Immunopathogenesis of Human Immunodeficiency Virus: Implications for Immune-Based Therapies

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Human immunodeficiency virus (HIV) infection leads to a state of CD4 lymphopenia and generalized immune activation with subsequent development of opportunistic infections and neoplasms. The use of highly active antiretroviral treatment has dramatically improved the clinical outcome for HIV-infected patients, but the associated cost and toxicity and the eventual development of drug resistance have underscored the need for additional therapeutic strategies. Immune-based therapies, such as treatment with cytokines or immunosuppressants, adoptive immunotherapy, and therapeutic immunizations, are being intensely investigated as potential supplements to antiretroviral therapy. Although much data have been generated as a result of these efforts, to date there has been little evidence of the clinical efficacy of these strategies. Randomized clinical studies remain critical in evaluating the clinical significance and the role of immune-based therapies in the therapeutic armamentarium against HIV.

At the turn of the century, an estimated 36.1 million individuals worldwide were living with HIV infection/AIDS [1]. Approximately 1 in every 100 adults aged 15–49 years is infected with HIV. The burden of this pandemic is disproportionately affecting the poorest nations in the world: >70% of infected individuals reside in sub-Saharan Africa and another 16% reside in South and Southeast Asia. Despite these grim statistics, important steps forward have been achieved with the use of highly active antiretroviral therapy (HAART), which has led to decreased mortality and morbidity rates among infected patients and to a dramatic decrease in transmission of HIV from mother to child [2, 3].

The limitations of HAART have been better realized during the past few years. Although the virus can be suppressed to levels of <50 copies/mL in plasma, eradication has not been achieved. In addition, the cost and complexity of HAART regimens, the ever growing list of long-term side effects, and the eventual development of resistance have underscored the immediate need for additional therapeutic goals and approaches. Considerable efforts are under way to complement HAART with manipulation of the immune system to improve overall clinical outcome.

A sound understanding of the immunopathogenesis of HIV infection is critical for the development and refinement of immunomodulating therapies. The new tools available for immunologic research have recently shed new light on areas that have remained controversial after 20 years of study of this complicated infectious disease of the immune system. Studies of primates that were infected with simian immunodeficiency virus (SIV) or chimeric SIV and HIV (SHIV), as well as studies of HIV-infected patients who have an enduring, intact immune system (hereafter known as “long-term nonprogressors”), have been fundamental to our understanding of the pathogenesis of HIV and the design and testing of immune-based therapies.

In the present review, we summarize some of the salient features of HIV immunopathogenesis from the perspective of identifying potential targets for immune-based therapies. We discuss aspects of acute primary infection, chronic infection, immune system activation, immune reconstitution after HAART,
the viral reservoir, HIV-specific immunity, and the CD4 T cell pool. We then review some of the immunologic strategies that have been directed toward these areas.

ACUTE PRIMARY HIV INFECTION

The clinical syndrome of acute primary HIV infection occurs at a median of 12 days after exposure in 40%–70% of infected individuals. It consists of a mononucleosis-like syndrome that can be easily missed if the history of exposure is not elicited or if appropriate testing is not done [4]. Symptoms include fever, myalgia, arthralgia, maculopapular rash, pharyngitis, lymphadenopathy, diarrhea, and (more rarely) aseptic meningitis [5]. This syndrome generally precedes seroconversion, and thus the most sensitive diagnostic test is detection of plasma HIV viremia by means of any of the methods of virus load measurement. The detection of p24 antigen is less sensitive (88.7%), but it may provide a more cost-effective method for large-scale screening because of its high specificity (100%) [6]. The diagnosis of HIV infection needs to be subsequently confirmed by means of standard ELISA and Western blot testing. Awareness of the acute seroconversion syndrome is important because patients with this syndrome may seek medical attention, and it is at this stage of the infection that intervention with antiretroviral therapy may preserve a more intact immune system and/or minimize dissemination of the virus.

Studies of primates have provided important insights regarding the events that occur soon after viral inoculation. Although tissue dendritic cells and macrophages are likely to be the first cellular targets of the virus [7], infected CD4+ T lymphocytes have been detected in the endocervix of SIV-infected animals as early as day 3 after intravaginal inoculation [8]. Soon after infection, viral replication has been observed in activated cells (human leukocyte antigen [HLA]-DR+ and Ki67+) as well as in resting, nonproliferating cells (HLA-DR- and Ki67-). Ki67 is a nuclear antigen that is present in cells during the late G1, S, G2, and M phases of the cell cycle, and HLA-DR is a cell-surface activation marker. In concordance with these data for animals, a cross-sectional study of tonsillar biopsy specimens obtained from patients who had acute HIV infection revealed a resting population of T lymphocytes that harbored virus [8]. Therefore, a cellular reservoir of the virus probably becomes established soon after primary infection and most likely consists of CD4+ T cells, dendritic cells, monocytes, and long-lived tissue macrophages (terminally differentiated cells that do not proliferate but that can support viral replication while being resistant to HIV cytopathicity). Intervention with HAART during acute seroconversion may diminish the size of this initial reservoir.

The hypothesis that the course of chronic retroviral disease could be favorably altered by early initiation of antiretroviral therapy was initially tested in the SIV-macaque model, in which treatment with antiretroviral agents during the period of acute infection led to control of viremia in the majority of animals after discontinuation of therapy [9]. In a more recent study of primates, in which animals were given intermittent antiretroviral therapy during early-stage SIV infection, viremia could be controlled after the treatment was discontinued [10]. A report by Rosenberg et al. [11] shows that this may also be the case for HIV-infected patients who initiate treatment during symptomatic acute primary HIV infection. At the time of their report, plasma viremia in 5 of the 8 patients who were included in the cohort that received continuous administration of HAART for 1–3 years had been under control for 5–9 months after the discontinuation of HAART. Despite their study’s small number of patients and short duration of follow-up, and despite the lack of a control group (which resulted in only historical data being available for comparison), this observation generates significant optimism with regard to the possible existence of a more favorable virological set point that can be established with early treatment. Whether this is due to preservation of elements of HIV-specific immunity, limitations in the degree of initial spread of the virus, or both remains unclear.

