Mechanisms that underly discordant CD4+ cell/virus load (VL) responses in patients who receive highly active antiretroviral therapy (HAART) were studied in 30 human immunodeficiency virus (HIV)–positive patients, in 3 groups. Discordant responders maintained CD4+ cell levels >200/mm3 with stable or increasing trend, despite sustained VLS of 500–5000 copies/mL, for >2 years. Treatment-success patients had CD4+ cell counts >200/mm3 with stable or increasing trend and VLS <50 copies/mL, for >2 years. Treatment-failure patients initially responded to HAART, followed by decreasing CD4+ cell counts and increasing VLS. Interferon-γ production to gag and noncytolytic CD8+ cell suppressive activity were greater in discordant responders. Cellular activation was greatest in patients with treatment failure. All discordant responders had non–syncytium-inducing (CCR5-tropic) viruses. Viruses from discordant responders and from patients with treatment failure had extensive resistance mutations; discordant responders had significantly lower viral replication capacities. These findings suggest that discordant responses may be related to enhanced HIV-directed immune responses, diminished cellular activation, decreased viral replication capacity, and preservation of non–syncytium-inducing virus strains.

Although current treatment guidelines stress the importance of the suppression of human immunodeficiency virus (HIV)–1 virus load (VL) below 50 copies/mL [1], not all patients are able to achieve or sustain this response with highly active antiretroviral therapy (HAART) [2–6]. Among patients who achieved undetectable VLs in the Swiss HIV Cohort study, 20% of treatment-naive and 35%–40% of treatment-experienced patients had virus rebound within 2 years [4]. Included in the group with incomplete virus suppression is a subset with discordant CD4+ cell/VL responses; the patients in this subset demonstrate increases in CD4+ cell counts for periods of 3–4 years, despite persistent viremia [7, 8]. Estimates of the prevalence of this phenomenon range from 7% to 55%, depending on how it is defined [3, 5, 7, 9–11]. Despite having ongoing detectable viral replication, patients with a dis-
discordant response have prolonged survival, immunologic benefit, and a reduced risk of AIDS complications [2, 4, 5, 9].

The mechanisms by which CD4+ cell counts are maintained in the face of ongoing viral replication are not known, but they may include decreased viral replication capacity [12], impaired viral replication in thymic tissue [13], decreased cell death by activation-induced apoptosis [14], or regeneration of HIV-specific immune responses, similar to those seen in long-term nonprogressors [15, 16].

The proper management of patients who experience a discordant response remains unclear; most treatment guidelines recommend that patients change therapy once virus rebound occurs, without regard to CD4+ cell counts [1]. However, this strategy entails risks, such as exposure to new adverse events or toxicities, decreased tolerance of or adherence to antiretroviral regimens, and depletion of future treatment options. However, continuing a regimen in the absence of complete virus suppression risks generation of increased resistance, including potential cross-resistance to other antiretroviral agents [17, 18]. Thus, a better understanding of the phenomenon of discordant CD4+ cell/VL responses may help define proper clinical management.

The purpose of this pilot study was to perform an in-depth analysis of a carefully defined group of patients with discordant CD4+ cell/VL responses sustained over several years of protease inhibitor (PI)–based HAART. Host factors, viral factors, treatment factors, and immune factors were investigated and were compared with responses from a group of patients who experienced both viral and immunologic failure during HAART, as well as with responses from a group of patients who achieved full virus suppression. We sought to determine which factors might play a role in prolonged discordant responses.

SUBJECTS AND METHODS

Subjects. Subjects were enrolled from the Duke University Medical Center Infectious Diseases Clinic. Inclusion criteria were as follows: >18 years old; confirmed diagnosis of HIV infection; current therapy with PI–based HAART; documented VL >50,000 copies/mL and/or CD4+ cell nadir <50 cells/mm3, while not on antiretroviral therapy; and meeting criteria for 1 of the 3 study groups as defined below. Patients were excluded from the study if therapy was initiated during primary HIV infection; if medications included immunomodulatory drugs, such as interleukin-2 or hydroxyurea; if nonadherence was documented by history or complete absence of genotypic resistance; or if vaccination or febrile illness occurred in the preceding 4 weeks.

