes decrease substantially (but does not normalize), in parallel with the increase in circulating CD4+ lymphocytes[18-20].

Concurrently, there is evidence of improved lymphocyte function as evidenced by improved delayed-type hypersensitivity and lymphoproliferative responses to recall antigens such as *Candida* and cytomegalovirus[15,21]. In summary, most of the decline in risk of opportunistic infections occurs at a time when the bulk of numerical and functional immune restoration is due to redistribution of lymphocytes from lymph nodes into the circulation after decreasing HIV-1 replication and its attendant proinflammatory state.

Second Phase Rise in CD4+ Lymphocytes. After the first 12 weeks of therapy peripheral blood CD4+ lymphocytes continue to rise, but a considerably slower pace[10,11,15,16,21-24]. This modest continued increase in CD4+ lymphocytes appears to persist for at least 4 years after HAART initiation, provided that HIV-1 replication remains controlled[25,26]. This second phase rise in CD4+ lymphocytes is mainly accounted for by an increase in *naïve* CD4+ lymphocytes, while memory CD4+ cells stabilize or increase only modestly[11]. Given that during this second phase there is a continued increase in the number of lymphocytes that carry T-cell receptor excision circles (TREC)[27,28], a marker of recent thymic emigration, it is thought that this phase represents continued thymic production. Whether thymic function improves after HAART or whether the increase in TREC+ cells just represents residual thymic production after removal of cellular trapping and destruction remains to be determined. In addition, since at least during the first year after HAART initiation CD4+ cells repopulating peripheral blood are programmed to traffic to lymph nodes, residual redistribution may contribute to the second phase CD4+ lymphocyte rise[29]. In contrast to the marked improvements in lymphocyte function seen during the initial weeks of HAART, immune function, as measured by lymphocyte proliferation or delayed-type hypersensitivity responses improve little, if at all, during the subsequent years of HAART administration[11].

Delayed HAART Initiation may Blunt or Delay Numerical CD4+ Cell Restoration

Several studies have been published describing the magnitude of immune restoration in patients receiving HAART for up to 4 years[10,11,15,16,22,23,26,30-34]. The table shows the magnitude of immune restoration in subjects who have controlled HIV-1 replication in 75% or more of the viral load determinations after initiation of therapy. This table shows that persons who start HAART earlier during the course of disease are more likely to attain normal numbers of peripheral blood CD4+ lymphocytes. In addition, the expansion of CD8+ lymphocytes, the increase in CD8+ cell activation, and the decrease in CD28 expression on CD4+ and CD8+ lymphocytes all approach normal values faster and get closer to values seen in HIV-uninfected subjects the earlier HAART is initiated. It is not known if with prolonged follow-up, patients who start HAART during advanced HIV infection will be able to normalize all lymphocyte phenotypic abnormalities. Two small studies suggested that after about one year of HAART the increase in CD4+ lymphocytes tend-

Table 1. *Immunologic changes in patients who maintain suppression of HIV-1 replication*

Characteristics/Ref.		[30]	[33]	[34]	[32]	[16]	[11]	HIV (-) [11]
Number of patients		8	28	39	32	19	20	19
Stage of infection		Acute	Acute	Early	Early	Advanced	Advanced	NA
CD4+*	PreHAART	451	470	792	756	170	226	NA
	PostHAART	761	758	1398	898	420	423	865
CD8+*	PreHAART	1880	1060	1392	1221	960	815	NA
	PostHAART	NS	NS	791	985	NS	771	595
Ratio*	PreHAART	0.4	NS	0.72	0.7	NS	NS	NA
	PostHAART	"Normal"	NS	2.02	1.2	NS	NS	NS
% normal CD4**		88%	93%	NS	NS	NS	35%	NA
Length of follow up		44 weeks	12 months	12 months	72 weeks	108 weeks	3 years	NA
Other markers		Normal CD28 on CD4	Trend to normalization of activation and CD28 expression	Persistent ↓ of CD28 on CD8	Trend to normalization of % of CD4 cells on lymph nodes	Persistent abnormal activation and ↓ expression of CD28	Persistent abnormal activation and ↓ expression of CD28	

*Values represent medians or means as presented in the original publications. CD4+ and CD8+ lymphocytes are expressed in cells/μl. **Normal was defined as above 500 CD4+ cells/μl. PHI: primary HIV infection. NS: not stated; NA: not applicable.

ed to stop in patients who started therapy during advanced HIV-1 infection[11,16]. A larger cohort study suggests a continuous rises in CD4+ lymphocytes after 4 years of therapy, even in patients who start HAART with fewer than 50 CD4+ lymphocytes/ml[26]. Whether CD4+ lymphocyte counts and other phenotypic abnormalities will eventually normalize in these subjects remains to be seen.

