MAJOR ARTICLE

HIV-Specific Cellular Immune Response Is Inversely Correlated with Disease Progression as Defined by Decline of CD4$^+$ T Cells in Relation to HIV RNA Load

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The average time between infection with human immunodeficiency virus (HIV) and development of acquired immune deficiency syndrome is ~8 years. However, progression rates vary widely, depending on several determinants, including HIV-specific immunity, host genetic factors, and virulence of the infecting strain. In untreated HIV-infected patients with different progression rates, we examined HIV-specific T cell responses in combination with host genetic markers, such as chemokine/chemokine-receptor (CCR) polymorphisms and human leukocyte antigen (HLA) genotypes. HIV-specific CD4$^+$ T cell responses and, to a lesser extent, HIV-specific CD8$^+$ T cell responses were inversely correlated with progression rate. Slower progression was not related to polymorphisms in CCR genes, HLA genotype, or GB virus C coinfection. These data suggest that HIV-specific T cell responses are involved in protecting the host from disease progression.

Disease-progression rates, between infection with HIV and the development of AIDS, vary greatly; although the median time is 8–10 years [1], this interval can vary from as little as 2–3 years (in subjects in whom disease progression is rapid) to 15–20 years or more (in subjects in whom disease progression is either absent or slow) [2]. Definitions of long-term nonprogression of disease and short-term progression of disease differ substantially [2] and depend on criteria such as the decline in CD4$^+$ T cell count, the absolute CD4$^+$ T cell count, and the HIV RNA level; however, it is clear that the disease-progression rate is determined by several factors, including HIV-specific cellular immune responses, host genetic factors, and viral factors.

The role that the HIV-specific cellular immune response plays in the constraint of HIV replication is still a matter of debate [3, 4]. In acute HIV infection, induction of HIV-specific CD8$^+$ T cell responses coincides with the control of viral replication [5–7]. Immune escape due to sequence variation in viral T cell epitopes is associated with increases in HIV RNA levels and implies that CD8$^+$ T cells play a central role [8–11]. Furthermore, depletion of CD8$^+$ T cells in monkeys

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infected with simian immunodeficiency virus has been reported to result in a rapid increase in viral replication [12].

In chronic HIV infection, inverse correlations between HIV RNA load in plasma (pVL) and HIV-specific T cell frequencies have been observed [13]. These initial observations have not been confirmed by subsequent studies examining the frequencies of HIV-specific $CD8^+$ T cells in relation to viremia [14–18]; however, qualitative differences in antigen-specific $CD8^+$ T cell responses of similar magnitude may play an important role in determining the efficiency with which viral replication is contained [14].

$CD4^+$ T cells, the main targets for HIV infection, play a key role in orchestrating cellular immune responses. HIV-specific $CD4^+$ T cell counts are higher in patients with long-term nonprogression of disease than in patients with progression of disease, but there is substantial overlap [19]. Especially in chronic HIV infection, strong HIV-specific $CD4^+$ T cell responses correlate with lower viral-replication levels and have been implicated as being beneficial for the maintenance of functional $CD8^+$ T cell responses [20–22].

Multiple host genes influence HIV's progression rate (reviewed in [23–25]) and also may contribute to the immune response; for example, a number of major-histocompatibility-complex class I alleles and polymorphisms within the CCR genes, such as CCR5, CCR2, SDF-1, CX3CR1, and RANTES, influence disease-progression rates. However, it should be noted that not all studies have reached similar conclusions regarding the effects of distinct polymorphisms [26, 27]. Additional factors that have been reported to affect the course of HIV disease include coinfections with GB virus C [28].

Viral factors can also affect the outcome of infection. Strains of HIV harboring unusual polymorphisms may explain the benign course of disease observed in a number of subjects with disease progression that is either slow or absent. HIV variants that are defective in the *Nef* gene [29] or that have either extension of the V2 region [30] or mutations in the gp41 transmembrane region [31] have been associated with slower disease progression.

The relatively few studies that have attempted to examine multiple parameters simultaneously that could contribute to disease progression within a single cohort of infected individuals have reached different conclusions [26, 27, 32]. In the present study, we analyzed a number of variables in a cohort of HIV-infected patients, in an attempt to dissect the relative contribution that these factors make to disease progression.

PATIENTS, MATERIALS, AND METHODS

Patients. We prospectively recruited and examined (1) 28 (patients 1–28) chronically HIV-infected white patients with a broad range of progression rates who were not being treated with antiretroviral therapy (ART) and (2) 10 patients (patients 29–38) being treated with ART (table 1). Accordingly, our inclusion criteria were as follows: (1) untreated patients had to have both documented HIV infection for 2–17 years and $CD4^+$ T cell counts >250 cells/μL, with longitudinal clinical data documenting $CD4^+$ T cell count and pVL; (2) ART-treated patients had to have HIV RNA levels and $CD4^+$ T cell counts that matched those in untreated patients with either no or slow disease progression; and (3) all patients had to be 18–75 years of age. We included only patients with HIV clade B infections (except for patients 1, 6, and 21, whose HIV clade could not be determined, because of low pVL). The study was approved by the Ethical Committee of the University Hospital of Zurich, Switzerland.

Quantification of pVL. pVL was quantified by the ultrasensitive Amplicor Monitortest, version 1.5 (Roche Diagnostics). The lower limit of detection was 50 copies of HIV RNA/mL.

