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IMPROVING THE MANAGEMENT OF HIV DISEASE®

VOLUME 7 ISSUE 3 JULY 1999

IN THIS ISSUE

Highlights From *The Science and Treatment of HIV: An Advanced CME Course for Clinicians* March 27–31, 1999

ABOUT THIS ISSUE...

The International AIDS Society–USA sponsored its first national CME course, *The Science and Treatment of HIV: An Advanced CME Course for Clinicians*, in March 1999. This issue of *Improving the Management of HIV Disease* features summaries of 4 basic science presentations given at the course.

Held over 4 days in Snowmass Village, Colorado, *The Science and Treatment of HIV* offered faculty-participant interaction in a relaxed setting that allowed for in-depth examinations of HIV disease management not possible in single day courses. Led by Course Co-Chairs Dr Scott M. Hammer and Dr Michael S. Saag, a group of 18 experts in HIV provided instruction to participants through lectures, workshops, case presentations, and small group discussions. Major topics covered included basic science and pathogenesis, current issues in antiretroviral therapy, and complications of HIV infection and antiretroviral therapy.

The course presentations summarized in this issue highlight recent research developments and their implications for management of antiretroviral therapy. Dr Bruce D. Walker discussed promising findings on the role of cellular immune response in controlling HIV-1 infection and the effects of therapy on the immune response. Dr Richard A. Koup examined the status of thymic function in HIV-1 infection and the potential implications for immune reconstitution. Dr Robert F. Siliciano presented new research on viral dynamics and the presence of latent viral reservoirs in patients on potent antiretroviral therapy, explaining prospects for reservoir eradication. Dr Eric Hunter outlined possible opportunities for preventing HIV-1 fusion with and entry into target cells, through strategies involving interaction of the HIV-1 envelope complex with receptors on the target cell.

The International AIDS Society–USA will sponsor the second annual *The Science and Treatment of HIV* course in March 2000. To receive information about the 2000 course, please call (415) 562-6720 or send e-mail to info@iasusa.org.

Upcoming issues of *IMHD* will continue to feature summaries of recent International AIDS Society–USA course presentations. Topics will include possible targets for intervention in early HIV infection, discordant responses to therapy, new antiretroviral drugs, and the status of AIDS in Africa.

For additional information on upcoming International AIDS Society–USA activities, please see pages 14–15 and 27.

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CORRESPONDENCE

IMHD welcomes editorial correspondence. Address letters to

Editor, *IMHD*
International AIDS Society–USA
Presidio of San Francisco
1001 B O'Reilly Avenue, Box 29916
San Francisco, CA 94129-0916
Phone: (415) 561-6720
Fax: (415) 561-6740
e-mail: info@iasusa.org

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Presentation Summaries

Announcements

Cellular Immune Response in HIV-1 Infection and Effects of Therapy on Immunologic Parameters

The role of the cellular immune response in HIV-1 infection in patients with long-term nonprogression of infection and the effects of potent antiretroviral therapy during early infection on immune function were discussed by Bruce D. Walker, MD.

HIV-1 infection is associated with progressive destruction of the immune system in the majority of patients. Some patients, however, exhibit no detectable viremia in available assays and no progression of disease over long-term follow-up in the absence of antiretroviral therapy. Attenuated virus and host genetic factors (eg, chemokine receptor polymorphisms) account for only a minority of cases of such long-term nonprogression. Accumulating data suggest that host cellular immune response plays a major role in containing HIV-1 infection in long-term nonprogressors, a mechanism characteristic of long-term control of infection with a number of other human viruses (eg, Epstein-Barr virus, cytomegalovirus, and herpes simplex virus). Recent data suggest a crucial role of HIV-1-specific T-helper cells in regulating effective immune response to HIV-1, including specific cytolytic CD8+ T-cell (CTL) activity, and indicate that this response can be preserved by early institution of potent antiretroviral therapy.

Recent data suggest that HIV-1-specific T-helper cell response can be preserved by early institution of potent antiretroviral therapy

IMMUNE RESPONSE TO HIV-1 INFECTION

Both humoral and cellular responses to HIV-1 have been detected in infected persons. Studies in long-term slow progressors have demonstrated that HIV-1-specific neutralizing antibody may be present in low levels to levels below the limits of detection, suggesting absence of a primary role of this mechanism in viral containment. However, a number of studies have now shown that CTL activity and number are associated with control of HIV-1 viremia and have suggested a central role for virus-specific CD4+ T-helper cells in regulating CTL response and activity.

CTLs kill infected cells via T-cell-receptor mediated recognition of processed viral protein presented in the context of MHC class I molecules on the surface of an infected cell (Figure 1). Studies of HIV-1 dynamics in vivo suggest a span of 2.6 days between new cell infection and budding of progeny virus; CTLs can identify and kill such cells during this period, thus preventing production of new virions, if the CTLs are present in sufficient number and in an appropriate activation state. In infected humans, CTLs are present at the earliest stages of acute infection, but decline in most individuals as infection progresses. Studies have been conducted to characterize the comparative activity of CTLs in rapid progressors versus nonprogressors by measuring lysis of cells expressing

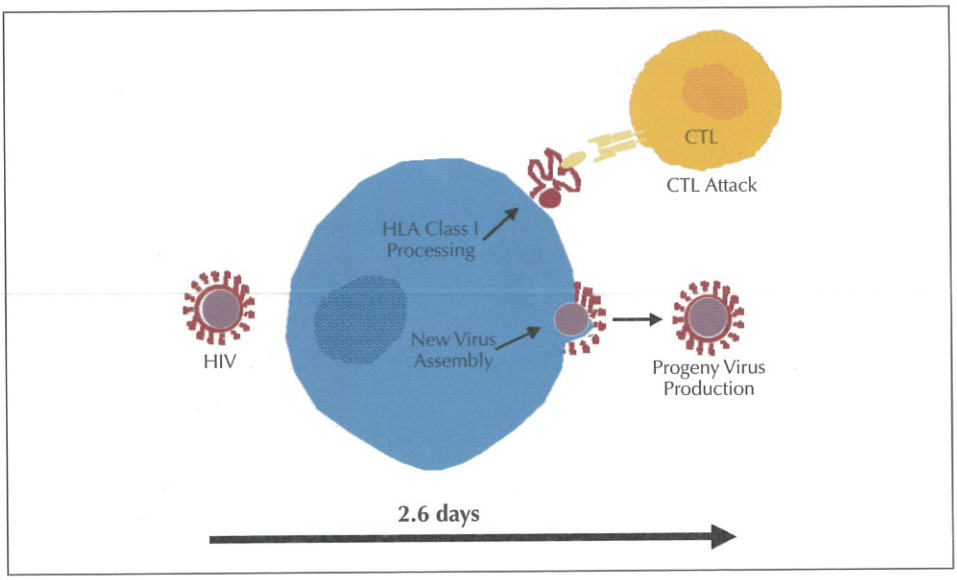


Figure 1. *CTLs recognize processed viral proteins expressed on the host cell surface. Rate of CTL activation and response may determine rate of virion production from newly infected cells and thus contribute to determination of viral set point in infected individuals.*

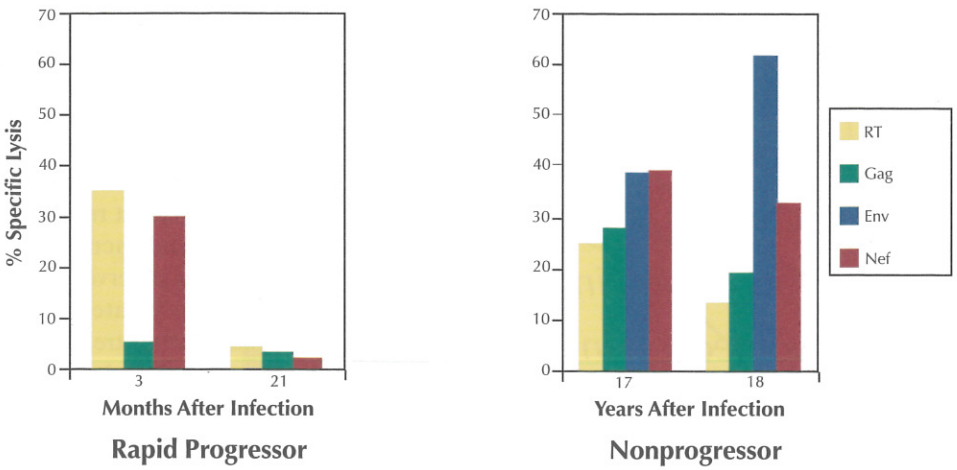


Figure 2. *HIV-1-specific CTL responses in rapid progressor and long-term nonprogressor. The rapid progressor exhibited a rapid CD4+ count decline and developed AIDS at 13 months with a consistently high plasma viral load (>300,000 copies/mL of HIV-1 RNA). The nonprogressor remains well at 19 years with a CD4+ cell count greater than 1000/μL and viral load less than 400 HIV-1 RNA copies/mL. Figure shows percent lysis by CTLs specific for HIV-1 reverse transcriptase (RT), Gag, Env, and Nef proteins.*

HIV-1 proteins. They have shown that although CTL response occurs in both after the acute infection period, this response appears to dissipate shortly thereafter in rapid progressors, while nonprogressors maintain a strong response broadly directed against multiple viral proteins (Figure 2). Additional studies have demonstrated that infected cells can be lysed prior to production of progeny virus. These studies have shown that addition of single CTL clones specific for single HIV-1 proteins in infected CD4+ cells in culture results in a 10,000-fold decrease in virion production compared with control experiments. The potential critical role of CTLs in controlling viremia has been supported by the recent finding that in vivo CD8+ cell depletion resulted in a dramatic increase in viremia in macaques infected with simian immunodeficiency virus. Attempts to restore CTL response in patients with chronic infection via infusion of HIV-1-specific CTLs have met with limited success; however, this finding is probably due to inability of the cells to achieve the appropriate activation state in vivo.

Findings showing a negative correlation between viral load and CTL activity have suggested a mechanism by which such activity might determine the viral set point in infected individuals. In brief, prompt CTL activation and response might prevent production of new virions, with progressively slower recognition and activation resulting in progressively higher rates of production and thus higher levels of viremia. A prime candidate for regulation of the activation of CTLs and magnitude of CTL response is the activity of CD4+ T-helper cells. These cells recognize antigen on cell surfaces via the T-cell receptor and the CD4 molecule on the helper cell surface, with the interaction stimulating lymphokine secretion and cell-cell interactions that regulate CTL activity, B cell function, antibody production, natural killer cell function, cytokine production, and antigen-presenting cell function (Figure 3). (Although it was generally believed that T-helper cells directly activated CTLs, it has recently been shown that activation of CTLs occurs through interaction with activated antigen-presenting cells. These latter cells are activated by contact with T-helper cells that have been activated by contact with the inactive antigen-presenting cells.) The crucial role of T-helper cells in maintaining effective immune responses in viral infection has been demonstrated in a number of models. For example, in the murine lymphocytic choriomeningitis virus infection model, viremia is controlled in association with a strong CTL response; however, in CD4+ cell-depleted or -knockout animals, CTL response wanes, and high-level viremia ensues after initial response.

