Longer-Term Immunologic Effects and Side Effects of Successful Antiretroviral Therapy

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With the development of more potent and better tolerated antiretroviral regimens, durable antiviral responses are being observed in an increasing fraction of patients. Substantial benefits are associated with these responses: Initially memory, then naive, CD4 cell counts may rise by 150–200 cells/mm³; CD8 cell numbers also rise sharply but then fall below pretreatment levels, presumably as antigenic stimuli driving the CD8 response decline; cellular activation markers decline; distortions in the T cell repertoire gradually lessen; and increases in proliferative responses to mitogens and recall antigens are more easily elicited. Clinical benefits directly accompany these immunologic benefits. Other than peripheral neuropathy, few long-term toxicities are associated with nucleoside analogue reverse transcriptase inhibitors. Recent reports, however, link protease inhibitors with hyperlipidemia, redistribution of body fat, and diabetes mellitus. As more human immunodeficiency virus type 1–infected persons receive long-term antiviral maintenance therapy, successful management of these toxicities will require more attention.

AIDS is characterized by the dysfunction and eventual deletion of CD4 lymphocytes [1–3]. This results in the progressive deterioration of many aspects of cellular immunity, predisposing infected persons to the array of opportunistic infections characteristic of advanced AIDS. Although suppression of HIV replication is a primary goal of antiretroviral therapy, the ultimate success of a chemotherapeutic regimen for AIDS depends on its ability to restore immune function [4]. Although earlier antiretroviral regimens, which consisted only of a single nucleoside reverse transcriptase inhibitor, demonstrated the short-term benefits of antiretroviral chemotherapy, more significant and more durable responses required the evolution of antiretroviral combinations that almost fully suppress viral replication. Despite the capacity, albeit limited, of less potent regimens to restore immune function, more significant immune reconstitution was not observed until the advent of newer multidrug antiretroviral regimens. By ensuring more complete and durable suppression of viral replication, these regimens provided for protection of the immune response for a long enough period of time to allow much more complete recovery of a coordinated, multifaceted immune response.

Rationale for Highly Active Antiretroviral Therapy (HAART)

Earlier regimens of lower potency allowed for the evolution of drug-resistant virus for two major reasons. First, ongoing high-level replication of a highly mutable agent in the presence of selective pressure was associated with the outgrowth of resistant quasispecies. Second, diverse viral quasispecies evolve even before the application of selective pressure, and hence, variants with single mutations associated with drug resistance are usually present even before therapy is initiated. More potent regimens of combination therapy prevent and/or delay the emergence of resistance by driving rates of viral replication to such low levels that selection is minimized and by requiring the virus to develop multiple mutations to grow in the simultaneous presence of multiple agents. When viral replication is driven to these low levels, the likelihood that a single virus variant with the required array of mutations will evolve becomes small.

HIV-1 protease inhibitors have significantly added to the armamentarium of antiviral drugs by providing a new molecular target requiring a novel complement of mutations for resistance at the same time that a major increase in virus suppression is applied. The HIV protease is a 99–amino acid homodimer, which is encoded in the 5' end of the pol gene and is expressed as part of the gag-pol polyprotein. The HIV protease cleaves residues in the gag and gag-pol polyproteins, a necessary step in RNA packaging and assembly and maturation of the virion [5, 6]. The addition of a protease inhibitor to one or more additional antiretroviral drugs [4, 6] reduces levels of plasma HIV-1 RNA below the limit of detection (20–50 copies/mL) in many but not all patients. Such regimens, called “highly active antiretroviral therapy” (HAART), delay or prevent the emergence of resistance [6].

The frequency of use of protease inhibitor–containing regi-
mens increased from <5% in 1995 to >80% by mid-1997 [5, 7]. A recent evaluation of 1,255 HIV-1-infected patients demonstrated that the use of combination antiretroviral therapy was associated with dramatic reductions in HIV-associated morbidity and mortality (figure 1) [7]. Among patients with CD4 cell counts of <100/mm³, mortality declined from 29.4 per 100 person-years in early 1996 to 8.8 per 100 person-years by mid-1997. In addition, the incidence of serious opportunistic infections (defined as either *Pneumocystis carinii* pneumonia, *Mycobacterium avium* complex disease, or cytomegalovirus retinitis) decreased from ~21.9 per 100 person-years in 1995 to 3.7 per 100 person-years by mid-1997. Thus, the most dramatic reductions in death and disease associated with AIDS coincided with the use of protease inhibitors [7].