Immune Function Remains Impaired, Even in Subjects who Normalize CD4+ Lymphocytes

Our group has been using the ability to respond to immunization as a gauge of immunologic competence. Conceptually, the development of an immune response to an immunogen is similar to the immune response needed to control an infectious agent. In both situations antigen-presenting cells recognize and present foreign antigens to lymphocytes, and these in turn generate cellular and humoral immune responses. In one case the outcome is resolution (or not) of the infection, which depends on the development (or failure) of an adequate immune response. In the case of immunization, we use the generation of humoral and cellular responses as the outcome. Using this approached we showed that even after more than 78 weeks of HAART, responses to immunization with recall and neo-antigens was impaired in HIV-infected patients, when compared to HIV-seronegative controls[12]. In addition, we showed that responses to immunization among HIV-infected subjects receiving HAART are heterogeneous yet predictable. Subjects with controlled viral replication, with higher *naïve* and memory CD4+ cells at the time of immunization, with higher expression of CD28 on CD4+ cells, and with decreased CD4+ cell activation were more likely to respond to immunization[12]. Using this approach we tested immune function in patients with the best responses to HAART: those with viral loads consistently below the limit of detection and who had achieved "normal" CD4+ cell counts[35]. At the time we studied these patents, they had been receiving HAART for longer than 3 years and had CD4+ lymphocyte counts in the normal range (median 730 cells/μl), not significantly different from counts in HIV-seronegative controls. Of note, the range of nadir CD4+ cell counts in the patients was very broad (20-506 cells/μl) and the median nadir CD4 was 250 cells/μl. Patients with a higher nadir CD4+ cell count and patients with increased expression of CD28 on CD4+ cells at the time of immunization were more likely to respond to immunization with recall and neo-antigens. No nadir CD4+ cell threshold was found above or below which the likelihood of response to immunization increased or decreased dramatically.

These findings have 2 important implications: even if patients normalize CD4+ cell counts, immunologic function may remain impaired if patients delay initiation of HAART. In addition, the earlier HAART is initiated, the more likely that immune phenotype and function will normalize. Recently, a large cohort study of patients starting HAART has shown that the 3-year prognosis of patients who start HAART with more than 350 CD4+ cells/μl is marginally but significantly better than those who start HAART with CD4+ ranging from 200 to 349 cells/μl, using an endpoint of death and new AIDS-defining events. Whether earlier initiation of HAART will ultimately be associated with clinical benefit after longer follow-up, or whether metabolic toxicities and antiretroviral failure will maim the benefits of early HAART initiation remains to be determined.

Hypothesis-Driven Clinical Trial Design will Help Identify Persistent Immunologic Lesions and May Offer Clinical Benefit

Potential explanations for incomplete immune restoration include residual viral replication, bone marrow insufficiency, thymic insufficiency, persistent trapping of cells in lymphoid organs, and irreversible damage to one or more lymphopoietic organs after prolonged uncontrolled HIV-1 replication. As we will discuss, we currently have immune-based therapeutics that can help us address the cause of incomplete immune restoration while performing studies aimed at magnifying the rise in CD4+ lymphocytes caused by controlling viral replication with HAART alone (Fig. 1). Many studies have shown that low level HIV-1 replication persists even in patients with plasma HIV-1 RNA levels below the limit of detection of currently available assays[36-38]. In addition, in one study patients with more residual replication, as measured by quantification of 2-LTR circles, had fewer peripheral blood CD4+ lymphocytes[37]. Trials of intensification of antiretroviral therapy currently underway will address whether residual HIV-1 replication accounts for the failure to completely restore CD4+ cell numbers, for the persistent increased immune activation, and for the persistent decrease in CD28 co-expression[11].