CHRONIC INFECTION, IMMUNE SYSTEM ACTIVATION, AND IMMUNE RECONSTITUTION AFTER HAART

The course of untreated HIV infection typically includes a long period of clinical latency (~8–10 years) before the development of AIDS, in association with decreasing CD4 cell counts and increasing levels of HIV viremia. Clinically, the hallmark of HIV infection is the development of opportunistic infections and neoplasms, the majority of which are now known to be of infectious etiology. The CD4 cell count has remained the best marker of the degree of immunodeficiency, and thus far it is the best guide for initiation or discontinuation of prophylaxis against opportunistic pathogens. The following immunologic strategies have been used or are being considered for use in an attempt to directly restore the CD4+ T cell pool:

1. IL-2
2. Granulocyte colony-stimulating factor (G-CSF)
3. Granulocyte-macrophage colony-stimulating factor (GM-CSF)
4. IL-16
5. IL-7
6. Adoptive immunotherapy

In addition to being characterized by immunodeficiency re-
lated to CD4⁺ T lymphocyte depletion, HIV infection is characterized by a state of profound immune activation that is manifested by increased turnover of T and B lymphocytes and natural killer (NK) cells and by increases in proinflammatory cytokines, such as IL-6 and TNF-α. A state of immunosuppression that is characterized by blunting of antigen-specific lymphoproliferative responses may be another direct consequence of immune system activation. Suppression of viremia by HAART can lead to the rapid emergence of some of these antigen-specific responses and to the decreased expression of activation markers on T lymphocytes [12, 13], which coincide clinically with improvement of AIDS-related opportunistic infections. This state of generalized immune system activation promotes the spread of HIV infection and has been the rationale behind the experimental use of the following immunosuppressive agents as immune-based therapies for HIV-infected patients:

1. Corticosteroids
2. IL-10
3. Cyclosporin A
4. Hydroxyurea
5. Thalidomide
6. Mycophenolate mofetil

Another potentially clinically relevant manifestation of immune system activation is elevation of CD8⁺ T cell counts during chronic HIV infection, with an accumulation of cells that have a highly activated phenotype (such as HLA-DR⁺ and CD38⁺, both of which are surface markers of CD8⁺ T cell activation). The fraction of CD8⁺ T cells that express bright CD38 (by high-fluorescence intensity, which denotes more molecules per cell) has been identified as an independent prognostic factor for disease progression [14]. This observation underscores the pathogenicity of immune activation per se in patients who have HIV infection and the importance of immune-based therapies directed at improving CD8⁺ T cell function.

The introduction of HAART has led to a significant improvement in the immune function of HIV-infected patients, with profound clinical impact. Increasing CD4 cell counts and suppression of viremia have been accompanied by a dramatic decrease in morbidity and mortality rates for patients with AIDS [15]. One could speculate that the rapid improvement seen within weeks of initiation of HAART in patients who have a variety of opportunistic illnesses suggests that decreases in immunosuppression, as opposed to a reversal of immunodeficiency, may be the primary mechanism of the immediate benefit. Regardless of the mechanism, the degree of immunologic improvement seen with HAART has allowed for discontinuation of primary or secondary prophylaxis for many opportunistic pathogens, including Pneumocystis carinii and cytomegalovirus [16]. Although the full spectrum of the T cell repertoire may not be restored as a consequence of antiretroviral treatment, it seems that it is adequate to cope with the most important pathogens involved in morbidity among HIV-infected patients.

Although the rate of incidence of opportunistic infections has dramatically decreased since the introduction of protease inhibitors, a similar degree of reduction in the incidence of lymphomas has not been observed [15, 17]. This suggests that earlier initiation or longer periods of treatment may be necessary to completely preserve or restore certain elements of the T cell repertoire, such as low-frequency clones that probably recognize rarely encountered peptides (figure 1) [18]. Introduction of HAART has also led to the emergence of unusual inflammatory responses, such as uveitis in patients with cytomegalovirus, lymphadenitis in patients with mycobacterial infections, or hepatitis flares in patients with chronic hepatitis C. These conditions occasionally are difficult to manage and at times necessitate immunosuppressive treatment or even temporary discontinuation of HAART [19]. They likely represent a rapid, unregulated increase in immune function due to reversal of immunosuppression, analogous to a type IV (T cell–mediated, delayed type) hypersensitivity reaction.

THE VIRAL RESERVOIRS: LATENT OR NOT?

The introduction of HAART for HIV infection was accompanied by optimism that 2–3 years of continuous treatment would lead to eradication of the virus [20]. This estimate was based on experimental observations and theoretical projections, including an assumption that no latent long-lived cells carried replication-competent virus and no residual viral replication took place when the plasma virus load was below the limits of detection of available assays. Unfortunately, a series of studies have clearly demonstrated persistent viruses and ongoing viral replication, even among patients who have HIV RNA levels of <50 copies/mL for extended periods [21–25]. In some of these studies, virus was cultured after activation of cells, whereas in others, PCR techniques demonstrated integrated and unintegrated proviral DNA, or spliced and unspliced viral RNA (figure 2). The most convincing evidence of persistence of replication-competent virus despite long-term HAART has come from studies in which HAART was discontinued for patients who had prolonged, successful suppression of the virus load. Of note are the rapid rebound of and tendency for plasma virus levels to return to pretreatment levels after discontinuation of HAART [26]. In these studies [27–29], the majority of patients experienced some degree of viral rebound within 2 weeks after discontinuation of HAART. Given the evidence that viral replication is persistent in many patients who have plasma virus loads of <50 copies/mL, use of the term
Figure 1. Chronic HIV infection is characterized by a state of immune activation that leads to immunosuppression and accelerated death of CD4+ T cells resulting in immunodeficiency due to CD4 lymphopenia. A preferential loss of naïve T cells is observed. HAART suppresses viral replication and quickly reverses the immunosuppression component of the immune dysfunction. The immunodeficiency component necessitates longer periods of treatment and may not be completely restored. Letters denote the receptor specificity of each of the T cells, Greek characters (in blue cells) denote the naïve phenotype, and Roman characters (in yellow cells) denote the memory phenotype.

“undetectable” to refer to virus load is imprecise and should be abandoned in clinical practice.

Efforts to diminish the HIV reservoir have been uniformly unsuccessful. Attempts to “purge” the virus by activating T lymphocytes with cytokines, such as IL-2, or with other immunostimulating methods, such as OKT3 (infusion of anti-CD3 monoclonal antibody), have not resulted in changes in this pool [30, 31]. This lack of success may reflect the importance of the tissue macrophages in preserving this reservoir, as was highlighted in a recent study of rhesus macaques that were infected with SHIV [32]. In these SHIV-infected animals, which had severe CD4 lymphopenia but sustained viremia, >95% of the virus-producing cells were tissue macrophages and <2% were T lymphocytes, as shown by in situ hybridization and immunohistochemical analysis of the lymph nodes, spleen, liver, and kidneys. Therefore, targeting the tissue macrophage reservoir with novel strategies, such as the use of proteasome inhibitors [33], seems to be an important approach that warrants investigation.

HIV-SPECIFIC IMMUNITY

Immunologic control of viral infections is a critical aspect of host defense. Against certain viruses—predominantly RNA viruses—the immune system is capable of launching an effective response that can lead to eradication of the offending agent. Unfortunately, this is not the case with many DNA viruses and retroviruses, such as HIV type 1 (HIV-1), that have DNA as an intermediate step in their replication cycle. The following immune-based strategies have been used or are being considered for use in attempts to improve the host immune response to HIV or to directly decrease viral replication:

1. IL-16
2. IL-12
3. GM-CSF
4. IFN-α
5. IL-15
6. Hydroxyurea
7. Adoptive immunotherapy
8. Therapeutic immunizations

After primary HIV infection occurs, a virological and immunologic set point is reached. This set point probably reflects a balance between the pathogenicity of the virus, the size of the initial viral reservoir, and the effectiveness of the host immune response. Extensive efforts with the use of older as well as the newest immunologic tools (table 1) have recently been directed toward achieving a better understanding of the nature of a
protective immune response to HIV. This better understanding has important implications for both vaccine development and treatment.