HIV-1-specific T-helper cell responses appear to occur early in infection, to be lost shortly thereafter in the majority of patients, and to not recover when they are lost. Studies of cell proliferation induced by HIV-1 antigen stimulation of peripheral blood lymphocytes from rapid progressors and long-term slow progressors have shown that specific T-helper cell responses to the

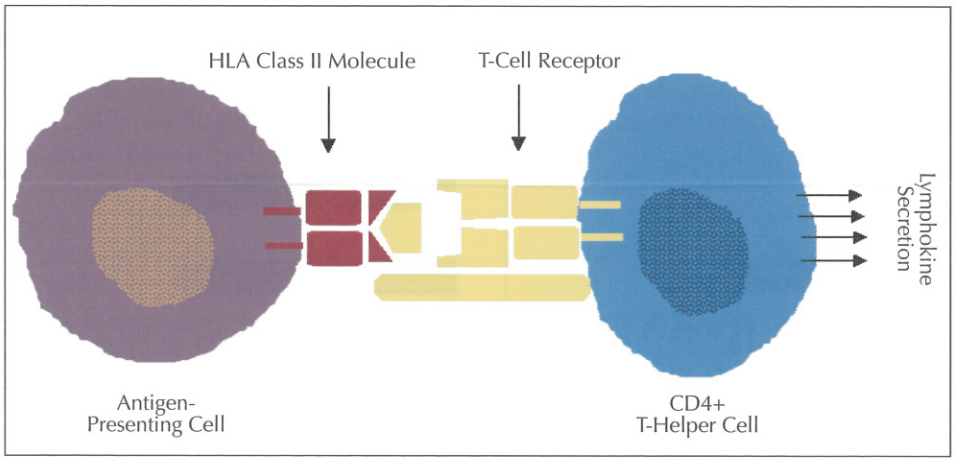


Figure 3. *Direct interaction of antigen-presenting cells and CD4+ T-helper cells results in T-helper cell lymphokine secretion and cell-cell interactions that regulate a variety of immunologic functions, including CTL activity and antigen-presenting cell function.*

viral proteins are lacking in the former, whereas the latter exhibit responses of large magnitude (Figure 4). Subsequently, it was shown in a group of treatment-naive patients with a wide range of viral load values (<400 to 300,000 plasma HIV-1 RNA copies/mL) that p24-specific T-helper cell responses were highly negatively correlated with level of viremia. Further, it was demonstrated that CTL response to HIV-1 Gag protein was significantly correlated with the level of HIV-1-specific T-helper cell activity. These findings indicate that HIV-1-specific T-helper cell response is associated with control of viremia and suggest both that loss of this response is associated with lack of CTL response in progressive disease and that preservation of response is associated with maintained control of viremia.

POTENTIAL EFFECT OF THERAPY ON IMMUNE FUNCTION

It is possible that HIV-1-specific T-helper cell response is lost in the earliest stages of acute infection due to activation of these CD4+ cells as part of initial immune response to infection—ie, activation of these cells serves to make them preferential targets of HIV-1 infection. Indeed, it has been observed that T-helper cells specific for such pathogens as cytomegalovirus are present when those specific for HIV-1 are absent in patients assessed in the early asymptomatic phase of chronic infection. The notion that loss of HIV-1-specific T-helper cells results from the dynamics of early infection has suggested the hypothesis that rapid initiation of potent antiretroviral therapy after initial infection might serve to protect developing T-helper cells and permit maturation of an effective immune response.

A strong T-helper cell response has been characteristic of the patients treated in early infection in initial studies

In ongoing studies, Dr Walker's group has identified persons with acute HIV-1 infection prior to seroconversion and instituted immediate treatment with triple drug potent antiretroviral therapy that includes a protease inhibitor, comparing p24-specific T-helper cell responses in these patients with those in untreated control patients with acute infection and with patients initiating potent antiretroviral therapy during chronic infection. Figure 5 shows the p24-specific response in one patient receiving early treatment; a marked increase in proliferative response was observed, with this increase being correlated with decreasing viral load during treatment. The development of a T-helper cell response has been characteristic of the patients treated in early infection; 11 of 12 patients studied have maintained a stimulation index response of greater than 10 over 6 months. In contrast, there is a minimal p24-specific T-helper cell response in patients with untreated acute infection over the course of 6 to 12 months. Restoration of the T-helper cell response has not been observed in patients given potent antiretroviral therapy for at least 12 months during chronic infection, whereas these responses remain detectable and elevated in the patients with very early initiation of treatment.

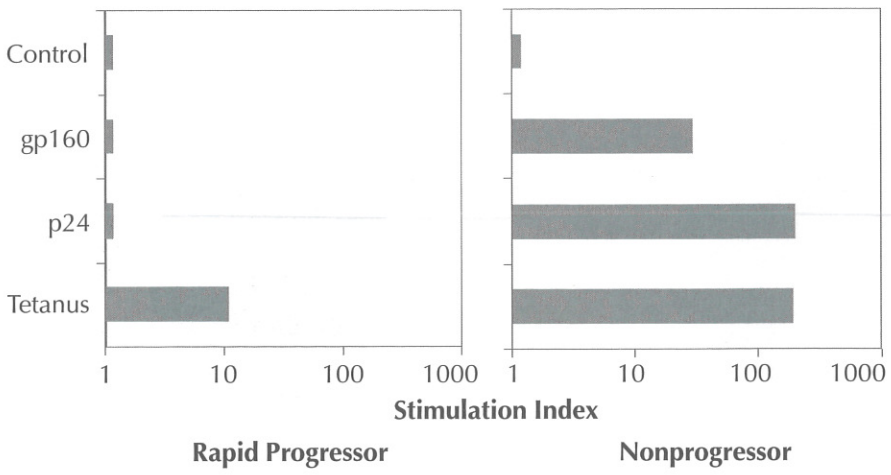
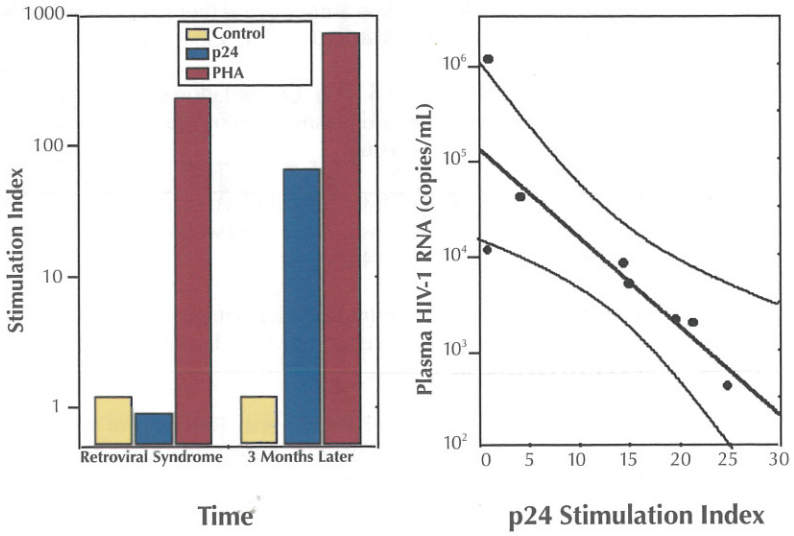


Figure 4. *HIV-1-specific T-helper cell response in rapid progressor and long-term nonprogressor (see Figure 2). Figure shows stimulation index as measure of proliferation of T-helper cells specific for HIV-1 gp160 and p24 compared with response in control condition and specific response to tetanus antigen.*

CAN THERAPY BE DISCONTINUED AFTER TREATMENT OF ACUTE INFECTION?

The apparent ability to preserve T-helper cell response with early potent antiretroviral therapy raises the questions of whether an effective immune response has been generated such that (1) viremia would remain controlled if therapy was withdrawn or (2) a rebound in viremia might prime effective immune response that subsequently controls the virus. A small number of cases have now been reported in which patients treated early in infection have maintained viral load below limits of detection or exhibited delayed return of viremia when treatment was stopped. In an ongoing controlled study, Dr Walker's group is assessing responses to stopping therapy in patients with viral loads below levels of detection who initiated potent antiretroviral therapy early in infection or during chronic infection. These studies will examine whether HIV-1-specific immune response can be boosted under selective conditions and



Emerging data indicate that the cellular immune response plays a critical role in containing HIV-1 replication, and case reports have indicated that immune containment of HIV-1 is an achievable goal

Figure 5. *HIV-1 p24-specific T-helper cell response in patient treated with early potent antiretroviral therapy in whom plasma viral load was reduced to levels below detection limits. Left: The p24-specific proliferative response compared with control condition and response to PHA (phytohemagglutinin) during acute retroviral syndrome and after 3 months. Right: Correlation of increased p24-specific response with decreasing plasma viral load. Curved top and bottom lines show 95% confidence intervals. Adapted from Rosenberg ES, et al.* Science. *1997;278:1447–1450.*

whether efforts should be undertaken to investigate this strategy with well-controlled, systematic studies.

CONCLUSIONS

Emerging data indicate that the cellular immune response plays a critical role in containing HIV-1 replication, and case reports have indicated that immune containment of HIV-1 is an achievable goal. Recent findings have indicated that HIV-1-specific T-helper cell response is inversely correlated with viral load, and that HIV-1 can induce strong virus-specific T-helper cell responses in individuals controlling viremia in the absence of antiretroviral therapy. These responses appear to be preserved in patients treated with potent antiretroviral therapy during acute infection but are not restored in the short term in patients treated during chronic infection. However, it should be noted that other recent evidence indicates that immune reconstitution may occur with continued therapy over the long term in patients with chronic infection. Data may suggest that the immune system can be harnessed more effectively in control of infection. Similarly, the findings indicating that immune reconstitution may occur in chronically infected patients suggest the opportunity for immunotherapeutic intervention to improve the ability of such patients to control infection.

Bruce D. Walker, MD, is Associate Professor of Medicine at Harvard Medical School and Director of the Partners AIDS Research Center at Massachusetts General Hospital in Boston.

SUGGESTED READING

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Status of the Thymus in Adult HIV-1 Infection: Implications for Immune Recovery

Evidence of continued thymic production of naive T lymphocytes and potential implications for immune reconstitution in HIV-1-infected individuals were discussed by Richard A. Koup, MD.

Potent antiretroviral therapy results in persistent and sustained increases in CD4+ T cells. The initial increase observed with institution of treatment consists of memory T cells, as indicated by the finding that the expanded T-cell population reflects the skewed T-cell repertoire present in individuals prior to therapy. After approximately 6 to 12 months, increases in naive CD4+ T cells are observed, occurring in both peripheral blood and lymphoid tissue (Figure 1). Although peripheral expansion of existing naive cells is likely, new findings indicate that increases in naive CD4+ T cells include cells recently produced by the thymus. Whereas expansion of existing cell populations cannot replace elements of the T-cell repertoire lost through HIV-1-associated depletion, continued thymic output may be expected to produce cells reflecting the full repertoire present prior to HIV-1 infection. These findings raise the possibility of immune reconstitution in patients with prolonged suppression of viral load.

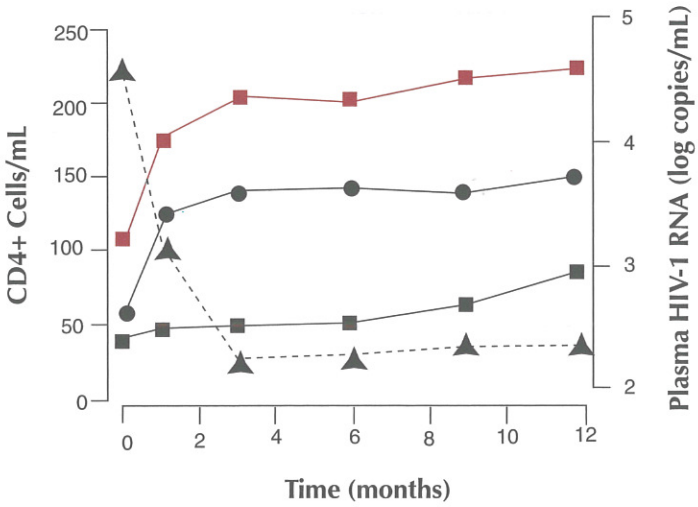

Figure 1. *Increase in CD4+ cell count with reduction in viral load on potent antiretroviral therapy showing late development of increase in naive CD4+ cells. Plasma HIV-1-RNA level is indicated with triangles. Red squares indicate overall CD4+ cell level, with circles showing memory CD4+ cell level and black squares showing naive CD4+ cell level. Adapted from Li TS, et al. Long-lasting recovery in CD4 T-cell function and viral-load reduction after highly active antiretroviral therapy in advanced HIV-1 disease.* Lancet. *1998;351:1682–1686. Copyright 1998 by The Lancet Ltd.*

THYMIC FUNCTION

The thymus is the major source of T-cell production in the body. In the thymus, bone marrow precursor cells with germline T-cell receptors undergo rearrangement of the T-cell receptor genes, alterations in surface phenotype (Figure 2), and proliferation. In cells that become T-cell receptor alpha/beta T cells, initial rearrangement of the beta gene triggers rearrangement of the alpha gene, preventing the possibility of use of the delta chain within the alpha gene. During this process, cells express both CD4 and CD8 surface antigens; differentiation of the cells into distinct CD4+ and CD8+ lineages occurs subsequent to and by processes independent of the T-cell receptor-gene rearrangement. This differentiation occurs just prior to release of the mature naive T cells into the peripheral circulation from the thymic medulla. It is estimated that 95% to 99% of all T-cell receptor rearrangements fail to recognize self-major histocompatibility complex and are eliminated by apoptosis in the thymus. Nevertheless, the remaining naive CD4+ and CD8+ T cells have T-cell receptor specificities reflecting a highly diverse repertoire estimated at 10^{16} different specificities.