The bone marrow is the source of T lymphocyte precursors. It is known that bone marrow function is impaired during untreated HIV-1 infection[39]. Bone marrow function improves after HAART administration[40,41]. Whether bone marrow insufficiency accounts for the slow and blunted increase in CD4+ lymphocytes in people who successfully control HIV-1 replication remains to be determined. In patients receiving HAART who had incomplete suppression of HIV-1 replication, administration of 250 μg of GM-CSF subcutaneously (SQ) thrice weekly produced an increase of only 19 CD4+ cells after 16 weeks, compared to no increase among placebo recipients[42]. It is unknown whether use of GM-CSF in HAART-treated patients with controlled viral replication or in patients receiving other cytokines, such as interleukin-2 (IL-2), will produce larger CD4+ lymphocyte rises.

Pre-T lymphocytes migrate to the thymic cortex, were they rearrange their T-cell receptor genes. Subsequently, CD4+CD8+ double positive lym-

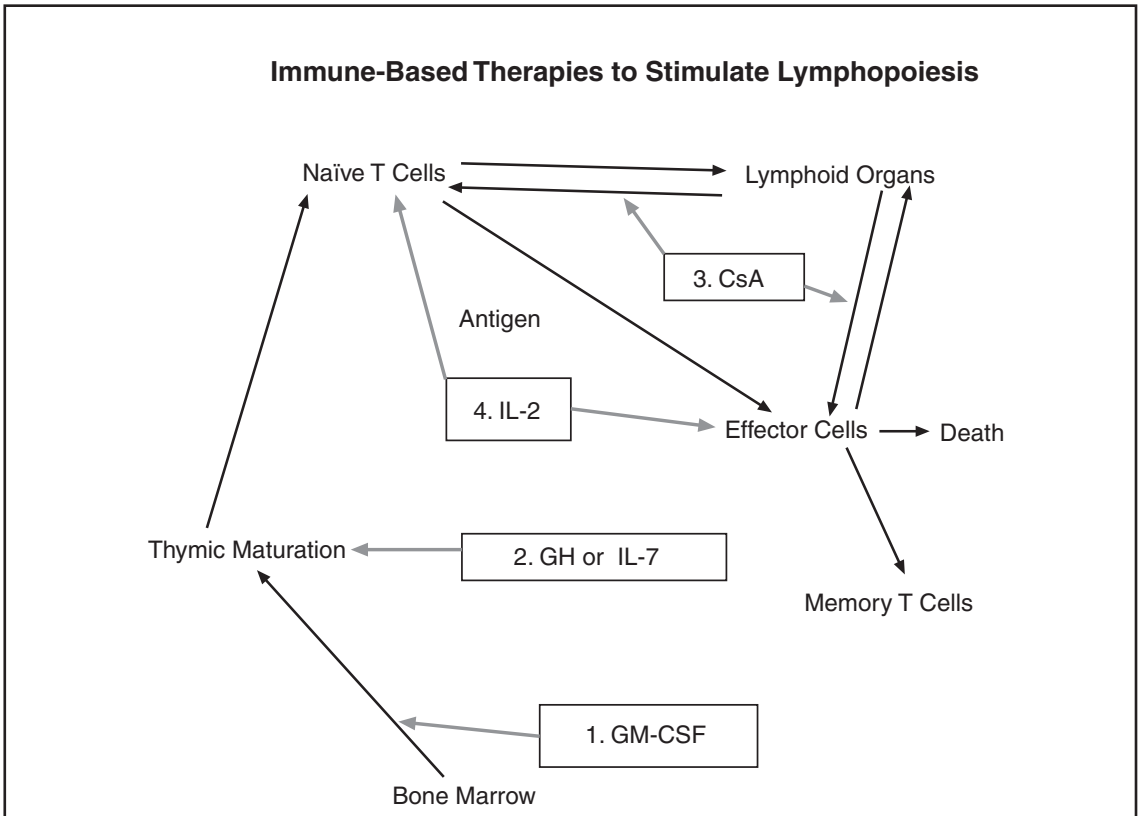

Figure 1. *Pre-T lymphocytes are produced in the bone marrow. They migrate to the thymus, where they undergo T-cell receptor rearrangement and positive and negative selection.* Naïve *T-cells are released and upon antigen encounter in lymph nodes, they differentiate into effector and memory cells. Most T-lymphocytes (around 98%) are in the lymphoid tissues not in peripheral blood. 1. Bone marrow function and pre-T lymphocyte production may be stimulated by using GM-CSF. 2. Growth hormone (GH) or Interleukin-7 (IL-7) are candidate stimulants of thymic function in humans. 3. Short courses of cyclosporine A (CsA) may result in decreased lymph node "trapping" of CD4+ and CD8+ cells. 4. Intermittent Interleukin-2 (IL-2) administration induces proliferation of CD4 and prolongs their survival, thus increasing the number of peripheral blood CD4+ cells.*