HLA genotyping. Genotyping was performed by use of either a commercially available kit, Cyclerplate System Protrans HLA A*/B*/DRB1* (Medizinische Diagnostische Produkte GMBH, Endotell AG) or polymerase chain reaction (PCR) with sequence-specific primers (Lymphotype ABC144; Biotest Schweiz AG) [33].

CCR genotyping. To analyze the CCR5Δ32-deletion polymorphism, the region encompassing the 32-nt deletion in CCR5 was amplified, and the 2 alleles were separated by PAGE [34]. Allelic variants of CCR2 V64I, CCR 559029G/A, and SDF-1 3′G/A were analyzed by 3 tetraprimer PCR assays [35]. In brief, for each assay, 4 primers were combined in a single tube, for initial amplification of the gene locus and subsequent allele-specific amplification. Products of the PCR amplification were separated by agarose-gel electrophoresis, for allelic discrimination.

GB virus C RNA. GB virus C RNA was extracted from plasma by use of the QIAamp Viral RNA Mini Kit (Qiagen), according to the manufacturer's instructions. Extracted RNA was reverse transcribed and amplified by nested PCR with primers specific for GB virus C. PCR products were quantified by gel electrophoresis [36] in which the lower limit of detection was 50–500 copies/mL.

HIV-specific T cells. Comprehensive analysis of HIV-specific $CD8^+$ T cell responses was performed directly ex vivo by interferon (IFN)–γ ELISpot analysis, as described elsewhere [37]. In brief, cryopreserved peripheral-blood lymphocytes (PBLs) were screened with pooled overlapping (by 10 amino acids) peptides, in which each peptide had a final concentration of 5 μg/mL and was 20 amino acid residues long. Peptides spanned the HIV reverse transcriptase, envelope (Env), p24 viral capsid antigen (p24 Gag), p17 Gag, and the accessory HIV proteins—Nef, Tat, and Rev (National Institute for Biological Standards and Control). Each peptide pool comprised 10 individual peptides. Phytohemagglutinin (PHA) was always included as a positive control, and the use of medium alone

Table 1. Data on untreated patients and on patients being treated with antiretroviral therapy (ART).

Patient (sex; age, years)	Yearly change		Duration, years		pVL		T cell count, cells/μL		HLA		CCR5				
	$CD4^+$ T cell count	$CD4^+$ T cell count/pVL	Infection	ART (infection before ART)	Untreated/ on ART	Median (range) during ART	$CD4^+$	$CD8^+$	A	B	CCR2 V64I	Δ32	59029 A/G	SDF-1 3′A	HGV RNA, copies/mL
Untreated															
1 (M; 43)	10.44	23.96	10		<20		665	1580	3, 32	35, 44	wt	wt	Hom	wt	Negative
2 (F; 41)	−1.06	18.91	16		<20		835	945	3, 24	35, 51	wt	wt	wt	Het	ND
3 (M; 41)	−19.63	14.36	5		457		1,151	1068	25, 32	57, 27	wt	Het	Hom	Het	Negative
4 (F; 34)	−69.43	6.42	13		522		1,364	1024	1, 2	44, 57	wt	wt	wt	Het	Negative
5 (M; 44)	−14.54	4.94	9		741		525	1236	3, 10	5, 49	wt	wt	wt	Hom	Negative
6 (M; 39)	12.95	4.18	15		<20		860	849	1, 3	57, 18	wt	wt	Het	wt	10^3
7 (M; 39)	−24.6	−0.54	14		15,335		332	374	3, 11	18, 55	wt	Het	Het	wt	10^6
8 (M; 37)	−7.13	−1.81	15		12,626		513	3149	2, 11	62, 51	wt	Het	Hom	Het	10^7
9 (F; 34)	−0.13	−2.06	10		160		777	1169	31, 80	27, 51	wt	Het	Het	wt	Negative
10 (F; 35)	−31.79	−3.259	3		9302		294	891	1, 3	8, 65	Het	wt	Het	wt	Negative
11 (M; 46)	20.96	−4.2	11		3038		388	734	23, 30	53, 65	wt	wt	Het	wt	10,000
12 (M; 48)	−57.97	−4.45	12		89		519	618	9, 10	52, 27	Het	wt	Hom	wt	Negative
13 (M; 34)	−16.93	−5.74	17		10,236		349	809	2, 3	13, 37	Hom	wt	Hom	wt	10^5
14 (M; 34)	−50	−6.3	6		741		626	1194	2, 3	13, 60	Het	Het	Hom	Het	Negative
15 (F; 38)	−31.57	−6.67	16		7322		297	1231	2, 32	13, 51	wt	wt	Hom	wt	10^6
16 (M; 42)	−11.3	−10.86	15		7662		536	551	2, 11	5, 27	wt	Het	Het	wt	ND
17 (M; 21)	−96.93	−12.28	2		47,269		275	741	24	51, 35	wt	Het	Het	wt	10^7
18 (M; 32)	−63.66	−13	5		3828		744	1047	24, 68	27, 36	wt	wt	wt	wt	Negative
19 (M; 36)	−64.39	−16.68	16		46,381		376	999	2, 24	15, 52	Het	wt	Het	wt	10^6
20 (F; 38)	−5.31	−18.68	15		8212		476	1145	1, 3	18, 57	wt	Het	Hom	wt	Negative
21 (M; 25)	−1.4196	−18.79	2		790		538	781	11, 34	61, 62	wt	wt	wt	wt	10^5
22 (M; 50)	−92.85	−19.22	2		14,803		466	981	1, 2	52, 62	wt	Het	Het	Het	Negative
23 (M; 38)	−71.8	−22.63	10		4507		493	1140	3, 24	57, 62	wt	wt	Het	wt	Negative
24 (M; 43)	−44.34	−29.89	17		1922		732	808	3, 24	51, 62	…[a]	…[a]	…[a]		Negative
25 (M; 37)	−185.52	−40.92	2		69,356		461	1310	2, 30	18, 44	wt	wt	Hom	wt	10^7
26 (M; 23)	−151.75	−44.22	2		12,810		384	1863	2, 3	18, 44	wt	wt	wt	Het	10^5
27 (M; 27)	−231.66	−49.85	2		27,707		497	694	2, 23	7, 60	wt	wt	Het	wt	10^7
28 (F; 40)	−47.54	−55.95	10		597		549	872	1, 2	27, 37	wt	Het	Hom	wt	10^6
Treated with ART															
29 (F; 34)	NA	NA		4.6 (9)	<20	20 (<20–246)	945	627	2, 3	7, 27	Het	wt	Hom	wt	10^7
30 (M; 35)	NA	NA		4.1 (?)	12,586	870 (32–12,586)	235	827	30, 31	44, 48	wt	wt	Hom	wt	Negative
31 (M; 35)	NA	NA		3.9 (6)	<20	<20 (<20–122)	343	880	2, 25	7, 51	wt	Het	Het	wt	Negative
32 (M; 51)	NA	NA		4.5 (8)	285	241 (<20–1590)	381	1,338	3, 32	38, 62	wt	wt	Het	wt	10^7
33 (F; 41)	NA	NA		4.7 (7)	<20	<20 (<20–1339)	634	544	23	44, 49	wt	Het	Het	wt	10^6
34 (F; 34)	NA	NA		2.7 (5)	13,151	16,568 (328–85,239)	336	378	1, 2	7, 44	wt	wt	Het	Het	Negative
35 (M; 41)	NA	NA		5.2 (?)	<20	<20 (<20–35,692)	354	816	2, 3	7, 62	Het	wt	Hom	wt	Negative
36 (M; 39)	NA	NA		4.4 (9)	621	924 (<20–4691)	415	804	2	51, 27	wt	wt	Het	Hom	10^6
37 (F; 35)	NA	NA		4.1 (6)	23	<20 (<20–78)	541	584	2, 24	7, 55	wt	Het	Het	Het	10^6
38 (M; 36)	NA	NA		3.8 (6)	1001	2238 (256–42,775)	431	537	2	7	wt	wt	Het	wt	Negative