### Relationships Among the Rate of Viral Replication, CD4 Cell Counts, and Prognosis

Because the rate of disease progression varies among HIV-1-infected patients, attempts have been made to determine exactly which clinical and laboratory measures can be used as prognostic markers of clinical outcome. Earlier studies identified the number of circulating CD4 cells as the best predictor of progression to AIDS [8, 9]. By use of newer, more sensitive techniques for detection of virus, the plasma virus burden, measured as the plasma HIV-1 RNA level, emerged as the single best predictor of progression to AIDS and death [10]. However, in a recent study of a large cohort of HIV-infected men, a regression tree analysis that used both plasma HIV-1 RNA measurements and CD4 cell counts provided better prognostic discrimination than did either variable alone [11]. Similar results were reported for a cohort of patients with symptomatic HIV-1 infection [12] and in a prospective study of 522 HIV-1-seropositive intravenous drug users [13]. Thus, plasma HIV-1 RNA levels and CD4 cell counts are useful indices of disease severity and would be predicted to serve as important markers of response to antiretroviral therapy.

Recent findings have also related prognosis to level of anemia in patients with HIV infection and are discussed in more detail elsewhere in this issue [14].

### Effects of Potent Antiretroviral Therapy on HIV-1-Associated Morbidity and Mortality

Several clinical studies have demonstrated that successful treatment with HAART results in marked reductions in virus load [15, 16]. In one study, the combination of lamivudine, zidovudine, and indinavir reduced the levels of HIV-1 RNA to <500 copies/mL for up to 1 year in 80% of patients (figure 2). Patients were previously treated with zidovudine and had baseline CD4 cell counts ranging from 50 to 400 cells/mm³ [17]. When the 32 patients who were originally randomized to receive simultaneous initiation of the three drugs were followed for 100 weeks, HIV-1 RNA levels decreased to <500 copies/mL in 25 (78%) of the patients [18]. Moreover, in 21 (66%) of the patients, virus load was reduced to <50 copies/mL for 2 years. In another study, 1 year after initiation of treatment with a combination of nelfinavir, zidovudine, and lamivudine, 80% of the patients had a virus load of <500 copies/mL [19]. Similarly, the combination of ritonavir, lamivudine, and zidovudine resulted in a reduction in plasma viral RNA to undetectable levels after 24 weeks of therapy [16].

Several studies have documented a prompt increase in CD4 cell counts following initiation of combination antiretroviral therapy (figure 3) [15, 17, 20, 21]. Although these increases reach only subnormal levels, they have been maintained for up to 3 years [22]. Moreover, significant clinical benefits have been demonstrated to parallel the decrease in plasma HIV-1 RNA levels and the increase in CD4 cell counts [21]. In a controlled trial of 1,156 patients with baseline CD4 cell counts of <200 cells/mm³, use of indinavir, zidovudine, and lamivudine demonstrated a 50% reduction (from 11% to 6%) in the progression to disease and death in patients with prior exposure to zidovudine [21]. Even when less complete suppression of viral replication is achieved, clinical benefits are readily de-
Although it has become clear that use of HAART is associated with increases in the numbers of circulating CD4 cells, more recent studies have addressed whether treatment with combination antiretroviral therapy results in other indicators of restoration of immune function [15, 20].