The naive phenotype of the cells emigrating from the thymus consists of the expression of the low-molecular-weight isoform of CD45 (CD45RA) and L-selectin (CD62L), and this phenotype can be maintained by circulating cells for many years. Upon exposure to their cognate antigen in secondary lymphoid tissue, the cells are stimulated to differentiate and proliferate, and acquire the memory T-cell phenotype characterized by expression of CD45RO. Although some memory cells may bear CD45RA, they do not express CD62L, with coexpression of CD45RA and CD62L being specific for the naive cell population.

The thymus is almost fully developed at birth. After an increase in weight over the first 6 months of life, it remains stable in size and exhibits a slight decrease in weight over the course of the individual's

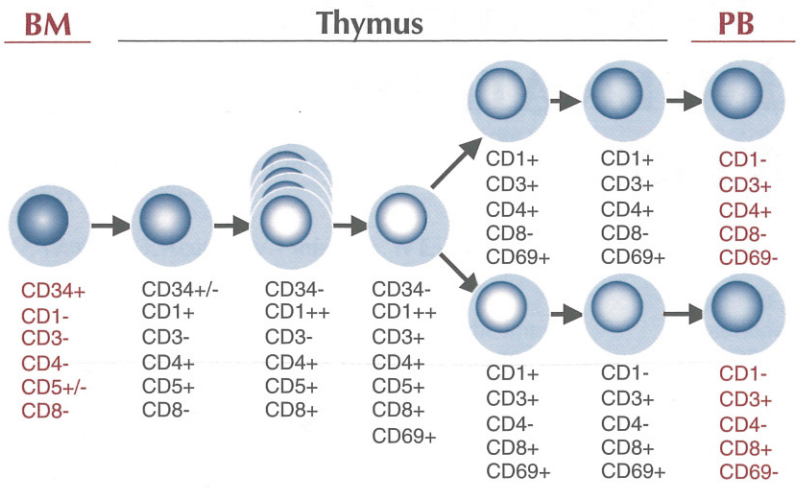

Figure 2. *Changes in phenotype with maturation from bone marrow (BM) precursors to mature naive T cells released from the thymus into the peripheral blood (PB).*

life. However, the thymus undergoes progressive pathologic changes (fairly linear from the first year through the fourth decade) characterized by replacement of perivascular spaces with adipose tissue and extreme loss of the cortical and medullary tissue responsible for production of new T cells. Although there is great interindividual variability in amounts of remaining thymic tissue by age, an average of approximately 70% to 80% of cortical and medullary tissue is lost by age 30, with very little functional thymic tissue remaining by late life (Figure 3). It was thus believed that the thymus was largely inactive in producing new T cells in mature individuals. However, it has been found that remaining cortical and medullary tissue in the aging thymus is histologically normal, and that phenotypes of the expected T-cell intermediates are present, with evidence of ongoing cell proliferation and T-cell receptor gene rearrangement. Thus, thymic tissue in adults appears to be functional, with the quantitative reduction in naive T-cell output (estimated at 10^9 in infancy to 6–7 x 10^7 by age 20) not implying a qualitative reduction.

CD4+ CELL CHANGES UNDER POTENT ANTIRETROVIRAL THERAPY

As noted, the importance of determining whether the thymus continues to function in producing new T cells in later life resides in the possibility of a global recovery of immune function. T cells emigrating from the thymus are programmed to detect a single antigen on a single pathogen. Expansion of existing naive cells would not serve to replace clones with defined specificities deleted through viral cytopathic effects and host cytolytic response, whereas thymic production could replenish the full complement of specificities. Evidence that some of the increase in naive CD4+ T cells is derived from peripheral expansion of existing cells comes from studies indicating increases in naive cell number in thymectomized patients receiving potent antiretroviral therapy. However, recent findings indicating the thymus as a source of some of this increase include studies showing that presence of CT-detected thymic tissue is associated with a greater CD4+ T-cell recovery in patients on potent antiretroviral therapy and studies in SCID-Hu mice showing recovery of function of implanted thymic tissue in HIV-infected animals treated with potent antiretroviral therapy. More direct evidence that cells constituting the later potent antiretroviral therapy-related increase in naive cell populations are derived from the thymus comes from recent studies utilizing a novel assay to identify recent thymic emigrants.

T-cell receptor-gene rearrangement in the thymus results in production of episomal DNA fragments, termed T-cell receptor rearrangement excision circles (TRECs), that can be used as markers of recent thymic emigration. These episomes are stable but do not replicate during mitosis, and are thus diluted with each round of cell division. The presence of TREC in naive T cells constitutes a surrogate marker of recent emigration since the episome(s) may also be present in cells that had emigrated at some time in the past but failed to replicate substantially. The assay exploiting the presence of

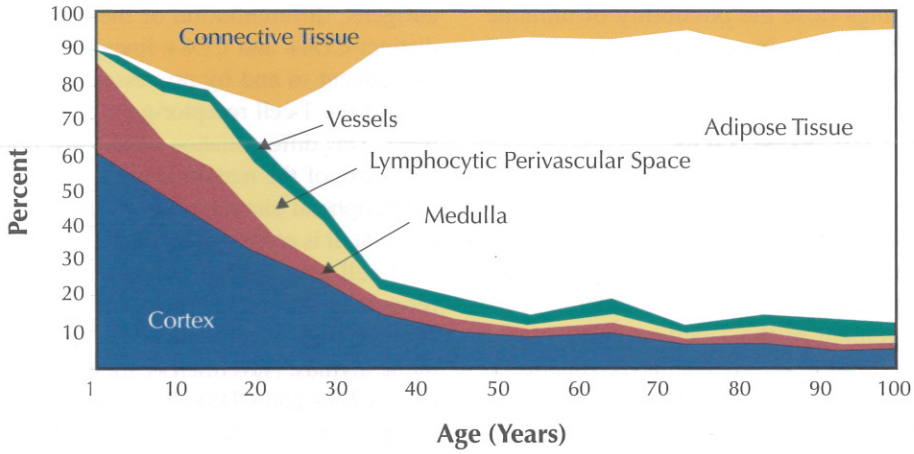

Figure 3. *Age-related changes of relative volumes (%) in tissue compartments of the thymus. Adapted from Steinmann GG, et al.* Scand J Immunol. *1985;22:563–575.*

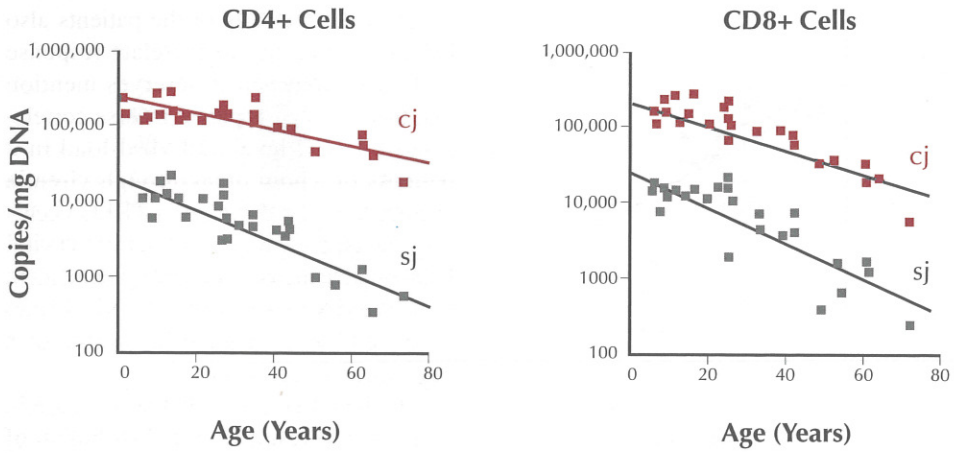

Figure 4. *Age-related changes in levels of cj and sj TREC in peripheral blood CD4+ and CD8+ cells. Adapted with permission from Douek DC, et al.* Nature. *1998;396:690–695.*

TREC is based on the recognition that the delta locus within the alpha gene of the alpha/beta T-cell receptor T cells is eliminated during maturation in the thymus; the elimination of the delta locus results in excision circles containing a signal joint (sj) and a coding joint (cj), both of which can be detected through polymerase chain reaction amplification. The sj and cj TREC are detected in phenotypically naive alpha/beta T cells (CD45RA+, CD45RO-) and not in memory T cells (presumably as a result of proliferation accompanying the conversion from naive to memory phenotype), B cells, or gamma/delta T cells. Use of the cj/sj TREC assay in peripheral blood from individuals ranging from birth to more than 70 years of age has shown that TREC levels decline with increasing age (Figure 4), as predicted from understanding of age-related changes in thymic output. In the context of a functioning thymus, the population of naive cells containing TREC represents a dynamic pool, with TREC levels being reduced by dilution through proliferation of these cells into subsequent generations of naive cells or proliferation and conversion into memory cells and variably replenished by different interindividual rates of thymic production of new naive cells. As shown in Figure 5, studies in normal and thymectomized individuals show that age-associated TREC levels are lower in the latter, with an approximately 1 log difference being observed at earlier ages and the difference decreasing with progressive age. These findings indicate that continued thymic output is required to maintain the persistent low level of TREC observed in adults.

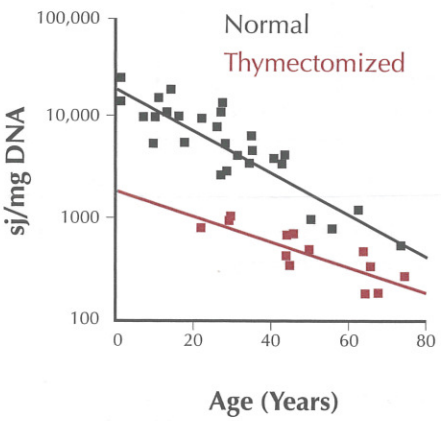

Figure 5. *TREC levels (sj) in CD4+ cells in normal and thymectomized individuals according to age. Adapted with permission from Douek DC, et al.* Nature. *1998; 396:690–695.*

In order to interpret findings using the TREC assay in HIV-1-infected individuals, additional disease-specific effects on naive cell levels need to be considered. HIV-1 would be expected to decrease TREC levels in the peripheral naive T-cell pool via infection and death of the cells and via dilution through stimulation of proliferation of these cells. In addition, a number of studies have identified effects of HIV-1 within the thymus. For example, there have been multiple reports of destruction of thymic architecture and limited thymopoietic potential in patients with later-stage HIV-1 disease, compared with a relatively low frequency of productively infected cells and relatively preserved histology in early infection. In addition, infection and killing of thymocytes bearing CD4 or other inhibition of thymocytes is likely to occur. Studies in the SCID-Hu mouse model have indicated that HIV-1 infects and depletes thymocytes double-positive for CD4 and CD8 molecules, and studies in the rhesus monkey/simian immunodeficiency virus model have indicated that infection results in inhibition of thymic precursors. Use of the TREC assay in HIV-1-infected individuals has demonstrated lower levels of TREC in the peripheral naive T-cell pool than in uninfected individuals (Figure 6). The relative contributions of viral effects in the thymus and viral effects in the peripheral pool remain unclear.

