Novel Therapies for HIV-1 Infection

Novel therapies for HIV-1 infection were discussed at the Atlanta conference by Robert T. Schooley, MD, from the University of Colorado School of Medicine in Denver. Dr Schooley's presentation focused primarily on immune-based therapies and included a brief overview of genetic therapies.

Recent enthusiasm over the development of new antiretroviral drugs and strategies that have been effective in decreasing viral replication must be tempered with the knowledge that there are limitations to the benefits from these currently employed treatments. Dose-limiting toxicities, the emergence of virus with reduced susceptibility to antiretroviral drugs, and incomplete control of viral replication all contribute to the failure of current therapies to completely restore immune responsiveness in patients with HIV disease. It has also been observed that the increases achieved in CD4+ cell counts appear to plateau even with very effective antiretroviral drugs, at least in patients who have already experienced significant quantitative loss of CD4+ cells. In this context, there remains interest in developing such alternative treatment approaches as immune-based and genetic therapies.

General Immune Modulation

There are two general approaches to immune-based intervention: (1) enhancement of HIV-1–specific immune-effector mechanisms; and (2) general augmentation or restoration of immune response. With regard to general immune modulation, there are continuing attempts to identify and use cytokines that are deficient in late-stage HIV infection (eg, interleukin [IL]-2) or that may enhance cellular immune function (eg, interferon [IFN]-α, IFN-γ, IL-2, IL-7, IL-12, and IL-15), as well as to down-regulate cytokines that may enhance HIV replication (eg, tumor necrosis factor [TNF] or IL-6).

Interleukin-2. Early in vitro studies showed that the ability to mount an Epstein-Barr virus (EBV)–specific cytotoxic T-cell (CTL) declined with advancing HIV infection. The ability to elaborate IL-2 in vitro was lost in association with this progressive decline in the induction of EBV-specific CTL activity. The addition of IL-2 to peripheral blood mononuclear cells (PBMCs) from AIDS patients restored EBV-specific cytolytic activity in most patients, except those with far advanced disease. In these patients, the addition of IL-2 augmented natural killer (NK) cell activity, but not EBV-specific CTL activity. Early clinical investigations at Stanford showed that IL-2 administration was associated with enhanced CTL activity against a variety of HIV gene products. In the most extensive clinical experience to date, investigators at the National Institutes of Health (NIH) administered intermittent courses of high-dose IL-2 (12–18 million units/d for 5 days every 4 weeks) to patients with HIV disease who were on standard single-agent nucleoside analogue therapy. It was found that 60% of those with initial CD4+ counts greater than 200 cells/μL showed substantial increases in their CD4+ counts after the infusions; those patients with lower CD4+ counts frequently exhibited no increase. Assessment of the effect of infusion on plasma HIV RNA using a sensitive branched-chain DNA (bDNA) assay showed that a burst of replication frequently accompanied the infusions. In patients with higher CD4+ counts, plasma HIV RNA levels typically decreased to their original levels after the acute increase, whereas plasma HIV RNA levels could remain elevated compared with pretreatment levels in some patients with lower CD4+ cell counts. Study of V_β-chain distributions before and after treatment with IL-2 has suggested that the CD4+ lymphocyte expansion is primarily limited to the existing repertoire at the time of treatment, with existing lineages being expanded rather than new lineages being added. Administration of IL-2 is associated with significant systemic side effects in the majority of patients, and with capillary leak syndrome in many. Issues in the clinical development of IL-2 that remain to be answered include the clinical significance of the rise in CD4+ cell counts induced by IL-2; the durability of these CD4+ cell count increases and the functioning of the cells produced under treatment; whether the viral burst accompanying treatment is deleterious in terms of augmenting viral diversity in the host; and whether this viral burst can be contained with more effective antiretroviral therapy.

A number of questions remain in the use of IL-2 in HIV disease: Does the viral burst observed with treatment augment viral diversity? How well do the CD4+ cells produced under treatment function?

Interleukin-12. Interleukin-12 induces production of IFN-γ in T- and NK cells and augments the cytotoxic activity of resting and cultured NK cells. Interleukin-12 is synergistic with IL-2 in stimulating CD8+ cell proliferation in animal models. Studies of samples in vitro from patients at various stages of HIV disease have shown the addition of IL-12 significantly enhances proliferative responses to recall antigens in samples from patients with CD4+ cell counts greater than 200/μL, but not in those patients with cell counts less than 200/μL. To date, clinical use of IL-12 has been associated with significant adverse reactions; however, some of the toxicities can be avoided by giving a priming dose.

Cytokine down-regulation. Early studies of the ability of different cytokines to modulate HIV replication included the observation that certain cytokines stimulated HIV replication in some cell lines. Those found to have an up-regulating effect in at least some cell lines include TNF, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Tumor necrosis factor up-regulates replication in several cell types, thus becoming a target for expression as a means of controlling TNF-induced replication. Drugs known to inhibit TNF production include pentoxifylline and thalidomide. Clinical study of the former thus far has demonstrated dose-related gastrointestinal (GI) toxicity, which has prevented administration of high enough doses of the drug to significantly alter TNF expression in vivo or to decrease HIV replication. In an ongoing placebo-controlled study (ACTG 267),

patients with CD4+ cell counts of 200/μL to 500/μL are being given thalidomide at 50, 150, or 300 mg/d to assess tolerability to the drug and the drug's effect on viral load.