Study groups were defined as follows: treatment success (group S), VL <50 copies/mL and CD4+ cell count >200 cells/mm3, with stable or increasing trend for >2 years; discordant response (group D), VL maintained between 500 and 5000 copies/mL and CD4+ cell count >200 cells/mm3, with stable or increasing trend for >2 years; treatment failure (group F), VL >400 copies/mL and increasing towards pretreatment baseline, with declining CD4+ cell count after an initial ≥3 log10 decrease in VL. Patients who met these group definitions were identified by a search of the Infectious Diseases Clinic database or by referral from their primary physician. CD4+ cell count and VL data, as well as treatment history, were obtained by retrospective chart review. HAART regimens were defined as 2 nucleoside reverse transcriptase inhibitors and 1 PI. Some patients also received a nonnucleoside reverse transcriptase inhibitor. Single-drug changes, because of side effects or to simplify dosing, were not considered to be a different HAART regimen. Efforts were made to match the subjects in terms of age, sex, and ethnicity, as well as to accurately reflect the population of our clinic.

Measurements. Blood was drawn at a single time for each patient. Measurements included quantitative analyses of CD38 antibodies bound per cell (ABC) on T cell subsets, by flow cytometry; CD4+ and CD8+ T cell receptor excision circle analysis (TRECs); CD8+ response to gag, rev, tat, vpr, and nef antigens, by interferon-γ (IFN-γ)–enzyme-linked immunospot (ELISpot) assay; noncytolytic CD8+ cell suppressive activity; serum IgG, IgA, and IgM responses to gp120, to gp41 peptide, and to HIV-1 MN V3 peptide, by ELISA; CCR5/CXCR1 coreceptor genotype; and high-resolution HLA typing, by reference strand–mediated conformation analysis [19]. Virus isolates were available for subjects in group D and group F; measurements included SI/NSI viral phenotype, drug susceptibility genotype and phenotype, and viral replication capacity.

Plasma HIV-1 RNA was measured by a reverse transcriptase–polymerase chain reaction (PCR) assay (Ultratrace Amplific HIV Monitor assay; Roche Diagnostic Systems; lower limit of detection, 50 copies/mL). For purposes of data analysis, all HIV-1 RNA values <50 copies/mL were assigned a value of 50 copies/mL.

Flow cytometry. Monoclonal antibodies (BD Biosciences) were added in the following 4-color phycoerythrin (PE), fluorescein isothiocyanate, peridinin chlorophyll protein, and allophycocyanin combinations: MsIgG/MsIgGv/CD4/MsIgG, CD38/CD45RA/CD4/CD62L, and CD38/HLA-DR/CD8/CD4. In addition, premixed Multitest CD3/CD8/CD45/CD4 was added. Cells were treated with FACS lysing solution (BD Biosciences), washed with PBS, fixed in PBS with 1% formaldehyde, and acquired immediately on a FACSCalibur cytometer (BD Biosciences). Quantitative immunofluorescence values, in units of fluorescence and low side scatter, for the Multitest tube and forward...
scatter/side scatter, with a sequential gate on CD4brt− or CD8brt− cells, for markers of lymphocyte activation and maturation. A minimum of 2500 events for each gate was acquired. Activated subsets are defined as CD38 HLA-DR+; naive subsets as CD62L−CD45RA+; and memory subsets as CD62L+CD45RA− and CD62L−CD45RA− [20, 21].

**Signal joint TREC**. Quantification of signal joint TREC in isolated CD4+ and CD8+ T cells was done by real-time quantitative PCR, by means of the 5′-nuclease (TaqMan) assay, with an ABI7700 system (Perkin-Elmer), as described by Douek et al. [22]. Real-time quantitative PCR was done on 5 µL of cell lysate (equivalent to 50,000 cells). Molecules of TREC were determined in cell lysates equivalent to 50,000 cells.

**IFN-γ ELISpot**. The IFN-γ ELISpot assay was done with pools of 122, 50, 23, 27, and 22 15-mer peptides overlapping by 11 amino acids, representing the gag, nef, tat, rev, and vpr gene products, respectively. Peptides were synthesized on the basis of the sequence of the HXB2 (gag) and consensus B (nef, tat, rev, and vpr) isolates. On the basis of results obtained with peripheral blood mononuclear cells (PBMCs) from 10 seronegative persons, results were considered positive only if the number of spots per million cells (spot-forming cells [SFCs]/106 cells) in the experimental wells was 3-fold higher than the background and was >20 SFCs/106 cells. Responses were considered mediated by CD8+ cells if the number of spots in the CD8+ cell–depleted population was <50% of the spots in the undepleted population. Responses were defined as CD4+ cell–mediated when the number of spots in the CD8+ cell–depleted population was >50% of the spots in the undepleted population. Results were reported as CD8+ cell–mediated with a CD4+ component if the spots in the CD8+ cell–depleted wells were <50% but were still positive, compared with the number of spots in the undepleted wells. Spots were counted by an independent source, with a CTL Imager reader (Cellular Technology Limited).