The role of CD8$^+$ T lymphocytes in the immunologic response to HIV has been the focus of intense study. Both cytolytic (CTL) activity and non-CTL activity, such as the production of chemokines, have been detected in response to HIV [34]. Inferential evidence suggests that CD8$^+$ T lymphocytes play an important role in the control or suppression of HIV viremia. In macaques that were infected with SIV, depletion of CD8$^+$ lymphocytes led to uncontrolled viremia, especially when the CD8 lymphopenia was of prolonged duration [35]. Reductions in levels of viremia in patients with acute HIV infection coincides with evolution of a CD8 response [36]. In rhesus macaques that have acute infection with cloned SIV, there is a temporal correlation between reductions in levels of viremia, evolution of a CD8 immune response, and simultaneous emergence of viral escape mutants with mutations of the epitopes recognized by the CD8$^+$ T cells [37].

The role of CD8$^+$ T lymphocytes in chronic infection is less well defined. In studies of patients with HIV who have progressive disease, CD8 responses to HIV antigens are still present in such patients to a degree similar to that in long-term non-progressors [38, 39]. Decreased responses have been reported in other studies of patients with advanced disease [40]. Typically, long-term HAART leads to a decrease in the CD8 HIV-specific responses, in association with a decrease in HIV RNA levels [41]. Recent treatment-interruption studies have shown that a resurgence of these responses occurs as levels of detectable viremia increase. Increased levels of CD8$^+$ HIV-specific T cells in patients with rising levels of detectable viremia show a direct correlation with levels of virus replication [42]. A series of studies have attempted to enhance CD8$^+$ T cell immunity by means of immunization with recombinant virus vectors or naked DNA or by administration of CD8$^+$ T cell clones. None of these approaches have led to significant changes in HIV RNA levels, which are the ultimate reflection of HIV-specific immunity.

The role of CD4$^+$ helper T cells in HIV-specific immunity is more complicated, since these cells are also a primary target of the virus. CD4 help is important for the generation and persistence of a successful CD8 response to viral infections [43, 44]. It has been suggested that a critical immunologic lesion is established when CD4$^+$ T cells with specificity for HIV encounter virus. This encounter leads the CD4$^+$ T cells to an activated state that renders them susceptible and permissive to HIV infection, thereby enabling their subsequent infection and death [45].

With the use of intracellular cytokine staining to detect IFN-γ production after stimulation with HIV antigens in a 6-h assay
Table 1. Methods used in the study of HIV-specific immunity.

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
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<tbody>
<tr>
<td>Lymphoproliferative assay</td>
<td>PBMCs are placed in culture wells with mitogenic or antigenic stimulation for 3-6 days; incorporation of ³H-thymidine is measured at the end of the culture.</td>
</tr>
<tr>
<td>Cytotoxicity assays</td>
<td>PBMCs, CD8⁺ T cells, or NK cells (effectors) are cultured for 4-6 h with cellular targets labeled with ⁵¹Cr; measurement of released ⁵¹Cr estimates how many targets were lysed.</td>
</tr>
<tr>
<td>Intracellular cytokine staining</td>
<td>PBMCs are cultured with targets (peptides, antigens, or EBV-transformed cells that express specific proteins) in the presence of a Golgi inhibitor for 6 h and then are stained for surface molecules and intracellular cytokines.</td>
</tr>
<tr>
<td>ELISPOT assay</td>
<td>PBMCs, purified CD4⁺ cells, or CD8⁺ T cells are cultured with targets for 24-48 h on cytokine capture plates; cytokine production is quantified as spot-forming units.</td>
</tr>
<tr>
<td>Tetramer staining</td>
<td>Tetrameric complexes of human leukocyte antigen and streptavidin plus specific peptides that can recognize and bind the corresponding T cell receptor.</td>
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NOTE. EBV, Epstein-Barr virus; NK, natural killer; PBMCs, peripheral blood mononuclear cells.

It has been shown that the CD4⁺ T cells that are able to recognize HIV antigens are present even in chronically infected patients, although typically to a degree lesser than that in long-term nonprogressors. Like HIV-specific CD8⁺ T cells, the frequency of these cells decreases after viral suppression with HAART [46]. Despite their ability to recognize HIV antigens and produce cytokines, they may not proliferate in a 5-day lymphoproliferative assay [47]. This could represent a quantitative defect (e.g., poor sampling representation of antigen-specific cells due to low precursor frequency) or a qualitative defect due to prior activation (e.g., anergy).

It has been suggested that different subsets of memory T cells are more prone to proliferative responses when they encounter antigen or peptide, whereas others are more efficient in effector functions, such as cytokine production or CTL activity. Therefore, it is prudent to interpret functional data in combination with careful subset characterization of T cells in the peripheral blood on the basis of the phenotypic profile [48, 49].

In a variety of studies, attempts have been made to enhance CD4⁺ T cell immunity to HIV by adoptive transfer of lymphocytes or immunization with HIV antigens (therapeutic vaccination). In vitro lymphocyte blast transformation to the p24 antigen of HIV has been said to be an important marker of HIV-specific immunity because it is present in long-term nonprogressors or patients who are treated during the period of acute infection [50]. Although these responses can be successfully induced by immune-based therapies, their induction has not been associated with lower levels of virus, suggesting that their detection per se in vitro may not always correlate with the level of control of HIV.

CD4⁺ T CELL POOL

The cardinal manifestation of HIV infection is the development of progressive CD4 lymphopenia, which disproportionately affects the naïve CD4⁺ T lymphocytes and leads to severe immunodeficiency. The CD4⁺ T cell count appears to be the most important prognostic factor and the most helpful determinant of the degree of immunodeficiency of an infected individual [51]. When combined with a measurement of virus load as a marker of immunosuppression, the overall level of immune function of an individual patient can be assessed.

Analysis of T cell turnover in patients with HIV infection has been controversial. The use of indirect methods and mathematical modeling, the cross-sectional nature of some studies, and issues of peripheral-blood-to-tissue distribution of lymphocytes introduce significant variability and complexity to this topic. A major issue of debate is the impact of HIV infection on CD4⁺ T cell production. At present, there are 2 schools of thought. The prevalent view is that HIV infection leads to decreased thymic function and decreased T cell production, with HAART leading to improved thymic function and increases in T cell production [52-54]. Our view is that HIV infection has little or no effect on thymic function and that it leads to polyclonal increases in T cell turnover. In this scenario, HAART leads to increases in the number of CD4⁺ T cells, despite a decrease in the rate of new CD4⁺ T cell production, as a result of an even greater decrease in the rate of HIV-mediated CD4⁺ T cell death [55-57].

