

The New England Journal of Medicine

©Copyright, 1995, by the Massachusetts Medical Society

Volume 332

JANUARY 26, 1995

Number 4

VIROLOGIC AND IMMUNOLOGIC CHARACTERIZATION OF LONG-TERM SURVIVORS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION

YUNZHEN CAO, M.D., LIMO QIN, M.D., LINQI ZHANG, PH.D., JEFFREY SAFRIT, PH.D., AND DAVID D. HO, M.D.

Abstract Background. In most subjects infected with human immunodeficiency virus type 1 (HIV-1), clinical or laboratory evidence of immunodeficiency develops within 10 years of seroconversion, but a few infected people remain healthy and immunologically normal for more than a decade. Studies of these subjects, termed long-term survivors, may yield important clues for the development of prophylactic and therapeutic interventions against the acquired immunodeficiency syndrome.

Methods and Results. We studied 10 seropositive subjects who remained asymptomatic with normal and stable CD4+ lymphocyte counts despite 12 to 15 years of HIV-1 infection. Plasma cultures were uniformly negative for infectious virus. However, particle-associated HIV-1 RNA was detected in four subjects with a sensitive branched-DNA signal-amplification assay, whereas in five others the levels of HIV-1 RNA were too low to detect. Infectious HIV-1 was detected in peripheral-blood mononuclear cells (PBMC) of three subjects by standard limiting-dilution cultures, and infectious virus was recovered from

another subject with use of a CD8-depleted culture. The other six subjects had no detectable infectious virus in their PBMC. A quantitative polymerase-chain-reaction assay revealed that all subjects had detectable but low titers of viral DNA in PBMC. Overall, the viral burden in the plasma and PBMC of long-term survivors was orders of magnitude lower than that typically found in subjects with progressive disease.

There was no *in vitro* evidence of resistance by host CD4+ lymphocytes to HIV-1 infection. However, long-term survivors had a vigorous, virus-inhibitory CD8+ lymphocyte response and a strong neutralizing-antibody response. In two subjects the kinetics of viral replication was consistent with the presence of a substantially attenuated strain of HIV-1.

Conclusions. Subjects who remain asymptomatic for many years despite HIV-1 infection have low levels of HIV-1 and a combination of strong virus-specific immune responses with some degree of attenuation of the virus. (N Engl J Med 1995;332:201-8.)

THE natural history and pathogenic processes of human immunodeficiency virus type 1 (HIV-1) infection are complex and variable, and they depend on a multitude of viral and host factors and their interactions.¹ Host factors may result in a variable susceptibility to HIV-1 infection and its pathogenic effects, whereas variation in the virus may account for differences in virulence and disease progression. Although symptoms related to the acquired immunodeficiency syndrome (AIDS) or laboratory evidence of immunodeficiency develops in a majority of infected persons within 10 years of seroconversion,²⁻⁵ a small number (approximately 5 percent) of infected persons, termed long-term survivors or persons with long-term nonprogressive disease, have remained clinically healthy and immunologically normal for more than a decade.⁶⁻¹¹ These long-term survivors have recently become the subject of intensive

investigation, because they may yield important information on the determinants of nonprogression that may be useful in designing new interventional strategies to contain the disease.

To obtain a balanced view of the pathogenic processes in long-term survivors, we examined host, immunologic, and virologic factors in a cohort of 10 subjects who have remained asymptomatic with normal and stable CD4+ lymphocyte counts despite 12 to 15 years of HIV-1 infection.

METHODS

Study Subjects

Ten HIV-1-seropositive subjects from the New York metropolitan area were referred to us because they met our working definition of long-term survivors of HIV-1 infection: they had no symptoms, normal and stable CD4+ lymphocyte counts, no prolonged use of antiretroviral agents, and at least 12 years of infection. The general clinical characteristics of the cohort are summarized in Table 1. The subjects ranged in age from 38 to 47 years, and all but one were men. Seven were infected through homosexual contact, two were infected through intravenous drug use, and the one woman was infected heterosexually. Their CD4+ lymphocyte counts have been consistently in the normal range, with no decline over time. The duration of HIV-1 infection was documented by the date of seroconversion in three subjects (Subjects 1, 4, and 9) who participated in a prospective

From the Aaron Diamond AIDS Research Center, New York University School of Medicine, 455 First Ave., New York, NY 10016, where reprint requests should be addressed to Dr. Ho.

Supported by grants (AI24030, AI25541, AI32427, and AI27665) from the National Institutes of Health (NIH) and an NIH contract on Correlates of Immune Protection, the Centers for AIDS Research of New York University, and the Aaron Diamond Foundation.