Remaining cortical and medullary tissue in the aging thymus is histologically normal, with evidence of ongoing cell proliferation and T-cell receptor gene rearrangement

To investigate whether recent thymic emigrants contributed to the reconstitution

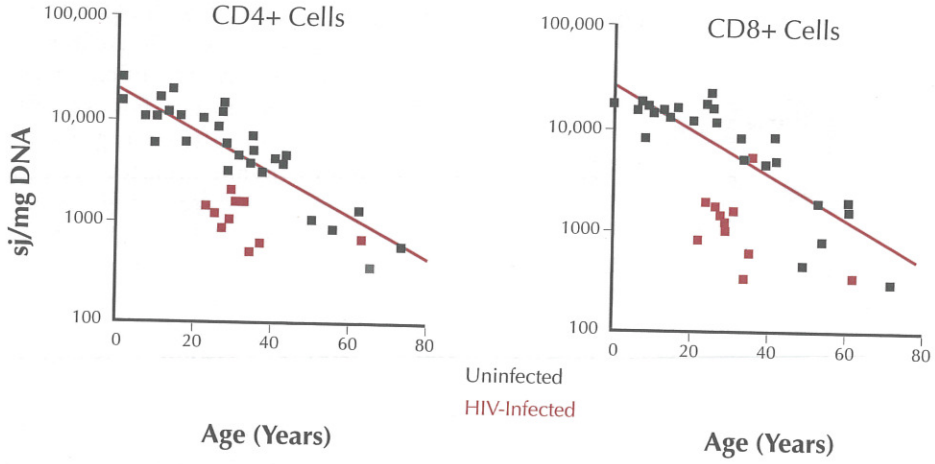

Figure 6. *TREC levels (sj) in CD4+ and CD8+ cells in uninfected individuals and patients with HIV-1 infection by age. Adapted with permission from Douek DC, et al.* Nature. *1998;396:690–695.*

of CD4+ T-cell populations in patients on potent antiretroviral therapy, TREC was quantified in peripheral blood CD4+ cells isolated from 10 patients at time points up to 9 months after initiation of treatment. TREC increases were observed in all but 1 of these patients after initiation of treatment and suppression of plasma viral load (Figure 7). Increases generally occurred within 4 to 16 weeks after treatment initiation, with 2 patients exhibiting large sustained increases to normal TREC levels. The sole patient in whom an increase was not observed was a 63-year-old individual who dropped out of the study early (all other patients were between the ages of 22 and 36 years); it is possible that an increase in TREC would have occurred with continued follow-up in this patient, or that the patient's thymus was not sufficiently functional to allow an increase to be detected. These increases in TREC were observed in the context of a general lack of significant increase in naive T-cell number; as reported, however, this increase characteristically is reported at 6 to 12 months after the beginning of therapy. The variability in the magnitude and kinetics of the TREC increases observed may reflect normal variability of thymic size and baseline TREC among individuals; the small number of patients studied did not allow for detection of a correlation of changes with pretreatment viral load or CD4+ cell count, with the age range of the majority of the patients also limiting the ability to correlate response with age. However, it deserves mention that there was an apparent inverse correlation of TREC level and viral load in 2 patients in whom breakthrough viremia occurred, with a decrease in TREC occurring coincident with increased plasma viral load, and an increase occurring coincident with the decrease in plasma viral load produced by subsequent institution of a new effective regimen.

It should be noted that an increase in TREC could result from redistribution of naive T cells from a sequestered site, such as lymphoid tissue. However, quantification of TREC in lymph nodes of patients actually indicates a decrease in TREC levels (Figure 8) compared with uninfected controls, suggesting the absence of such a

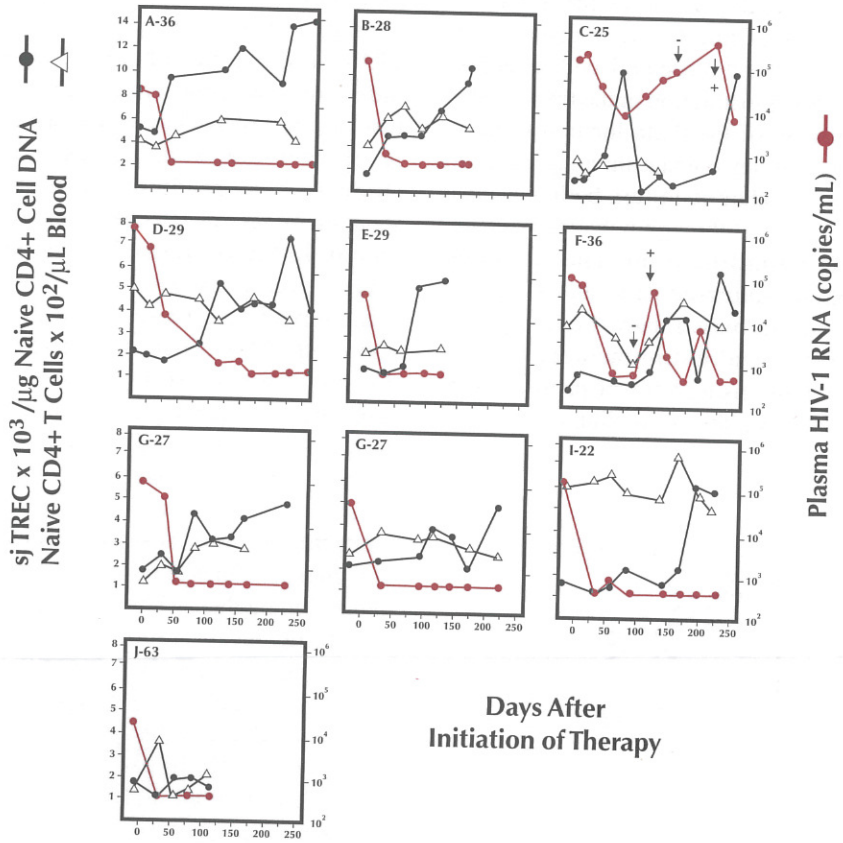

Figure 7. *Changes in TREC level (sj), naive CD4+ T-cell count, and plasma HIV-1 RNA level in 10 patients after initiation of potent antiretroviral therapy. TREC levels increased in all patients except a 63-year-old individual who discontinued the study early (J-63). Two patients with breakthrough viremia (C-25, F-36) exhibited decreased TREC in association with increased viral load and increased TREC in association with subsequent suppression of viral load upon switching therapy. Adapted with permission from Douek DC, et al.* Nature. *1998;396:690–695.*

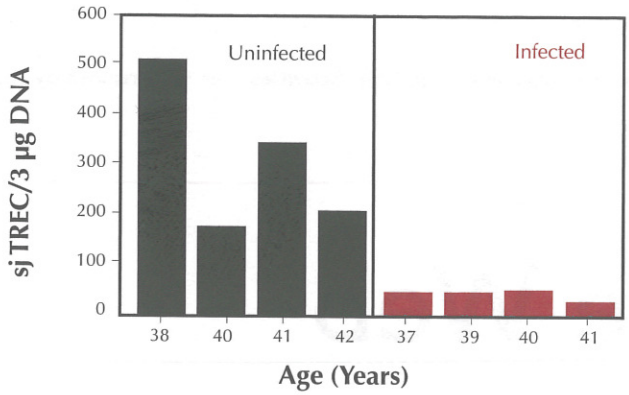

Figure 8. *Comparison of TREC levels (sj) in lymph nodes of uninfected individuals and HIV-1-infected individuals of similar age. Adapted with permission from Douek DC, et al.* Nature. *1998;396:690–695.*

reservoir. Thus, overall, these early findings indicate that the thymus is active in HIV-1-infected patients aged 20 to 40 years, and that thymopoiesis is contributing to the naive T-cell recovery observed in patients taking potent antiretrovirals.

CONCLUSIONS

Studies of TREC in adult thymectomy patients have shown that the thymus is functional, albeit at reduced capacity, late in life. The reduced levels of TREC observed in HIV-1 infection indicate that the virus adversely affects thymic output or causes dilution of TREC within recent thymic emigrants, or both. The finding that TREC increases after initiation of potent antiretroviral therapy indicates that the thymus is functioning and producing new naive T cells and thus that some of the increase in naive CD4+ T cells observed in patients during potent antiretroviral therapy is derived from this source. These findings, however, do not refute that peripheral expansion of naive T cells is also operative in maintaining CD4+ T-cell homeostasis.

Together, these data raise the possibility that prolonged viral suppression in the presence of a functioning thymus could result in replacement of clones of naive CD4+ T cells that had been deleted by HIV-1, which could eventually lead to complete reconstitution of a virus-ravaged immune system. Although available short-term data do not show any significant return of HIV-1-specific CD4+ T-cell activity in patients initiating potent antiretroviral therapy during chronic infection, there are some indications of improvement, and it is unclear what will occur with longer-term follow-up during continued viral suppression. Other recent data have indicated that recovery of proliferative responses to antigens and repair of lymph node architecture can be observed later in the course of potent therapy, indicating a recovery of overall immune function. It currently is unclear what the time frame of reconstitution of immune function might be, and how this process is affected by age and by individual variations in thymic function. As in the case of prospects for restoring HIV-1-specific immune function by combining potent antiretrovirals with immunotherapy, the demonstration of a functioning thymus in adult patients raises the possibility of employing interventions to enhance thymic output to hasten immune reconstitution.

Richard A. Koup, MD, is the Jay P. Sanford Professor of Infectious Diseases and Chief of the Division of Infectious Diseases at University of Texas Southwestern Medical Center in Dallas.

SUGGESTED READING

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- What are effective strategies for antiretroviral failures?
- Is there a role for resistance testing in the clinic?
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Format

Each CME activity will focus on a complex issue in HIV disease management and will include a didactic introduction to the issue and several caselettes with interactive sets of clinical decision points. Each caselette uses a clinical model to explore a selected topic and issues of controversy or confusion in HIV medicine. Also included will be a suggested reading list and links to other relevant educational resources on the Web.

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Editor in Chief
Michael S. Saag, MD, Professor of Medicine
Director, AIDS Outpatient Clinic, University of Alabama at Birmingham

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Meg Newman, MD, Assistant Professor of Medicine
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First CME Activity
"Clinical Management Issues in Antiretoviral-Experienced Patients"
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Director, Positive Health Program, San Francisco General Hospital

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New Developments in the Pathogenesis of HIV-1 Infection: Viral Dynamics and a Reservoir for HIV-1

Characteristics of latent HIV-1 infection in resting CD4+ cells and implications for antiretroviral treatment were discussed by Robert F. Siliciano, MD, PhD. The following article summarizes his presentation.

With the advent of potent antiretroviral therapy and the ability to reduce plasma viral load levels below the limits of detection of available sensitive assays for prolonged periods, it was initially hoped that prolonged treatment could eradicate HIV-1 from the body. Initial studies showing that rapid and dramatic decreases in plasma viral load could be achieved with single potent antiretroviral drugs provided important information on viral and cellular dynamics of infection. These studies indicated that HIV-1 infection is a dynamic process consisting of new rounds of infection and replication in susceptible cells with rapid decay of both free virus and productively infected cells. The rapid decline in viral load suggested that new infection of cells was prevented, revealing the intrinsic decay rate of the various cellular compartments of the virus. Analysis of decay rates in these studies and in subsequent studies using potent antiretroviral therapy showed that an initial rapid decay phase was followed by a slower decay phase, with the second phase being attributed to turnover of chronically infected cells (Figure 1). With the ability of potent antiretroviral therapy to maintain plasma viral load below limits of detection of available assays, it was hypothesized that given continuation of this second decay rate, virus could potentially be completely eliminated with 2 to 3 years of therapy.

However, the nature of viral dynamics when plasma viral load is below assay detection limits remained undefined. Evidence of ongoing viral replication in patients on potent antiretroviral therapy comes from the following observations: (1) not all patients on therapy achieve plasma viral loads below limits of detection and (2) virologic failure can occur in patients fully suppressed on therapy upon switching treatment to a less intense regimen. Such findings suggest that although current potent antiretroviral regimens can reduce plasma viral load below detection limits, ongoing viral replication persists. One form of viral persistence consistent with these observations would be a low-level 'smoldering' infection in CD4+ cells. Other mechanisms that might account for viral persistence consist of reservoirs of persistent HIV-1. Identified reservoirs include follicular dendritic cells, located in the germinal centers of peripheral lymphoid tissue. These cells can retain antigenic material on their surface for prolonged periods, with half-life of this reservoir having been estimated at approximately 2 weeks. Persistently infected macrophages constitute a second potential reservoir; since HIV-1 infection does not kill these cells, they may continue to produce virus for their life span. The half-life of macrophages is approximately 2 weeks, and mathematical models have suggested that the second phase of decay in plasma viral load under antiretroviral treatment is due to turnover of these cells. The most significant of viral reservoirs appears to be resting memory CD4+ cells bearing an integrated copy of the viral genome. Since these cells have an exceptionally long life span, this reservoir would appear to represent a major barrier to the goal of viral eradication.

