Immune Reconstitution Strategies in HIV Infection

The immunopathogenic mechanisms of HIV-1 infection involve multiple complex interactions of the virus with the host's immune response to infection. Delineation of some of these mechanisms has led to development of immunologic strategies to better control HIV infection and to augment immune reconstitution observed with potent antiretroviral therapy. Strategies for improving or hastening immune reconstitution in the setting of potent antiretroviral therapy were discussed at the Los Angeles course by Ronald T. Mitsuyasu, MD.

IMMUNE DEFECTS IN HIV INFECTION

The primary immunologic defect in HIV-1 infection is the decline in CD4+ cell number and function, with decreased CD4+ cell function indicated by decreased delayed-type hypersensitivity reaction to recall antigens, decreased lymphoproliferative (LPA) responses to antigens, and decreased production of T_H1 cytokines, including interleukin-2, interleukin-12, and interferon gamma. Other defects include chronic immune activation resulting from ongoing viral replication, as evidenced by increased numbers of CD38+ and HLA-DR+ CD4+ T cells and CD8+ T cells, and increased production of proinflammatory cytokines (eg, interleukin-1, interleukin-6, and tumor necrosis factor). Perturbations of CD4+ and CD8+ T-cell repertoires may reflect clonal deletion or clonal exhaustion with ongoing HIV infection. The CD4+ cell repertoire abnormalities are relatively limited during early infection and worsen over time. The CD8+ cell repertoire appears to expand during early infection, possibly as a result of differentiation in response to HIV antigens, but subsequently exhibits a dramatic reduction. The decline in CD4+ cell count consists of declines in both memory (CD45RO+) and naive (CD45RA+, CD62L+) phenotypes. An increase in CD8+ cells during early infection, which is likely attributable to an increase in activated cells, includes an increase in memory cells and is followed by declines in overall cell number and both memory and naive phenotypes.

IMMUNE CHANGES WITH POTENT ANTIRETROVIRAL THERAPY

Potent antiretroviral therapy is associated with significant increases in CD4+ cell count. Ongoing investigation into immune reconstitution in this setting is examining the degree of immune reconstitution with continued viral suppression, specific quantitative and qualitative immune changes during therapy, the degree to which improved host immunity might play a role in regulating HIV replication and spread, and whether host immunity to HIV and other pathogens can be enhanced by specific immune interventions.

Compelling clinical evidence of functional improvement in host immunity is provided by data showing significant decreases in the incidence of opportunistic infections and mortality with the use of protease inhibitor-containing combination regimens (Figure 1). The significant increase in CD4+ cell count with potent antiretroviral therapy has now been shown by many investigators to be biphasic, consisting of an initial rapid increase (4 to 12 weeks after initiation of treatment) followed by a more gradual increase (Figure 2). It appears that the first phase increase is attributable to redistribution of cells from lymphoid tissue and to decreased activation-induced apoptosis; this initial increase is due largely to increased numbers of

The second phase increase in CD4+ cells appears to be due to increased proliferation characterized by a gradual and persistent increase in naive cells

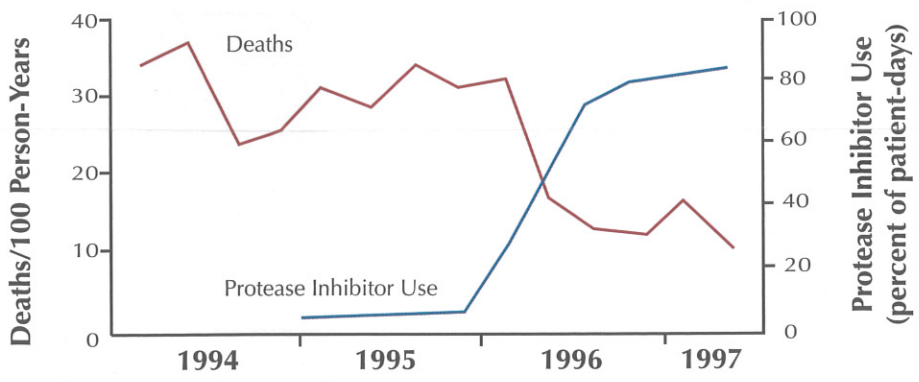

Figure 1. *Impact on mortality of the use of protease inhibitor-containing potent antiretroviral regimens among HIV-infected patients with CD4+ cell counts of less than 100/µL. Adapted from Palella F Jr, et al.* N Engl J Med. *1998;338:853–860. Copyright 1998 Massachusetts Medical Society. All rights reserved.*