Table 1. Clinical Characteristics of Long-Term Survivors.*

SUBJECT†	AGE (YR)/ SEX	ROUTE OF INFECTION	RANGE OF CD4 COUNTS (CELLS/mm ³)	YEARS OF INFECTION	HLA TYPE	
					CLASS I	CLASS II
1 (+)	38/M	Homosexual sex	600–1200	15	A3; B57; Cw3	DR1,7; DQ(ND)
2 (○)	38/M	IV drug use	500–700	≥12	A24,32; B51,52; Cw1,2	DR15,11,52; DQ6,7
3 (△)	46/M	Homosexual sex	560–740	≥13	A2,3; B7,14; Cw7,8	DR15,6,52; DQ1(6)
4 (□)	40/M	Homosexual sex	500–1200	14	A1,2; B51,57; Cw2,6	DR7,11,52,53; DQ7,2
5 (◇)	41/M	IV drug use	800–1000	≥12	A2,26; B38; Cw3	DR11,13,52; DQ6,7
6 (●)	38/M	Homosexual sex	560–860	12	A2,19; B44; Cw3,5	DR52; DQ1
7 (▲)	42/F	Heterosexual sex	500–850	13	A1,2; B8,58; Cw3,7	DR15,3,52; DQ6,2
8 (■)	44/M	Homosexual sex	400–800	≥15	A2,24; B18,51; Cw1,7	DR52; DQ1,3
9 (×)	47/M	Homosexual sex	600–1100	14	A11,26; B62; Cw3	DR4,6; DQ(ND)
10 (◆)	47/M	Homosexual sex	550–850	≥14	A1,25; B18,37; Cw6	DR15,11,52; DQ6,2

*IV denotes intravenous, and ND not done.

†Each subject is identified by a number and a symbol, which is also used in Figures 1, 2, and 4.

study of the natural history of the disease at the New York Blood Center. Subject 7 had given birth to an infected child 13 years before the start of our study, and Subject 8 had had unexplained hypergammaglobulinemia and lymphoid hyperplasia on biopsy 15 years before the study began. The duration of infection in the other subjects was determined on the basis of the year in which they discontinued high-risk behavior, such as intravenous drug use (Subjects 2 and 5) or unprotected homosexual sex (Subjects 3, 6, and 10). None had received antiretroviral agents for a prolonged period, although some had received short courses of zidovudine (Subjects 2 and 7), didanosine (Subject 2), or recombinant gp160 (Subject 3). No subject was receiving antiretroviral therapy at the time of this study. As Table 1 shows, these long-term survivors had a range of HLA class I and II phenotypes as determined by standard serologic typing (Blood Systems Laboratory, Scottsdale, Ariz.), indicating that they did not share a common HLA type. In addition, the 10 subjects were not found to have epidemiologic features in common.

Quantitation and Isolation of HIV-1

Infectious HIV-1 in plasma and peripheral-blood mononuclear cells (PBMC) was quantitated as described elsewhere.¹²⁻¹⁴ Particle-associated RNA in plasma was quantitated with a modification of the branched-DNA signal-amplification assay,^{15,16} with freshly collected samples. This ultrasensitive assay has a typical detection limit of approximately 630 copies of RNA per milliliter of plasma.¹⁷ HIV-1 DNA in PBMC was quantitated by the polymerase chain reaction (PCR) as described elsewhere.¹⁸ Briefly, proviral DNA was initially studied with limiting dilutions to a point at which less than 25 percent of the resulting PCR products were positive. The number of proviral copies was then estimated by the formula $-\ln(F)$, where F is the fraction of negative reactions, assuming that the incidental appearance of positive PCR products follows a Poisson distribution. Appropriate positive and negative controls were included in all quantitative assays.

When the initial attempt to isolate HIV-1 was unsuccessful, subsequent attempts were made with up to 5 million PBMC that had been subjected to CD8+ lymphocyte depletion with immunomagnetic beads (Dyna, Great Neck, N.Y.).

Susceptibility of PBMC from Long-Term Survivors to HIV-1 Infection in Vitro

To assess the susceptibility of cells from long-term survivors to HIV-1 infection in vitro, PBMC (2 million cells) from each of eight study subjects (all except Subjects 1 and 9) and two normal controls were inoculated with 3000 median tissue-culture infective doses (TCID₅₀) of the HIV-1 isolate JRCSF.¹⁹ The cultures were then washed extensively on the second day, and the expression of p24 antigen in the supernatant was determined by an immunoassay (Abbott Laboratories, Abbott Park, Ill.) on days 4, 7, and 14 of culture. Similar experiments were carried out in parallel with PBMC that had

been largely (>98 percent) depleted of CD8+ lymphocytes by the immunomagnetic-bead method. In these experiments as well as those described immediately below, PBMC from subjects with progressive disease could not be studied in parallel for comparison, because such cells harbor another infectious isolate of HIV-1 that would have clouded the interpretation of the results.

HIV-1–Inhibitory Activity Mediated by CD8+ Lymphocytes

A series of experiments were conducted to quantitate the inhibitory activity of CD8+ lymphocytes on HIV-1 replication in CD4+ lymphocytes. CD4+ and CD8+ lymphocytes were each purified (to 98 percent purity) from PBMC of long-term survivors and normal controls with immunomagnetic beads. CD4+ lymphocytes were then stimulated for three days by the addition of phytohemagglutinin (2 μg per milliliter), and CD8+ lymphocytes were stimulated for three days with an anti-CD3 monoclonal antibody (12F6), irradiated allogeneic feeder cells, and interleukin-2 (100 units per milliliter). Two million CD4+ lymphoblasts were then inoculated with 3000 TCID₅₀ of the JRCSF isolate alone or together with variable numbers of autologous activated CD8+ lymphocytes (from 1 million down to 320 in fivefold dilutions). The expression of p24 antigen in the culture supernatant was monitored periodically during the ensuing 14 days.