The T lymphocyte pool is continuously renewed as a result of thymic input and peripheral expansion. The thymus is a critical source of new naïve T lymphocytes early in life and decreases in function with age. When T cells are generated from stem cells under the influence of the thymus, circular pieces of DNA are excised as a by-product of TCR gene rearrangement so that the levels of these T cell–receptor excision circles (or TRECs) become markers of recent thymic emigrants. They are found predominantly in the naïve T cells and decrease gradually with age [54, 58]. These DNA circles do not replicate during cell division, and they are passed to 1 of the 2 daughter cells. Therefore, increases in cell proliferation or decreases in thymic input can lead to decreased levels of TRECs. Similarly, increased levels of TRECs can be due to either an increase in thymic...
output or a decrease in the rate of proliferation of the T cells that contain TRECs.

A mathematical model suggested by Hazenberg et al. [59], which was based on longitudinal data from T cell turnover studies and TREC levels, proposed a decrease in the rate of turnover of naïve T lymphocytes as the main explanation for the increasing levels of TRECs seen after initiation of HAART. Data that support this hypothesis have been published recently [55, 57].

Regardless of the mechanism, replenishment of the CD4+ T cell pool is a logical approach for immune-based therapies. The adoptive transfer of cells and the use of T cell growth factors, such as IL-2, are 2 strategies that have been vigorously studied.

**IMMUNE-BASED STRATEGIES FOR TREATMENT**

**Cytokines**

**IL-2.** IL-2, a cytokine that is produced by activated CD4+ T cells, leads to proliferation and differentiation of T lymphocytes. In the United States, IL-2 is licensed for the treatment of patients who have metastatic melanoma and renal cell carcinoma. Its clinical use for patients who have HIV infection is currently being evaluated in 2 international phase III trials. IL-2 has been extensively studied in phase I and phase II studies. In the majority of these studies, a 5-day cycle of IL-2, 1.5–7.5 million IU (MIU), was given every 8 weeks, with administration done twice daily by means of sc injection. This approach has led to substantial increases in CD4+ T cell counts in HIV-infected patients [60–64]. Similar results have been generated in previous studies of 5-day continuous iv administration of IL-2 (figure 3) [65].

Most patients typically have flulike symptoms during and for a few days after the end of a 5-day cycle. More recent studies that have been done in the HAART era and that have involved the intermittent use of IL-2 continue to demonstrate CD4+ T cell expansions of a magnitude greater than those achieved by use of HAART alone. In a randomized trial that involved 511 patients who had CD4+ T cell counts of ≥300 cells/µL and who were receiving a combination of antiretroviral agents, the change in the CD4+ T cell count (from baseline levels) was greater for patients who were treated with IL-2 than for control patients, with a mean cell difference of +251 cells/µL at month 12 after initiation of therapy \( P = .0001 \) [66]. At 12 months, there was no difference between the patients who were treated with IL-2 and the control patients with respect to the percentage of patients who had a virus load of <50 copies/mL \( P = .55 \) or the change in virus load from the baseline level \( P = .63 \).

In another randomized trial that involved 82 patients who had CD4 cell counts of 200–500 cells/µL, the majority of whom were receiving HAART, those patients who were randomized to receive IL-2 had a 113% increase in CD4+ T cell counts, compared with a 13% increase noted among control patients [67]. For patients who had recently begun receiving HAART and who had CD4+ T cell counts of 50–300 cells/µL and HIV RNA levels of <10,000 copies/µL, another randomized study showed a 156% increase in CD4+ T cell counts among patients who were treated with IL-2, compared with a 35% increase in CD4+ cell counts in control patients after 60 weeks of follow-up [68].

The expansion of the CD4+ T cell pool after intermittent administration of IL-2 is polyclonal and is not associated with significant changes in CD8 or NK cell counts. A preferential expansion of CD4 cells with the naïve phenotype is observed despite the fact that memory cells typically express higher levels of the high-affinity IL-2 receptor complex. Despite in vitro evidence of enhanced HIV replication by IL-2 and transient, occasional 6-fold increases in virus load at the end of an IL-2 cycle [69], long-term follow-up of patients treated with IL-2 has shown that, overall, the virus load is not adversely affected and is even slightly lower in IL-2 recipients than it is in patients treated with antiretroviral agents alone [70]. Studies are currently under way to examine the effects of IL-2 used as a single mode of therapy in HIV-infected patients with early-stage disease [71].

Patients who respond to intermittent administration of IL-
2 can maintain a high CD4 cell count with 1 IL-2 cycle given every 16 months on average (R. Davey, personal communication). The key remaining question in the study of IL-2 is the degree of clinical benefit associated with these expansions in the number of CD4+ T cells. Two large-scale, phase III, multicenter, international studies (A Study of IL-2 in People with Low CD4+ T Cell Counts on Active Anti-HIV Therapy [SILCAAT] and Evaluation of Subcutaneous Proleukin in a Randomized International Trial [ESPRIT]) are addressing this issue by assessing clinical events as the primary end point in a cohort of 5400 patients; results of these studies are anticipated within the next 5 years.

**Low-dose continuous IL-2.** Use of IL-2 has also been studied among HIV-infected patients who were given a daily injection of 62,500–250,000 IU/m² for 6 months. This regimen typically is well tolerated without significant toxicity. In a nonrandomized trial, low-dose continuous IL-2 was shown to lead to increases in the number of NK cells, although it did not lead to significant increases in the number of CD4+ T cells in comparison with baseline levels [72]. In a more recently published randomized study of continuous (6-month) sc IL-2 given at a higher dosage (1.2 MIU/m²) to 115 patients who had CD4 cell counts of <300 cells/μL (median CD4 cell count, 184 cells/μL), a significant increase in the number of NK cells was noted in patients who received IL-2, compared with that in control patients (156 cells/mL vs. 19.9 cells/mL, respectively; \(P < .001\)). Although a small increase from the baseline level was noted with regard to the percentage of naïve CD4+ T cells in patients who were treated with IL-2, compared with that in control patients (3.52% vs. 1.33%, respectively; \(P < .001\)), both groups experienced a decrease from the baseline level with regard to the percentage of memory CD4+ T cells; this decrease was more pronounced in patients who were treated with IL-2 (4.49% vs. 1.11%; \(P < .001\)) [73]. A significant rate of withdrawal was noted for the patients who were treated with IL-2 (23 of 56 patients) in this study. It appears that the cycles of rest and restimulation that are associated with intermittent administration of IL-2 are required for significant long-term expansion of the number of CD4+ T cells. Finally, the role of IL-2 as a CTL-inducing vaccine adjuvant is also being investigated, and promising results have been observed in animal model studies [74, 75].

**G-CSF.** G-CSF is a hematopoietic growth factor that is critical for the maintenance of the function and number of circulating neutrophils. Leukopenia is frequently seen in HIV-infected patients as part of treatment-related toxicity or as a manifestation of concurrent disease. Neutrophil dysfunction with accelerated apoptosis, decreased chemotaxis, and decreases in the respiratory burst have also been reported [76]. Administration of G-CSF was initially studied in patients who had AIDS and was found to be effective in preventing neutropenia and decreasing the rates of infection [77, 78]. In a randomized placebo-controlled trial, 30 HIV-infected patients who had CD4+ T cell counts of <350 cells/μL while receiving stable HAART for at least 24 weeks were randomized to receive G-CSF or placebo 3 times per week for 12 weeks. At the end of the study, the treatment group had increases in the memory CD4+ T cell pool (approximately 20 cells/μL) and the CD8+ T cell pool (approximately 100 cells/μL), including an increase in the number of CD8+ T cells that expressed the activation marker CD38. Virus load remained stable, and all immune parameters returned to baseline levels at the 24-week follow-up. A decrease in the in vitro proliferative responses to IL-2, phytohemagglutinin antigen, and Candida antigen was also noted among treated patients [79]. The lack of detrimental effect on HIV virus load and CD4 cell count provides reassurance of the safety of the use of G-CSF for patients with HIV infection when such use is dictated by treatment or infection-induced neutropenia.

