Combination of HIV-1–Specific CD4 Th1 Cell Responses and IgG2 Antibodies Is the Best Predictor for Persistence of Long-Term Nonprogression

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Background. Strong T cell and antibody responses to human immunodeficiency virus (HIV), low virus production, and some genetic traits have been individually associated with nonprogression of HIV infection, but the best correlate with protection against disease progression remains unknown.

Methods. We prospectively followed 66 untreated long-term nonprogressors and analyzed relationships between HIV-1–specific CD4 T helper (Th) 1 and CD8 T cell responses and HIV-1–specific antibodies, HIV-1 RNA and proviral DNA loads, host genes, and CD4 Th1 cell counts at entry into the study and 4 years later.

Results. HIV-1 p24–specific CD4 Th1 cell proliferation, interferon (IFN)–γ production, and IFN-γ–producing cell frequencies at entry significantly and negatively correlated with HIV-1 RNA and proviral DNA loads and were independent of CD4 Th1 cell counts and host genes. HIV-1 Gag–specific IFN-γ–producing CD8 T cell frequencies correlated with HIV-1 proviral DNA loads but not with RNA loads. Only high frequencies of HIV-1 p24–specific CD4 Th1 cells combined with HIV-1 gp41–specific IgG2 antibodies significantly predicted persistence of high CD4 Th1 cell counts.

Conclusion. HIV-1–specific CD4 Th1 responses combined with IgG2 antibodies and IFN-γ–producing CD4 Th1 cells are better predictors of long-term nonprogression than are virus parameters, host genes, or HIV-1–specific CD4 Th1 or CD8 T cell proliferation.

Nonprogression (NP) in HIV infection is a rare event characterized by a tight host-virus equilibrium in which there are stable, high CD4 Th1 cell counts and low viral replication in the absence of treatment [1–6]. Factors put forward to explain this status [3–5, 7–9] raise the question of whether long-term nonprogression (LTNP) essentially results from a particular set of genes or whether there is also a benefit from strong immune responses that are independent of these genes. Analysis of this question is relevant for the vast majority of the HIV-infected population, because neither antiretroviral therapy [10] nor the present vaccine strategies [11] can eradicate the virus—they only limit disease progression. Genetic factors that affect disease progression involve (1) HIV variants encoding for defective viruses [8, 12], which are too rare, however, to explain most cases of LTNP [13]; (2) variants of genes encoding for chemokines and chemokine receptors limiting HIV entry [9, 14–17]; or (3) some HLA alleles governing immune responses [16, 18–20]. A particular set of these host genetic factors can, indeed, help classify two-thirds of the cases of LTNP [16].

Host immune responses to HIV are central in controlling the infection, as is demonstrated by the viral
escape after depletion of CD8 T cells in simian immunodeficiency virus–infected macaques [21], but immune correlates of NP are still unclear. All CD8 T cell functions—including cytotoxicity [5, 23–25] and production of soluble factors [4], chemokines [22], and cytokines—have been observed in LTPN. The vigorous anti–HIV-1 responses by CD4 Th1 cells found in LTPN [26–29] support a central role for CD4 Th1 cells in the control of viral replication [30, 31]. The strong frequencies of HIV–1–specific CD4 Th1 or CD8 T cells that produce interferon (IFN)–γ found in LTPN appear to be less consistently correlated with control of viral replication [32, 33] than are memory cytotoxic CD8 T cells that have proliferative potential [34–36], which raises a question about the contribution of IFN-γ production to control of viral replication and suggests that control of viral replication might be better associated with the proliferative potential of memory T cells [37]. Similarly, the capacity of HIV–1–specific CD4 Th1 cells to proliferate and produce interleukin (IL)–2 in association with IFN-γ was shown to better correlate with NP than was their capacity to produce IFN-γ alone [38–43]. Finally, because CD4 Th1 cells are a preferential target for HIV–1 [44, 45], the strong frequencies of these HIV–1–specific cells observed in LTPN might reflect only protection against HIV–1 infection rather than control of viral replication. Therefore, a major step in understanding LTPN would be to determine if these immune responses make an independent contribution from that of the host’s genes in the control of viral replication.