In one study, eight previously untreated adults with advanced HIV-1 disease were treated with ritonavir, started as monotherapy for the first 2 weeks and then combined with zidovudine and zalcitabine for a total of 12 months [20]. After the initial 2 weeks of ritonavir treatment, there was a rapid decrease in plasma HIV-1 RNA. A low virus load was maintained throughout the 12-month treatment period and was below the level of detection in three of the eight patients. The first 2 weeks of treatment resulted in a twofold increase (to a mean ± SEM of 327 ± 74 cells/μL of plasma) over baseline (164 ± 86 cells/μL) in circulating CD4 cells. The early rise in CD4 cells was primarily of the CD4⁺CD45RO⁺ (memory) phenotype. During the next 12 months of treatment, CD4 cell numbers slowly continued to increase; this increase comprised primarily cells of the CD4⁺CD45RA⁺ (naive) surface phenotype. In addition, circulating CD4 cells of the CD45RA⁺/L-selectin⁺ (naive) phenotype were very low both before treatment and during the first 4 months of treatment [20]. After 4 months, plasma levels of naive CD4 cells rose steadily to reach an overall twofold increase from baseline. Since L-selectin facilitates movement of lymphocytes from the bloodstream into lymph nodes, changes in this population of naive CD4 cells may more accurately reflect a more functional subgroup [20].

The pattern of increases in CD4 cells (i.e., first memory cells and then naive cells) corresponded with a decrease in CD4 cell activation markers [20]. From day 15 through month 12, there was a continuous decrease in activated CD4 cells displaying major histocompatibility complex class II molecules (HLA-DR). By 12 months, the number of activated CD4 cells reached normal values. This decline in cellular activation markers is likely to represent, at least in part, lower levels of virus-mediated destruction. A transient rise at day 15 in naive CD4 cells displaying the α-chain of the IL-2 receptor (CD25⁺) was followed by a reduction to normal values at 4 months. During the last 6 months of treatment, there was a significant increase in such cells.

Treatment with HAART had a different effect on CD8 lymphocytes. During the first 2 weeks of treatment, there was an increase in CD8 cells, although of lower amplitude than that

Evidence for Restoration of T Cell Function During Combination Antiretroviral Therapy

HIV infection results in alterations in specific phenotypic subsets of CD4 and CD8 T cells. Following combination antiretroviral therapy, decreases in surface markers associated with cellular activation are observed as CD4 cells of the memory and naive phenotypes increase in the peripheral blood. Although it has become clear that use of HAART is associated with increases in the numbers of circulating CD4 cells, more recent studies have addressed whether treatment with combination antiretroviral therapy results in other indicators of restoration of immune function [15, 20].

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Table 1. Major immunologic changes with successful highly active antiretroviral therapy in patients infected with HIV-1.

<table>
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<th>Changes</th>
<th>Description</th>
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<tr>
<td>CD4 and CD8 cells increase (rapidly, then slowly)</td>
<td>First, memory phenotypes increase</td>
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<tr>
<td>— First, memory phenotypes increase</td>
<td>Later, naive phenotypes increase</td>
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<tr>
<td>Markers of T cell activation or turnover decrease</td>
<td>Response to recall antigens and mitogens improves</td>
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<td>Distortions in CD4 T cell repertoire decrease</td>
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observed for the CD4 cell subset [20]. At day 15, the number of circulating CD8 cells increased from a mean ± SEM of 1,168 ± 427 cells/μL at baseline to 1,387 ± 619 cells/μL. During the next 12 months, CD8 cell numbers declined to a mean ± SEM of 966 ± 480 cells/μL. This value was still above normal (550 ± 165 cells/μL plasma) for six of the eight patients.

Another recent study of 33 previously untreated HIV-infected adults included detailed analyses of T cell subsets [15]. Patients received ritonavir, zidovudine, and lamivudine for 36 weeks. Plasma HIV-1 RNA levels decreased to below the limit of detection at 36 weeks. The mean number of CD4 cells increased to ~145–196 cells/μL at 36 weeks. The recovery rates for both CD4 and CD8 cells were biphasic (figure 4). The most rapid recovery of CD4 T cells was during the first 3 weeks. Most of this early recovery in CD4 cells was in memory cells (i.e., CD45RO+) and not in naive cells (CD45RA+/CD62L−). The most rapid rates of recovery of CD8 cells were observed during the first 6 weeks [15]. Total numbers of CD8 cells declined during the latter phases of treatment, approaching baseline values. Since most of this decrease is the result of loss of memory cells, it is possible that this decline is due to diminished antigenic stimulation following decline of HIV-1-associated antigenic stimulation.