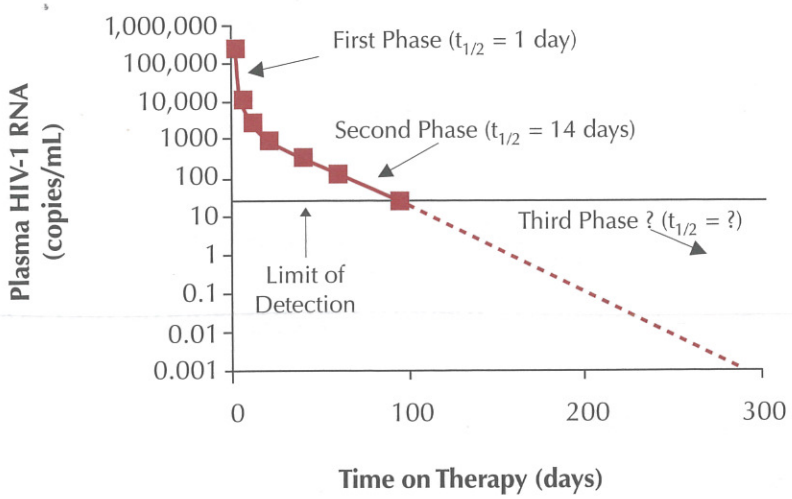

Figure 1. *Representation of 2 identified phases of plasma HIV-1 RNA decay with introduction of potent antiretroviral therapy and reduction of viral load to levels below detection limits of the assay. The first phase is attributed largely to turnover of infected activated CD4+ cells and the second largely to turnover of infected macrophages or other chronically infected cells. Adapted with permission from Finzi D, et al.* Cell. *1998;93(5):665–671. Copyright 1998 Cell Press.*

ESTABLISHMENT OF LATENT INFECTION IN CD4+ CELLS

CD4+ T cells emerge from the thymus and enter the circulation as naive cells. Upon encountering antigen, they are activated and proliferate, carrying out various immunologic functions in response to the initiating antigen (Figure 2). After several rounds of division, some of these cells revert to an inactive state as memory T cells that permit an immune response in subsequent cases of exposure to the activating antigen. Each of these stages of T-cell development is permissive for HIV-1 infection. Whereas infection of activated CD4+ cells results in productive infection, latent infection may be established in resting cells via 2 mechanisms (Figure 3). Preintegration latency is a transient form of latency in which nuclear import of transcribed viral DNA does not occur after fusion of the virion with a resting CD4+ cell and reverse transcription of viral RNA. If the cell becomes activated before HIV-1 genomes in the cytoplasm are degraded (a process occurring over hours to days), nuclear import, integration into chromosomes, virus gene expression, and assembly and release of infectious virus can occur. The more stable postintegration latency can occur when activated CD4+ cells, in which integration of proviral DNA has occurred, revert to a resting state with minimal transcription of viral genes; such cells must survive both the cytopathic effects of the infection and cytolytic host effector mechanisms. As discussed below, studies have now definitively established the existence of a pool of such cells in infected individuals. In theory, activation of these cells would result in productive infection at low levels; although virus produced from such a reservoir would constitute only a small proportion of plasma virus in untreated individuals, persistence of the virus in this reservoir assumes considerable importance in patients in whom viral load is profoundly suppressed with potent antiretroviral therapy.

Postintegration latency can occur when activated CD4+ cells, in which integration of proviral DNA has occurred, revert to a resting state with minimal transcription of viral genes

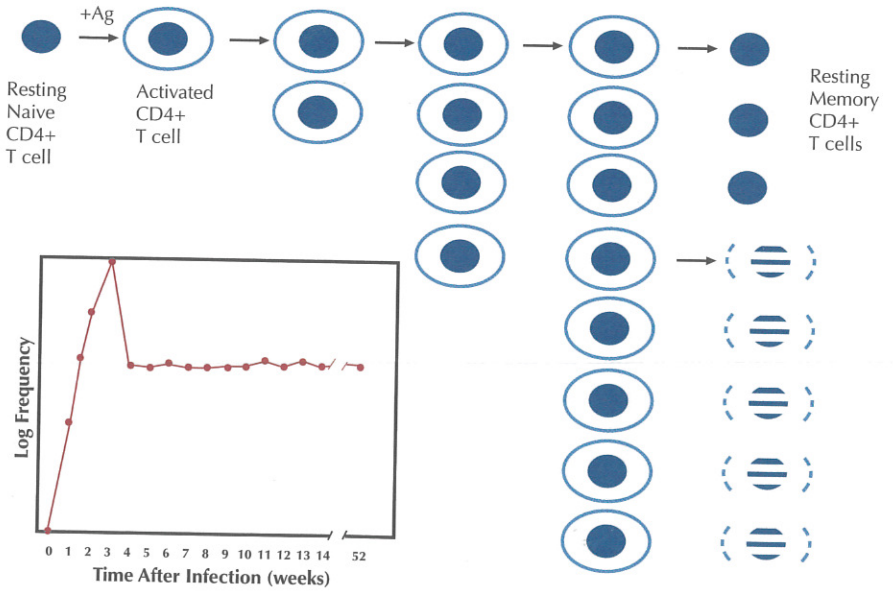

Figure 2. *Representation of activation, proliferation, and deletion or reversion to inactive (memory) state of CD4+ T cells in response to infection. Inset shows frequency of antigen-specific cells during and after immune response to infection.*

LATENT RESERVOIRS IN BLOOD AND LYMPHOID TISSUE

Studies to detect resting CD4+ T cells with integrated viral DNA were conducted by isolating high-purity populations of resting cells and utilizing an inverse polymerase chain reaction to amplify a segment of the proviral DNA. Studies in peripheral blood from HIV-1-infected donors showed that cells with integrated viral DNA constituted less than 0.01% of resting CD4+ cells. Additional studies of samples of peripheral blood and lymph nodes from patients with asymptomatic HIV-1 infection showed that the frequencies of such cells were similar in blood and lymph node tissue, ranging from less than 16 to 410 cells per million resting cells in the latter, and were not correlated with CD4+ count, plasma HIV-1 RNA level, or antiretroviral therapy. Subsequent studies, utilizing a novel quantitative culture assay to determine what proportion of these cells could produce infectious virus, showed mean frequencies of replication-competent virus of 5 and 7 per million resting cells in lymph node and blood, respectively, with these reduced estimates suggesting the presence of defective viral DNA in some cells (Figure 4). Estimates of the total body number of resting cells with integrated viral DNA based on these data indicate a range of from 4.6×10^6 to 3.4×10^7, with a mean of 1.2×10^7.

PERSISTENCE OF LATENT INFECTION IN PATIENTS ON POTENT ANTIRETROVIRAL THERAPY

Although the size of the pool of resting CD4+ cells with replication-competent virus is small, the long life of these cells

suggests that the reservoir represents an enduring potential for rekindling of infection. In a recent study, Dr Siliciano's group evaluated the presence of latent virus in a selected group of 22 patients who were highly adherent to their antiretroviral therapy regimen. These patients had received a 3- to 5-drug regimen including a protease inhibitor for up to 30 months, had exhibited increased CD4+ cell count and a rapid decline in plasma HIV-1 RNA to levels below detection (<200 copies/mL) that was consistently maintained over the course of treatment, and were heterogeneous with respect to age, sex, risk factor for infection, disease stage, prior treatment, and current antiretroviral regimen. Dr Siliciano's group used a culture method in which resting CD4+ T cells from these patients were exposed to optimal conditions for activation prior to co-culture. This method showed that replication-competent virus was present in all 18 cases in which a sufficient number of resting cells were available for evaluation, with frequencies ranging from 0.2 to 16.2 cells per million. Cross-sectional analysis (Figure 5) showed that the frequency of resting cells with replication-competent virus did not decrease according to time on therapy, suggesting relative stability of this reservoir with potent antiretroviral therapy.

In a subsequent study, Dr Siliciano's group attempted to directly measure the decay rate of latently infected cells by following patients longitudinally. In a group of 42 patients receiving potent antiretroviral regimens, latently infected resting cells were detected in 33 of 35 who had responded to therapy with a decrease in plasma HIV-1 RNA level to less than 200 copies/mL and maintenance of viral load below this detection limit. After an initial decline in frequency of latently infected cells due to turnover of those cells with preintegration latency during the initial weeks of treatment, frequencies clustered around a value of 1 cell per million (Figure 6). These remaining cells appear to decay quite slowly, with longitudinal follow-up indicating a half-life value on the order of 44 months. At this decay rate, eradication of this reservoir would require 60 years based on estimates of the total body number of resting cells with latent replication-competent virus. Of note, this estimated half-life has been repeatedly revised upward with continued follow-up as subsequent measurements in patients with an apparent more rapid decay have indicated return of the frequency value to that of previous measurements. This finding is likely attributable to the fact that measured values are generally close to the assay detection limits and that there is a wide 95% confidence interval surrounding each individual measurement. Thus it is likely that little or no change in actual number of latently infected cells has occurred. In addition, low levels of ongoing replication (occurring below the level of detection of the assays) could contribute to the generation of new latently infected cells with prolongation of the apparent half-life of this reservoir. It should also be noted that another group has reported a more rapid decay in some patients, with a half-life of approximately 6 months being reported in a small subset of patients who were treated within 90 days of infection.

The pool of cells with postintegration replication-competent virus appears to decay quite slowly, with longitudinal follow-up indicating a half-life value of approximately 44 months

To determine whether virus from this reservoir had developed resistance to combination treatment, nucleotide sequences of the HIV-1 *pol* gene were assessed in isolates from a subgroup of

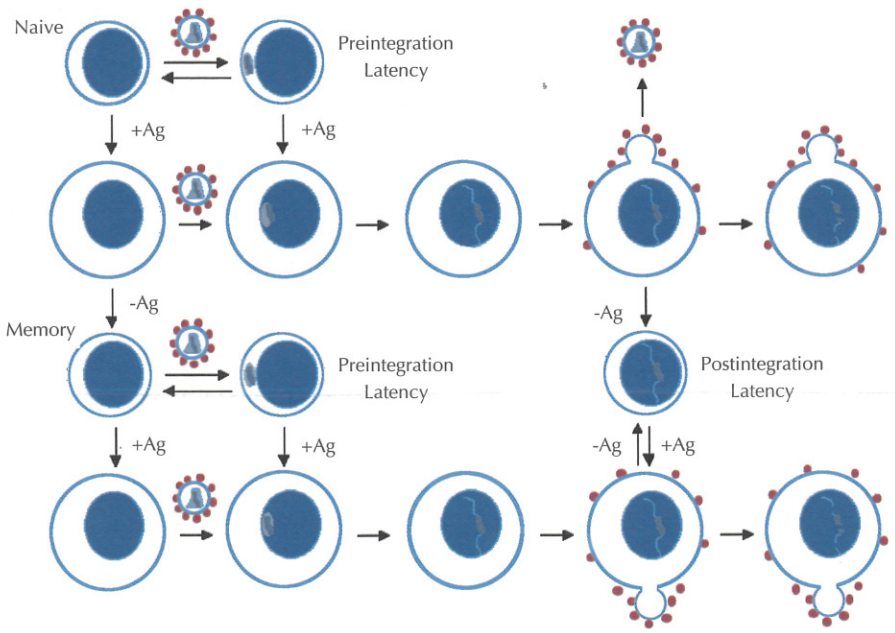

Figure 3. *Schematic of preintegration latency and postintegration latency of HIV-1 in resting memory cells. Activation by reexposure to antigen may result in productive infection in both cases. Adapted with permission from Finzi D, et al.* Cell. *1998; 93(5):665–671. Copyright 1998 Cell Press.*

patients, with results indicating that the virus from these cells were of drug-susceptible genotypes. These findings support the notions that potent antiretroviral therapy was successful in suppressing viral replication and selection of resistant mutants and that the isolates are derived from long-lived cells infected prior to initiation of potent therapy—notions consistent with the hypothesis that these cells serve as a stable, enduring reservoir for HIV-1. This scenario is also supported by observations of a patient in whom viral load under therapy with ritonavir/ saquinavir/zidovudine/lamivudine after initial failure of ritonavir monotherapy has occasionally been above detection limits. To test the hypothesis that the reservoir of latently infected cells might be reseeded by ongoing active infection, genotypic analysis of isolates was performed to detect the presence of resistant variants that might be expected to accumulate in these cells in the setting of ongoing replication. It was found that each set of isolates from this patient contained predominantly wild-type sequences with some variants that may have emerged under initial ritonavir therapy, although the frequency of the latter has remained unchanged over time. Such findings suggest that this reservoir is established during primary infection with largely wild-type virus and is relatively stable thereafter. The idea that early treatment might prevent establishment of this reservoir is not supported by findings to date; several of the patients assessed in these studies were treated during primary infection, including a patient in whom treatment was started within 48 hours of presentation with acute retroviral syndrome, and latently infected cells have been identified in all of these patients.