phocytes are positively and negatively selected in the thymus, and the positively selected cells give rise to CD4+ and CD8+ *naïve* lymphocytes, that are then released into the circulation. The thymus involutes with age[43] and thymic architecture is disrupted in HIV-1 infection[44]. Although in many HAART treated patients there is evidence of thymic function after treatment with HAART[27,28,45], it is not known whether thymic function actually improves after HAART. As has been shown in CD4 restoration after chemotherapy[46], our group has shown that among some patients who fail to increase CD4+ lymphocytes after controlling HIV-1 replication there is evidence of impaired thymic function[47]. Whether this impaired thymic function is the result of true thymic failure or whether it is the result of inadequate supply of pre-T cells by the bone marrow remains to be seen. Growth hormone (GH) has been shown to improve thymic function in mice with atrophic thymuses[48]. More recently, GH has been shown to increase thymic volume and number of *naïve* CD4+ cells after administration to a small number of HIV-1 infected humans[49].

Interleukin-7 (IL-7) is a cytokine that induces survival and proliferation of immature thymocytes[50]. In murine models of radiation-induced lymphopenia, IL-7 administration enhances thymopoiesis. This cytokine is being developed for human use. Studies using GH or interleukin-7, in HIV-1 infected patients with incomplete immune restoration will address whether improving thymic function will result in peripheral blood CD4+ lymphocyte rises.

Shortly after initiation of HAART there is a decrease, but not normalization, of the inflammatory state that causes lymphocyte trapping in lymphoid organs[18,19]. *In vivo*, cyclosporine A decreases proinflammatory cytokine and adhesion molecule expression[51,52]. In patients who started HAART during acute HIV-1 infection Rizzardi et. al. have shown that an 8 week course of cyclosporine A used to attain plasma levels similar to those used for organ transplantation resulted in larger CD4+ lymphocyte rises than those seen in historical acute HIV-1 seroconverters treated with the same HAART regimen[53]. Cyclosporine-treated patients had higher CD4+ lymphocytes than historic controls even one year after cessation of cyclosporine administration. Whether cyclosporine administration will result in augmentation of the CD4+ lymphocyte rise caused by HAART in patients with chronic HIV-1 infection and the mechanisms whereby cyclosporine A induces CD4+ lymphocyte rises are currently being studied in a controlled trial.

IL-2 is a T-cell growth factor that induces CD4+ cell proliferation and prolongs CD4+ survival[54]. High-dose, intermittent IL-2 administration has been shown to increase peripheral blood CD4+ lymphocyte counts when given to HIV-1 infected patients with high CD4+ lymphocyte counts or to HAART-treated patients with low circulating CD4+ cell counts[55-57]. Whether the increase in CD4+ lymphocyte counts induced by IL-2 will translate into clinical benefit is currently being studied in 2 large, randomized clinical trials.

Will the Increase in CD4+ Lymphocytes Conferred by the Use of Immune-Based Therapies result in Clinical Benefit?

Due to the success of HAART in decreasing morbidity and mortality in developed countries[7], testing the clinical efficacy of new interventions will require either the performance of multiple large and long-duration clinical trials using clinical endpoints or the development of surrogate markers. Requiring clinical endpoint studies for immune-based therapies will considerably slow the development of these agents. In addition, due to the different mechanism of action of these biologicals, the demonstration that the increase in CD4+ lymphocytes by one agent such as IL-2 will not accelerate the development of different agents, resulting in the need for designing large, long, and expensive trials for every candidate. For antiretroviral therapies a decrease in plasma HIV-1 RNA level is an accepted surrogate marker of clinical benefit. No such surrogate exists for immune-based therapies.