The effects of antiretroviral therapy on CD4 T cell responses to recall antigens have also been examined [20]. Six previously untreated adults with advanced HIV disease were treated with ritonavir, zidovudine, and zalcitabine. Before treatment was begun, no detectable in vitro proliferative responses to recall antigens from cytomegalovirus and Mycobacterium tuberculosis were found. Within weeks after the initiation of treatment, in vitro proliferation responses to these antigens were detected. Between 1 and 6 months after treatment was started, a significant increase in stimulation indices against cytomegalovirus and tuberculin antigens occurred in all patients. For three of the six patients, the antigen-specific proliferation reached normal ranges after 3–6 months of treatment, indicating that CD4 T cell proliferation to recall antigens can be partially restored in about half of patients [20].

Proliferative responses to HIV antigens return slowly, if at all, following HAART. A recent study was undertaken to determine whether aggressive antiviral treatment initiated during primary infection could preserve this aspect of immunity [36]. In three patients treated with combination antiviral therapy (including use of a protease inhibitor) before seroconversion, the virus load was suppressed to undetectable levels, which correlated with a gradual generation of virus-specific proliferative responses. It has been suggested that preservation or restoration of proliferative responses of CD4 cells to HIV antigens is an essential feature of immunologic support for HIV-specific cytotoxic T cell activity [36]. In addition, a preliminary report of patients treated for >2 years with HAART demonstrated increases in HIV-specific cytotoxic T lymphocytes in patients with advanced infection [37].

Evidence for Restoration of the T Cell Repertoire

In addition to effects on surface phenotypic characteristics of CD4 and CD8 cells and decreases in the response to recall

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Figure 4. Kinetics of CD4 and CD8 T cell subgroups in response to ritonavir, zidovudine, and lamivudine therapy in HIV-1-infected patients. Bars are SEM. From [15] (used with permission).
mitogens and recall antigens, HIV-1 infection is associated with perturbations in the T cell repertoire of antigen receptors [38]. Effects of antiviral therapy on perturbations in the T cell antigen receptor repertoire have also been examined [3, 39]. In one study of 11 patients, disruptions in T cell receptor β-chain, variable region subfamilies were significantly higher in HIV-infected patients than in eight uninfected controls [3]. After 6–15 months of treatment with a regimen that included indinavir and IL-2, there was no detectable restoration of normal patterns of variable region subfamilies of T cell receptors, despite increases in CD4 T cell counts.

In another study of seven HIV-infected patients who received either no previous antiretroviral therapy or only sequential monotherapy, CD8 cell repertoires were drastically altered at all stages of infection, regardless of clinical status, CD4 and CD8 cell counts, or virus load (table 2) [39]. CD4 cell repertoires from patients with high viremia or decreased CD4 cell counts had severely disturbed distribution of lengths of the complementarity-determining region 3 compared with that in seronegative controls or patients with low viremia and high CD4 cell counts. After 6 months of successful treatment with HAART (as manifested by decreased virus load), CD8 cell perturbations remained high, whereas CD4 cell repertoires were less perturbed after 3–6 months of antiviral treatment. In 8 of 11 patients, perturbations in the CD4 T cell receptor repertoire decreased to the normal range [39], whereas in two cases of unsuccessful therapy, the disruptions in the CD4 cell repertoire persisted [40]. The measurable increase in diversity may have reflected the elimination of highly expanded HIV-specific activated T cells, resulting in the net normalization of the T cell receptor repertoire and reduction in size of expanded ones [39, 41]. Several other studies of the effects of antiretroviral chemotherapy on the T cell repertoire are underway. These studies have been complicated by the absence of an optimal approach to the quantitative evaluation of the distribution of T cell receptors.

### Long-Term Toxicities of Antiretroviral Therapy

Important side effects associated with use of protease inhibitors have been recently reviewed [5, 42]. Now that patients are living longer as a result of HAART, and the risk of HIV-associated mortality has diminished, the issue of long-term side effects has become relevant. Long-term use of HIV-1 protease inhibitors has been associated with hyperlipidemia [43], increased glucose levels [44], redistribution of body fat [45–48], and emergence of diabetes mellitus (table 3) [49]. Preliminary reports have described a syndrome of “fat wasting of the face and limbs with relative central adiposity,” or “lipodystrophy” [45, 47, 48]. In one report of 116 HIV-positive patients taking at least one protease inhibitor, 64% of the participants developed lipodystrophy after a mean of 10 months [45]. Other studies have demonstrated much lower incidences of this complication, however. Patients who developed lipodystrophy also had significantly higher levels of plasma triglyceride, cholesterol, insulin, and C-peptide and higher insulin resistance scores. Three patients developed new or worsening diabetes.