Evidence suggests that the latent reservoir is established during primary infection with largely wild-type virus and is relatively stable thereafter

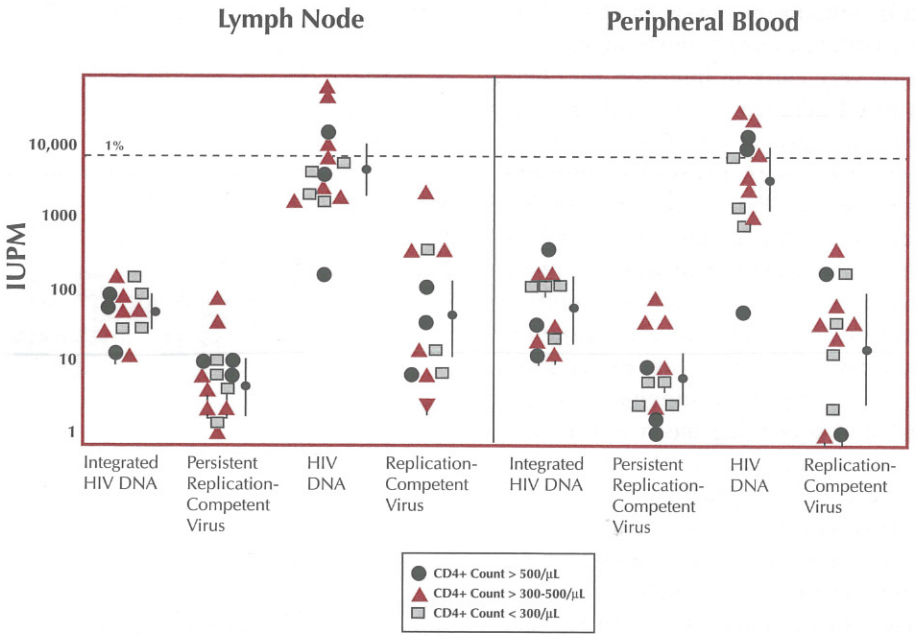

Figure 4. *Frequency of integrated HIV-1 DNA and replication-competent virus in resting CD4+ T cells in lymph node tissue and peripheral blood. IUPM indicates infectious units per million cells. Adapted with permission from Chun T-W, et al.* Nature. *1997;387:183–188.*

PROSPECTS FOR ERADICATION OF THE LATENT RESERVOIR

A recent report by investigators from the National Institutes of Health suggests that eradication of the latently infected CD4+ cells may be possible with the addition of interleukin-2 (IL-2) to antiretroviral therapy to activate the resting cells, resulting in cell death from viral cytopathic effect or from immune mechanisms. In this report, latently infected cells became undetectable in 3 of 14 patients treated in this manner. In addition to the possibility that latently infected cells persisted in these patients at a frequency below assay detection limits, there are a number of issues regarding the potential for success of strategies involving IL-2 use. One is that resting cells (ie, CD25-phenotype) do not express high-affinity IL-2 receptors (but do express low-affinity receptors), leaving it generally unclear how the effects of IL-2 in activating the cells might be mediated. Second, it is unclear what fraction of these cells might become activated by IL-2, an important consideration given that all of the cells must become activated for eradication to be achieved. Finally, the fate of the cells that become competent for transcription of the latent provirus is also unclear, with the mechanism of clearance in vivo remaining undefined. Continued investigation of the effects of IL-2 or other methods of activating latently infected cells in this setting will provide a better idea of the potential utility of such an approach.

It is also possible that the potential adverse consequences of the existence of this reservoir might be avoided through methods to enhance effector immune response. Studies in long-term nonprogressors with HIV-1 infection indicate the presence of a strong HIV-1-specific

cellular immune response, and there have been isolated case reports of patients treated very early in infection who have exhibited delayed or no viral rebound upon withdrawal of treatment in apparent association with preserved immune response. Dr Siliciano's group has detected latently infected resting CD4+ cells in one such patient who has maintained a plasma viral load below limits of detection for 33 months after stopping treatment, suggesting that under some conditions immune mechanisms are capable of preventing this reservoir from rekindling infection in the absence of continued antiretroviral therapy.

It should be noted that attempts to identify latently infected cells have not been successful in all patients, including subsets of patients who were rescued from advanced disease by potent antiretroviral therapy, who had a low number of such cells prior to therapy, or who were long-term nonprogressors, in addition to those patients receiving antiretrovirals plus IL-2 mentioned above. Although it is probably the case that in each of these instances levels of latently infected cells simply are below limits of detection, it has been the experience of Dr Siliciano's group that it is often difficult to identify such cells in patients who initiated effective antiretroviral therapy in very advanced disease—eg, at CD4+ cell counts below 10/μL—and responded with CD4+ cell count increases of large magnitude. It is possible that immune reconstitution in such patients includes new cells that do not acquire latent infection, raising the hope that generation of latent infection may not be a general consequence of immune reconstitution.

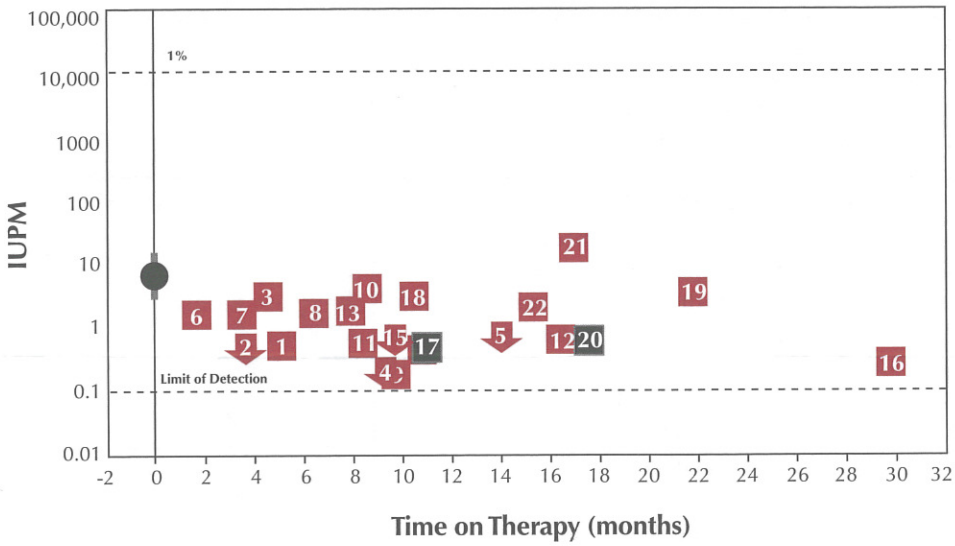

Figure 5. *Cross-sectional analysis of frequency of latently infected resting CD4+ T cells in individual patients by time on potent antiretroviral therapy. IUPM indicates infectious units per million cells. Adapted with permission from Finzi D, Hermankova M, Pierson T, et al.* Science. *1997;278:1295–1300. Copyright 1997 American Association for the Advancement of Science.*

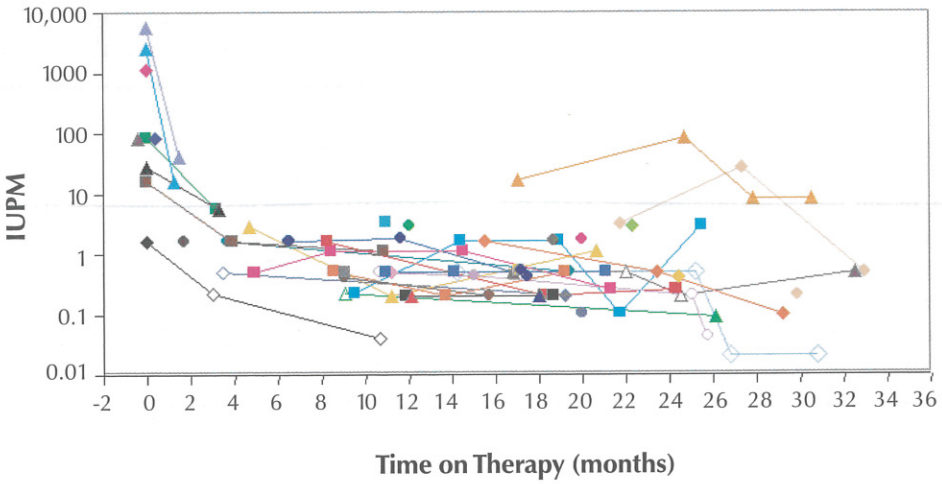

Figure 6. *Longitudinal analysis of frequency of latently infected resting CD4+ T cells by time on potent antiretroviral therapy. IUPM indicates infectious units per million cells. Adapted from Finzi D, et al.* Nat Med. *1999;5(5):512–517.*

CONCLUSIONS

The findings discussed indicate that HIV-1 can establish a state of latent infection in long-lived resting memory CD4+ T cells, with this reservoir persisting in patients taking potent antiretroviral regimens who have maintained plasma viral load below limits of detection for prolonged periods. This reservoir appears to be established early in infection and to be relatively stable over time; the current estimate of the half-life of this reservoir is 44 months, indicating the potential survival of these cells for the lifetime of infected individuals. Although it is known that memory T cells are long-lived, the life span of such cells in uninfected individuals has not been established. It is known that functional memory for viral infections can persist for at least 60 years, although it is unknown whether this long-term memory reflects survival of memory cells. Studies of memory T cells with dicentric chromosomal lesions, which die during mitosis, have indicated an intermitotic half-life of approximately 5 months. However, given the significant disruption of T-cell homeostasis that occurs in HIV-1 infection, it is unclear whether these findings can be applied to

infected individuals. Additional studies to define more clearly the decay rate of this reservoir are needed.

The implications of the long-term persistence of latently infected cells include the possibility of a rekindling of infection if antiretroviral therapy is stopped. On the other hand, the early findings indicating that the virus in this reservoir in patients on potent antiretroviral therapy represents drug-susceptible virus indicates that therapy is capable of completely suppressing active viral replication and emergence of resistant variants; thus, continued suppression of virus from the latent reservoir should be possible with continued treatment. Additional investigation of methods for eradication of virus from latent reservoirs is needed.

Robert F. Siliciano, MD, PhD, is Professor of Medicine and Molecular Biology and Genetics and Director of the Graduate Program in Immunology at The Johns Hopkins University School of Medicine in Baltimore, Maryland.

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Novel Targets for Antiretroviral Therapy: Pushing the *ENV*elope

Eric Hunter, PhD, discussed prospects for preventing HIV-1 fusion with and entry into target cells. Such prospects are based on the current understanding of the interaction of components of the viral envelope (Env) glycoprotein complex with CD4 and chemokine receptors on the target cell. The following article is a summary of Dr Hunter's presentation.

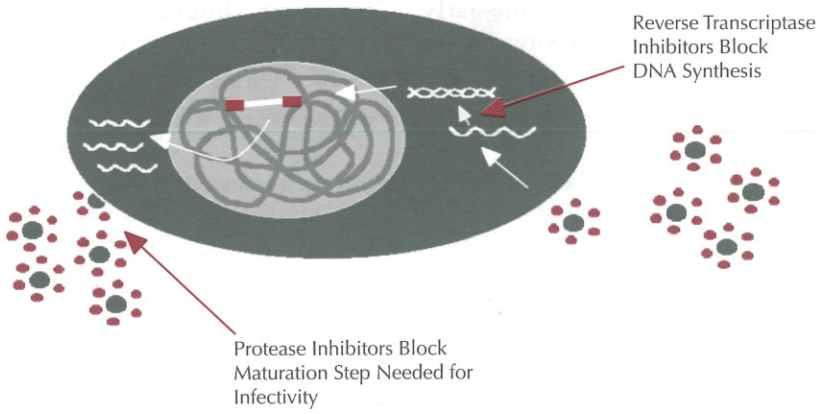

Figure 1. *Actions of currently available antiretroviral drugs in preventing viral replication.*

Currently available antiretroviral drugs act at one of 2 distinct phases of the life cycle of HIV-1 by inhibiting the viral reverse transcriptase or the protease enzymes (Figure 1). The reverse transcriptase inhibitors (RTIs) act to block viral DNA synthesis carried out by the enzyme. Nucleoside RTIs (nRTIs) act as analogues of the nucleosides used in viral DNA synthesis, whereas nonnucleoside RTIs (NNRTIs) act as allosteric inhibitors that modify the structure of the enzyme to render it inactive. The second stage of the life cycle shown to be particularly sensitive to inhibition is a stage in the maturation of the virion when the long precursor proteins that function to assemble virions at the plasma membrane are converted into protein shells that encase the viral genetic information and replicative enzymes. This process is accomplished by proteolytic cleaving of the individual precursor proteins to activated individual protein domains by the HIV-1 protease. Protease inhibitors act to inhibit this enzyme and block the virus from maturing into an infectious form. Both basic types of drugs currently used must thus enter the host cell in order to be functional against virus that has already entered the host cell; this requirement also results in cellular side effects due to cross-reactivity of the compounds with other enzymes within the cell and through other poorly defined mechanisms. The prospect of preventing viral entry is thus inviting as a means of more directly blocking the viral replicative cycle. Increased understanding of the mechanisms of viral entry into host cells has allowed the formulation of a number of approaches by which this end might be achieved.