Addressing these questions requires the long-term follow-up of cohorts in which these parameters have been measured at entry. We prospectively measured both HIV–1–specific CD4 Th1 and CD8 T cell responses in 66 nonprogressors from the Asymptomatiques à Long Terme (ALT) cohort in which viral characteristics [13, 46], host genes [16, 17], and antibody responses to HIV–1 [47] have been extensively studied and for whom a 4-year prospective follow-up after entry into the study was available. We focused on HIV–1–specific CD4 Th1 cell responses evaluated at entry into the study by proliferation of, level of production by, and quantification of IFN-γ–producing CD4 Th1 cells, and we also measured the frequencies of HIV–1 Gag–specific CD8 T cells. A multivariate analysis incorporating the host chemokine/chemoreceptor gene variants, HLA alleles [16], and anti–HIV-1 IgG2 antibodies previously associated with NP in this cohort [47] was used to determine which parameter could best predict persistence of stable CD4 Th1 cell counts over time and LTPN.

PATIENTS, MATERIALS, AND METHODS

Patients

A total of 66 HIV–1–infected nonprogressors from the ALT cohort [13] for whom an HIV–1–specific T cell proliferation and/or IFN-γ enzyme-linked immunospot (ELISPOT) assay were performed at entry into the cohort were studied. Inclusion criteria in the ALT cohort were HIV–1 seropositivity for at least 8 years, CD4 Th1 cell count >600 cells/mm³ for the preceding 5 years, no clinical symptoms, and no receipt of antiretroviral therapy [13]. Plasma HIV–1 RNA load, which was not routinely available at the time of entry into the study (1994–95), was not an inclusion criterion. The Pitié-Salpêtrière Hospital Institutional Review Board approved the protocol, and each patient provided written, informed consent. Patient characteristics are shown in table 1. Patients were prospectively followed up through yearly evaluations of CD4 Th1 cell counts and plasma HIV–1 RNA loads. Ten patients were lost to follow-up, and 20 patients initiating antiretroviral therapy in accordance with the criteria recommended in 1996–2000 [48–51] were excluded from follow-up (8, 6, 3, and 3 patients at years 1, 2, 3, and 4, respectively).

CD4 Th1 Cell Counts

All absolute CD4 Th1 cell counts were conducted in a single laboratory. Counts were performed on fresh blood by 4-color flow cytometry (Coulter) and were determined in accordance with a standard internal control (mean ± SD reference value, 858 ± 260 cells/mm³).

Parameters of HIV–1 Production

HIV–1 RNA. HIV–1 virions were quantified in fresh plasma samples by use of the NASBA assay (Organon-Teknika; limit of detection, 800 RNA copies/mL) and the ultrasensitive HIV–1 Amplicor-Monitor assay (Roche-Diagnostic Systems; limit of detection, 20 copies/mL), which, when combined, gave a limit of detection of <800 RNA copies/mL. All tests were conducted in a single laboratory [13].

HIV–1 proviral DNA. The level of HIV–1 proviral DNA was determined in frozen peripheral blood mononuclear cells (PBMCs) by use of a modified Amplicor Monitor assay (Roche Laboratories) with an internal HIV–1 proviral DNA standard provided by S. Kwok [52]. All tests were conducted in a single laboratory [53], and results were expressed as copies of HIV–1 proviral DNA/10⁸ PBMCs.