**Noncytolytic CD8+ cell suppression of HIV replication**. Noncytolytic CD8+ cell antiviral activity was measured by means of a CD8+ cell suppression assay that involved a single cycle of HIV replication, as described elsewhere by T omaras et al. [23]. Briefly, CD4+ cell–enriched PBMCs from seronegative donors were infected with HIV that had been pseudotyped with a laboratory-adapted CXCR4-tropic HIV envelope (NL4–3). Pseudotyped viruses were produced by transfection of plasmid DNA (pNL4-3 LUCR+E), obtained from the National Institutes of Health AIDS Research and Reference Reagent Program; and pNL4-3env; gift of T. Dragic) into the human embryonic kidney cell line, 293T, by means of a modified calcium phosphate method [24]. The laboratory-adapted viral envelope NL4–3 was used to evaluate β-chemokine–independent effects of CD8+ cell antiviral activity. CD4+ primary T lymphocytes were infected for 2 h at 37°C, with 100 µL of pseudotyped virus in the presence of 20 µg/mL DEAE, in a flat-bottomed 96-well tissue culture plate. The number of CD4+ T cell targets for infection was 0.9 × 105 cells. After the 2-h incubation, control CD8+ T cells or patient-derived CD8+ T cells were added at an effector:target cell ratio of 2:1; 72 h after the start of infection, the samples were assayed for infectivity, with the Promega Luciferase Assay System and with a luminometer (MicroLumat Plus; EG&G Berthold). Noncytolytic antiviral CD8+ T cell–mediated activity was scored by comparison with the well-described effector CD8+ T cell line (HS-HVS CD8+ T cells) and with control CD8+ cells from HIV-seronegative persons. For this study, subjects were considered to have a positive score for CD8+ cell suppression if the CD8+ cells, at a 2:1 (effector:target cell) ratio, caused at least a 1-log reduction in HIV replication.

**ELISA**. Antigen–specific end-point antibody titers were determined by ELISA, as described elsewhere [25, 26], except that black ELISA plates (microfluor 2; DYNEX; Thermo Labsystems) and the fluorescent alkaline phosphatase substrate Atto phos (Roche Molecular Biochemicals) were used. Samples were considered positive for antigen–specific antibody when the number of relative light units for the sample dilution was at least 3-fold higher than the number of relative light units for a naive sample at the same dilution.

**SI/NSI viral phenotyping**. HIV was cultured from PBMCs by techniques published elsewhere [27]. Syncytium formation was determined with MT-2 cells [28].

**Genotype, phenotype, and replication capacity**. Genotypes were determined by population sequencing of resistance test vector pools, by means of dye-terminator chemistry and analysis, on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Full-length protease and amino acids 1–305 of reverse transcriptase were analyzed. Translated protease and reverse transcriptase amino acid sequences were compared with the NL4–3 reference strain. Genotypes were interpreted on the basis of published guidelines and an analysis of phenotype-genotype data in the ViroLogic database. Mutations that were judged to be conferring resistance to nucleoside reverse transcriptase inhibitors were as follows: M41I, E44D, K65R, D67N, T69D/N/S/A, K70R, L74V, V75T/M/A/I, V118I, Q151M, M184V, L210W, T215Y/F, and K219Q/E/N. Primary mutations that were associated with resistance to PIs were as follows: D30N, G48V, T215Y/F, and K219Q/E/N. Secondary mutations that were associated with PIs were as follows: L10I/F/R/V, K20I/M/R/T, L24I, V32I, L33F/V, M36I/L/V, M46I/L/V, I47V, F53L, I54L/V, L63X, A71I/L/T/V, G73A/S/T, V77I, N88D, and I93L/M (http://www.hivresistanceweb.com).

Phenotypic testing of drug susceptibility was done on stored frozen plasma samples, by means of a rapid recombinant virus assay (PhenoSense HIV; ViroLogic) [29]. The replication capacity of HIV was measured with a modified version of this rapid assay. Test vectors were constructed by insertion of pa-
tient-derived reverse transcriptase and protease sequences into a modified retrovirus vector that contained a luciferase indicator gene that allows quantification of viral replication [29]. After normalization of the output of the assay, on the basis of the virus inoculum, the ability of the recombinant virus to replicate in the absence of an antiretroviral drug was measured. The replication capacity, expressed as a percentage, was calculated as the ratio of the normalized luciferase activity from viruses that contained patient-derived sequence to that from viruses that contained wild-type sequences. A ratio of <100% reflects a reduced replication capacity, compared with that of the NL4–3 reference strain.