NOTE. Het, heterozygous for mutation (for CCR2 V64I, V64/I64; for CCR5 Δ32, +/Δ32; for CCR5 59029 A/G, A/G; for SDF-1 3′A, 3′A/+); HGV, hepatitis G virus; Hom, homozygous for mutation (for CCR2 V64I, I64/I64; for CCR5 Δ32, Δ32/Δ32; for CCR5 59029 A/G, G/G; for SDF-1 3′A, 3′A/3′A); NA, not applicable; ND, not done; pVL, HIV RNA load in plasma; wt, wild-type sequence (for CCR2 V64I, V64/V64; for CCR5 Δ32, +/+; for CCR5 59029 A/G, A/A; for SDF-[1] 3′A, +/+);

[a] No results from polymerase chain reaction were available.

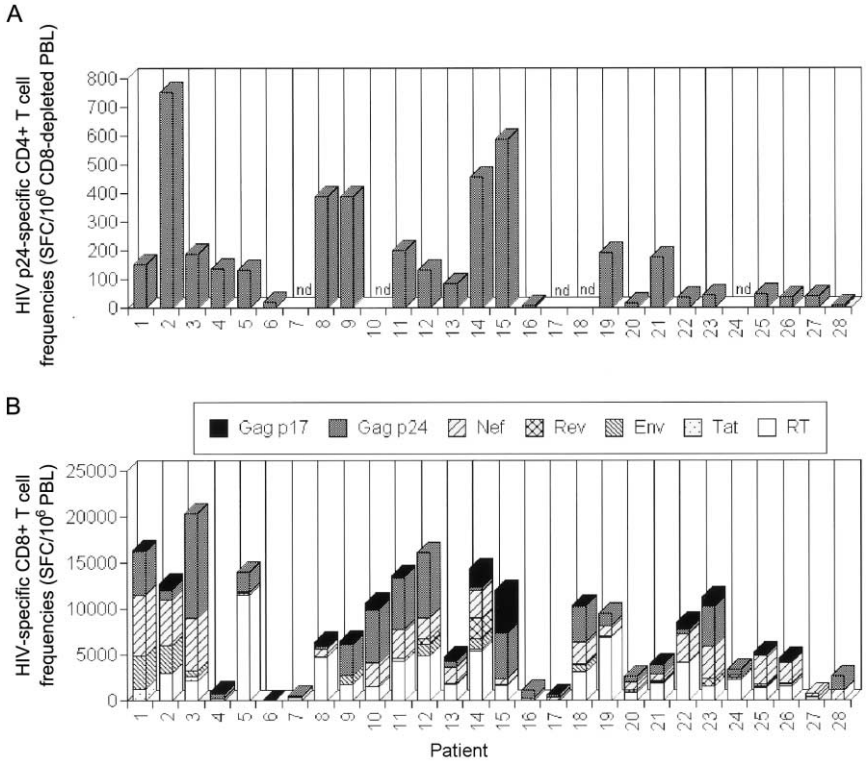

Figure 1. HIV p24-specific CD4$^+$ *(A)* and HIV-specific CD8$^+$ *(B)* T cell frequencies, in untreated patients. nd, not determined.