**GM-CSF.** GM-CSF is a glycoprotein that is produced by T lymphocytes, fibroblasts, and endothelial cells. It predominately regulates the growth and function of neutrophils and macrophages. In vitro studies have suggested that GM-CSF suppresses replication of HIV in macrophages [80]. As with G-CSF, GM-CSF was initially used for treatment of neutropenia in patients with HIV infection.

A phase III, randomized, double-blind, placebo-controlled trial evaluated the effects of GM-CSF on incidence of and time to opportunistic infection or death as well as the effects of GM-CSF on plasma virus loads and CD4 cell counts in patients who were receiving stable antiretroviral therapy and who had CD4 cell counts of ≤50 cells/μL or ≤100 cells/μL plus a prior AIDS-defining illness [81]. GM-CSF, 250 μg given sc 3 times per week, significantly reduced the overall incidence of infection (78% for patients given placebo vs. 67% for patients given GM-CSF; \(P = .03\)), but it did not reduce that of AIDS-related clinical events or death. There was also a delay in the time to first infection for the GM-CSF recipients, in comparison with that for the placebo recipients (median time to infection, 97 days for GM-CSF recipients vs. 56 days for placebo recipients; \(P = .04\)). GM-CSF recipients had higher mean CD4 cell counts at 12 months (mean CD4 cell count, 152 cells/μL vs. 102 cells/μL in control patients). There were no differences in changes in virus load in the 2 groups.

The safety and efficacy of GM-CSF used in combination with protease inhibitors were studied in a randomized trial of 20 HIV-infected patients who were receiving stable HAART. This study documented good tolerance and no increases in inflammatory cytokines or virus load [82]. In a randomized study of 105 patients who were receiving nucleoside analogue treatment and who had CD4 cell counts of <300 cells/μL, a greater reduction in the mean virus load was noted in GM-CSF recipients.
(−0.60 log for GM-CSF recipients vs. −0.07 log for placebo recipients; \(P = .02\)) at 6 months. No significant differences between the 2 groups were noted with regard to the incidence of opportunistic infection or the CD4+ T cell counts [83]. Current evidence supports the use of GM-CSF when it is clinically indicated for the management of neutropenia in patients with HIV infection, although available data are not yet sufficient enough to suggest that it has a clinically significant, additional immunomodulating role.

**IFN-α.** IFN-α suppresses HIV replication in infected T cells in vitro by interfering with the assembly and release of progeny viruses. IFN-α was initially studied as a therapeutic agent for Kaposi’s sarcoma (dosage, 3–35 MIU per day), with reported response rates of 20%–67% [84, 85]. A strong correlation between clinical response and CD4+ T cell count was observed, suggesting an immune system–mediated antitumor effect [84–86]. Similarly, the antiretroviral effect in vivo also correlated with the level of immune function, as measured by the CD4+ T cell count, suggesting that IFN-α was acting as an immune enhancer rather than as a direct antiviral agent. At present, IFN-α is licensed for administration to patients with Kaposi’s sarcoma. The toxicities associated with its use include flu-like symptoms; neutropenia that can be pronounced when the agent is combined with other myelosuppressive medications, such as zidovudine; and elevated transaminase levels [87]. The superiority of the combination of IFN-α and ribavirin, in comparison with IFN-α monotherapy, for the treatment of chronic hepatitis C has also been demonstrated in HIV-coinfected patients [88]. Low-dose oral IFN-α has failed to produce any clinical benefit in large randomized trials [89].

**IL-10.** IL-10 is a potent anti-inflammatory cytokine that is produced by monocytes, T lymphocytes, B lymphocytes, and keratinocytes. IL-10 is critical in the control of systemic inflammation by the inhibition of proinflammatory cytokines and the down-regulation of surface expression of major histocompatibility complex II and costimulatory molecules. IL-10 enhances B lymphocyte proliferation and immunoglobulin production. Previous in vitro data have suggested a potential anti-HIV role for IL-10, probably mediated through the suppression of IL-6 and TNF-α production by macrophages [90]. The effects of IL-10 on HIV replication in vitro were later found to be variable and dependent on the presence of other cytokines as well as the type of infected cells. The most convincing anti-HIV effects were observed in acutely infected macrophages [91]. A recently published randomized study of sc administration of IL-10 (given in dosages of either 1 μg/kg/day or 4 μg/kg/day, or 8 μg/kg given 3 times per week for 4 weeks) to HIV-infected patients who were receiving antiretroviral agents and who had CD4+ T cell counts of >200 cells/μL failed to show any virological benefit [92]. Elevated serum levels of IL-10 and high expression of CD40L on T cells have been detected in HIV-infected patients, in comparison with HIV-seronegative control patients, and have been correlated with the serum levels of immunoglobulins; this finding suggests that IL-10 may have a central role in the hypergammaglobulinemia seen in patients with HIV infection [93]. The role of IL-10 in therapy against HIV infection is currently less clear.

**IL-12.** IL-12 is a heterodimeric cytokine that can enhance the CTL activity of NK cells as well as the proliferation and activation of CTL CD8+ T lymphocytes and NK cells. IL-12 is instrumental in the development of Th1-type immune responses and has been more extensively investigated as a potential immunotherapeutic agent for patients with renal cell carcinoma [94, 95]. Exogenous IL-12 has been associated with increases in HIV and non-HIV lymphoproliferative responses in vitro [96]. In a phase I study, 47 patients with HIV infection and CD4 cell counts of 100–500 cells/μL received a single sc dose of recombinant human IL-12 (3, 30, 100, 300, or 1000 ng/kg) or placebo and were followed for 14 days [97]. Significant adverse events occurred in the patients in the 2 highest dose groups. Increases in serum IFN-γ correlated with toxicity. No significant effects on CD4+ T cell counts or virus load were noted. Increases in CD8 and NK cell counts were observed in patients who received a dose of 30–300 ng/kg. Given the role of IL-12 in fostering Th1-type responses, it may play a role as an adjuvant of HIV vaccines [98]; its role in an immune-based therapy against HIV is less clear.