INTERACTION OF HIV-1 ENV COMPLEX WITH CD4 AND CHEMOKINE RECEPTORS

It is now known that HIV-1 interacts with 2 molecules encoded by the target cell to gain cell entry: the high-affinity receptor CD4, which is essential for both viral entry and cell-cell fusion in syncytium formation, and 1 of a group of 7-transmembrane protein molecules that function as receptors for cellular chemokines. Interaction of the HIV-1 surface envelope glycoprotein with both of these surface molecules appears to be required for the process of fusion of the viral membrane and cell membrane that permits viral entry (Figure 2).

The role of the CD4 molecule in viral entry was recognized many years ago on the basis of the observation that the virus primarily infected cells with significant levels of surface CD4 molecules; it was subsequently shown that monoclonal antibodies to CD4 blocked infection of susceptible cells. After the gene encoding CD4 was isolated, it was further demonstrated that transfection of human cells with the gene rendered the cells susceptible to infection. Initial evidence for a second receptor for viral entry was provided by studies showing that transfection of mouse cells with CD4 genes did not render the cells susceptible to infection. Studies also showed that heterokaryons formed by fusion of the CD4-expressing mouse cells with human

Increased understanding of the mechanisms of viral entry into host cells has allowed the formulation of a number of approaches for preventing entry

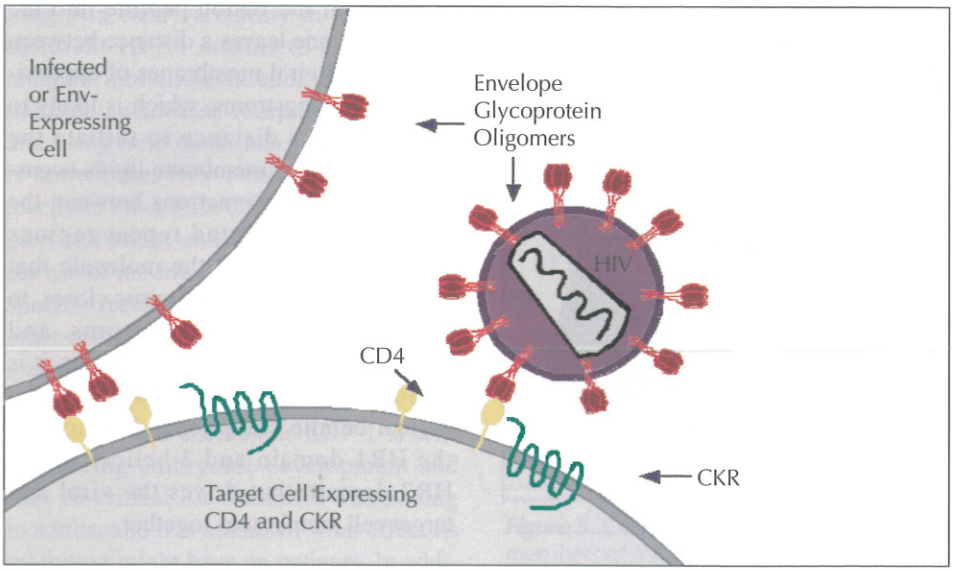

Figure 2. *Interaction of HIV-1 envelope (Env) complex with CD4 receptors and chemokine receptors (CKR).*

cells lacking CD4 were susceptible to viral infection. This finding indicated that a component of the human cells in addition to CD4 was active as a viral receptor. Prior to the identification of this second receptor, it was found that HIV-1 comprised macrophage-tropic and T-cell-tropic variants; although both types of virus efficiently bind CD4, it was found that neither infected the alternative target-cell type in culture.

Investigators at the National Institutes of Health subsequently identified a human gene that in conjunction with CD4 permitted entry of T-cell-tropic virus into mouse cells and did not permit entry of macrophage-tropic virus into the same cell. The gene encoded a previously cloned 7-transmembrane G-protein-binding chemokine-receptor-like molecule that was subsequently identified as the chemokine receptor CXCR4, a receptor that binds the chemokine SDF-1 and is involved in lymphocyte response to antigen stimulation. SDF-1 is a potent chemoattractant for lymphocytes and progenitor cells and appears to be constitutively expressed in many tissues.

The recognition that the chemokines RANTES, MIP-1α, and MIP-1β produced by CD8+ cells could block entry of macrophage-tropic but not T-cell-tropic virus led to the demonstration that the gene encoding the receptor (CCR5) for these chemokines conferred susceptibility to the former but not the latter viral variants. Additional evidence that the CCR5 receptor is involved in macrophage entry came from studies showing that peripheral blood cells with homozygous deletions in CCR5 genes are resistant to infection by macrophage-tropic virus.

ROLE OF GP120 AND GP41 IN VIRUS-CELL FUSION

Evidence has accumulated to show that binding with CD4 induces conformational changes in the glycoprotein components of the HIV-1 envelope complex. The changes permit binding to the coreceptor and viral and cell membrane fusion. Studies employing a monoclonal antibody as a pseudoreceptor believed to bind to HIV-1 at the same site as the extracellular portions of the chemokine receptor have permitted the determination of the 3-dimensional structure of the core of HIV-1 gp120, the viral envelope receptor-binding protein (Figure 3). Determination of the structure of the core as a complex with the CD4 molecule and the monoclonal antibody revealed that CD4 was bound specifically into a cleft in gp120, with it being hypothesized that the binding of CD4 resulted in exposure of the chemokine receptor-binding site. It was also shown that soluble CD4 induces conformational changes in both gp120 and gp41, the 2 components of the HIV-1 envelope

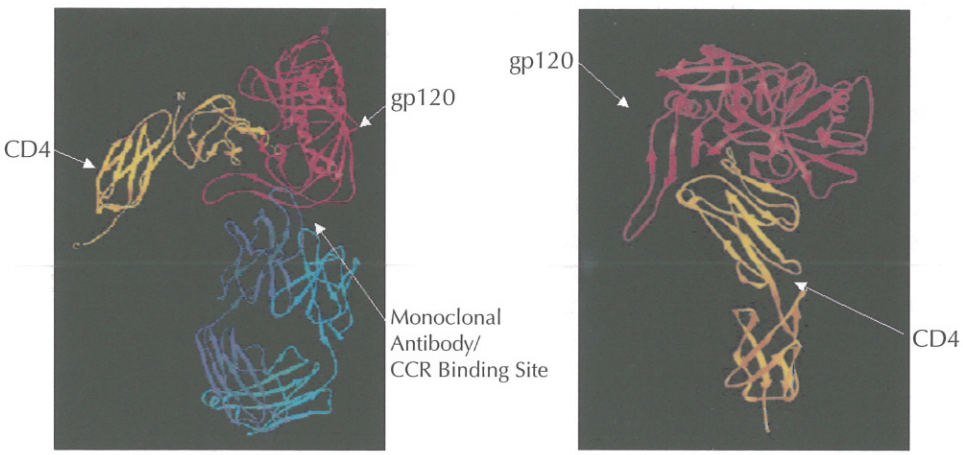

Figure 3. *Left: Overall structure of HIV-1 gp120 (shown in red) determined as a complex with CD4 (yellow) and a monoclonal antibody occupying a site believed to bind the chemokine receptor (light blue indicates light chain; purple, heavy chain). The ribbon diagram lacks many of the variable loop domains. Right: CD4 gp120 interactions. Ribbon diagram shows gp120 (red) binding to CD4 (yellow). Adapted from Kwong PD, et al.* Nature. *1998;393:648–659.*

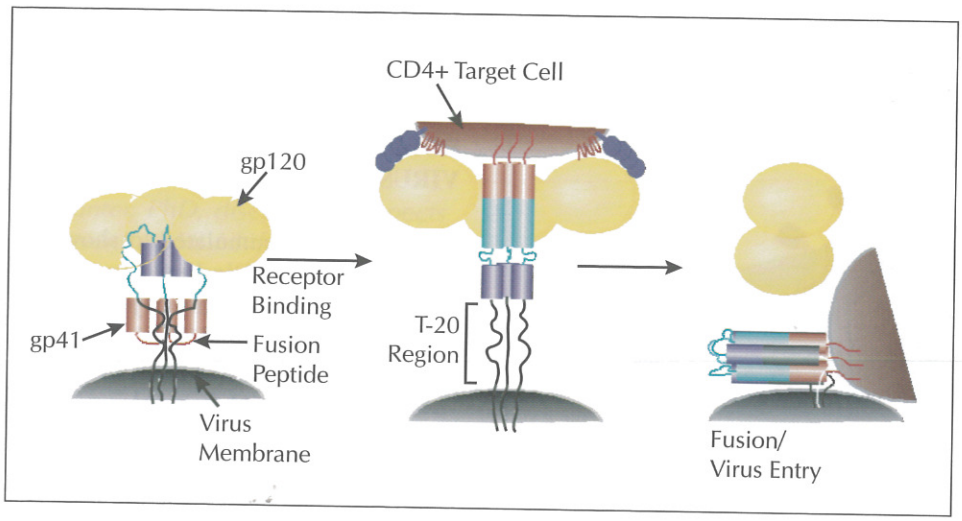

Figure 4. *Model for trimeric HIV-1 Env complex. Left: One gp120 molecule is cut away to show N-terminal heptad repeat constrained in an unstructured form. Middle: Following binding of the receptor complex, the extended-chain heptad repeat region can assemble into a trimeric coiled-coil, thereby allowing the fusion peptide to insert into the target cell membrane. Right: A second step in the conformational change is the formation of the 6-helix, coiled-coil bundle that results in apposition of the viral and target cell membranes. Adapted from Kilby JM, et al.* Nat Med. *1998;4(11):1302–1307.*

glycoprotein complex. As a result of binding with CD4, gp120 binds the chemokine receptor with a 100- to 1000-fold increased affinity, with normally hidden antigenic epitopes in both gp120 and gp41 being exposed.

These findings suggested that the conformational changes in the viral envelope were similar to those observed in the trimeric hemagglutinin molecules on the surface of the influenza virus upon exposure to the acidic environment of the cell endosome. In this case, rearrangement of the hemagglutinin HA2 region results in formation of a helical domain from an unstructured protein chain, with the coiled-coil formation acting as a structured rod to force a hydrophobic peptide, termed the fusion peptide, into the target cell membrane. It is believed that the changes occurring in HIV-1 gp120 and gp41 result in alteration of gp41 structure analogous to that observed with the hemagglutinin HA2 domain. In addition to 3 gp120 molecules, the HIV-1 envelope complex consists of 3 gp41 molecules; the extracellular regions of gp41 consist of an amino terminal hydrophobic peptide and 2 heptad repeat regions (HR1 and HR2).

Receptor binding results in formation of a coiled-coil structure from the unstructured HR1 domain of gp41, similar to that observed in the corresponding influenza hemagglutinin region, that forces the fusion peptide into the host cell while the host CD4 and chemokine receptors remain bound (Figure 4). The insertion of the fusion peptide into the cell membrane leaves a distance between the cell and viral membranes of approximately 100 angstroms, which is likely to be too large a distance to initiate the actual mixing of membrane lipids occurring in fusion. Interactions between the HR1 and HR2 heptad repeat regions result in a folding of the molecule that brings the target cell and virus closer, to within less than 20 angstroms, and allows fusion to occur. In particular, it is believed that it is the formation of a helical bundle comprising 3 helices of the HR1 domain and 3 helices of the HR2 domain that drives the viral and target cell membranes together.