Coreceptor genotyping. Genomic DNA was isolated from PBMCs and was amplified for CCR5 by PCR by the methods described by Samson et al. [30]. A portion of the CXCR1 gene was amplified from genomic DNA by PCR by techniques described by Faure et al. [31].

Statistical analysis. Nonparametric methods were used to analyze the data. Medians and interquartile ranges were used to describe continuous variables. Categorical variables were expressed as the number or percentage per group. Comparisons between and among groups were made with the Wilcoxon rank sum test or with the Kruskal-Wallis test, for continuous variables, and with the Fisher’s exact test, for categorical variables. Age was controlled for in the analysis of CD4+ and CD8+ TREC. All P values are 2-sided. Statistical significance was defined as α = .05. All statistical analyses were done with SAS, version 8.2 (SAS Institute).

RESULTS

Study population. Ten patients were enrolled in each of the 3 groups over a period of 6 months; demographic and clinical characteristics were similar across groups, except that group D had more white individuals (table 1; P = .05, Fisher’s exact test). CD4+ cell counts increased and low VLs were sustained for group D subjects over periods that approached 60 months (figures 1 and 2). The median duration of treatment failure for group F patients was 5 months.

Host factors. HLA types known to influence disease progression [32, 33] and CCR5/CXCR1 coreceptor genotypes did not differ among groups (data not shown). Known duration of HIV infection was greatest for group D patients (table 2), but not significantly so.

Treatment factors. All patients were taking a regimen that consisted of 2 nucleoside reverse transcriptase inhibitors and 1 PI; a few patients were also taking 1 nonnucleoside reverse transcriptase inhibitor. Nelfinavir was the most commonly used PI, especially in group D (8 vs. 4 each in group S and group F). Prior to their current regimen, patients had been treated with a wide variety of antiretroviral agents, which is reflective of changes in treatment strategies and in the availability of new drugs. Four patients had single-drug changes in their HAART regimen, because of side effects or to simplify dosing; this was not considered a new HAART regimen. Group F patients were more likely to have had prior HAART regimens and had been treated with a greater number of antiretroviral agents, but these differences were not significant (table 2).

Immune factors. Immune function was assessed on the basis of nadir CD4+ T cell counts; serum antibody responses to gp120, to gp41 peptide, and to HIV-1MN V3 peptide; TREC; CD8+ responses to gag, rev, tat, vpr, and nef antigens; noncytolytic CD8+ suppressive activity; and measures of cellular activation, including expression of CD38+HLA-DR+ and CD38 ABC quantitation of T cell subsets. Serum IgA and IgM responses were usually absent to all tested antigens. Serum IgG responses for all 3 groups were clustered in a narrow range, and no differences were observed. CD4+ and CD8+ TREC showed large within-group variations, and group-to-group comparisons did not differ, after age was controlled for.

Nadir CD4+ cell counts were unavailable for 8 subjects because they presented to our clinic after having initiated therapy.

### Table 1. Demographic and clinical characteristics of groups, in study of discordant CD4+ cell and virus load responses, during highly active antiretroviral therapy.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group F: patients with treatment failure (n = 10)</th>
<th>Group D: patients with discordant response (n = 10)</th>
<th>Group S: patients with treatment success (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>45 (40–49)</td>
<td>46 (37–51)</td>
<td>39 (38–41)</td>
</tr>
<tr>
<td>Male, %</td>
<td>80</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>White, %</td>
<td>40</td>
<td>90</td>
<td>40</td>
</tr>
<tr>
<td>African American, %</td>
<td>60</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>Study-visit virus load, copies/mL</td>
<td>33,855 (20,516–74,273)</td>
<td>1506 (1134–3400)</td>
<td>50 (50–50)</td>
</tr>
<tr>
<td>Study-visit CD4+ cell count, absolute, cells/mm³ (%)</td>
<td>133 (9)</td>
<td>480 (21)</td>
<td>454 (29)</td>
</tr>
</tbody>
</table>

**NOTE.** Values for age and virus load are medians (25th–75th quartiles). Ethnicity differed significantly among groups (P = .05, Fisher’s exact test). In pairwise comparisons, all P values were obtained with Wilcoxon rank sum test. The following differed significantly between group F and group D: study-visit virus load (P < .0001); study-visit CD4+ cell count (absolute, P = .003; %, P = .009). Between group S and group D, study-visit virus load differed significantly (P < .0001).
Among the remainder, group F subjects had a significantly lower CD4+ cell nadir (absolute or percentage) than did group D subjects (table 3; absolute, \(P = .001\); percentage, \(P = .003\), Wilcoxon rank sum test).