**IL-15.** IL-15 is a cytokine that shares with IL-2 some activities as well as 2 of the 3 chains of its trimeric cellular receptor (they share the β and γ chains, and each cytokine uses its own specific α chain). IL-15 is produced by activated monocytes and macrophages, and it supports the proliferation of activated T and B lymphocytes and the CTL function of CD8+ T cells and NK cells. IL-15 was found to enhance the proliferative capacity of peripheral mononuclear cells and purified CD4+ T cells of HIV-infected patients in vitro upon stimulation with mitogens, recall (tetanus) antigens, and HIV-specific antigens [99]. IL-15 was also found to enhance expansion of HIV-specific cytotoxic CD8+ T lymphocytes [100] in vitro, to a degree similar to that of IL-2 and of the CTL activity of NK cells from HIV-infected patients [101].

It is not clear how the virus load may be affected during in vivo administration of IL-15 to HIV-infected patients, given (1) the observed in vitro up-regulation of CCR5, the coreceptor of macrophage (M)-tropic HIV, on lymphocytes [102] by IL-15 and (2) evidence that suggests increased HIV replication of M-tropic viruses. IL-15 can also enhance in vitro the production of IFN-γ and IL-12 during an immune response and thus is a candidate for testing as an adjuvant of CTL-inducing vaccines.

**IL-16.** IL-16 is a proinflammatory cytokine that is syn-
thesized predominantly by CD8+ T cells and, to a lesser extent, by CD4+ T cells, eosinophils, and monocytes. Pro-IL-16 is first cleaved by caspase 3 and then autoaggregates in its tetrameric active form. IL-16 is a natural ligand for the CD4 receptor; however, it can also bind to lymphocytes of CD4 knockout animals [103]. It is a chemoattractant for both activated and resting CD4+ T cells. IL-16 leads to enhanced expression of the α and β chains of the IL-2 receptor, enhancing T cell proliferation in the presence of IL-2 and IL-15. T cells that respond to IL-16 secrete other proinflammatory cytokines, such as GM-CSF, IL-6, and TNF-α. They also become unresponsive to antigen and thus are less susceptible to activation-induced death.

In vitro, IL-16 was found to have an inhibitory effect on HIV replication at the level of viral transcription [104]. The same effect was reported on naturally infected CD8-depleted peripheral blood mononuclear cells from HIV-infected patients. In a cross-sectional comparison of IL-16 serum levels, higher levels were seen in asymptomatic HIV-infected patients than in healthy volunteers, and lower levels were noted in patients with more advanced disease [105]. While differences in absolute CD8+ T cell counts could have explained some of these observations, they were not reported in this cohort. More recent in vitro data suggest that monocyte and dendritic cells are protected from HIV replication in the presence of IL-16, secondary to decreased viral entry [106]. The unique properties of IL-16, including its anti-HIV activity, increased sensitivity of lymphocytes to the proliferative effects of IL-2 and IL-15, and protection from activation-induced death, make this molecule a particularly attractive candidate for further study with regard to HIV infection [107].

**IL-7.** IL-7, which is produced by stromal cells of the bone marrow and the thymus, is essential in early T cell development. IL-7 can also stimulate the proliferation of mature peripheral T cells. An antiapoptotic effect via increased expression of bcl-2, in addition to enhanced proliferation, probably contributes to the positive effects of IL-7 on naïve T cell expansion prior to T cell–receptor rearrangement. IL-7 induces secretion of other cytokines, such as IL-6, and enhances generation of cytotoxic T lymphocytes and lymphokine-activated killer cells [108]. The most anticipated potential clinical usefulness of IL-7 is the stimulation of lymphoid regeneration in settings of congenital or acquired lymphopenia, such as severe combined immune deficiency, HIV infection, or myeloablative therapy [109]. Recent data have shown an inverse correlation between serum levels of IL-7 and CD4 cell counts in both adults and children who are infected with HIV, which raises the possibility of a compensatory mechanism [110, 111]. This observation, along with the known effect of IL-7 on naïve T cell proliferation, has heightened the interest for testing of this cytokine in patients with HIV infection and other lymphopenic states.

**Immunosuppressants**

**Hydroxyurea.** Hydroxyurea has been used extensively for the treatment of patients who have hematologic malignancies and sickle cell anemia. Hydroxyurea is a free radical quencher that acts by inhibiting the cellular enzyme ribonucleotide reductase, leading to decreases in intracellular deoxynucleotide triphosphates (dNTPs) that are necessary for cellular division as well as HIV replication [112]. Hydroxyurea exerts a cytostatic effect that leads to cell-cycle arrest in the early S phase and a decrease in cellular activation. Hydroxyurea inhibits the synthesis of proviral DNA in T lymphocytes and has been shown to act synergistically with dideoxynosine (ddI). In vitro, macrophages are extremely sensitive to the antiviral activity of hydroxyurea, and they respond to concentrations lower than those required for suppression of virus in T lymphocytes. The interest in administration of this agent to patients with HIV infection stems from its low potential for resistance (since a cellular enzyme is targeted), low cost, fairly good tolerability, and potential enhancement of salvage regimens [113]. Enthusiasm was generated by an anecdotal case that involved an HIV-infected patient (known as the “Berlin patient”) in whom viral suppression was achieved after discontinuation of antiretroviral agents; the patient had been treated with a regimen that included hydroxyurea during the period of acute infection [114].

Hydroxyurea has been studied as part of combination regimens; it has been given with ddI, for chronically infected patients, and with indinavir and ddI, for those patients who have acute primary HIV infection. To date, there have been no reports of controlled, randomized trials that have involved patients who have acute primary HIV infection, for which hydroxyurea has been anecdotally reported to be of greater benefit than are regimens that do not contain hydroxyurea [115]. Several controlled, randomized studies that involve patients who have chronic HIV infection have been done.

In a placebo-controlled, double-blind trial, 145 patients (80% of whom were antiretroviral naïve) who had established HIV infection were randomized to receive ddI, stavudine, and hydroxyurea (500 mg given twice per day) or ddI with stavudine alone [116]. After 12 weeks, the group of hydroxyurea recipients who have acute primary HIV infection, for which hydroxyurea has been anecdotally reported to be of greater benefit than are regimens that do not contain hydroxyurea [115]. Several controlled, randomized studies that involve patients who have chronic HIV infection have been done.

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Despite these positive effects on virus load, the blunting of the increases in CD4+ T cells, along with the toxicity (particularly pancreatitis) that has been reported when hydroxyurea is combined with antiretroviral agents in addition to ddI, has introduced uncertainty for the ultimate role and safety of this agent in the treatment of HIV infection [118, 119]. A more promising area may be the use of hydroxyurea or newer analogues as part of a treatment regimen for acute primary infection, as suggested by the encouraging results observed for SIV-infected macaques [10].

**Corticosteroids.** Administration of corticosteroids is the standard of care for HIV-infected patients who have moderate or severe *Pneumocystis carinii* pneumonia [120]. Corticosteroids have also been evaluated in immunomodulating treatment for HIV infection. Potential mechanisms of action include decreases in proinflammatory cytokines and rescue of CD4+ T cells from activation-induced death caused by HIV [121]. For decreases in proinflammatory cytokines and rescue of CD4+ T cells, along with the toxicity (particularly pancreatitis) that has been reported when hydroxyurea is combined with antiretroviral agents in addition to ddI, has introduced uncertainty for the ultimate role and safety of this agent in the treatment of HIV infection [118, 119]. A more promising area may be the use of hydroxyurea or newer analogues as part of a treatment regimen for acute primary infection, as suggested by the encouraging results observed for SIV-infected macaques [10].