Neutralizing Activity of Plasma against Primary HIV-1 Isolates

Serial dilutions of plasma samples from nine long-term survivors (all subjects except Subject 1) and four subjects with progressive disease were tested for neutralizing activity against a panel of 13 primary HIV-1 isolates (each containing 100 TCID₅₀) obtained after short-term culture of PBMC from long-term survivors or from U.S. patients who had the acute infection syndrome, were asymptomatic, or had AIDS. The assays were performed according to a published protocol,^{20,21} with 2 million activated PBMC from a normal donor used as target cells and the expression of p24 antigen in the supernatant used as a measure of HIV-1 replication. Extreme care was taken to ensure the complete removal of all added plasma from the cultures before the measurement of p24 antigen, because residual plasma might contain anti-p24 antibodies capable of interfering substantially with the p24 antigen assay, resulting in false evidence of virus neutralization.

Kinetics of Replication and Cytotoxicity of HIV-1 Isolates

HIV-1 was successfully isolated from the PBMC of three subjects (Subjects 8, 9, and 10) by a standard procedure,¹²⁻¹⁴ whereas in a fourth (Subject 7) isolation required CD8+ lymphocyte depletion. The kinetics of replication in 2 million PBMC from a normal donor was determined for each viral isolate (3000 TCID₅₀) by serial monitoring of p24 antigen expression in the culture supernatant, as described elsewhere. Viral cytotoxicity in purified cultures of CD4+

lymphocytes was assessed by serial counting of viable cells by light microscopy.¹⁴

RESULTS

Levels of HIV-1 in PBMC and Plasma

The levels of HIV-1 in the PBMC and plasma of long-term survivors were determined by several techniques. First, plasma cultures were uniformly negative for infectious virus (<1 TCID₅₀ per milliliter) in tests of nine subjects involving up to 1 ml of sample (Fig. 1). However, particle-associated HIV-1 RNA was detectable in four subjects (Subjects 4, 6, 8, and 10) by an ultrasensitive branched-DNA assay¹⁵⁻¹⁷; the values ranged from 839 to 11,549 copies of RNA per milliliter of plasma. The other five subjects had HIV-1 levels below the limit of detection of the assay (<630 RNA copies per milliliter). Overall, the amount of HIV-1 in the plasma of these nine long-term survivors was orders of magnitude lower than that found in subjects with progressive disease. In our experience,¹² asymptomatic persons with progressive disease had titers of infectious HIV-1 ranging from 5 to 100 TCID₅₀ per milliliter of plasma (mean, 30), whereas patients with AIDS had titers ranging from 5 to 5000 TCID₅₀ per milliliter (mean, about 1000). Similarly, subjects with progressive disease in a recent study of ours had plasma counts of viral RNA ranging from 4000 to 90 million copies per milliliter. The mean values were 580,000 copies per milliliter among patients with CD4+ cell counts below 200 per cubic millimeter and 71,000 copies per milliliter among those with counts ranging from 200 to 500 per cubic millimeter.¹⁶

Levels of HIV-1 were also determined in the PBMC of long-term survivors. Infectious HIV-1 was detected

and quantified in three subjects (0.2, 50, and 5 TCID₅₀ per million cells in Subjects 8, 9, and 10, respectively) by a standard limiting-dilution assay¹²⁻¹⁴ (Fig. 1). In contrast, seven subjects had no detectable infectious virus in 10 million PBMC (<0.1 TCID₅₀ per million cells). These negative results are particularly striking because in other settings we have isolated HIV-1 from PBMC at a rate approaching 100 percent.¹²⁻¹⁴ Therefore, additional attempts to recover infectious HIV-1 were made with 2 million to 5 million PBMC depleted of CD8+ lymphocytes, because this maneuver has been shown to improve the efficiency of HIV-1 isolation.²²⁻²⁵ The cultures for Subjects 1 through 6 remained negative, although an HIV-1 isolate was obtained from Subject 7 by this method (data not shown).

The amount of HIV-1 proviral DNA in the PBMC of long-term survivors was determined by an established method of quantitative PCR.¹⁸ As Figure 1 shows, all the subjects had detectable viral DNA, but the copy numbers were generally quite low, ranging from 10 to 100 copies per million PBMC, except in Subjects 9 and 10, who had 296 and 1783 copies per million PBMC, respectively. These two subjects also had the highest titers of infectious HIV-1 in PBMC (Fig. 1). Once again, the amount of HIV-1 in the PBMC of long-term survivors as measured by these two techniques appeared to be substantially lower than the levels that we¹²⁻¹⁴ and others^{18,26-28} have found in patients with progressive disease; such patients had a mean infectious titer of about 1700 TCID₅₀ per million PBMC and a mean count of about 5000 copies of proviral DNA per milliliter.