CD8+ cell IFN-\(\gamma\) ELISpot responses to rev, to vpr, and to nef did not differ significantly among groups. Responses to gag were significantly greater for group D subjects than for group S subjects (table 3; CD8+ + CD4+ cell response, \(P = .003\); CD8+ cell–depleted response, \(P = .05\), Wilcoxon rank sum test). A detectable CD4+ cell component in the ELISpot response to gag was seen in 6 of 10 group D subjects, in 3 of 8 group F subjects, and in 0 of 10 group S subjects. Group D subjects also had a greater response to tat than did subjects in group S, although this was not statistically significant. There were no significant differences in ELISpot response between group F and group D.

Noncytolytic CD8+ cell activity was assessed by an HIV suppression assay. The proportion of subjects with CD8+ cell–mediated noncytolytic HIV suppressive activity differed among groups. Suppressive activity against CXCR4-tropic virus was more common in group D (7/10) and in group S (6/10) than in group F (2/9) (group D vs. group F, \(P = .07\), Fisher’s exact test).

Immune activation was measured by quantification of levels of circulating activated CD4+ and CD8+ lymphocytes, defined by expression of CD38+ HLA-DR+ (table 3). Patients in group S (suppressed virus) had significantly less activation than did patients in group D (CD4+ cells, \(P = .005\); CD8+ cells, \(P = .001\), Wilcoxon rank sum test). Patients in group F had the highest levels of activation, whereas patients in group D had levels intermediate between these 2 extremes. Immune activation also was assessed at the single-cell level, by quantification of CD38 ABC expression on the surface of CD4+ or CD8+ lymphocytes. By this assay, no difference was observed between group D and group S. Group F had significantly higher levels of CD38 ABC than did group D for all T cell subsets, including activated CD4+ and activated CD8+ lymphocytes (activated CD4+ cells, \(P = .04\); activated CD8+ cells, \(P = .008\), Wilcoxon rank sum test).

**Viral factors.** Pretreatment VLs were obtained, when possible, for all subjects (table 4). In cases in which these were not available, a VL that had been determined when the patient was not undergoing therapy was used as the baseline value. Neither value was available for 11 subjects, because therapy was initiated before the availability of VL testing. All such patients had documented nadir CD4+ cell counts <50 cells/mm\(^3\). Median baseline VLs differed significantly between subjects in group F and group D (\(P = .04\), Wilcoxon rank sum test).
Genotypic and phenotypic drug-susceptibility testing of patients in group D and group F demonstrated high levels of resistance to virtually all components of each subject’s HAART regimen, and numbers of mutations per patient did not differ between groups (table 4). Group F and group D differed in their viral replication capacity, relative to the reference virus NL4–3, and viruses from discordant responders had significantly lower replication capacities ($P = .04$, Wilcoxon rank sum test). For the 15 subjects from whom virus could be cultured, all group D patients had non-syncytium-inducing (CCR5-tropic) viruses, whereas patients in group F had predominantly syncytium-inducing (CXCR4-tropic) viruses (group D: NSI = 6, SI = 0; group F: NSI = 2, SI = 7; $P = .007$, Fisher’s exact test).

**DISCUSSION**

In this study we sought to determine the role that various host factors, treatment factors, immune factors, and viral factors play in the phenomenon of discordant CD4$^+$ cell/VL responses among patients who received PI-based HAART. The VLs in discordant responders do not return to pretreatment levels, despite accumulation of extensive resistance mutations. Potential mechanisms for this sustained partial virus suppression are identified in our study. Compared with group S patients (patients with full virus suppression), group D patients had increased CD8$^+$ responses to gag and tat antigens, by IFN-γ ELISPOT, and a detectable CD4$^+$ response to gag was present in more than half of the group D subjects. These results are similar to findings from studies of long-term nonprogressors and differ from results seen in patients rapidly progressing to AIDS [34]. Interestingly, in a study of chronically infected adults, subjects with VLs that range from 1000 copies/mL to 10,000 copies/mL had the highest proportion of gag-specific CD4$^+$ cells [35]. Additionally, discordant responders in our study had enhanced noncytolytic CD8$^+$ suppressive activity, compared with patients who experienced treatment failure.