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In another study, 4 patients who received steroids for wasting syndrome had a mean weight gain of 3.5 kg in addition to a transient decrease in virus load [123]. The recently described increased incidence of avascular hip necrosis occurring in association with corticosteroid use among HIV-infected patients has raised concerns about the safety of corticosteroids in the HAART era, and at present, there is little enthusiasm for conducting further studies of prednisone as a primary treatment modality for patients with HIV infection [124]. Short-term use of steroids for patients who have severe inflammatory reactions that occur upon initiation of HAART may be of benefit [125].

**Cyclosporin A.** Cyclosporin A is a potent immunosuppressive medication that suppresses T cell activation by blocking IL-2 release and IL-2–dependent proliferation and differentiation. It is also an inhibitor of activation-induced death of T lymphocytes [126]. Cyclosporin A forms a complex with cyclophilin A, which is necessary for gag processing and final virion maturation, leading to a direct anti-HIV effect that results in the production of noninfectious HIV particles [127].

Despite promising in vitro antiviral activity, initial in vivo studies that involved asymptomatic HIV-infected patients prior to the introduction of HAART had disappointing results. Significant toxicity prohibited administration of cyclosporin A to patients with AIDS [128].

Use of cyclosporin A was studied in 7 rhesus macaques with acute SIV infection [129]. Transient decreases in proviral DNA and gag protein p27 levels were observed. A delay in the typical decrease in the CD4/CD8 ratio was noted in 5 of the animals that were studied, whereas the other 2 animals had rapid progression to lethal disease. A pilot study of cyclosporin A is under way in a group of 8 patients with primary HIV infection; the study involves administration of cyclosporin A for 8 weeks, along with continuous HAART [130]. Randomized trials in the setting of HAART will be needed to determine whether this approach is of any value.

**Mycophenolate mofetil.** Mycophenolic acid and its ester mycophenolate mofetil (MMF) selectively inhibit the de novo synthesis of guanosine nucleotides by competing with inosine monophosphate dehydrogenase. They potentiate the antiviral activity of guanosine inhibitors of reverse transcriptase, such as abacavir [131]. In addition, they induce the apoptosis of activated T cells and inhibit proliferative responses to mitogenic or antigenic stimuli. Mycophenolic acid has been shown to suppress HIV replication in vitro at concentrations similar to those used in transplant recipients. These virological and immunologic properties (similar to those of hydroxyurea), have made it a candidate for in vivo studies of patients with HIV infection.

In a nonrandomized pilot study, HIV-infected patients who had high CD4 cell counts and virus loads of <5 copies/μL received MMF (0.5 g given twice per day for the first 4 weeks and 1 g given twice per day thereafter) in addition to an antiretroviral regimen of abacavir and amprenavir. During 24 weeks of follow-up, no renal or liver toxicity was noted, and CD4+ and CD8+ T cell counts remained stable. It is too early to speculate on the role of MMF in immune-based therapy for HIV infection.

**Thalidomide.** For some time, thalidomide has been evaluated as an anti-HIV agent because of its ability to interfere with the production of TNF-α. TNF-α is known to stimulate nuclear factor–κB, a cellular transcription factor that is important for HIV replication [132]. In vivo studies have yielded conflicting results with regard to the effects of thalidomide on TNF-α levels (decreases, increases, and no effect have been noted). In general, there has been a positive correlation between TNF-α levels and HIV RNA levels. Given the inconsistent effects of thalidomide on TNF-α levels plus the overall poor tolerance and teratogenicity of this agent, its clinical use is mostly limited to settings in which it has proved to be most successful.

In a randomized, placebo-controlled trial of 57 patients, thalidomide, 200 mg/day given for 4 weeks, increased the percentage of patients whose oral aphthous ulcers completely healed (55% of thalidomide recipients vs. 7% of placebo recipients) [133]. Of note, this moderate clinical benefit was associated with median HIV RNA levels that increased by 0.42 log₁₀ copies/mL in thalidomide recipients, compared with increases of 0.05 log₁₀ copies/mL in placebo recipients (P = .04). TNF-α levels also unexpectedly increased by 12.16 pg/mL in the treated patients versus –0.31 pg/mL in the control patients.
Therapeutic Immunizations

From the very beginning of the AIDS epidemic, the need for a vaccine has been clear, as have been the challenges posed to its development. The development of an HIV vaccine has proceeded along 2 pathways: preventive and therapeutic. The goal of the therapeutic immunization is to enhance and broaden the immune system’s ability to recognize HIV. This would lead to better immunologic control of HIV and, potentially, to an ability to reduce or, ideally, discontinue use of antiretroviral agents. Therapeutic vaccines are not currently used for any other chronic infectious disease. For comparison and exchange of ideas, the most similar area is the experimental field of cancer immunotherapy. The idea of using active immunotherapy for HIV infection [135] has its origin in the days before it was recognized that HIV infection is not associated with a state of true microbiological latency.

The evaluation of therapeutic vaccines for HIV infection has been particularly problematic because there exists no clear surrogate marker that clearly represents protective or beneficial HIV-specific immunity in the patient who has chronic infection. To date, the best sources for guidance have been studies of long-term nonprogressors. These are patients with a long history of HIV infection who have maintained high CD4+ T cell counts and low HIV RNA levels in the absence of therapy [136]. Even in these studies it is sometimes difficult to distinguish markers of immune protection. The mere fact that a T cell responds to a given antigen in vitro does not guarantee that this is a relevant HIV-specific response in vivo. It has been very difficult to identify laboratory measurements of HIV reactivity that correlate with plasma levels of HIV-1 RNA across the spectrum of HIV infection.

The immunogenicity of vaccines can be studied in clinical trials and in the laboratory. However, clinically relevant immunogenicity can be determined only from data that indicate the effect of vaccination on validated markers of progression of HIV disease, such as virus load and CD4+ T cell count, and/or the impact of the intervention on clinical end points. The introduction of HAART, with the dramatic reduction in the number of AIDS-defining events and mortality rates, has posed an additional challenge to the clinical testing of therapeutic vaccines in particular and immune-based therapies in general. To ensure adequate responses to immunogenicity, the enrollment criteria for most of these studies select for patients who have early-stage disease and high CD4+ T cell counts.

In this regard, a recently published study showed that, among vaccine recipients of 2 HIV-1 envelope vaccines, the patients who developed new lymphoproliferative responses predominantly had CD4 cell counts that were in a higher stratum (>350 cells/μL) [137]. Therefore, correlation of lymphoproliferative responses with clinical outcome is complicated by the fact that responsiveness selects for a group of patients who would be expected to do well. This further highlights the need for randomized, controlled trials that are of sufficient sample size and duration with clinical end points.