It therefore appears that in the long-term survivors we studied, HIV-1 replication is well controlled in vivo. What accounts for this finding? The experiments de-

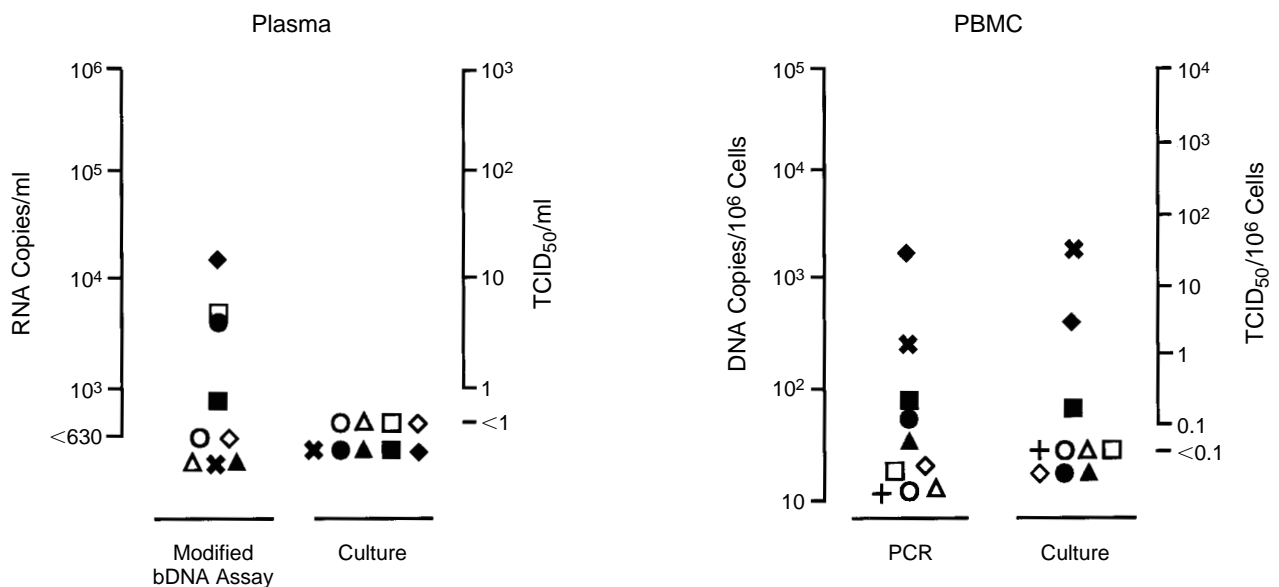


Figure 1. Levels of HIV-1 in Plasma and PBMC of Long-Term Survivors.

Each symbol represents a study subject, identified in Table 1. TCID₅₀ denotes median tissue-culture infective dose, and bDNA branched DNA. The detection limit of the bDNA signal-amplification assay was 630 copies of RNA per milliliter of plasma.

scribed below pursue three possible explanations: that these patients' CD4+ lymphocytes are less susceptible to HIV-1 infection; that they have stronger HIV-1-specific immune responses; and that they harbor strains of HIV-1 that are defective or attenuated.

Susceptibility of PBMC from Long-Term Survivors to HIV-1 Infection in Vitro

PBMC from eight long-term survivors (all except Subjects 1 and 9) and two normal controls were examined for their in vitro susceptibility to infection by JRCSF,¹⁹ an exogenous strain of HIV-1, since no infectious virus was found in cells from any of the subjects except Subjects 8 and 10. As Figure 2 shows, the virus replicated efficiently to high levels (>10,000 pg of p24 antigen per milliliter) in the PBMC of two normal donors, whereas its replication in the PBMC of long-term survivors was substantially less. In fact, only one culture (of cells from Subject 4) reached a level of p24 antigen expression ≥ 1000 pg per milliliter. At first glance, these results suggest that cells from long-term survivors were more refractory to HIV-1 infection in vitro. However, when the cultures were depleted of CD8+ lymphocytes, the remaining CD4-enriched PBMC from each long-term survivor supported HIV-1 replication at levels in excess of 1000 pg of p24 antigen per milliliter, and in five subjects the level exceeded 10,000 pg per milliliter (Fig. 2). On average, CD8+ lymphocyte depletion produced a 22-fold increase in peak HIV-1 replication in PBMC from the eight long-term survivors, as compared with a 3-fold increase in the two normal controls. Although CD4-enriched PBMC from several

long-term survivors (for example, Subjects 6 and 8) were less efficient in replicating the JRCSF isolate (Fig. 2), these cells did support the efficient growth of other primary HIV-1 isolates (data not shown). On the basis of the findings shown in Figure 2, we conclude that the CD4+ lymphocytes from long-term survivors had no gross intrinsic resistance to HIV-1 infection in vitro. Instead, there is strong evidence to suggest that CD8+ lymphocytes from these survivors had substantial HIV-1-inhibitory activity.

Detection and Quantitation of HIV-1-Suppressive Activity of CD8+ Lymphocytes

To show conclusively that CD8+ lymphocytes from long-term survivors indeed mediate potent suppression of HIV-1 replication in vitro, a series of experiments were performed in which CD8+ lymphocytes were added back to the sample. The results of three such experiments are shown in Figure 3. In CD4-enriched PBMC from a normal donor, the JRCSF strain of HIV-1 replicated efficiently to high levels, and the addition of 320 to 1 million autologous CD8+ lymphocytes resulted in only slight reductions in viral replication. In contrast, for Subjects 2 and 5, the addition of autologous CD8+ lymphocytes led to marked reductions (by about two orders of magnitude) in HIV-1 replication. In quantitative experiments of this type, we were able to determine the minimal number of autologous CD8+ lymphocytes required to inhibit peak HIV-1 replication by 90 percent for eight long-term survivors, as follows: Subject 2, 40,000 CD8+ lymphocytes; Subject 3, 200,000; Subject 4, 200,000; Subject 5, less than 300;