We propose that the ability or failure to respond to immunization with different antigens could be an adequate surrogate marker for functional immune competence. We have shown that HIV-1 infected patients receiving HAART and HIV-1 uninfected individuals have subnormal responses to immunization[12,35]; in addition, responses to immunization are heterogeneous among HAART-treated patients, even when CD4+ cell counts are comparable[35]. More recently we have shown that in patients who start HAART with or without IL-2 during moderately advanced HIV-1 disease, the increase in CD4+ lymphocytes in recipients of HAART plus IL-2 is not associated with an increased ability to respond to immunization when compared to responses in patients treated with HAART alone[58]. This finding is notable given that IL-2 recipients had twice as many peripheral blood CD4+ cells at the time of immunization and suggests that the increase in CD4+ lymphocytes in this patient population will not necessarily result in improved immune function.

For the ability to respond to immunization to be considered an adequate surrogate of clinical benefit of an immune-based intervention, the ability to respond to immunization needs to explain the clinical benefit. Although small studies suggest that HIV-1 infected patients who respond to immunization have a more favorable course of HIV disease[59,60], a more formal demonstration of the association of response to immunization and clinical benefit is needed. Nevertheless, we consider that incorporating immunization studies in early phase trials of immune-based therapies will allow

us to learn about functional immunity, even if the formal link to clinical benefit is so far lacking.

Restoration of HIV-Specific Immune Responses

The generation of SIV-specific CD8+ immune responses after immunization is associated with control of SIV replication in macaque models[61]. In addition, the development of immune-escape mutants is temporally associated with loss of control of SIV replication[62]. CD8+ lymphocyte depletion using anti-CD8 antibodies results in an increase in SIV replication[63-65]. All this evidence strongly suggests that HIV-specific CD8+ lymphocytes mediate immune control of HIV-1 replication. HIV-1 specific CD8+ responses decrease after administration of HAART[66]. HIV-1 specific lymphoproliferative responses also have been associated with control of HIV-1 replication[67], but whether these responses are cause or consequence of low levels of HIV-1 replication remains unknown. These responses are recovered after initiation of HAART in a variable proportion of patients, the sooner after HIV-1 infection HAART is initiated, the more likely a patient will be to exhibit HIV-specific lymphoproliferative responses[22,68-70].

In conclusion, after initiation of HAART HIV-1 specific CD8 responses decrease, and although HIV-specific CD4+ lymphoproliferative responses return in some patients, the likelihood that patients will be able to achieve immune control of HIV-1 replication without additional intervention is remote. Again, immune-based therapies such as therapeutic immunization are needed to generate HIV-specific responses and to ascertain whether patients who start HAART during chronic HIV-1 disease will be able to achieve immune control of HIV-1 replication. The fact that many patients with chronic HIV-1 infection have broad HIV-specific immune responses[71] and the ability of HIV-1 to rapidly evade these responses[72] creates a formidable challenge for HIV-1 therapeutic immunization.

Conclusions

Immune restoration after HAART is incomplete but sufficient to protect patients from common opportunistic infections. Whether incomplete immune restoration will be associated with late onset complications such as malignancies remains to be determined. Immune-based therapies to magnify immune restoration after HAART and creative ways to test them will teach us about the residual immune lesions after treatment of HIV-1 infection while potentially offering patients clinical benefit.

Acknowledgments

The author is supported by a research grant from the NIH (AI50501-01).