allowed nonspecific background to be determined. HIV-specific CD4$^+$ T cell counts were determined ex vivo by IFN-γ ELISpot analysis; PBLs were depleted of CD8$^+$ T cells by anti-CD8$^+$ monoclonal antibody conjugated to magnetic beads (Dynal UK) before stimulation with overlapping pooled peptides (20-mer peptides overlapping by 10 amino acids) that spanned the full-length HIV p24 protein (5 μg/mL [National Institute for Biological Standards and Control]) [37].

The HIV-unrelated recall antigen streptokinase/streptodornase (SK/SD) (200 U/mL [Lederle]) and nonspecific PHA stimulation (5 μg/mL) were used as positive controls, and medium alone was used as the negative control. Results were expressed as the number of specific spot-forming cells (SFCs) per 10^6 CD8$^+$ T cell–depleted PBLs. Before normalization, background values were subtracted from the specific response.

A positive response was defined as one that was >3 SD above background. Spot quantification was automated and standardized by use of an ELISpot plate reader, software version 2.1 (Autoimmun Diagnostika). All assays were performed in duplicate.

Statistics. Data were analyzed by either SPSS 11.0 linear regression or Spearman's rank correlations. The Mann-Whitney *U* test and *T* test were used for comparative analysis of the groups of untreated and ART-treated patients.

RESULTS

HIV-specific T cell responses correlate with disease-progression rates in untreated patients. We examined a cohort of 28 chronically HIV-infected, antiretroviral-naive patients with documented varying course of pVL and CD4$^+$ T cell count over time (table 1). To examine HIV-specific CD8$^+$ T cell frequencies, PBLs were screened by IFN-γ ELISpot analysis with overlapping peptides spanning the HIV proteins Env, Gag, Pol, Nef, Tat, and Rev. We determined the composition of the HIV-specific CD8$^+$ T cell response to each individual protein. Most of the responses were directed against the HIV Gag p24, Nef, and Pol proteins (figure 1*B*). The magnitude of the total response (defined as the sum of all responses to individual pools) correlated with the number of recognized pools per patient (figure 2*A*), indicating that the greater the HIV-specific CD8$^+$ T cell count, the broader the recognition profile. These findings corroborate previously reported observations [17, 18].

To determine the HIV-specific CD4$^+$ T cell frequencies, CD8-depleted PBLs were stimulated with the pooled overlapping peptides spanning HIV Gag p24 (figure 1). The results showed that, in general, patients with high HIV-specific CD8$^+$ T cell frequencies also exhibited detectable or substantial HIV-specific CD4$^+$ T cell frequencies (figure 2*B*); indeed, the frequencies of these 2 types of cells were positively correlated (figure 2*B*).

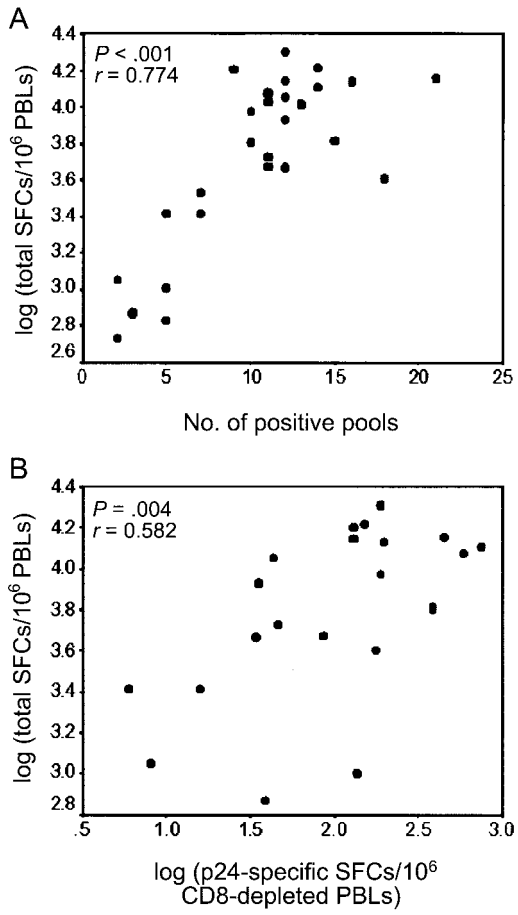

Figure 2. *A,* Total HIV-specific $CD8^+$ T cell response and no. of recognized peptide pools, in 28 untreated patients. The magnitude of the total HIV-specific $CD8^+$ T cell response (defined as the sum of individual responses) correlated with the no. of recognized peptide pools. *B,* HIV-specific $CD4^+$ and HIV-specific $CD8^+$ T cell frequencies. The log of the total HIV-specific $CD8^+$ T cell frequencies correlates with the log of the HIV p24-specific $CD4^+$ T cell frequencies. Each black dot (●) represents data from an individual patient. *P* and *r* values for linear regression are shown.