HIV–1–Specific CD4 Th1 Cell Responses

An HIV–1 antigen–specific T cell proliferation assay was performed on fresh whole PBMCs from 41 patients after depletion by anti–CD8 monoclonal antibody (MAb) magnetic beads (Dynabeads; Dynal), as described elsewhere [54]. Cells were incubated for 6 days with the recombinant HIV–1 LAI p24 protein (0.25 μg/mL; donated by Transgène), which was previously tested in 10 seronegative donors with negative results. Control antigens were purified protein derivative (Serum-Statens Institute) and candidin (Sanofi-Diagnostic-Pasteur) at appropriate concentrations. Positive controls were anti–CD3 and anti–CD28 MAbs (Im-
Table 1. Characteristics of the cohort at entry into the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Sex, %</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>76</td>
</tr>
<tr>
<td>Women</td>
<td>24</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>At HIV-1 diagnosis</td>
<td>28.1 (23.0–33.0)</td>
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<tr>
<td>At entry into the study</td>
<td>36.7 (32.8–41.6)</td>
</tr>
<tr>
<td>Risk group, %</td>
<td></td>
</tr>
<tr>
<td>Homosexual</td>
<td>56</td>
</tr>
<tr>
<td>Intravenous drug user</td>
<td>17</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>11</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>13</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td>Duration of HIV-1 seropositivity, years</td>
<td>9.0 (8.1–10.0)</td>
</tr>
<tr>
<td>CD4 Th1 cell count, cells/mm³</td>
<td>666 (510–794)</td>
</tr>
<tr>
<td>HIV-1 RNA load, copies/mL</td>
<td>6100 (200–46,500)</td>
</tr>
<tr>
<td>HIV-1 proviral DNA load, copies/10⁶PBMCs</td>
<td>208 (45–949)</td>
</tr>
<tr>
<td>HIV-1 p24–specific CD4 Th1 cell proliferation, stimulation index (n = 41)</td>
<td>2.0 (1.3–5.0)</td>
</tr>
<tr>
<td>HIV-1 p24–specific IL-2 production, pg/mL (n = 37)</td>
<td>7 (0–13)</td>
</tr>
<tr>
<td>HIV-1 p24–specific IFN-γ production, pg/mL (n = 37)</td>
<td>92 (21–188)</td>
</tr>
<tr>
<td>HIV-1 p24–specific CD4 Th1 cell count, sfc/10⁶ PBMCs (n = 55)</td>
<td>In total group 70 (30–200)</td>
</tr>
<tr>
<td>HIV-1 Gag–specific CD8 T cell count, sfc/10⁶ PBMCs (n = 43)</td>
<td>In total group 2986 (1476–4663)</td>
</tr>
<tr>
<td>HIV-1 gp41–specific IgG2 antibody response, no. (%) (n = 71)</td>
<td>18 (27)</td>
</tr>
</tbody>
</table>

**NOTE.** All data are median (interquartile range), unless otherwise indicated. IL, interleukin; IFN, interferon; PBMCs, peripheral blood mononuclear cells; sfc, spot-forming cells; stimulation index, counts per minute of (cells + stimuli):(cells + medium).

munotech). After labeling with tritiated thymidine (CEA), positive responses were defined as >3000 cpm and stimulation index ≥3 (counts per minute of [cells + stimuli]:[cells + medium]), as described elsewhere [54].

**Cytokine production assay.** Fresh PBMCs from 37 of these 41 patients were stimulated for cytokine production, as described elsewhere [54, 55], by use of the same HIV-1 LAI p24 protein and anti-CD3 and anti-CD28 MAbs as described above. Culture supernatants were collected at day 2 after incubation, in accordance with previously established kinetics [55], and were cryopreserved before batch analysis with the IL-2 (Immunotech) and IFN-γ (Endogen) ELISA kits. The cytokine production from CD8-depleted PBMCs showed a persistence of 90% of the cytokine production from nondepleted PBMCs.

**ELISPOT Assay for Quantification of HIV-1–Specific IFN-γ–Producing CD4 Th1 Cells**

A sensitized IFN-γ ELISPOT assay derived from one described elsewhere [36, 54] was performed on frozen PBMCs from 55 nonprogressors with available samples that had a viability after thawing of >85% (Trypan-Blue exclusion). Briefly, recombinant HIV-1 LAI p24 protein (2 μg/mL; Protein-Sciences) was added in triplicate wells for a previously optimized 40-h incubation period to ensure maximal specific IFN-γ release and low background (median, 2 spot-forming cells [sfc]/well). Depletion of CD4 Th1 cells (Dynabeads; Dynal) abolished 85% of the IFN-γ production (data not shown). Negative and positive controls were medium alone and phytohemagglutinin (1 μg/mL; Murex), respectively. Spots were counted using an ELISPOT reader (Zeiss), and data were expressed as sfc/10⁶ PBMCs. Results were considered to be positive if there were >50 sfc/10⁶ PBMCs after subtraction of the mean background value obtained without incubation with antigen. Counts of HIV-1 p24–specific cells were found to be similar in fresh and frozen PBMCs from 5 patients (P = .34).