References

1. Birx D, Brundage J, Larson K, et al. The prognostic utility of delayed-type hypersensitivity skin testing in the evaluation of HIV-infected patients. Military Medical Consortium for Applied Retroviral Research. J Acquir Immune Defic Syndr 1993; 6:1248-57.
2. Gottlieb M, Schroff R, Schanker H, et al. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981;305:1425-31.
3. Mildvan D, Mathur U, Enlow R, et al. Opportunistic infections and immune deficiency in homosexual men. Ann Intern Med 1982;96:700-4.
4. Dolan M, Clerici M, Blatt S, et al. *In vitro* T cell function, delayed-type hypersensitivity skin testing, and CD4+ T cell subset phenotyping independently predict survival time in patients infected with human immunodeficiency virus. J Infect Dis 1995;172:79-87.
5. Cameron D, Heath-Chiozzi M, Danner S, et al. Randomised placebo-controlled trial of ritonavir in advanced HIV-1 disease. The Advanced HIV Disease Ritonavir Study Group. Lancet 1998;351:543-9.
6. Hammer S, Squires K, Hughes M, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with HIV infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N Engl J Med 1997;337:725-33.
7. Palella F Jr, Delaney K, Moorman A, et al. Declining morbidity and mortality among patients with advanced HIV infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338:853-60.
8. Valdez H, Chowdhry T, Asaad R, et al. Changing spectrum of mortality due to HIV: analysis of 260 deaths during 1995-1999. Clin Infect Dis 2001;32:1487-93.
9. Yeni P, Hammer S, Carpenter C, et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. JAMA 2002;288:222-35.
10. Tarwater P, Margolick J, Jin J, et al. Increase and plateau of CD4 T-cell counts in the 3(1/2) years after initiation of potent antiretroviral therapy. J Acquir Immune Defic Syndr 2001;27:168-75.
11. Valdez H, Smith K, Lederman M, et al. Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. AIDS 2002 (in press).
12. Valdez H, Smith K, Landay A, et al. Response to immunization with recall and neoantigens after prolonged administration of an HIV-1 protease inhibitor-containing regimen. ACTG 375 team. AIDS Clinical Trials Group. AIDS 2000;14:11-21.
13. Storek J, Gooley T, Witherspoon R, Sullivan K, Storb R. Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. Am J Hematol 1997;54:131-8.
14. Socie G, Stone J, Wingard J, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. N Engl J Med 1999;341:14-21.
15. Connick E, Lederman M, Kotzin B, et al. Immune reconstitution in the first year of potent antiretroviral therapy and its relationship to virologic response. J Infect Dis 2000;181:358-63.
16. Notermans D, Pakker N, Hamann D, et al. Immune reconstitution after 2 years of successful potent antiretroviral therapy in previously untreated HIV type 1-infected adults. J Infect Dis 1999;180:1050-6.
17. Lederman M, Connick E, Landay A, et al. Immunologic responses associated with 12 weeks of combination antiretroviral therapy consisting of zidovudine, lamivudine, and ritonavir: results of AIDS Clinical Trials Group Protocol 315. J Infect Dis 1998;178:70-9.
18. Andersson J, Fehniger T, Patterson B, et al. Early reduction of immune activation in lymphoid tissue following highly active HIV therapy. AIDS 1998;12:123-9.
19. Bucy R, Hockett R, Derdeyn C, et al. Initial increase in blood CD4(+) lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues. J Clin Invest 1999;103:1391-8.