The main antigens that have been tested in large clinical trials are recombinant HIV-1 envelope gp160 vaccine (VaxSyn; MicroGeneSys), inactivated virions stripped of gp120 (HIV-1 Immunogen or Remune; Immune Response), a yeast-expressed combination of the gag proteins p24 and p17 (p24-VLP; British Biotech) and recombinant gp120 protein from the MN virus (MN-rgp120; Genentech). The recombinant gp160 vaccine has been tested in several clinical trials in different countries. In the United States, in a randomized, placebo-controlled study that had a 5-year follow-up, Birx et al. [138] studied 608 patients who had CD4 cell counts of >400 cells/μL. A primary series of 6 monthly shots was followed by 6 booster immunizations every 2 months. Despite clear evidence of persistent immunogenicity in the form of lymphoproliferative response to envelope proteins, no clinical benefit was documented with respect to disease progression.

In Germany, Goebel et al. [139] enrolled 208 patients who had CD4 cell counts of >200 cells/μL in a randomized placebo-controlled trial and recorded their CD4 cell counts, plasma viral RNA levels, and proviral DNA levels for 24 months. The vaccine induced new gp160 lymphoproliferative responses and new delayed-type hypersensitivity skin reactions to gp160 but failed to show any positive changes in CD4 cell counts or decreases in HIV RNA levels. Similarly, in Sweden, 835 patients who had CD4 cell counts of >200 cells/μL were enrolled in a randomized, placebo-controlled study by Sandstrom and Wahren [140]. The patients received placebo or vaccine every 3 months for 3 years, in addition to the optimal available therapy. No clinical benefit was found, with 63 of 416 of the vaccine recipients and 61 of 419 of the placebo recipients reaching a primary clinical end point (i.e., death or AIDS-defining illness). The time to event did not differ in a comparison of the 2 groups. Similar outcomes were reported by Tsoukas et al. [141] in Canada, who performed a 3-year study of 278 patients who had CD4 cell...
counts of >500 cells/μL, and by Pontesilli et al. [142] in Italy, who studied for 2 years 99 patients who had CD4 cell counts of >600 cells/μL.

The HIV-1 Immunogen (or Remune) vaccine has an excellent safety profile, and multiple studies have documented its immunogenicity with generation of new p24 lymphoproliferative responses and induction of p24-specific CD4+ T cells, as detected by IFN-γ and TNF-α production [143, 144]. A large-scale, multicenter, randomized trial studied the effect of HIV-1 Immunogen that was given to 2527 HIV-infected patients who had CD4 cell counts of 300–549 cells/μL [145]. In each group, there were 53 patients who reached the primary end point of clinical progression (i.e., opportunistic infection or malignancies, and death; RR, 0.97; 95% CI, 0.66–1.42). There was no difference between the 2 groups with regard to the overall mortality rate, with 23 deaths occurring among the HIV-1 Immunogen recipients and 19 deaths occurring among the control patients. Despite the ability of HIV-1 Immunogen to generate lymphoproliferative responses to p24 antigen in multiple studies [146, 147], no differences in virus load were noted in a comparison of the 2 patient groups in this trial.

The aim of another randomized, placebo-controlled trial was to study the efficacy of MN-rgp120 in 573 patients who had CD4 cell counts of >600 cells/μL. There was no difference in the rate of disease progression that was noted for the 2 patient groups after a 15-month follow-up [148].

The impact of vaccination with p24-VLP (viruslike particles), with or without zidovudine, was studied in Australia in a 52-week randomized clinical trial of HIV-infected patients who had CD4 cell counts of >400 cells/μL. A statistically significant augmentation and/or broadening of the CTL responses, in comparison with baseline levels (P = .04), was noted in the 9 patients who received p24-VLP with zidovudine, in comparison with the patients who received zidovudine or p24-VLP alone [149]. The enhanced CTL activity, however, did not lead to a decrease in virus load or to changes in lymphocyte counts.

Therefore, most of the therapeutic vaccines that have been tested to date have demonstrated excellent safety, tolerability profiles, and immunogenicity. The disappointing conclusion is that, despite evidence of induction of reactivity to HIV antigens by a variety of methods (e.g., lymphoproliferative response, neutralizing antibodies, CTL or delayed-type hypersensitivity), neither a change in the most important parameter of HIV-specific immunity (virus load) nor clinical benefit has been achieved.

Adoptive Lymphocyte Immunotherapy

The adoptive transfer of autologous or syngeneic lymphocytes has been investigated most intensively by use of cells with specificity for cytomegalovirus or Epstein-Barr virus in patients with cancer and in bone marrow transplant recipients [150]. Despite slow progress, this is a fascinating area that holds potential for future applications in patients with HIV infection or other states of immunodeficiency [151]. As noted below, small studies of adoptive immunotherapy in HIV-infected patients have been performed using a variety of approaches.

The lymphocyte transfers that have been given to HIV-infected patients have included CD8+ or CD4+ T cells, or a combination of the 2. The cells have been resting or activated and have been either native or gene modified. In the majority of these studies, cells obtained from the patient or from an HLA-matched donor are expanded in vitro, either nonspecifically or with enrichment of HIV epitope–recognizing clones [152]. In some studies, the transferred CD4+ T cells have been genetically modified to resist HIV infection [153].

In studies of HIV-infected patients, a common observation has been that the lymphocyte infusions are well tolerated. The infused cells have been traced by different techniques and have been shown to be able to survive for long periods [154]. CD8+ CTL clones that recognize HIV epitopes appear to have the ability to localize to areas of viral replication, as demonstrated in lymph node biopsy specimens [155], but they tend to be short-lived, dying of apoptosis at the time of encounter with their target [156, 157]. The presence of CD4+ T cells that have T cell reactivity similar to that of the CD8+ T cells seems to be a critical component for improved survival of CD8+ T cells after transfer [154]. In one study, coadministration of IL-2 led to an increase in the CD4+ T cell count in the group of patients who received IL-2 along with the lymphocytes, but it did not lead to longer survival of the infused CD8+ T lymphocytes [153].

It is important to note that none of these studies have demonstrated convincing beneficial effects of these interventions on plasma virus load or total CD4+ T cell counts. A dramatic increase in the virus load was noted on 1 occasion, in a study of a nef-CTL clone. This was attributed to the emergence of escape mutants that developed secondary to the selection pressure imposed by the transferred CTL clone [158].

Further technological advances, particularly with regard to the ability (1) to identify relevant antigen-specific T cells or (2) to genetically modify cells to improve resistance to infection or confer second specificities with chimeric receptors, are likely to further boost the interest and research in this field.

**SUMMARY**

HIV infection is a chronic disease of the immune system that affects individuals of any age, including children and young adults. Therapeutic strategies should be developed with consideration given to the fact that eradication of the virus is not a realistic target and that prolonged administration of HAART is associated with significant toxicity. The merits and limitations
of HAART have brought into focus the study of immune-based therapies as a potential supplement to antiretroviral medications. Although the field of immune-based therapies is rapidly evolving and has enhanced our comprehension of HIV immunopathogenesis, only a few of the tested strategies have thus far emerged as viable options and have advanced to testing in phase III clinical trials. Demonstration of clinical efficacy in appropriately powered, randomized, controlled clinical trials will be essential if immune-based therapies are to become part of the therapeutic arsenal against HIV disease.

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