**Quantification of HIV-1–Specific CD8 T Cells by IFN-γ ELISPOT Assay**

An IFN-γ ELISPOT assay derived from one described elsewhere [36, 54] was performed on frozen PBMCs from 43 nonprogressors with available samples that had a viability after thawing of >85% (Trypan-Blue exclusion). Cells in triplicate were stimulated by pools of 9–11 synthetic 15-mer peptides overlapping by 11 aa and spanning the entire HIV-1 gag sequence (each
Figure 1. Relations between disease parameters and HIV-1 p24–specific CD4 Th1 cell proliferation and interferon (IFN)–γ production at entry into the study. A, HIV-1 p24–specific CD4 Th1 cell proliferation and lack of correlation with CD4 Th1 cell count. B, Correlation between HIV-1 p24–specific CD4 Th1 cell proliferation and plasma HIV-1 RNA load. C, Correlation between HIV-1 p24–specific IFN-γ production and plasma HIV-1 RNA load. D, Correlation between HIV-1 p24–specific IFN-γ production and proviral DNA copies/10^6 peripheral blood mononuclear cells (PBMCs). Lymphoproliferative responses against HIV-1 p24 were tested on fresh CD8-depleted PBMCs from 41 nonprogressors and were compared with disease parameters that were measured simultaneously. The HIV-1 p24–specific CD4 Th1 IFN-γ production was evaluated at entry into the study in supernatants from fresh PBMCs from 37 of the 41 nonprogressors tested for lymphoproliferation. Black symbols, patients with CD4 Th1 cell counts ≥600 CD4 Th1 cells/mm³; white symbols, patients with CD4 Th1 cell counts <600 CD4 Th1 cells/mm³; stimulation index, counts per minute of (cells + stimuli):(cells + medium).

peptide, 2 µg/mL; Neosystem). The 18-h incubation had been previously optimized under these conditions. Positive responses were defined as described above. The CD8-depleted PBMCs (anti-CD8 Dynabeads; Dynal) from 20 samples tested against 87 Gag peptide pools yielding positive results eliminated a median of 90% of the spot-forming cells.

CCR5, CCR2, SDF1, and HLA Typing

The chemokine receptor/chemokine gene variants (CCR5-D32, CCR2-64I, and SDF1-3′A) and HLA phenotypes of the ALT cohort have been reported elsewhere [16]. They were used in the statistical analysis and were incorporated into the multivariate analysis.

Isotype Characterization of Antibodies Directed against HIV-1

Anti–HIV-1 antibody isotypes in the ALT cohort have been described elsewhere [47]. These data were incorporated into the multivariate analysis.

Statistical Analysis

Data were analyzed using SPSS software (version 11; SPSS). Associations between continuous variables were tested using the
nonparametric Spearman’s rank correlation test, the Mann-Whitney U test, or the Kruskal-Wallis test. The $\chi^2$ test or Fisher’s exact test were used for categorical variables.

To investigate factors independently associated with LTNP (defined as persistent CD4 Th1 cell count >600 cells/mm$^3$ during follow-up), we used time-to-event analysis, a method developed specifically to handle censored data. Thus, patients initiating antiretroviral therapy or lost to follow-up were considered to be censored at the time of the last evaluation before these events. We prepared univariate Cox models of time to progression, and the characteristics significantly associated ($P < .20$) with control of viral replication at entry into the study were entered into a multivariate backward Cox model. Continuous variables were either entered as continuous variables or converted to categorical variables. We used tertiles to test whether associations were linear (continuous model), because 3 classes represent the minimum number that can detect a deviation from linearity in limited sample sizes [56]. For example, the tertiles for HIV-1 p24–specific CD4 Th1 cells were 0–40, 40–170, and >170 sfc/10$^6$ PBMCs, with the latter tertile defining high counts of HIV-1–specific CD4 Th1 cells. The variables that had the best likelihoods of an association with LTNP were used in later analyses. In addition to the parameters tested in the present study, we incorporated the chemokine/