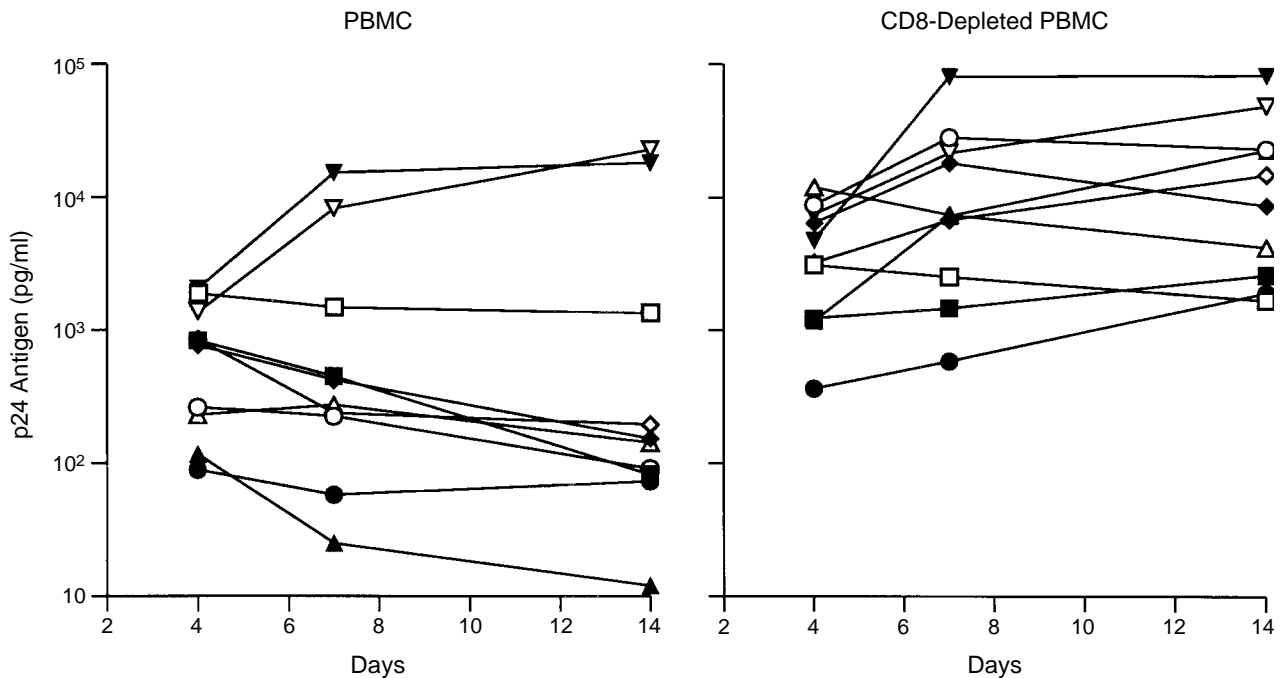


Figure 2. Kinetics of Replication of HIV-1 Isolate JRCSF in PBMC and CD8-Depleted PBMC from Eight Long-Term Survivors and Two Normal Controls.

Each symbol represents a study subject identified in Table 1. Two normal donors are also shown (∇ and \blacktriangledown).

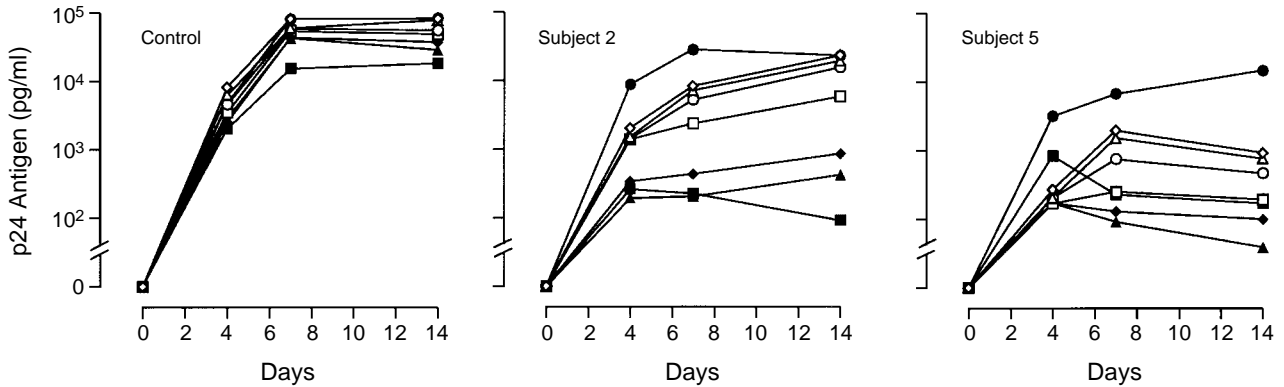


Figure 3. Kinetics of Replication of HIV-1 Isolate JRCSF in Unfractionated PBMC (■) from a Normal Control and Subjects 2 and 5, as Well as in CD8-Depleted, CD4-Enriched PBMC without CD8+ Lymphocytes (●) and with 1 Million (▲), 200,000 (◆), 40,000 (□), 8000 (○), 1600 (△), and 320 (◇) Autologous CD8+ Lymphocytes Added Back to the Cell Culture.