The aforementioned assessment of $CD8^+$ T cell frequencies included $CD4^+$ T cell responses, because $CD4^+$ T cells were not depleted before stimulation. Herein, we have referred to this total response as "$CD8^+$ T cell response" because its contribution to the total response is 20–1000-fold greater than that of the $CD4^+$ T cell response. The positive correlation between the HIV-specific $CD8^+$ T cell response and the p24-specific $CD4^+$ T cell response also held when the p24-specific $CD4^+$ T cell response was subtracted from the total response prior to the analysis ($P = .007$; data not shown).

We next sought to correlate, in our cohort of HIV-infected patients, the magnitude of the immune responses to the different disease-progression rates. The slope for $CD4^+$ T cells is very powerful for the prediction of the disease-progression rate [38]. Furthermore, integration of both HIV RNA and $CD4^+$ T cell count into a single parameter provides a better predictor of disease progression than does either measurement alone [39, 40]. For each patient, we calculated 2 parameters that reflected the disease-progression rate. First, regression analysis of all available total $CD4^+$ T cell counts before the analysis yielded a slope that characterized the yearly change in $CD4^+$ T cell count until the time of analysis. Second, we refined this parameter by integrating the evolution of pVL into it; at each time point, the ratio of $CD4^+$ T cell count to the corresponding log pVL was used for regression analysis, to yield the yearly change in total $CD4^+$ T cell count/log pVL (hereafter termed "normalized yearly change in $CD4^+$ T cell count"). A median of 10 (range, 4–20) values each for $CD4^+$ T cell count and for HIV RNA were available for the calculation of the slopes.

We then compared the 2 slopes to the magnitude of each patient's HIV-specific T cell response (figure 3). Although it was not statistically significant, there was an inverse correlation between the total HIV-specific $CD8^+$ T cell frequencies and the normalized yearly change in $CD4^+$ T cell count ($P = .054$; figure 3*C*). In contrast, no correlation, either direct or inverse, was observed between either the yearly change in $CD4^+$ T cell count or the normalized yearly change in $CD4^+$ T cell count and the total $CD8^+$ T cell count ($P = .969$ and $P = .767$, respectively) or between the total HIV-specific $CD8^+$ T cell count and pVL ($P = .137$).

The normalized yearly change in $CD4^+$ T cell count was inversely correlated with the HIV p24-specific $CD4^+$ T cell frequencies (figure 3*D*; $P = .003$) but not with the HIV-unrelated antigen SK/SD-specific $CD4^+$ T cell frequencies ($P = .723$). No correlation between actual pVL and the HIV p24-specific $CD4^+$ T cell frequencies was observed ($P = .374$).

These results indicate that stronger and broader HIV-specific $CD8^+$ T cell responses and greater HIV-specific $CD4^+$ T cell frequencies were associated with slower disease-progression rate as defined by the normalized yearly change in $CD4^+$ T cell count before the analysis. In contrast, no relation between the duration of infection and the magnitude of the HIV-specific T cell response was observed (data not shown).

Influence of host factors on time course of HIV infection. Various host genetic factors are associated with distinct HIV progression rates [23, 24]. We therefore analyzed whether, in our cohort of HIV-infected patients, our parameters for disease progression (i.e., yearly change in $CD4^+$ T cell count and yearly normalized change in $CD4^+$ T cell count) were correlated with either different host genetic factors or coinfection with GB virus C. We used Spearman's rank correlations to analyze the following host factors:

1. HLA class I genotype: Based on the HLA genotype of each patient, a relative risk hazard was calculated on the basis of the relative HLA hazard values reported in a study by O'Brien et al. [41] (see details in the footnotes to table 2). Only HLA