Another study was undertaken to determine whether enlargement of the dorsocervical fat pad or “buffalo hump” is attributed to the use of protease inhibitors [48]. Although the accumulation of adipose tissue in this region has been associated with Cushing’s syndrome, no other signs of this syndrome were observed in this study [47, 48]. All eight of the HIV-infected patients were receiving antiretroviral drug regimens at the time of study; however, only four of the eight patients were receiving a protease inhibitor [48]. Since the development of a buffalo hump was not unique to the patients taking protease inhibitors, it raises the question of whether this fatty tissue accumulation is protease inhibitor–specific. The mechanisms underlying the formation of buffalo hump in Cushing’s syndrome as well as during HIV-1 infection remain unclear but may be a reflection of subclinical changes in endocrine and metabolic function. A slight elevation in triglyceride levels was observed in patients with buffalo hump compared with levels in HIV-positive controls; however, this difference was not significant [48].

Other preliminary reports indicate that although protease inhibitor–induced hyperglycemia is uncommon, it can be extremely severe when it does occur [44, 49]. No associated risk factors for diabetes were present. Because of the relatively recent development of these drugs, information on the long-term side effects of protease inhibitors is limited. It is clear that further studies are needed to determine the pathophysiological mechanisms and clinical significance of the changes associated with long-term use of protease inhibitors.

### Summary

The introduction of protease inhibitors in the management of HIV disease has resulted in durable antiviral responses (HIV-1

### Table 2. Perturbation in CD4 and CD8 T cell repertoires in patients with HIV-1 infection.

<table>
<thead>
<tr>
<th>In CD4 cell repertoire</th>
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<tr>
<td>• Perturbation not significant in early HIV infection</td>
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<tr>
<td>• Worsened with disease progress</td>
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<tr>
<td>• Normalized in those responding to highly active antiretroviral therapy</td>
</tr>
<tr>
<td>In CD8 cell repertoire</td>
</tr>
<tr>
<td>• Major restrictions during progression to AIDS</td>
</tr>
<tr>
<td>• Persisted during 6 months of highly active antiretroviral therapy</td>
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</table>

### Table 3. Long-term side effects associated with antiretroviral therapy in patients with HIV-1 infection.

- Hyperlipidemia: triglycerides and cholesterol increase
- Hyperglycemia: new or worse diabetes mellitus
- Lipodystrophy: fat wasting of face and limbs; central adiposity increases
- “Buffalo hump”?

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RNA below the level of detectability) and increases in CD4 cell numbers (mean of 150–200 cells/µL) in many patients able to adhere to medication regimens. Suppression of viral replication has resulted in dramatic decreases in morbidity and mortality. Emerging studies indicate that successful antiretroviral therapy halts damage to the immune system and leads to partial restoration of immune function. The studies reported here suggest that the following sequence of events is involved in the restoration of immune competence. First, after successful antiretroviral therapy, there is a rapid increase in circulating CD8 and mature CD4 memory T cells with less prominent evidence of cellular activation [20]. After initial recovery of CD4 memory T cells, there is a slow but continuous recovery of naive CD4 cells, possibly of the L-selectin+ subset. This effect, which occurs most significantly after 4 months of antiviral treatment, suggests a mechanism by which the T cell repertoire can become diversified [15, 20]. In addition, there is a partial but significant improvement of memory CD4 T cell reactivity to recall antigens after 3 months of antiviral therapy [20]. This reactivity suggests that the immune defects that are characteristic of advanced HIV disease are at least partially reversible by combination antiretroviral therapy. Although the data are incomplete, some evidence suggests that the increases in CD4 cells observed over the longer term are also associated with increased T cell diversity [39].

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References