Subject 6, 1 million; Subject 7, less than 40,000; Subject 8, 200,000; and Subject 10, 200,000. These findings show that long-term survivors had a quantitatively greater HIV-1-suppressive response to CD8+ lymphocytes than did subjects with progressive disease, in whom 1 million autologous CD8+ lymphocytes were generally required to induce substantial inhibition of viral replication.²²⁻²⁴

Neutralizing Activity of Plasma against HIV-1

Having detected evidence of a strong cellular immune response, we next turned our attention to the neutralizing activity of plasma samples from nine long-term survivors against a diverse panel of primary HIV-1 isolates. The results are shown in Table 2. Al-

though primary HIV-1 isolates are known to be relatively resistant to neutralization by antibody and soluble CD4,^{21,29} plasma samples from our long-term survivors had broad neutralizing activity in general, especially when compared with the lack of neutralizing activity of plasma samples from subjects with progressive disease. These findings suggest that the long-term survivors had vigorous functional antibody responses directed against HIV-1.

Characterization of the Biologic Properties of HIV-1 Isolates in Vitro

As has been mentioned, infectious HIV-1 could not be isolated from six subjects despite multiple attempts using optimal protocols. Nevertheless, HIV-1 was re-

Table 2. Effectiveness of Plasma from Long-Term Survivors and Controls with Progressive Disease in Inhibiting Infection by Primary HIV-1 Isolates.

SOURCE OF PLASMA (SUBJECT)*	PRIMARY HIV-1 ISOLATE												
	FROM LONG-TERM SURVIVORS			FROM PATIENTS WITH ACUTE INFECTIONS				FROM ASYMPTOMATIC PATIENTS		FROM PATIENTS WITH AIDS			
	From Subject 7	From Subject 9	From Subject 10	VS	RA	JP	A	WM	N-70	JRCSF	JRFL	LS	AC
<i>reciprocal of plasma dilution that produced ≥90% inhibition of HIV-1 infection</i>													
Long-term survivors													
2	16	—	—	—	—	—	—	16	—	—	—	32	32
3	8	16	—	64	64	64	64	32	32	32	64	256	>256
4	64	—	64	8	64	16	16	128	8	—	32	16	—
5	256	16	8	16	64	256	32	64	16	16	8	64	32
6	256	16	—	64	64	64	64	16	64	256	64	256	>256
7	128	8	—	8	32	32	16	32	—	64	—	64	32
8	16	—	8	16	8	8	128	128	8	64	128	64	8
9	—	—	—	—	256	256	16	256	8	—	—	—	—
10	32	8	—	—	32	>64	8	16	—	—	16	—	32
Controls with progressive disease													
A	—	—	—	—	—	—	—	—	8	—	—	—	—
B	—	—	—	—	—	—	—	—	—	8	32	—	—
C	—	—	—	—	—	—	—	—	—	—	—	—	—
D	—	—	—	—	—	—	—	—	—	—	—	8	8

*Subject 1 was not included because of insufficient plasma samples. The control subjects included two asymptomatic carriers with low CD4+ cell counts (Subjects A and B), a patient with AIDS-related complex (Subject C), and a patient with AIDS (Subject D).

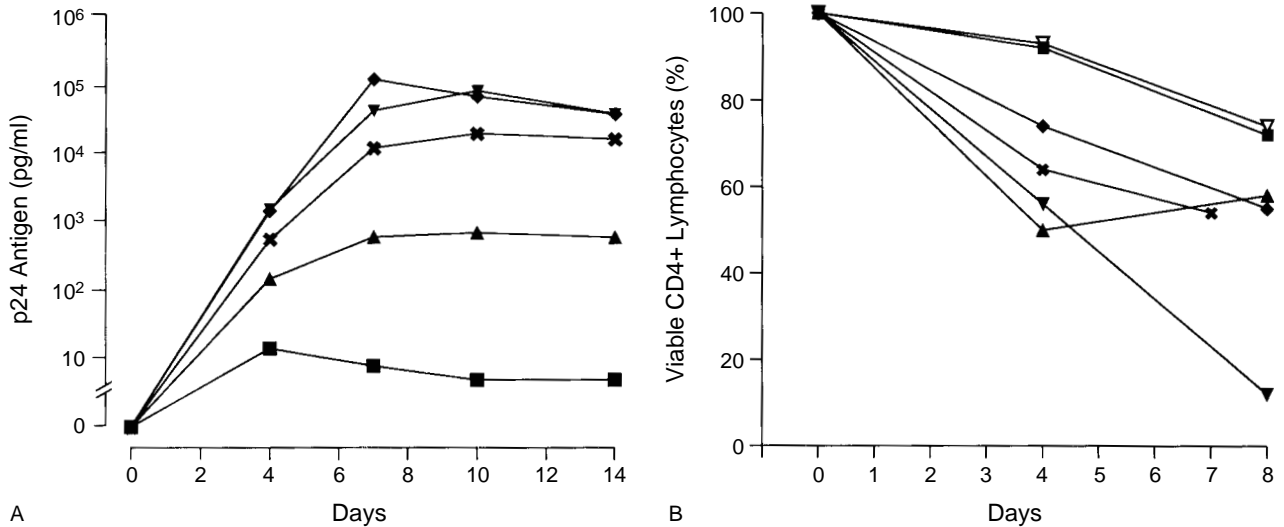


Figure 4. Kinetics of Replication (Panel A) and Cytotoxic Effect on CD4+ Lymphocytes (Panel B) of HIV-1 Isolates from Long-Term Survivors and of the JRCFSF Isolate (▼).