An interesting, although not unexpected, finding in our study is the lack of correlation between the 2 types of CD8$^+$ responses that were measured—IFN-γ production and noncytolytic virus suppression—when the discordant subjects were compared with the other 2 groups. It has been shown that noncytolytic CD8$^+$ activity correlates with disease stage [36]. Patients with an intact immune system (i.e., long-term nonprogressors or patients early in the course of infection) have stronger noncytolytic CD8$^+$ cell activity. The results of our study agree with
these previous findings, because group S and group D had stronger activity and a more intact immune system, as evidenced by CD4\(^+\) cell counts. It is known that cytolytic CD8\(^+\) responses and/or IFN-\(\gamma\) production, as measured by the ELISpot assay, are enhanced if there is viral replication to stimulate these responses [37, 38]. This may be the reason that patients with discordant responses to treatment and patients with treatment failure have increased CD8\(^+\) cell activity, as measured by the IFN-\(\gamma\) ELISpot assay. It may be that, for noncytolytic responses, the presence of strong CD4\(^+\) cell help or other unknown immunologic factors, and not viral replication, stimulates and maintains this response. It has been shown that CD8\(^+\) cells can have both cytolytic and noncytolytic activity, but some cells have only 1 of these responses [39, 40]. Thus, the cytolytic (or IFN-\(\gamma\)--producing) responses and noncytolytic responses of CD8\(^+\) cells may be in some ways independent of each other, which would explain why discordant responders are more similar to patients with treatment failure in terms of IFN-\(\gamma\) production and are more similar to patients with full suppression in terms of noncytolytic activity.

In untreated patients and in patients who experience treatment failure, CD4\(^+\) cell counts generally decline in proportion to viral replication [41], and current data implicate activation-induced apoptosis [14] as a major mechanism that underlies CD4\(^+\) cell death. Direct cytopathic effects of HIV may also play a role, but these effects cannot account for the total losses that occur [42]. Cellular activation was measured in 2 ways in this study. The first, CD38\(^+\)HLA-DR\(^+\) expression, is a qualitative measurement that determines whether or not a cell is activated. The second, CD38 ABC on CD8\(^+\) cells has been shown to be a better predictor of progression to AIDS and death than have either CD38\(^+\)HLA-DR\(^+\) expression or CD4\(^+\) cell count [43]. The decreased cellular activation in patients with discordant responses, relative to those with treatment failure, as measured by CD38 ABC, may contribute to maintenance of CD4\(^+\) cell counts, because less activation-induced apoptosis is likely occurring.

Although thymic contribution to immune reconstitution in HIV-infected patients treated with HAART has been demonstrated elsewhere [22, 44, 45], no such effect was seen in our study. The limited sample size may have contributed to this result, but others have reported similar findings [46].

Patients with multidrug-resistant virus who continue therapy may have virus with decreased replication capacity, relative to wild-type HIV [12, 47]. In our study, virus from patients with a discordant response to treatment had significantly lower replication capacities than did virus from patients with treatment failure, a finding that suggests a role for reduced viral fitness in maintenance of CD4\(^+\) cell counts. Specific mutations that are associated with decreased viral replication capacity include D30N in protease, and the reverse transcriptase mutation M184I/V [48, 49]. Group D had a higher proportion of patients with D30N than did group F, but this difference was not significant. Viral phenotype may also affect the pace of CD4\(^+\) cell change. Syncytium-inducing (CXCR4-tropic) virus strains replicate more efficiently than do non-syncytium-inducing (CCR5-tropic) virus strains [50], and this enhanced pathogenicity is significantly associated with an increased risk of progression to AIDS and death [51]. Among our patients, none of the 6 discordant responders had syncytium-inducing virus, compared with 7 of 9 patients with treatment failure.

The generation of resistance mutations that decrease viral replication capacity suggests a role for the maintenance of a patient’s current HAART therapy, in some situations, despite the presence of multidrug-resistant virus. Interestingly, 1 group D patient who discontinued therapy after the study visit ex-
permitted rapid virus rebound, to a VL >300,000, within 7 weeks. This patient had broad and robust IFN-γ cell activity. The impact of enhanced IFN-γ production, at least in this patient, was modest at best in the maintenance of virus suppression, after treatment interruption. These findings suggest that reduced replication capacity plays an important role in allowing control of drug-resistant virus. For this patient, reinstitution of HAART reestablished the discordant treatment response.

Numerous studies have demonstrated the importance of host factors, such as HLA type and coreceptor genotype, in HIV progression [31–33, 52]. Although our sample size was small, we included these factors to ensure that the observed differences were not due to uneven distribution of confounding variables. No differences among groups were observed.