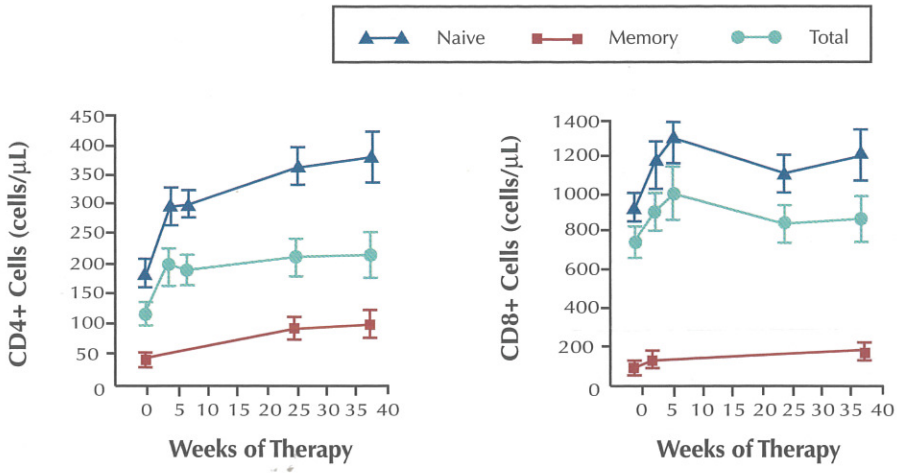

Figure 2. *Changes in total, memory, and naive CD4+ and CD8+ T cells in response to potent antiretroviral therapy. Bars show SEM. Adapted from Pakker NG, et al.* Nat Med. *1998.*

memory cells. The second phase increase appears to be due to increased proliferation of cells, characterized by a gradual and persistent increase in naive cells. The CD8+ cell population also exhibits a rapid early increase of both memory and naive cells, followed by a decline in total and memory CD8+ cell number but a persistent increase in naive CD8+ cells.

A number of findings now indicate that the increase in CD4+ cell number following the initial rapid rise reflects true expansion. Along with the increase in number of cells of naive phenotype, an increase in the number of cells positive for Ki67 (a marker of rapid cell proliferation) and an expansion of CD4+ T-cell repertoire have been demonstrated. Increases in newly-produced T cells after initiation of potent antiretroviral therapy have also been demonstrated using assays to measure incorporation of deuterated glucose into DNA of newly-produced cells.

Other recent studies have shown that there is a T-cell progenitor defect in HIV infection with an increase in thymic-derived cells after initiation of antiretroviral therapy, suggesting that effective treatment may be associated with improved thymic function. These studies use T-cell receptor rearrangement excision circles (TREC), produced during processing of T cells in the thymus, as a marker of thymic output. They have shown that (1) although thymic function declines with age, there is substantial output into late adulthood; (2) HIV infection is associated with decreased TREC levels in peripheral blood and lymphoid tissue; and (3) potent antiretroviral therapy results in a rapid and sustained increase in thymic output in the majority of patients. Other studies have shown that increases in thymic mass detected by computed tomography are associated with increases in naive CD4+ T cells in patients receiving potent antiretroviral therapy. The latter findings suggest both the potential contribution of the thymus to overall immune reconstitution and the potential for reconstitution of elements of the T-cell repertoire that may be lost through HIV infection. Available evidence indeed suggests that CD4+ T-cell repertoire normalizes to some degree during follow-up of patients receiving potent antiretroviral therapy; as noted, it has been observed that an initial expansion of the T-cell repertoire in CD8+ T cells is followed by a reduction in repertoire that does not improve during 6 months of subsequent follow-up.

CD4+ CELL FUNCTION

Functional improvement of CD4+ T-helper cells with potent antiretroviral therapy has been demonstrated in a number of studies. Research has shown improved delayed-type hypersensitiyity responses and increased proliferative responses to some recall antigens, including cytomegalovirus (CMV), *Candida*, and tuberculin antigens. However, functional reconstitution in patients with chronic HIV infection is incomplete with regard to LPA response to HIV antigens. For example, in a study of 39 patients taking potent antiretroviral therapy for 3 years with plasma HIV RNA maintained below 10,000 copies/mL, improved recall response to CMV, tuberculin, and *Candida* antigens but not to tetanus or HIV antigens was observed. Another study in patients with prior CMV retinitis and CD4+ cell counts below 100/µL has shown after 5 to 6 months of potent antiretroviral therapy improved LPA responses for CMV and *Candida* (in 70% of patients), *Mycobacterium avium* complex (in 50% of patients), and *Toxoplasma* and herpes simplex virus (in 20% of patients). One group of investigators has proposed that improved LPA response to mitogens and recall antigens may be due to a transient burst in interleukin-2 production seen with the rapid decrease in viral replication upon initiation of potent antiretroviral therapy. They proposed that initiation of antiretroviral therapy may produce a switch in predominance from T_H2 cytokines (interleukin-4 and interleukin-10) to T_H1 cytokines.