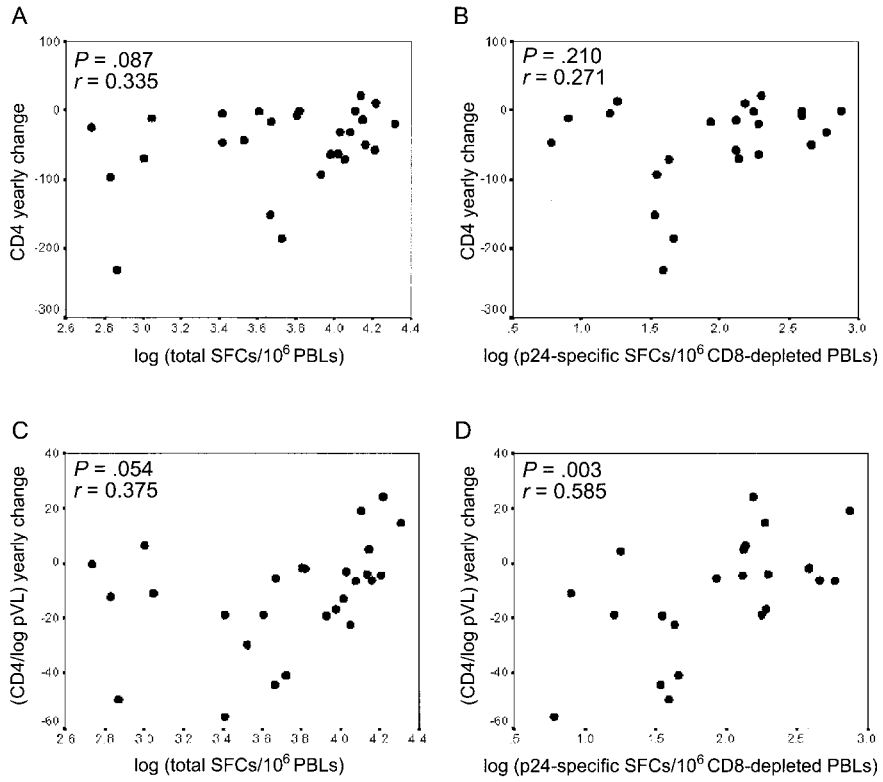

Figure 3. Correlations between disease progression and HIV-specific $CD4^+$ and HIV-specific $CD8^+$ T cell frequencies, in 28 untreated patients. *A,* Yearly decreases in $CD4^+$ T cell counts, showing a trend toward an inverse correlation with the log of the total HIV-specific $CD8^+$ T cell frequencies. Total HIV-specific $CD8^+$ T cell frequencies are the sum of all responses measured against individual peptide pools, and yearly change in $CD4^+$ T cell counts was calculated by regression through all available measurements of $CD4^+$ T cell counts. *B,* Yearly change in $CD4^+$ T cell counts, which are not correlated with the log of HIV p24-specific $CD4^+$ T cell frequencies. *C,* Yearly change in $CD4^+$ T cell count/log of virus load in plasma (log pVL), showing a trend toward an inverse correlation with the log of total HIV-specific $CD8^+$ T cell frequencies. The yearly change in $CD4^+$ T cell count/log pVL was calculated by regression through the quotients of total $CD4^+$ T cell count and log pVL. *D,* Yearly change in $CD4^+$ T cell count/log pVL, which is inversely correlated with the log of HIV p24-specific $CD4^+$ T cell frequencies. Each black dot (●) represents data from an individual patient. *P* and *r* values for linear regression are shown. PBLs, peripheral-blood lymphocytes; SFCs, spot-forming cells.

alleles B*58, B*27, B*57, B*51, A*11, A*68, B*35, and B*53 (all of which have a significant relative hazard) were used for the calculation. No correlation between HLA genotype and disease-progression rate was found (table 2).

2. CCR2/CCR5 polymorphism: Based on the CCR2-CCR5 genotype of each patient, a relative risk hazard was calculated on the basis of the relative hazard values reported in a study by Ioannidis et al. [42] (see details in the footnotes to table 2). No correlation between the combined CCR2-CCR5 genotype and disease-progression rate was found (table 2).

3. CCR2/CCR5 polymorphism and coinfection with GB virus C: We combined the relative hazard values calculated in item 2 with the relative risk factor inferred on the basis of coinfection with GB virus C, as reported by Xiang et al. [28] (see details in the footnotes to table 2). No correlation between the combined CCR2-CCR5 genotype plus GB virus C coinfection and disease-progression rate was found (table 2).

4. CCR2/CCR5 polymorphism, coinfection with GB virus C, and HLA genotype: We combined all the relative hazard values calculated in items 1 and 3; no correlation between this combined relative hazard value and disease-progression rate was found (table 2).

In summary, we found no correlation between our measures of disease-progression rate and either host genetic factors or GB virus C coinfection; however, because the size of our cohort of HIV-infected patients is very small compared with those normally used for genetic analyses, our results do not exclude the possibility that host genetic factors are involved in disease progression.

HIV-specific T cell responses in untreated subjects and in treated patients who had comparable viremia and $CD4^+$ and $CD8^+$ T cell counts. We hypothesized that the association between stronger and broader HIV-specific $CD4^+$ and HIV-specific $CD8^+$ T cell responses and a slower disease-progression rate was a causal relationship and not just a consequence of a generally lower pVL in patients with slower disease progression.

Table 2. Spearman's rank correlations between disease-progression rate and host genetic factors, GB virus C coinfection, and immunological parameters.

	Yearly change		T cell frequency			T cell count		Relative-risk hazard based on			
Yearly change	$CD4^+$ T cell count[a]	$CD4^+$ T cell count/log pVL[b]	Total HIV-specific, SFCs/10^6PBLs	p24-specific $CD4^+$, SFCs/10^6 CD8-depleted PBLs	pVL[c]	$CD4^+$[c]	$CD8^+$[c]	HLA genotype[d]	CCR2/CCR5 polymorphism[e]	CCR2/CCR5 polymorphism and HGV infection[f]	CCR2/CCR5 polymorphism, HGV infection, and HLA genotype[g]
$CD4^+$ T cell count		0.61 (.001)	NS	NS	−0.571 (.002)	NS	NS	NS	NS	NS	NS
$CD4^+$ T cell count/log pVL	0.61 (.001)		0.44 (.021)	0.54 (.008)	−0.55 (.003)	NS	NS	NS	NS	NS	NS

NOTE. Data are correlation (*P* value). HGV, hepatitis G virus; NS, not significant; PBLs, peripheral-blood lymphocytes; pVL, HIV RNA load in plasma; SFCs, spot-forming cells.