Each symbol represents a study subject, identified in Table 1. In panel B, ▽ denotes a control culture without virus.

covered from PBMC of three subjects (Subjects 8, 9, and 10) by a standard method and from a fourth (Subject 7) by a CD8-depleted coculture. The kinetics of replication in these four isolates was assessed in normal activated PBMC, as shown in Figure 4A. The isolates from Subjects 9 and 10 replicated to levels similar to that of a wild-type isolate (JRCFSF); in contrast, the isolates from Subjects 7 and 8 both replicated to maximal levels of p24 antigen expression that were below 1000 pg per milliliter, a finding consistent with substantial attenuation of growth. None of the four isolates were capable of infecting a number of T-cell lines, including MT-2 cells, and thus none were considered to have a syncytium-inducing phenotype. The cytotoxicity of these isolates against normal purified CD4+ lymphocytes was also examined in vitro. As Figure 4B shows, the isolate from Subject 8 had no cytotoxic effect at all, whereas those from Subjects 7, 9, and 10 were somewhat cytotoxic, though less so than JRCFSF, which is generally not considered to be one of the more cytotoxic variants among well-characterized HIV-1 isolates. On the basis of these findings, we believe that viral attenuation was evident in Subjects 7 and 8.

DISCUSSION

In the asymptomatic long-term survivors we have described, HIV-1 replication appeared to be well controlled, with the viral load in plasma and PBMC orders of magnitude lower than those typically found in subjects with progressive disease (Fig. 1). Recently, low levels of HIV-1 in lymphoid tissues of other long-term survivors have also been reported.³⁰ These findings are consistent with those of a large number of published reports suggesting that disease progression is driven by an increasing viral burden.^{12,14,16,18,26-28,31-33}

In eight of our long-term survivors (all except Subjects 9 and 10) there were distinctive virologic features. Repeated attempts to isolate infectious HIV-1 from six

of the eight subjects were unsuccessful (Fig. 1); in the other two (Subjects 7 and 8), viral attenuation was evident (Fig. 4). These findings, coupled with the extremely low viral loads observed, suggest that these subjects may not merely represent one extreme end of the normal distribution of patients with HIV-1 infection. These eight subjects were phenotypically similar to the long-term survivor reported by Greenough et al.¹¹ In contrast, Subjects 9 and 10, who had higher viral burdens and wild-type-like viruses, were more similar to the subjects with long-term nonprogressive disease studied by Pantaleo et al.,³⁰ who found higher viral burdens (mean plasma RNA copy number, 70,000 per milliliter) and obtained higher rates of virus isolation than those reported here. It is not clear that such subjects, although they meet the current clinical definition of long-term survivors,⁶ will not have progressive disease in the coming years.

We also studied three potential mechanisms that would account for the low viral load in long-term survivors: host-cell resistance, strong immunity, and weakened virus. Our study of the CD4+ lymphocytes in our subjects did find efficient replication of HIV-1 in vitro and the absence of gross intrinsic resistance to the virus. However, varying degrees of efficiency in viral replication were observed among the CD4+ lymphocytes from these subjects, as we³⁴ and others³⁵ have seen with cells from normal donors.

In a manner consistent with an earlier report showing that persons with long-term nonprogressive disease have higher levels of HIV-1-specific antibodies and CD8+ lymphocytes,³⁶ we found evidence of vigorous immune responses to HIV-1. Figures 2 and 3 show a substantial HIV-1-suppressive response by CD8+ lymphocytes in all the subjects studied. This suppressive effect of CD8+ lymphocytes was not restricted to cells with HLA compatibility and was more efficient if there was cell-to-cell contact (data not shown). Therefore, it

is likely that these CD8+ lymphocytes were qualitatively similar to those with the virus-inhibitory characteristics described by Walker et al.²²⁻²⁴ Although the nature of these inhibitory cells remains elusive, it is known that clones of cytotoxic T lymphocytes, when activated in vitro, can mediate similar inhibition.³⁷ Cytotoxic T lymphocytes that recognize specific HIV-1 envelope, core, and polymerase products have been detected in samples of PBMC from Subjects 1 through 7 and 10 (unpublished data).

These long-term survivors also had potent and broad neutralizing-antibody responses against a diverse panel of primary HIV-1 isolates (Table 2). This was in distinct contrast to findings in subjects with progressive disease²⁹ (and Table 2). The presence of a high level of neutralizing-antibody activity and a vigorous CD8+ lymphocyte response indicates that the immune system of the long-term survivors must have been continually exposed to viral antigens.

We obtained mixed results with respect to HIV-1 attenuation in long-term survivors. Subjects 9 and 10 had higher viral loads and harbored HIV-1 isolates that replicated as efficiently as wild-type viruses. In contrast, isolates from Subjects 7 and 8 showed markedly reduced rates of replication in vitro. We speculate that the degree of viral attenuation may have been even higher in the subjects from whom we could not isolate the virus. The viral genome of these long-term survivors is currently being characterized in order to elucidate the possible genetic basis of such attenuation. To date, no evidence of a gross *nef* defect has been found in our 10 subjects,³⁸ although one such case has been identified by others.³⁹ Recently, defects in the NFκB and Sp1 sites within the viral long-terminal repeats have been found in two of our subjects (unpublished data).