CD8+ HIV replication suppressor factor. Attempts to identify and isolate the putative soluble factor(s) mediating the anti-HIV effect of CD8+ cells have been ongoing for several years. A number of molecules have recently been suggested as candidates. One (IL-16) has been shown to produce only modest suppressive effects on HIV replication. Another group of investigators has suggested that this in vitro activity is mediated by the three chemokines RANTES, MIP-1α, and MIP-1β. Although these molecules were reported to have a significant effect in curtailing HIV replication, the lack of information on the specific experimental procedures used in the publication of the findings makes the results difficult to interpret. High concentrations of these chemokines were required for in vitro activity.

Cytokine caveats. Although many of the findings involving cytokine modulation are intriguing, it is important to remember that cytokines are autocrine or paracrine molecules that normally operate in a complex and tightly regulated network. Thus, attempts to achieve isolated effects on one of the factors may be unsuccessful due to compensatory responses by other cytokines. In addition, there is the possibility of detrimental effects occurring distally in the network. The findings on how the cytokines mediate functions important in HIV disease often are based on highly artificial experimental systems that are incapable of capturing the complex effects of this modulation. It is also true that "bad" cytokines are not necessarily all bad; for example, it is known that blocking TNF effects in murine mycobacterial infection models results in increased mortality.

Modulation of HIV-specific Effector Mechanisms

HIV-specific immune effector mechanisms are likely to play a key role in controlling virus replication in the early phases of HIV infection and may be a major determinant of the natural history of HIV disease. It remains unclear as to whether the cellular or humoral immune response to infection is the most critical or whether both are important in controlling virus replication. It is known that during clearance of initial viremia that there is emergence of CTL activity, which occurs before neutralizing antibodies appear; it is also known that long-term nonprogressors have greater CTL activity against HIV and may also have more potent antibody response. However, these phenomena may simply be characteristics of a good host immune response to infection rather than being directly involved in controlling viral replication. Once infection has been established, the viral population within an individual is constrained by a very potent immune response, but can overcome these constraints by generation of extraordinary genetic diversity. This tremendous genetic diversity is one of the major challenges to immunomodulatory approaches to treatment of HIV.

A number of approaches to enhance humoral and cellular HIV-specific immune responses are under investigation. With regard to humoral mechanisms, a number of monoclonal antibodies that neutralize primary HIV isolates have been produced and an AIDS Clinical Trials Group (ACTG) study of a few of these antibodies is scheduled to begin in the near future.

The ability of CTLs to detect and destroy cells expressing HIV antigens has been recognized for many years. As part of the host's immune response to HIV, an individual develops CTL clones specific for a number of detectable HIV gene products. Figure 1 illustrates one study in which an HIV-infected person was shown to exhibit specific response to 10 different HIV epitopes derived from three different genes. However, a single amino acid change in one of the gene products can render cells bearing the epitope unrecognizable to a CTL clone that was previously effective in detecting and killing such cells. Attempts to use CTLs in therapeutic regimens will have to consider the ever-increasing heterogeneity of target viral strains.

Progressively sophisticated attempts to create and expand CTLs and reintroduce them into the patient have not yet yielded evidence that such a strategy is effective in reducing viral replication. The initial approach of polyclonal expansion and reinfusion of CD8+ cells was not associated with detectable changes in viral load, but with the number of cells infused, no substantial increase in HIV-specific CTL activity would be expected. More recently, studies in which HIV-specific CTLs that have been expanded ex vivo have been initiated, but studies of changes in viral load in this setting have been inconclusive. Another approach that appears to hold significant promise involves genetically modifying the T-cell receptor complex. A technique for fusing the extracellular domain of the CD4 molecule with the zeta chain of the T-cell receptor complex has been developed. Cells thus modified bind the gp120 molecule on the surface of cells undergoing infection with HIV and lyse these cells, effectively functioning as a universal killer of HIV-envelope–expressing cells. In an ongoing study at the NIH, retroviral vectors are used to insert the CD4 molecule into harvested CTLs of an uninfected identical twin to convert them into universal killer cells; these cells are then infused into the HIV-infected twin in an attempt to better control HIV.