Figure 2. Frequencies of CD4 Th1 cells specific for HIV-1 p24 and HIV-1 viral production. A, Frequencies of spot-forming cells/10$^6$ peripheral blood mononuclear cells (PBMCs) and correlation with plasma HIV-1 RNA load. B, Frequencies of sfc/10$^6$ PBMCs and correlation with HIV-1 proviral DNA load. An interferon-$\gamma$ enzyme-linked immunospot assay was performed on samples collected from 55 nonprogressors at entry into the study. Black symbols, patients with CD4 Th1 cell counts >600 CD4 Th1 cells/mm$^3$; white symbols, patients with CD4 Th1 cell counts ≤600 CD4 Th1 cells/mm$^3$. 
chemoreceptor gene variants and HLA alleles (A3, B5, B12, B14, B17, B27, DR6, and DR7) in this cohort that were shown elsewhere to be significantly associated (P < .02) with nonprogressors [16], when their effect in progressors was compared. Furthermore, the IgG2 antibodies against HIV-1 p55, p24, p68, gp160, gp120, and gp41 that were shown to be significantly associated with plasma HIV-1 RNA loads at entry into the study [47] were entered in a first backward multivariate analysis showing that only anti-gp41 IgG2 antibody was an independent predictor of NP (P < .05). A second backward multivariate model was built that included anti-gp41 IgG2 antibody and the other variables retained in univariate analyses. Life-table estimators, adapted for once-yearly follow-up, were used to assess proportions of LTNP at various time points in the subgroups defined on the basis of the factors independently associated with LTNP.

RESULTS

HIV-1 p24–specific CD4 Th1 cell proliferation and IFN-γ production in association with low plasma HIV-1 RNA and cell-associated HIV-1 proviral DNA loads. Lymphoproliferative responses to HIV-1 p24 were tested on fresh samples obtained at entry into the study from 41 patients (median CD4 Th1 cell count, 676 cells/mm³ [interquartile range [IQR], 525–846 cells/mm³]; median HIV-1 RNA load, 4200 copies/mL [IQR, 190–35,000 copies/mL]). Responses were detected in whole and CD8-depleted PBMCs from 6 subjects (15%) and 13 subjects (32%), respectively. These responses were independent of CD4 Th1 cell counts (r = 0.12; P = .45) (figure 1A) but were negatively correlated with plasma HIV-1 RNA loads (r = −0.396; P = .01) (figure 1B) and cell-associated HIV-1 proviral DNA loads (r = −0.363; P = .02) measured simultaneously. The proliferative responses against tuberculin and candidin, which were observed in higher proportions of samples (87% and 43%, respectively, in whole PBMCs), were not correlated with HIV-1 RNA or proviral DNA loads (data not shown).

The levels of IFN-γ and IL-2 directed against HIV-1 p24 were simultaneously quantified in culture supernatants from 37 of these 41 patients, and a 10-fold higher level of IFN-γ than of IL-2 (median, 92 vs. 7 pg/mL) was observed. HIV-1 p24–specific IFN-γ production, but not IL-2 production, was negatively correlated with plasma HIV-1 RNA loads (r = −0.583; P = .0001) (figure 2A) and HIV-1 proviral DNA loads (r = −0.529; P = .001) (figure 2B), but it was not negatively correlated with CD4 Th1 cell counts (r = 0.13; P = .44) measured at entry into the study. In contrast, IL-2 production, but not IFN-γ production, was positively correlated with the HIV-1 p24–specific proliferative responses (r = 0.41; P = .04). The 5 patients with both IL-2 and IFN-γ production had a 25-fold lower median plasma HIV-1 RNA load (median, 187 vs. 3300 copies/mL) than the 27 patients with IFN-γ production only, although the difference was not statistically significant.

To evaluate whether these correlations between responses to HIV-1 p24 and virus parameters might reflect protection of HIV-1–specific CD4 Th1 cells against HIV-1 entry, we studied their relationship with the chemokine/chemoreceptor gene variants previously linked to this cohort—the CCR5-Δ32 deletion and the CCR2-64I and SDF1-3′A mutations [16]—but found no association (data not shown). Altogether, these data show that the capacity of CD4 Th1 cells to proliferate and produce IFN-γ against HIV-1 is strongly associated with low HIV-1 RNA and proviral DNA loads and is independent of CD4 Th1 cell counts and genetic resistance to virus entry.