Adherence was not directly assessed in this study, although every effort was made to include only subjects who were highly adherent to their treatment regimen. Differences in adherence also may play a role in discordant responses, because it has been shown that CD4+ cell counts increase at an adherence level that is lower than the level required for complete virus suppression [53]. Indeed, what constitutes an appropriate clinical target for “undetectable” VL is unclear. For example, recent data on patients treated with lopinavir/ritonavir show no difference in outcome among patients whose VL was suppressed to <3 copies/mL, compared with patients whose VL was detectable between 3 and 50 copies/mL [54]. Current recommendations may underestimate the clinical and immunologic impact and the durability of incomplete virus suppression. However, resistance mutations have been shown to develop in patients with transient virus relapse [55] but not in patients whose VL is suppressed to <50 copies/mL [56].

The use of parameters other than just VL measurements, to make decisions regarding antiretroviral therapy, may be warranted. Such parameters may include CD4+ cell counts (or trends), cellular-activation measurements, or viral replication capacity. Prospective randomized trials in which management decisions incorporate these parameters may be worth considering. In addition, the continuing debate over when to start therapy may be of direct relevance. Although the majority of our patients had a CD4+ cell nadir <200 cells/mm³, the CD4+ cell nadir was significantly lower and baseline VL values were significantly higher for patients with treatment failure than for patients with discordant responses. Discordant responders had values similar to those of patients who achieved full virus suppression, a finding that indicates a potential relationship between the level of pretreatment viremia and cellular destruction and the ability to achieve long-term immunologic success with HAART.

Our study had several limitations. First, given the intensity

Table 3. Immune factors among groups, in study of discordant CD4+ cell and virus load responses, during highly active antiretroviral therapy.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Group F: patients with treatment failure (n = 10)</th>
<th>Group D: patients with discordant response (n = 10)</th>
<th>Group S: patients with treatment success (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nadir CD4+ cell count, cells/mm³ (%)</td>
<td>20 (2)</td>
<td>160 (11)</td>
<td>124 (14)</td>
</tr>
<tr>
<td>IFN-γ ELISpot results, SFCs/10⁶ cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gag</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8+ and CD4+</td>
<td>760 (123–1081)</td>
<td>1198 (705–2623)</td>
<td>434 (153–818)</td>
</tr>
<tr>
<td>CD8+ cell–depleted</td>
<td>21 (15–74)</td>
<td>49 (18–80)</td>
<td>19 (13–30)</td>
</tr>
<tr>
<td>tat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8+ and CD4+</td>
<td>41 (8–104)</td>
<td>55 (15–75)</td>
<td>16 (0–50)</td>
</tr>
<tr>
<td>CD8+ cell–depleted</td>
<td>4 (3–9)</td>
<td>3 (0–5)</td>
<td>3 (3–5)</td>
</tr>
<tr>
<td>Presence of CXCR4 suppressive activity, no.</td>
<td>2/9</td>
<td>7/10</td>
<td>6/10</td>
</tr>
<tr>
<td>Activated CD4+ cells, cells/mm³ (%)</td>
<td>15 (1)</td>
<td>44 (11)</td>
<td>26 (6)</td>
</tr>
<tr>
<td>Activated CD8+ cells, cells/mm³ (%)</td>
<td>192 (37)</td>
<td>312 (31)</td>
<td>113 (14)</td>
</tr>
<tr>
<td>CD38 ABC on activated CD4+ cells</td>
<td>17,297 (14,865–31,626)</td>
<td>11,119 (8918–17,653)</td>
<td>12,344 (11,719–13,991)</td>
</tr>
<tr>
<td>CD38 ABC on activated CD8+ cells</td>
<td>20,357 (11,923–29,293)</td>
<td>8413 (6670–10,115)</td>
<td>7829 (6795–10,661)</td>
</tr>
</tbody>
</table>