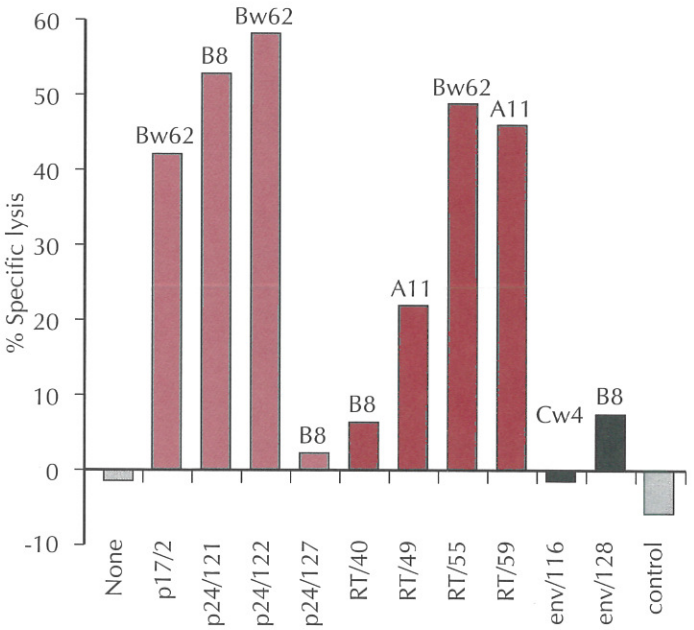

Figure 1. HIV-1 MHC-class I–restricted CTL epitopes recognized by unstimulated PBMCs from an HIV-infected individual. Adapted with permission from Johnson RP, Walker BD. Curr Top Microbiol Immunol. *1994;189:44.*

Genetic Therapies

Genetic therapies for HIV infection are based on the premise that genetic alterations in T-lymphocytes or hematopoietic stem-cells may render them resistant to HIV infection and provide an opportunity to reconstitute normal host immunity with cells not depleted by ongoing viral replication. The methods currently being investigated consist of inserting intracellular genes or proteins that interfere with the viral replication cycle. One approach involves the intracellular expression of RNA sequences that bind either with viral messenger RNA (mRNA) or with viral genomic DNA (antisense approach), or that can cleave specific viral sequences (ribozymes). Another RNA-based approach, ie, the "decoy" approach, involves expressing a defective regulatory viral RNA sequence. Similar strategies have been employed using expression of defective viral proteins instead of RNA. In addition to these transdominant mutant approaches, there are strategies that attempt to exploit the fact that viral production or assembly can be hampered by intracellular antibody fragments directed at viral elements. In yet another approach, termed the "suicide-gene" approach, genes that express proteins that are toxic to mammalian cells, such as diphtheria toxin or ricin, are placed under the control of elements that are up-regulated when HIV replication is initiated. Each of these approaches has several significant practical barriers to successful clinical application. For example, one difficulty is devising vectors for genetic material that can enter all of the cell types capable of being infected by HIV; another barrier is that the genetic diversity of the population of virus within an individual may hinder the success of any single approach whose effectiveness depends on recognition of specific viral sequences.

Despite the potential obstacles to genetic therapies, these approaches continue to hold much scientific interest. As noted by Dr Schooley, apart from the potential direct therapeutic benefits, the study of genetic therapies will provide an opportunity to study the molecular pathogenesis of HIV infection and to work out the barriers to gene therapies for a wide variety of other diseases.

Robert T. Schooley is Professor of Medicine at the University of Colorado School of Medicine in Denver.

Suggested Readings

Chehimi J, Starr SE, Frank I, et al. Impaired interleukin-12 production in human immunodeficiency virus-infected patients. *J Exp Med.* 1994;179:1361–1366.

Clerici M, Lucey DR, Berzofsky JA, et al. Restoration of HIV-specific cell-mediated immune responses by interleukin-12 in vitro. *Science.* 1993;262:1721–1724.

Cocchi F, DeVico AL, Garzino-Demo A, et al. Identification of RANTES, MIP-1a and MIP-1b as the major HIV suppressive factors produced by CD8+ T cells. *Science.* 1995;270:1811–1815.

Kalams SA, Johnson RP, Trocha AK, et al. Longitudinal analysis of T-cell receptor (TCR) gene usage by human immunodeficiency virus 1 envelope-specific cytotoxic T lymphocyte clones reveals a limited TCR repertoire. *J Exp Med.* 1994;179:1261–1271.

Koup RA. Virus escape from CTL recognition. *J Exp Med.* 1994; 180:779–782.

Kovacs JA, Baseler M, Dewar RJ, et al. Increases in CD4 T lymphocytes with intermittent courses of interleukin-2 in patients with human immunodeficiency virus infection. *N Engl J Med.* 1995;332:567–575.

Teppler H, Kaplan G, Smith K, et al. Efficacy of low doses of the polyethylene glycol derivative of interleukin-2 in modulating the immune response of patients with human immunodeficiency virus type 1 infection. *J Infect Dis.* 1993;167:291–298.

Torpey D III, Huang XL, Armstrong J, et al. Effects of adoptive immunotherapy with autologous CD8+ T lymphocytes on immunologic parameters: lymphocyte subsets and cytotoxic activity. *Clin Immunol Immunopathol.* 1993;68:263–272.

Wahren B, Bratt G, Persson C, et al. Improved cell-mediated immune responses in HIV-1 infected asymptomatic individuals after immunization with envelope glycoprotein gp160. *J Acquir Immune Defic Syndr.* 1994;7:220–229.

Walker BD. The rationale for immunotherapy in HIV-1 infection. *J Acquir Immune Defic Syndr.* 1994;7(suppl 1): S6–S13.

Walker BD, Chakrabarti S, Moss B, et al. HIV-1 specific cytotoxic T-lymphocytes in HIV seropositive individuals. *Nature.* 1987;328:345–348.