[a] Slope in $CD4^+$ T cell count before analysis.

[b] Slope in $CD4^+$ T cell count/log pVL before analysis.

[c] At the time of analysis.

[d] Relative-risk hazards for HLA B*58 (0.38), B*27 (0.42), B*57 (0.52), B*51 (0.68), A*11 (0.69), A*68 (1.2), B*35 (1.5) and B*53 (2.1) were taken from O'Brien et al. [41] and were calculated by multiplication of individual HLA relative-risk hazards values.

[e] Relative-risk hazards for CCR5 Δ32 (0.7) and CCR2 V64I (0.88) were taken from Ioannidis et al. [42] and were calculated by multiplication of individual relative-risk hazards.

[f] Relative-risk hazards for CCR5 Δ32 (0.7) and CCR2 V64I (0.88) were taken from Ioannidis et al. [42], and the relative-risk hazard associated with HGV coinfection (0.27) was taken from Xiang et al. [28]; all were calculated by multiplication of individual relative-risk hazards.

[g] Combination of relative-risk hazard based on HLA genotype and relative-risk hazard based on CCR2/CCR5 polymorphism and HGV infection.

Therefore, we compared the HIV-specific $CD8^+$ T cell frequencies in 10 untreated subjects (individual 1, 2, 3, 5, 9, 11–13, 21, and 26) versus those in 10 ART-treated patients (individuals 29–38) who had comparable plasma viremia and total $CD4^+$ and total $CD8^+$ T cell counts (figure 4). The total HIV-specific $CD8^+$ T cell frequencies, the number of recognized peptide pools, and the HIV p24-specific $CD4^+$ T cell frequencies in the untreated group were significantly higher than those in the treated group ($P = .001$–$.006$; figure 4), despite the absence of any significant difference in the total $CD4^+$ T cell counts. This lends support to speculation that stronger HIV-specific cellular immunity in untreated patients is at least partly responsible for this clinical phenotype and that it is not merely a consequence of lower viral replication. Alternatively, the most drug-resistant and, therefore, probably "less-fit" virus that exists in ART-treated patients with detectable viremia may induce fewer specific immune responses that are of lesser magnitude.

DISCUSSION

In the present study, we have examined the HIV-specific cellular immune response and host genetic factors in a cohort of HIV-infected patients. To reduce the heterogeneity in this cohort, we included only patients with a follow-up of ⩾2 years and excluded all patients infected with documented HIV strains other than clade B. We sought to define individual disease-progression rates on the basis of the evolution of $CD4^+$ T cell count and pVL. For each patient, we calculated a slope through all available $CD4^+$/pVL ratios. We found that HIV-specific $CD4^+$ T cell responses and, to a lesser extent, HIV-specific $CD8^+$ T cell responses were inversely correlated with disease-progression rate defined as the rate of $CD4^+$ T cell change in relation to pVL. No correlations between the HIV-specific cellular immune response and either the HIV RNA level or the total $CD4^+$ T cell count at the time of analysis were seen. However, we did observe that the yearly change in the total $CD4^+$ T cell count was correlated with pVL at the time of analysis (i.e., the higher the pVL at the time of analysis, the greater the slope of the change in total $CD4^+$ T cell count before analysis ($P = .002$).

The cellular immune response in chronic HIV infection is the product of past interactions between HIV and the immune system, rather than the result of an immediate event. Thus, comparisons between the decline in $CD4^+$ T cell count (i.e., yearly change in $CD4^+$ T cell count) and HIV-specific cellular immune response seem more appropriate than correlations with a single $CD4^+$ T cell count. The slope for $CD4^+$ T cells is very powerful for predicting the disease-progression rate [38]. Integration of both the HIV RNA level and the $CD4^+$ T cell count into a single parameter provides a better predictor of disease progression than does the use of either measurement alone [40]. In consideration of this, we sought to integrate the evolution of both $CD4^+$ T cell count and pVL into one param-