Strategies to block the processes of viral fusion and entry include blockade of CD4-receptor or chemokine-receptor binding and inhibition of gp41-mediated fusion

STRATEGIES FOR BLOCKING FUSION AND ENTRY

Based on current knowledge of the viral fusion and entry processes, a number of strategies to block these processes have been formulated, including blockade of CD4-receptor or chemokine-receptor binding and inhibition of gp41-mediated fusion.

More than a decade ago, it was shown that soluble CD4 molecules could block HIV-1 infection of cells and syncytium formation in vitro, with it being shown that its activity in this regard was synergistic with nRTIs. However, although the use of soluble CD4 was associated with minimal toxicity, clinical investigation revealed an absence of effect on viral replication in vivo. Moreover, it was shown that soluble CD4 did not inactivate uncultured patient isolates of HIV-1, and it has since been demonstrated that it can potentiate entry of infecting strains in vivo.

The prospects for inhibition of chemokine receptors remain uncertain. It is known that chemokines capable of binding these receptors, such as RANTES and the CCR5 macrophage coreceptor, can block entry of virus. Further, the pharmaceutical industry has experience with 7-transmembrane receptors, and it has been shown that variants of RANTES, for example, can compete for the receptor-binding site with gp120 without triggering functional activity of the lymphocyte (ie, without triggering G-protein-coupled stimulation). However,

there is a clear variability in response of different HIV-1 strains to chemokine-receptor inhibition. In addition, there are multiple chemokine receptors (Figure 5), and many bind more than 1 chemokine; it is known that HIV-1 can use chemokine receptors other than CXCR4 and CCR5 to gain cell entry, and it is unclear whether the use of modified chemokines to block a specific receptor might drive virus to use other receptors. Finally, the potential clinical consequences of blocking chemokine pathways are unknown; for example, it is known that the chemokine SDF-1 is critical during embryonic development and may be critical for continued functioning in adults, and it is unknown what effect its inhibition might have on patients. In addition to the RANTES derivatives and other beta chemokines that have been shown to inhibit HIV-1 in vitro, 2 small-molecule inhibitors that block gp120 binding with the CXCR4 receptor for T-cell-tropic virus recently have been described; both ALX40-4C and the bicyclam AMD3100 have been found to be potent inhibitors of HIV-1 replication in vitro. Small-molecule inhibitors of the viral envelope interaction with CCR5 receptors are in preclinical development.

The potential promise of strategies for preventing viral entry by inhibiting gp41-mediated fusion was suggested by initial findings indicating that mutations in the gp41 HR1 region rendered virus noninfectious. Subsequent investigation has shown that peptides corresponding to the HR1 and HR2 regions (DP107 and DP178) can inhibit membrane fusion and virus infectivity if they are present at the time of virus-cell interaction, with a correlation being found between inhibition of viral replication and the ability of the peptides to form helical coiled-coil structures. The DP178 peptide, now known as T-20, is the most potent of these peptides, and has entered early phase clinical study.

It is postulated that T-20 peptide works by blocking the formation of the helical bundle that brings the viral and cellular membranes together. Hence, the peptide would prevent the gp41 HR2 domain from folding into the already formed triple helix coiled-coil structure of the altered HR1 domain. T-20 was shown to be active at a concentration of 1 ng/mL (IC_{50}) and to inhibit infectivity of a variety of primary HIV-1 isolates in in vitro studies and to reduce viral RNA to levels below limits of detection in SCID-Hu mouse models of HIV-1 infection. In an initial dose-escalation trial, T-20 was administered intravenously twice daily at doses of 3, 10, 30, and 100 mg for 15 days, and the effect on viral load was observed. As shown in Figure 6, changes in plasma viral load were dose dependent, with patients receiving the highest dosage of 200 mg/d having viral load below limits of detection by the end of treatment. It is noteworthy that the kinetics of reduction in viral load in the latter patients resemble those observed in patients receiving potent combination antiretroviral therapy. Subsequent early phase studies of subcutaneous administration of T-20, which was shown to result in blood concentrations and trough levels comparable to those observed with intravenous administration, have yielded promising results. Although use of even a relatively small peptide could generate an anti-peptide immunogenic response, anti-

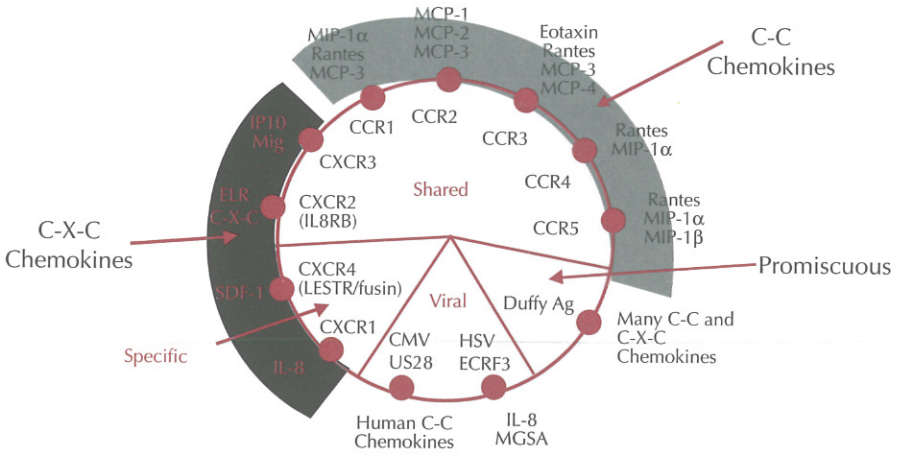

Figure 5. *Classification of chemokines and their receptors. Chemokine receptors are members of a 7-transmembrane G-protein-binding family of proteins. The receptors are broadly classified as C-C and C-X-C receptors based on amino acid structure. Adapted from Premack BA, et al.* Nat Med. *1996; 2(11):1174–1178.*

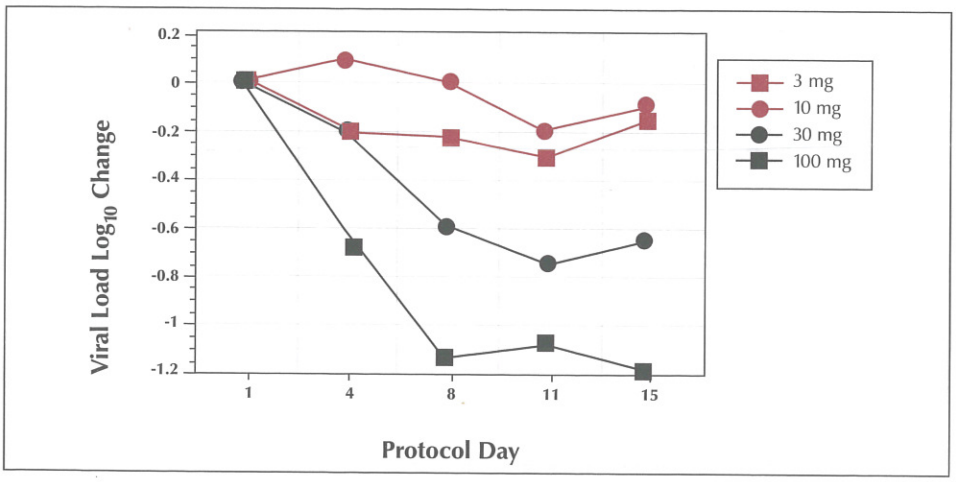

Figure 6. *Median plasma viral load change in patients receiving T-20 3, 10, 30, or 100 mg intravenously twice daily. Adapted from Kilby JM, et al.* Nat Med. *1998;4(11):1302–1307.*

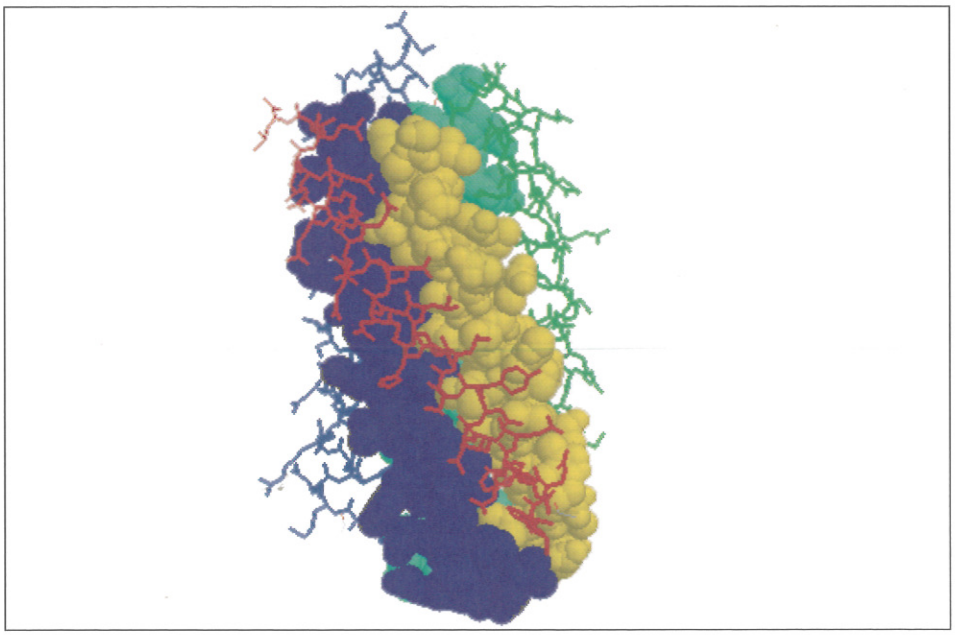

Figure 7. *Three-dimensional structure of the helical bundle formed between the HR1 (N-terminal) and HR2 (C-terminal) domains of gp41 showing HR2 fitting into a binding groove on the HR1 coiled-coil structure.*

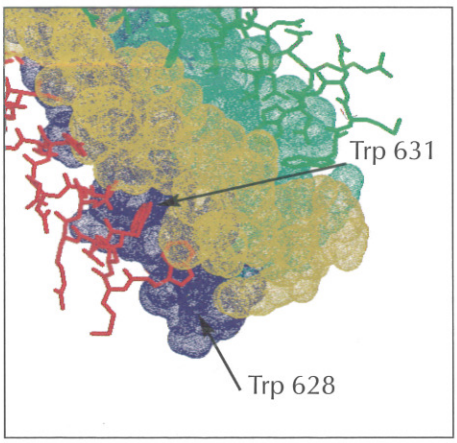

Figure 8. *C-terminal heptad repeat pocket in HR1 structure into which tryptophans (Trp) of the HR2 domain are embedded.*

bodies to T-20 do not appear to inhibit T-20 activity in HIV-1-infected patients or in vitro. Specific antibody responses to T-20 have not been observed thus far in treated patients, including at least 1 patient treated for more than 1 year. Early viral rebound has been observed in some patients in an early phase II study in apparent association with viral resistance to T-20; testing in vitro indicates that mutations can arise in the region of the viral genome encoding the HR1 region of gp41 that forms the groove into which the T-20 molecule fits. These changes presumably prevent contact of T-20 but allow function of the corresponding wild-type glycoprotein sequence. Overall, however, these early findings indicate that fusion inhibition may be a feasible strategy for inhibiting viral replication in vivo and could form part of a divergent treatment strategy.

Given these encouraging findings and the proposed mechanisms of virus-cell fusion, it is plausible that small-molecule inhibitors of fusion might be developed. Analysis of the 3-dimensional structure of the helical bundle formed between the gp41 HR1 and HR2 domains as part of the fusion process indicates that the HR2 peptide fits into a deep binding groove on the surface of the HR1 coiled-coil structure (Figure 7); within this groove is a deep pocket into which the tryptophans of the HR2 domain are embedded (Figure 8). Small molecules that can inhibit the fusion process at this site are now actively being sought.

CONCLUSIONS

HIV-1 entry into host cells requires the presence of both CD4 and a chemokine receptor protein on the host cell. Viral binding with the target cell induces conformational changes in the gp120 and gp41 components of the HIV-1 envelope complex that mediate viral and cell membrane fusion. Receptor binding and the protein-protein interactions involved in the conformational change appear to be suitable targets for therapeutic intervention. The early clinical evaluations of the fusion inhibitor T-20 suggest that targeting interventions at the fusion stage will be a useful strategy. Additional investigation of small-molecule fusion inhibitors is needed.

Eric Hunter, PhD, is Professor of Microbiology and Director of the Center for AIDS Research at The University of Alabama at Birmingham.

Suggested Reading

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