20. Badley A, Dockrell D, Algeciras A, et al. *In vivo* analysis of Fas/FasL interactions in HIV-infected patients. J Clin Invest 1998;102:79-87.
21. Li T, Tubiana R, Katlama C, Calvez V, Ait Mohand H, Autran B. Long-lasting recovery in CD4 T-cell function and viral-load reduction after highly active antiretroviral therapy in advanced HIV-1 disease. Lancet 1998;351:1682-6.
22. Angel J, Parato K, Kumar A, et al. Progressive HIV-specific immune recovery with prolonged viral suppression. J Infect Dis 2001;183:546-54.
23. Kaufmann G, Bloch M, Zaunders J, Smith D, Cooper D. Long-term immunological response in HIV-1-infected subjects receiving potent antiretroviral therapy. AIDS 2000;14:959-69.
24. Autran B, Carcelain G, Li T, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease (see comments). Science 1997;277:112-6.
25. Renaud M, Katlama C, Mallet A, et al. Determinants of paradoxical CD4 cell reconstitution after protease inhibitor-containing antiretroviral regimen (in process citation). AIDS 1999;13:669-76.
26. Hunt PS, Rodríguez B, Valdez H, et al. Continued CD4+ T-cell count gains in patients with 4 years of suppressed viral load on HAART. 9th CROI. Seattle 2002 [abstract].
27. Zhang L, Lewin S, Markowitz M, et al. Measuring recent thymic emigrants in blood of normal and HIV-1-infected individuals before and after effective therapy. J Exp Med 1999;190:725-32.
28. Douek D, McFarland R, Keiser P, et al. Changes in thymic function with age and during the treatment of HIV infection (see comments). Nature 1998;396:690-5.
29. Hengel R, Jones B, Kennedy M, Hubbard M, McDougal J. CD4+ T cells programmed to traffic to lymph nodes account for increases in numbers of cd4+ T cells up to 1 year after the initiation of highly active antiretroviral therapy for HIV type 1 infection. J Infect Dis 2001;184:93-7.
30. Carcelain G, Blanc C, Leibowitch J, et al. T cell changes after combined nucleoside analogue therapy in HIV primary infection. AIDS 1999;13:1077-81.
31. Bisset L, Cone R, Huber W, et al. Highly active antiretroviral therapy during early HIV infection reverses T-cell activation and maturation abnormalities. Swiss HIV Cohort Study. AIDS 1998;12:2115-23.
32. Bart P, Rizzardi G, Tambussi G, et al. Immunological and virological responses in HIV-1-infected adults at early stage of established infection treated with highly active antiretroviral therapy. AIDS 2000;14:1887-97.
33. Kaufman G, Zaunders J, Cunningham P, et al. Rapid restoration of CD4 T cell subsets in subjects receiving antiretroviral therapy during primary HIV-1 infection. AIDS 2000; 14:2643-51.
34. Plana M, García F, Gallart T, et al. Immunological benefits of antiretroviral therapy in very early stages of asymptomatic chronic HIV-1 infection. AIDS 2000;14:1921-33.
35. Lange H, Medvik K, Asaad R, Wild M, Kalayjian R, Lederman M. Earlier initiation of HAART better preserves immune competence even in persons who achieve "normal" CD4+ T-cell counts. XIV International AIDS Conference. Barcelona 2002 [abstract].
36. Pilcher C, Miller W, Beatty Z, Eron J. Detectable HIV-1 RNA at levels below quantifiable limits by amplicor HIV-1 monitor is associated with virologic relapse on antiretroviral therapy. AIDS 1999;13:1337-42.
37. Dornadula G, Nunnari G, Vanella M, et al. Human immunodeficiency virus type 1 - infected persons with residual disease and virus reservoirs on suppressive highly active antiretroviral therapy can be stratified into relevant virologic and immunologic subgroups. J Infect Dis 2001;183:1682-7.
38. Sharkey M, Teo I, Greenough T, et al. Persistence of episomal HIV-1 infection intermediates in patients on highly active antiretroviral therapy. Nat Med 2000;6:76-81.
39. Schooley R, Mladenovic J, Sevin A, et al. Reduced mobilization of CD34+ stem cells in advanced human immunodeficiency virus type 1 disease. J Infect Dis 2000;181:148-57.
40. Isgro A, Mezzaroma I, Aiuti A, et al. Recovery of hematopoietic activity in bone marrow from HIV type 1-infected patients during highly active antiretroviral therapy. AIDS Res Hum Retroviruses 2000;16:1471-9.
41. Huang S, Barbour J, Deeks S, et al. Reversal of HIV type 1-associated hematosuppression by effective antiretroviral therapy. Clin Infect Dis 2000;30:504-10.
42. Jacobson M, Spritzler J, Valdez H, et al. The effects of GM-CSF on plasma HIV-1 RNA and CD4 lymphocyte counts in HIV-1 infected subjects receiving concomitant potent antiretroviral therapy. 9th CROI. Seattle 2002 [abstract].
43. Steinmann G, Klaus B, Muller-Hermelink H. The involution of the ageing human thymic epithelium is independent of puberty. A morphometric study. Scand J Immunol 1985;22:563-75.
44. Haynes B, Hale L. The human thymus. A chimeric organ comprised of central and peripheral lymphoid components. Immunol Res 1998;18:175-92.
45. Smith K, Valdez H, Landay A, et al. Thymic size and lymphocyte restoration in patients with HIV infection after 48 weeks of zidovudine, lamivudine, and ritonavir therapy. J Infect Dis 2000;181:141-7.
46. Mackall C, Fleisher T, et al. Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. N Engl J Med 1995;332:143-9.
47. Teixeira L, Valdez H, McCune J, et al. Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. AIDS 2001;15:1749-56.
48. Binz K, Joller P, Froesch P, Binz H, Zapf J, Froesch E. Repopulation of the atrophied thymus in diabetic rats by insulin-like growth factor I. Proc Natl Acad Sci USA 1990;87:3690-4.
49. Napolitano L, Lo J, Gotway M, et al. Increased thymic mass and circulating *naïve* CD4 T cells in HIV-1-infected adults treated with growth hormone. AIDS 2002;16:1103-11.
50. Fry T, Mackall C. Interleukin-7: from bench to clinic. Blood 2002;99:3892-904.
51. Molossi S, Clausell N, Sett S, Rabinovitch M. ICAM-1 and VCAM-1 expression in accelerated cardiac allograft arteriopathy and myocardial rejection are influenced differently by cyclosporine A and tumour necrosis factor-alpha blockade. J Pathol 1995;176:175-82.
52. Petzelbauer P, Wolff K. Effects of cyclosporin A on resident and passenger immune cells of normal human skin and UV-induced erythema reactions. Br J Dermatol 1992;127:560-5.
53. Rizzardi G, Harari A, Capiluppi B, et al. Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy. J Clin Invest 2002;109:681-8.
54. Kovacs J, Vogel S, Metcalf J, et al. Interleukin-2 induced immune effects in HIV-infected patients receiving intermittent interleukin-2 immunotherapy. Eur J Immunol 2001;31:1351-60.
55. Kovacs J, Baseler M, Dewar R, et al. Increases in CD4 T lymphocytes with intermittent courses of interleukin-2 in patients with HIV infection. A preliminary study. N Engl J Med 1995;332:567-75.
56. Kovacs J, Vogel S, Albert J, et al. Controlled trial of interleukin-2 infusions in patients infected with the HIV. N Engl J Med 1996;335:1350-6.
57. Mitsuyasu R, Gelman R, Weng D. Prospective, randomized, controlled phase II study of HAART with continuous intravenous or subcutaneous Interleukin-2 (IL-2) in HIV-infected patients with CD4+ counts of 50 to 350 cells/mm^3: ACTG 328 Final Results at 84 weeks. 8th CROI. Chicago 2001 [abstract].
58. Valdez H, Pollard R, Sahner D, et al. Intermittent cycles of IL-2 increase CD4+ T-cell counts but do enhance antibody responses to immunization. XIV International AIDS Conference. Barcelona 2002 [abstract A5046s].
59. Loke R, Murray-Lyon I, Coleman J, Evans B, Zuckerman A. Diminished response to recombinant hepatitis B vaccine in homosexual men with HIV antibody: an indicator of poor prognosis. J Med Virol 1990;31:109-11.
60. Redfield R, Birx D, Ketter N, et al. A phase I evaluation of the safety and immunogenicity of vaccination with recombinant gp160 in patients with early human immunodeficiency virus infection. Military Medical Consortium for Applied Retroviral Research. N Engl J Med 1991;324:1677-84.