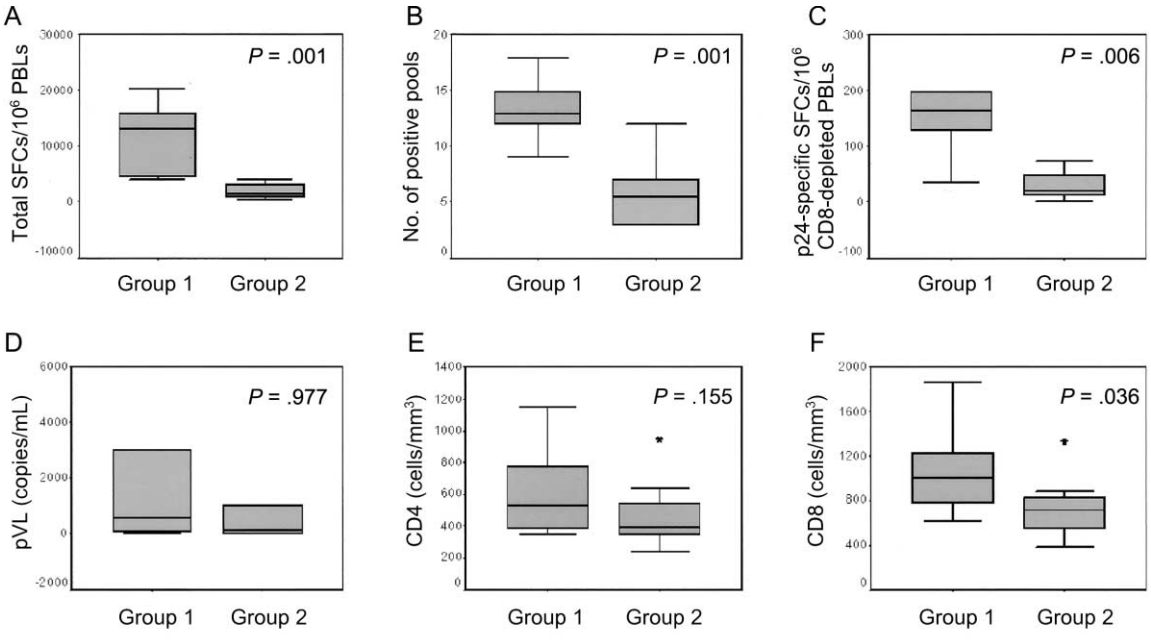

Figure 4. HIV-specific $CD4^+$ and HIV-specific $CD8^+$ T cell responses, in untreated patients (group 1) and in patients being treated with antiretroviral therapy (group 2) who had comparable plasma viremia and total $CD4^+$ and total $CD8^+$ T cell counts. The gray boxes denote interquartile ranges; the black bars denote medians; the whiskers extend to adjacent values, and the asterisks denote outliers. *A,* Total HIV-specific $CD8^+$ T cell frequencies. *B,* No. of peptide pools inducing a positive $CD8^+$ T cell response. *C,* HIV p24-specific $CD4^+$ T cell frequencies. *D,* Virus load in plasma. *E,* Total $CD4^+$ T cell counts. *F,* Total $CD8^+$ T cell counts.

eter. To do this, we calculated the ratio between the absolute $CD4^+$ T cell count and the log of HIV RNA at each available time point for each patient. Regression through these data points yielded a slope that correlated inversely with the HIV-specific $CD4^+$ T cell frequencies and that showed a tendency toward an inverse correlation with the HIV-specific $CD8^+$ T cell frequencies. We conclude that the evolution of viremia and $CD4^+$ T cell count before the time of analysis is related to the magnitude of the HIV-specific cellular immune response. Thus, an efficient HIV-specific cellular immune response is characteristic of patients with either stable $CD4^+$ T cell counts or stable HIV RNA levels, and a weak or absent HIV-specific cellular immune response is characteristic of patients with either increasing viremia or a dramatic decrease in $CD4^+$ T cell count.

Clearly, factors other than the magnitude of the HIV-specific cellular immune response are involved in disease progression. For example, patient 6 had no detectable HIV RNA during the preceding 2 years and had no detectable HIV-specific cellular immune response; in contrast, patients 1 and 2 also had no detectable HIV RNA but did have prominent HIV-specific cellular immune responses (respective $CD8^+$ T cell responses, 16,421 SFCs/10^6 PBLs and 12,970 SFCs/10^6 PBLs). Thus, mechanisms governing the disease-progression rate in patients are most likely very diverse. HIV-specific cellular immunity might be crucial in some patients, but other factors might be decisive in others.

Various host genetic factors are associated with distinct HIV disease–progression rates [23, 24]. Convincing data exist for the involvement of certain HLA class I genotypes (reviewed in [25]) and polymorphisms of CCR5 and CCR2 [42]. We sought to assess these host genetic factors in our cohort of HIV-infected patients and to determine whether disease progression is better correlated with these factors or with the HIV-specific cellular immune response. Because coinfection with GB virus C is predictive of longer survival [28], we also looked at this parameter. Because our cohort of HIV-infected patients was small, our results must be considered with caution. Slower disease progression, as defined by either the yearly change in $CD4^+$ T cell count or the yearly normalized change in $CD4^+$ T cell count, was not related to polymorphisms in CCR genes, to HLA alleles, or to coinfection with GB virus C.

We also compared the HIV-specific cellular immune response in untreated patients with that in ART-treated patients. The 2 groups of patients were matched for their absolute $CD4^+$ T cell counts and HIV RNA levels at the time of their recruitment. We found that HIV-specific $CD4^+$ T cell frequencies and HIV-specific $CD8^+$ T cell frequencies were substantial in all patients with detectable viremia; however, the virus-specific T cell frequencies were significantly higher in the untreated patients than in the ART-treated patients. This suggests that the magnitude of the HIV-specific immune response is not merely reflecting antigenic drive. However, the nadir of the absolute $CD4^+$ T cell count in the ART-treated patients was very low, with a median of 34 cells/μL (range, 0–609 cells/μL), before the initiation of ART. Because of the marked decrease in $CD4^+$ T cell counts in these patients, the immune system may have been irreversibly damaged [4].

In summary, we have ranked untreated patients within a spectrum of disease progression, according to the evolution of their total $CD4^+$ T cell count and pVL. Using this approach, we have found that the magnitude of the HIV-specific cellular immune response is inversely related to the previous evolution of total $CD4^+$ T cell count and pVL. These findings add weight to the argument that HIV-specific cellular immunity is protective.

SWISS HIV COHORT STUDY MEMBERS

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