Functional reconstitution in chronic HIV infection is incomplete, including absence of significant improvement in LPA response to HIV antigens

The absence of a significant improvement of responses to HIV antigens over

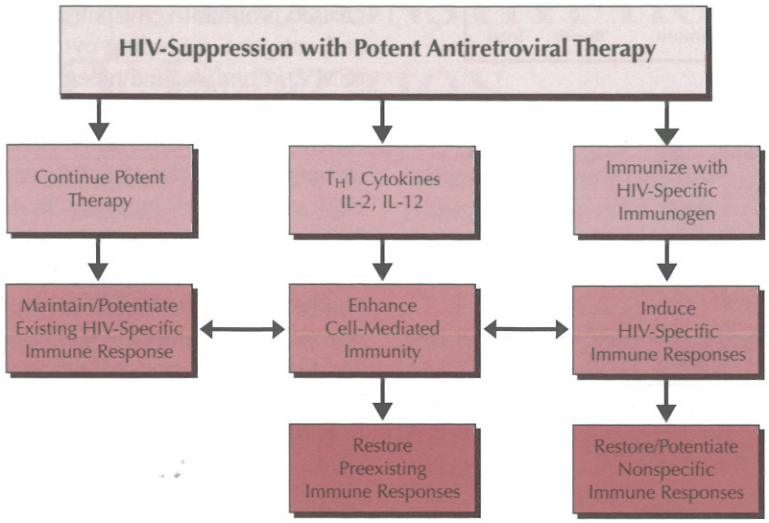

Figure 3. *Strategies for enhancing HIV-specific immunity.*

time on therapy raised concern that HIV-specific CD4+ T-helper cell and CD8+ cytolytic T-cell (CTL) responses may be irretrievably lost in HIV infection. Rosenberg and colleagues and Walker and colleagues have postulated that HIV-specific T-helper cell responses to HIV core and envelope antigens are lost shortly after acute infection in most patients, although HIV-specific responses remain strong in the context of preserved CD4+ cell count in HIV-infected long-term nonprogressors and have been shown to be preserved in patients initiating potent antiretroviral therapy during acute infection. Although data from some labs suggest that restoration of HIV-specific T-cell responses in patients with chronic infection is unlikely to occur with potent therapy alone, a number of additional findings suggest the possibility that such responses are not completely lost and that strategies to restore them may be feasible. One such finding is the observation that although CTL responses to a number of HIV antigens were reduced in association with reduction in viral load after initiation of potent antiretroviral therapy, CTL precursors appeared to increase in number. This finding suggests that CTL response may initially decline with decreased HIV antigen stimulation, but that appropriate stimulation of CTL precursors may permit restoration of this response over time. Similarly, it has been reported that interruption of antiretroviral therapy with a subsequent increase in viremia may result in increased HIV-specific CD4+ T-helper cell and CTL responses in some patients, suggesting that the capacity for heightened HIV-specific immune response may be stimulated with reexposure to viral antigens in vivo.

STRATEGIES FOR ENHANCING HIV-SPECIFIC IMMUNITY

It is possible that maintained effective antiretroviral therapy might ultimately allow reconstitution of HIV-specific immune responses; however, based on available data in patients followed for relatively long periods, this prospect seems unlikely in patients with chronic HIV infection. Two additional strategies for enhancing or hastening reconstitution of such responses are (1) immunizing with HIV-specific immunogen(s) and (2) using T_H1 cytokines to improve cell-mediated immunity (Figure 3). Improved control of viral replication over the long term may also permit better overall immune restoration.