61. Barouch D, Santra S, Schmitz J, et al. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science 2000;290:486-92.
62. Barouch D, Kunstman J, Kuroda M, et al. Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature 2002;415:335-9.
63. Jin X, Bauer D, Tuttleton S, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J Exp Med 1999;189:991-8.
64. Metzner K, Jin X, Lee F, et al. Effects of *in vivo* CD8(+) T cell depletion on virus replication in rhesus macaques immunized with a live, attenuated simian immunodeficiency virus vaccine. J Exp Med 2000;191:1921-31.
65. Schmitz J, Kuroda M, Santra S, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 1999;283:857-60.
66. Ogg G, Jin X, Bonhoeffer S, et al. Decay kinetics of HIV-specific effector cytotoxic T lymphocytes after combination antiretroviral therapy. J Virol 1999; 73:797-800.
67. Rosenberg E, Billingsley J, Caliendo A, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia (see comments). Science 1997;278:1447-50.
68. Al-Harthi L, Siegel J, Spritzler J, Pottage J, Agnoli M, Landay A. Maximum suppression of HIV replication leads to the restoration of HIV-specific responses in early HIV disease. AIDS 2000;14:761-70.
69. Blankson J, Gallant J, Siliciano R. Proliferative responses to HIV-1 antigens in HIV-1 - infected patients with immune reconstitution. J Infect Dis 2001;183:657-61.
70. Rosenberg E, Altfeld M, Poon S, et al. Immune control of HIV-1 after early treatment of acute infection. Nature 2000;407:523-6.
71. Betts M, Ambrozak D, Douek D, et al. Analysis of total HIV-specific CD4(+) and CD8(+) T-cell responses: relationship to viral load in untreated HIV infection. J Virol 2001;75:11983-91.
72. Altfeld M, Rosenberg E, Shankarappa R, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. J Exp Med 2001;193:169-80.