HIV-1 p24–specific IFN-γ–producing CD4 Th1 cells and control of the plasma HIV-1 RNA and cell-associated HIV-1 proviral DNA loads. The HIV-1 p24–specific IFN-γ–producing CD4 Th1 cells were evaluated at entry into the study by ELISPOT on frozen PBMCs from 55 nonprogressors (median CD4 Th1 cell count, 679 cells/mm³ [IQR, 511–812 cells/mm³]; median HIV-1 RNA load, 6600 copies/mL [IQR, 350–36,000 copies/mL]) and were detected in 31 subjects (56%) with a median frequency of 188 sfc/10⁶ PBMCs (IQR, 103–317 sfc/10⁶ PBMCs) and a tertile distribution of 0, 40, and 170 sfc/10⁶ PBMCs. These frequencies were negatively correlated with the plasma HIV-1 RNA loads (r = −0.304; P = .026) (figure 2C) and the cell-associated HIV-1 proviral DNA loads (r = −0.461; P = .0001) (figure 2D) but not with the CD4 Th1 cell counts (r = 0.215; P = .12) measured at entry into the study.

Table 2. Associations between HIV-1 p24–specific CD4 Th1 cell proliferation, interferon (IFN)–γ producing CD4 Th1 cells, plasma HIV-1 RNA load, and HIV-1 proviral DNA load.

<table>
<thead>
<tr>
<th>Patients with HIV-1 p24–specific CD4 Th1 cell response</th>
<th>CD4 Th1 cell proliferation, SI</th>
<th>IFN-γ-producing CD4 Th1 cell count, sfc/10⁶ PBMCs</th>
<th>Plasma HIV-1 RNA load, copies/mL</th>
<th>HIV-1 proviral load, DNA copies/10⁸ PBMCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation and IFN-γ–producing cells (n = 7)</td>
<td>5 (3–43)</td>
<td>220 (30–2187)</td>
<td>230 (20–4400)</td>
<td>47 (2–437)</td>
</tr>
<tr>
<td>Proliferation or IFN-γ–producing cells (n = 15)</td>
<td>2 (1–11)</td>
<td>153 (26–3770)</td>
<td>7200 (90–162,000)</td>
<td>410 (8–2035)</td>
</tr>
<tr>
<td>No proliferation and no IFN-γ–producing cells (n = 10)</td>
<td>2 (0.3–6)</td>
<td>20 (0–42)</td>
<td>6000 (30–186,000)</td>
<td>616 (53–9756)</td>
</tr>
</tbody>
</table>

NOTE. Data are median (range). SI, stimulation index, defined as counts per minute of (cells + stimuli):(cells + medium); sfc, spot-forming cells.

a P = .193, Kruskal-Wallis test.

b P = .039, Kruskal-Wallis test.

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We further evaluated the relationship between viral parameters and combined proliferation and IFN-γ production in the 32 patients in whom ELISPOT and proliferation assays against HIV-1 p24 could be performed in parallel. Significantly lower HIV-1 proviral DNA loads—but not plasma HIV-1 RNA loads, although these were lower—were observed in the 7 patients with both proliferative responses and strong frequencies of IFN-γ–producing cells, compared with those in the 15 patients with either proliferative responses or strong frequencies of IFN-γ–producing cells and with those in the 10 patients with no responses (table 2). Altogether, these data show a stronger correlation between frequencies of HIV-1–specific CD4 Th1 cells and frequencies of cells harboring HIV-1 proviral DNA than between frequencies of HIV-1–specific CD4 Th1 cells and plasma HIV-1 RNA loads, and they also show that this association is reinforced by the presence of CD4 Th1 cell proliferative responses to HIV-1, which are independent of CD4 Th1 cell counts, at entry into the study.

**HIV-1 Gag–specific CD8 T cells and cell-associated HIV-1 proviral DNA.** The HIV-1 Gag–specific IFN-γ–producing CD8 T cells were quantified at entry into the study by ELISPOT on 43 available samples of frozen PBMCs (median CD4 Th1 cell count, 676 cells/mm³ [IQR, 508–790 cells/mm³]; median HIV-1 RNA load, 6600 copies/mL [IQR, 350–56,000 copies/mL]). We used pools of 15-mer synthetic peptides overlapping by 11 aa and spanning the entire HIV-1 gag sequence. High frequencies of HIV-1 Gag–specific CD8 T cells were observed (median, 2986 sfc/10⁶ PBMCs [IQR, 1476–4663 sfc/10⁶ PBMCs]) that significantly correlated with frequencies of HIV-1 p24–specific CD4 Th1 cells (r = 0.439; P < .005) (figure 3A) but not with CD4 Th1 cell counts at entry into the study. In contrast to the CD4 Th1 cell reactivity to HIV-1 p24 that correlated with both plasma and cell-associated HIV-1 RNA loads, the frequencies of HIV-1 Gag–specific CD8 T cells did not correlate with plasma HIV-1 RNA loads (data not shown) but did correlate with cell-associated HIV-1 proviral DNA loads (r = −0.383; P = .009) (figure 3B) measured simultaneously at entry into the study.