**NOTE.** Values are medians (25th–75th quartiles), unless otherwise stated. In pairwise comparisons, P values were obtained with Wilcoxon rank sum test. The following differed significantly between group F and group D: nadir CD4+ cell count (P = .001); nadir CD4+ cell percentage (P = .003); X4 suppressive activity (P = .01; Fisher’s exact test); activated CD4+ cell count (P = .004); activated CD8+ cell count (P = .03); CD38 antibodies bound per cell (ABC) on activated CD4+ cells (P = .04); CD38 ABC on activated CD8+ cells (P = .008). Between group S and group D: interferon-γ IFN-γ enzyme-linked immunospot assay (ELISpot) results (gag: CD8+ and CD4+ cells, P = .003; CD8+ cell–depleted, P = .05); activated CD4+ cell count (P = .0003); activated CD8+ cell count (P<.0001) and percentage (P = .01). Cellular activation was defined by CD38+HLA-DR+ expression. SFCs, spot-forming cells.
Table 4. Viral factors among groups, in study of discordant CD4+ cell and virus load responses, during highly active antiretroviral therapy.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Group F: patients with treatment failure (n = 10)</th>
<th>Group D: patients with discordant response (n = 10)</th>
<th>Group S: patients with treatment success (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline or off-treatment virus load, copies/mL</td>
<td>170,203 (144,495–203,681)</td>
<td>63,607 (31,414–144,000)</td>
<td>64,255 (43,237–180,000)</td>
</tr>
<tr>
<td>Presence of CXCR4 viral phenotype, no.</td>
<td>7/9</td>
<td>0/6</td>
<td>NA</td>
</tr>
<tr>
<td>Mutations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Associated with reverse transcriptase</td>
<td>5 (2–6)</td>
<td>5 (4–5)</td>
<td>NA</td>
</tr>
<tr>
<td>M184V, no.</td>
<td>7/10</td>
<td>9/9</td>
<td>NA</td>
</tr>
<tr>
<td>Protease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>2 (1–2)</td>
<td>1 (1–2)</td>
<td>NA</td>
</tr>
<tr>
<td>D30N, no.</td>
<td>2/10</td>
<td>5/9</td>
<td>NA</td>
</tr>
<tr>
<td>Secondary</td>
<td>6 (4–7)</td>
<td>5 (4–6)</td>
<td>NA</td>
</tr>
<tr>
<td>Replication capacity, %</td>
<td>22 (16–47)</td>
<td>12 (9–14)</td>
<td>NA</td>
</tr>
</tbody>
</table>

**NOTE.** Values are medians (25th–75th quartiles), unless otherwise stated. Viral replication capacity is % relative to that of wild-type reference virus (NL4–3). Resistance mutations are defined in text. Significant differences were noted in pairwise comparisons between group F and group D: baseline or off-treatment virus load (P < .04, Wilcoxon rank sum test); replication capacity (P = .04, Wilcoxon rank sum test); and X4 viral phenotype (P = .007, Fisher’s exact test). NA, not applicable.

of the evaluations done, our sample size was necessarily small. This clearly limited our ability to detect differences among the 3 groups. Second, this was not a randomized study, although efforts were made to match the groups in terms of age, sex, and ethnicity. Third, treatment decisions were determined by patients’ primary physicians, thus leading to some variability in regimens and management choices. Last, the statistical analyses did not account for multiple comparisons, because this was a hypothesis-generating study.

The characteristics of the patients in our study raise an interesting question: Why were the patients who experienced a discordant response not prescribed a different regimen once virus rebound occurred? The answer is patient-specific, but for the most part, this was the patient’s choice. As a group, the discordant responders had been infected with HIV for an extremely long period of time. Although this was the first HAART regimen for most of these patients, many had previously been treated with single- or dual-drug therapy. Many of them had finally found a regimen that they were able to tolerate and take consistently. They chose not to change unless the VL continued to increase or unless they began to experience immunologic decline.

In summary, our observations demonstrate that some patients maintained on a PI-based regimen, despite detectable viral replication, maintain and increase CD4+ cell counts for periods exceeding 3–4 years. These patients have resistance profiles similar to those of patients with treatment failure but have a very different clinical outcome. Viruses in these patients are predominantly CCR5-tropic and have decreased replication capacity. These low levels of virus and the presence of relatively intact immune function may provide sufficient stimulus for the immune system to generate important HIV-specific immune responses. This, combined with a decreased replication capacity and a less-pathogenic viral phenotype, may contribute to continuing control of HIV replication and at least partial immune reconstitution, perhaps via diminished levels of cellular activation–induced apoptosis.

On the basis of these observations of a group of patients whose discordant responses are sustainable for periods of at least 3–4 years, it may not be necessary to switch PI-based HAART regimens, as long as CD4+ cell counts are maintained and viremia does not increase beyond an as-yet-unspecified level. Although a larger population-based study has shown that 65% of patients with persistent plasma HIV RNA levels >500 copies/mL eventually experience immunologic failure, despite continued treatment, the group in that study was quite diverse overall [8]. Certain subsets within this population almost certainly resemble our patients, whose long-term outcome may be much more favorable. Identification of a group of patients with prolonged discordant responses, understanding of the mechanisms behind it, and determination of the best management strategy for such patients will be important foci of future work.

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