Early investigation of therapeutic vaccination in combination with dual nucleoside reverse transcriptase inhibitor (nRTI) treatment yielded disappointing results regarding augmentation of immune response. However, a recent study of an HIV-specific, gp120-depleted, whole virus inactivated vaccine (Remune), in conjunction with potent antiretroviral therapy, has suggested the potential for significant immunologic improvements. The vaccine is an inactivated (heat- and chemical-treated and irradiated), whole virus immunogen derived from the HZ321 HIV strain (clade A Env and clade G Gag). The vaccine exhibits broad immune cross-reactivity with several viral subtypes, including clades A, B, and E. In a study reported by Valentine and colleagues, 43 patients with CD4+ cell counts above 350/μL were randomized to receive vaccine that contains incomplete Freund's adjuvant or adjuvant alone at weeks 4, 16, and 28 after initiation of potent antiretroviral therapy. At week 32, patients in the vaccine group exhibited significantly greater LPA responses to gp120-depleted HZ321 (P=0.005), native p24 antigen (P=0.0002), and clade B whole virus (P=0.007), as well as an increase in levels of the beta chemokine, MIP-1β, and a significant increase in delayed-type hypersensitivity skin test responses to p24 antigens compared with controls. The increase in MIP-1β may further decrease HIV spread in these vaccine-stimulated patients. Responses to HIV antigens were at least equal to those observed in long-term nonprogressors. Further, although the study was not designed to show differences in effect on viral load, a significantly greater proportion of vaccine recipients exhibited reduction of plasma HIV RNA to below 40 copies/mL (94% vs. 75%, P=0.0007). This

Proliferative responses to HIV antigens in vaccine recipients were at least equal to those observed in long-term nonprogressors

study has provided one of the first demonstrations of the ability to improve HIV-specific immune response through immune intervention in the presence of effective viral suppression. Additional study of this approach is needed to determine durability of response, effect on HIV-specific CTLs, and potential long-term virologic and clinical effects. Additional trials with this vaccine and several others are planned.

Other recent studies have assessed the effects of administering interleukin-2 with potent antiretroviral therapy. Early studies of interleukin-2 treatment indicated significant increases in CD4+ cell count but no additional effect on plasma viral load compared with antiretroviral therapy alone. In a recent study reported by Davey and colleagues, 78 patients with CD4+ cell counts of 200 to 500/μL were randomized to potent antiretroviral therapy with or without interleukin-2 at a dose of 7.5 MU twice a day for 5 days every 8 weeks for 6 cycles. At the end of treatment, mean CD4+ cell count had increased by 112% (from 355 to 739/μL) in interleukin-2 recipients compared with 18% (from 341 to 405/μL) in control patients, with similar magnitudes of increase being observed at all strata of baseline CD4+ cell count. Further, there was a trend toward reduction of viral load to below the limits of assay detection in a greater proportion of interleukin-2 recipients. The proportion of patients with plasma HIV RNA levels below 50 copies/mL increased from 39% before treatment to 65% after treatment in the interleukin-2 group and from 31% to 36% in the control group. Other recently reported studies of interleukin-2 dose and schedule in this setting have also indicated sizable increases in CD4+ cell count compared with antiretroviral therapy alone, with some studies also suggesting improved virologic response.

A report from the National Institutes of Health indicates that prolonged interleukin-2 treatment may result in an inability to recover viral DNA from pooled peripheral cells in some patients. The long-term durability of this effect will need to be addressed. Substudies of the fully accrued ACTG 328, a randomized controlled trial of interleukin-2 in patients on potent antiretroviral therapy with CD4+ cell counts of 50 to 350/μL, will evaluate the long-term benefit of interleukin-2 on CD4+ cell quantitative and functional recovery, effects on latently-infected cells, durability of viral suppression, frequency and rapidity of development of antiretroviral drug resistance, and expansion of naive CD4+ cells and TREC-positive cells. Other trials of interleukin-2 in combination with potent antiretroviral therapy have been initiated to evaluate whether improvements in quantitative or functional immune response translate into prolonged virologic effect and clinical benefit.

CONCLUSIONS

Preserving and restoring host immunity is important to regulating HIV expression and preventing opportunistic infections in HIV-infected individuals. Antiretroviral therapy increases CD4+ T-cell populations and reduces chronic activation of T cells, but does not appear to fully reconstitute normal T-cell function in patients treated during chronic infection. Studies with therapeutic immunization in the setting of potent antiretroviral therapy suggest that HIV-specific immune response may be improved with immune-based interventions. Studies combining interleukin-2 treatment with antiretroviral therapy also suggest that HIV-specific immunity may be enhanced through exogenous T_H1 cytokine treatment. Ongoing investigation will provide additional information on the characteristics of these responses, and future studies are likely to combine these approaches in an attempt to achieve more effective immune restoration and better control of HIV.

Dr Mitsuyasu is Associate Professor of Medicine at the University of California Los Angeles and Director of the UCLA CARE Center.

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