**HIV-1 p24–specific CD4 Th1 cell responses and LTNP.** Finally, we sought to identify which of the above parameters related to control of viral replication after a median 9 years of HIV-1 seropositivity could predict persistence of LTNP status as assessed by persistence of entry criteria—that is, CD4 Th1 cell count ≥600 cells/mm³—over the course of 4 years of follow-up.

Neither HIV-1 p24–specific proliferative responses at entry into the study nor frequencies of HIV-1–specific IFN-γ–producing CD8 T cells were predictive of persistence of LTNP. We then evaluated the predictive value of the other variables associated with NP ([16, 47] and see Patients, Materials, and Methods) in the 53 patients with HIV-1 p24 ELISPOT entry data and follow-up data available (median follow-up, 21 months [IQR, 12–48 months]). Only HIV-1 p24–specific CD4 Th1 cell counts >170 sfc/10⁶ PBMCs (i.e., within the highest tertile); HIV-1 RNA and proviral DNA loads; IgG2 antibodies against HIV-1 p55, p24, p68, gp160, gp120, and gp41 [47]; and HLA allele B12 [16] yielded P < .02. A first backward multivariate analysis of the 6 patients with IgG2 antibody responses showed that only anti–HIV-1 gp41–specific IgG2 antibodies were independent predictors of LTNP (P < .05), and this variable was then incorporated into the multivariate analysis with the other retained variables. Only high HIV-1 p24–specific cell counts
Figure 4. Persistence of long-term nonprogression (LTNP) as assessed by the number of HIV-1 p24–specific CD4 Th1 cells combined with HIV-1 gp41–specific IgG2 antibodies. Results of the multivariate analysis showed high HIV-1 p24–specific CD4 Th1 cell frequencies combined with presence or absence of IgG2 antibodies against HIV-1 gp41 measured at entry into the study in 53 patients who had a median follow-up of 21 months. The cutoff of 170 spot-forming cells/10⁶ PBMCs was determined by the third tertile of HIV-1 p24–specific CD4 Th1 cells. Persistence of LTNP status is defined as persistence of CD4 Th1 cell counts >600/mm³ without symptoms or receipt of antiretroviral therapy. The probabilities of remaining NP were estimated on the basis of results of the life-table analysis.

DISCUSSION

This unique overall analysis of the various aspects of immune responses to HIV-1 and viral and host gene parameters in a cohort of nonprogressors clearly demonstrates that the combination of high frequencies of anti–HIV-1 p24–specific CD4 Th1 cells and anti–HIV-1 gp41–specific IgG2 antibodies are better predictors for persistence of LTNP—defined as CD4 Th1 cell count >600 cells/mm³ for >10 years—than are IFN-γ–producing CD8 T cell responses to HIV-1 Gag, plasma and cellular virus parameters, host chemokine/chemoreceptor gene variants, and HLA alleles. The data also provide novel evidence for a strong association between the frequencies of HIV-1 p24–specific CD4 Th1 cells and the frequencies of HIV-1–infected cells when measured simultaneously, and this association was even potentiated by an HIV-1 p24–specific CD4 Th1 cell proliferation or IL-2 production that was independent of the depletion of CD4 Th1 cells.

CD4 Th1 cell responses, which were independent of the CD4 Th1 cell counts, were strongly correlated with virus parameters at entry into the study. The large sample size of the ALT cohort and the wide ranges of viral production at entry into the study may explain the differences between our results and those of previous studies in which the selected viral parameters were in a much less broad range but did not correlate with the CD4 Th1 cell responses to HIV-1 [33, 39–41]. Discrepancies with other studies, in which no such associations were found [38–43], may also be explained by several technical factors, although...
all assays used to measure CD4 Th1 cell responses in the present study were concordant at indicating correlation with virus parameters, particularly stimulation with a whole recombinant p24 protein instead of peptides overlapping the whole gag sequence, the duration of incubation, and the threshold of detection in our ELISPOT assay.

In accordance with the results of previous studies [42, 43], we found that both IL-2 and IFN-γ production against HIV-1 p24 was associated with lower levels of HIV-1 RNA in plasma and proviral DNA in PBMCs, although IFN-γ production alone was not. However, neither HIV-1–specific proliferation nor IL-2 production predicted NP, as assessed by stable CD4 Th1 cell counts. Such features might reflect the exquisite sensitivity to HIV-1 infection of CD4 Th1 cells capable of proliferation and IL-2 production, whereas IFN-γ–producing CD4 Th1 cells might be more resistant. However, only NSI R5 viruses of low cytopathogenicity were found in the ALT cohort [37, 46]. In addition, we have shown here for the first time that these responses were independent of the host chemokine/chemoreceptor gene variants known to limit HIV-1 entry [14–20]. These data suggest that an association between strong HIV-1–specific CD4 Th1 cell responses and low virus parameters does not reflect a genetic protection from HIV-1 infection in this cohort but rather reflects an immunity-based control of the virus.

In contrast, frequencies of HIV-1 Gag–specific IFN-γ–producing CD8 T cells were correlated with CD4 Th1 cell frequencies but not with plasma HIV-1 RNA loads. This latter finding, in accordance with the results of several studies [33–34], does not exclude the possibility, however, that effector [57] or memory cytotoxic T lymphocytes (CTLs) might better correlate with LTNP than does the immediate IFN-γ production of CD8 T cells tested in the present study. Indeed, we reported elsewhere that the number of HIV-1–specific memory CTLs, but not the number of HIV-1–specific IFN-γ–producing CD8 T cells, was significantly higher in nonprogressors from the ALT cohort than in progressors [36]. We also showed elsewhere that HIV-1–specific memory CTLs were negatively correlated with HIV-1 RNA loads in progressors [35]. However, the correlation we found in the present study between the frequencies of IFN-γ–producing CD8 T cells specific for HIV-1 proviral DNA is novel and suggests that those cells control HIV-1–infected cells better than soluble virions do, and this finding is in accordance with the reactivity that major histocompatibility complex class I–restricted CD8 T cells have with cell-associated antigens.

The multivariate analysis compared, in 55 nonprogressors, the predictive value of all parameters that were associated even roughly with control of viral replication in plasma in a univariate analysis. Viral factors and host chemokine/chemoreceptor genes and HLA alleles were less predictive than were high frequencies of HIV-1 p24–specific CD4 Th1 cells combined with anti–HIV-1 gp41–specific IgG2 antibodies for the persistence of CD4 Th1 cell counts >600 cells/mm³ over the course of 4 years. The sample size did not allow us to test whether these 2 parameters could also predict long-term control of viral replication, because only 26 patients had an HIV-1 RNA load <10,000 copies/mL and only 14 patients had an HIV-1 RNA load <400 copies/mL at entry into the study. We cannot exclude the possibility that viral and host gene parameters would have a predictive value in a larger sample size, but our data illustrate the more potent effect of the CD4 Th1 cell parameters. Whether the predictive value of anti–HIV-1 gp41–specific IgG2 antibodies reveals a specific immune protection, as a recent study suggests [58], remains to be determined. In the present study, we considered IgG2 antibody levels to reflect CD4 Th1 cell functions, because, in humans, as in mice, IgG2 antibody production certainly depends on IFN-γ production [59–62]. Indeed, although IgG1 antibodies predominate in HIV-1–specific responses [63], IgG2 antibodies to various HIV-1 antigens had been previously found in patients in the ALT cohort with low HIV-1 RNA loads [47] and were positively correlated with HIV-1 p24–specific CD4 Th1 cell responses, suggesting that they might be used as a soluble marker of CD4 Th1 cell responses to HIV-1, as has been proposed in mice.

In conclusion, although progression of HIV-1 disease likely depends on several factors, the CD4 Th1 cell responses to HIV-1 are the strongest correlate of the persistence of NP, thus emphasizing the key contribution that CD4 Th1 cells and IgG2 antibodies specific for HIV-1 p24 and gp41, respectively, make to the long-term control of HIV-1 disease. The independence of the HIV-1–specific CD4 Th1 cell responses from genetic factors of NP provides hope that efforts to design vaccines and immune-based treatment strategies will succeed in conferring nonprogressor status to all HIV-infected individuals.

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References