Two previous reports strongly support the notion that viral characteristics have a critical role in long-term nonprogressive infection. First, Learmont et al.¹⁰ described six recipients of blood transfusions from one HIV-1-infected donor who have remained well and immunologically stable despite a decade of infection. The blood donor has also remained healthy. This cluster of long-term survivors suggests the possibility that an attenuated virus was transmitted. Second, experiments carried out by Kestler et al.⁴⁰ showed that monkeys experimentally inoculated with simian immunodeficiency virus with deletions in the *nef* gene had no signs of disease and maintained low viral burdens along with normal CD4+ T-cell counts. This study showed conclusively that viral attenuation can result in long-term nonprogressive infection.

In summary, the long-term survivors we studied had low levels of HIV-1 in the presence of strong virus-specific immune responses combined with some degree of viral attenuation, thereby tipping the balance in favor of the infected host. The level of virus and the degree of immunity observed in these subjects could serve as important guideposts for our therapeutic and prophylactic efforts against AIDS. Ideally, therapies should aim to reduce the burden of HIV-1 to the levels seen in long-term survivors or below, and vaccines should at-

tempt to induce the type of immunity found in these subjects. Most important, perhaps, long-term survivors of HIV-1 infection provide a ray of hope indicating that it is possible to live with the virus for prolonged periods without harm.

We are indebted to the study subjects for their participation, to R. Koup and J. Moore for helpful suggestions, and to W. Chen for the preparation of the figures.

REFERENCES

- Pantaleo G, Graziosi C, Fauci AS. The immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med* 1993;328:327-35.
- Lifson AR, Rutherford GW, Jaffe HW. The natural history of human immunodeficiency virus infection. *J Infect Dis* 1988;158:1360-7.
- Muñoz A, Wang M-C, Bass S, et al. Acquired immunodeficiency syndrome (AIDS)-free time after human immunodeficiency virus type 1 (HIV-1) seroconversion in homosexual men. *Am J Epidemiol* 1989;130:530-9.
- Jason J, Lui K-J, Ragni MV, Hessel NA, Darrow WW. Risk of developing AIDS in HIV-infected cohorts of hemophiliac and homosexual men. *JAMA* 1989;261:725-7.
- Rutherford GW, Lifson AR, Hessel NA, et al. Course of HIV-1 infection in a cohort of homosexual and bisexual men: an 11 year follow up study. *BMJ* 1990;301:1183-8.
- Schrager LK, Young JM, Fowler MG, Mathieson BJ, Vermund SH. Long-term survivors of HIV-1 infection: definitions and research challenges. *AIDS* 1994;8:Suppl 1:S95-S108.
- Buchbinder SP, Katz MH, Hessel NA, O'Malley PM, Holmberg SD. Long-term HIV-1 infection without immunologic progression. *AIDS* 1994;8:1123-8.
- Keet IPM, Krol A, Klein MR, et al. Characteristics of long-term asymptomatic infection with human immunodeficiency virus type 1 in men with normal and low CD4+ cell counts. *J Infect Dis* 1994;169:1236-43.
- Sheppard HW, Lang W, Ascher MS, Vittinghoff E, Winklestein W. The characterization of non-progressors: long-term HIV-1 infection with stable CD4+ T-cell levels. *AIDS* 1993;7:1159-66.
- Learmont J, Tindall B, Evans L, et al. Long-term symptomless HIV-1 infection in recipients of blood products from a single donor. *Lancet* 1992;340:863-7.
- Greenough TC, Somasundaran M, Brettler DB, et al. Normal immune function and inability to isolate virus in culture in an individual with long-term human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses* 1994;10:395-403.
- Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N Engl J Med* 1989;321:1621-5.
- Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N Engl J Med* 1991;324:961-4.
- Connor RI, Mohri H, Cao Y, Ho DD. Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals. *J Virol* 1993;67:1772-7.
- Pachl C, Todd JA, Kern DG, et al. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA (bDNA) signal amplification assay. *J Acquir Immune Defic Syndr* (in press).
- Cao Y, Ho DD, Todd J, et al. Clinical evaluation of branched DNA (bDNA) signal amplification for quantifying HIV-1 in human plasma. *AIDS Res Hum Retroviruses* (in press).
- Fultz T, Todd J, Hamren S, et al. Quantitation of plasma HIV-1 RNA using an ultra-sensitive branched DNA (bDNA) assay. Presented at the 2nd National Conference on Human Retroviruses and Related Infections, Washington, D.C., January 29-February 2, 1995. abstract.
- Simmonds P, Balfe P, Peutherer JF, Ludlam CA, Bishop JO, Leigh Brown AJ. Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers. *J Virol* 1990;64:864-72.
- Koyanagi Y, Miles S, Mitsuyasu RT, Merrill JE, Vinters HV, Chen ISY. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. *Science* 1987;236:819-22.
- Ho DD, McKeating JA, Li XL, et al. Conformational epitope on gp120 important in CD4 binding and human immunodeficiency virus type 1 neutralization identified by human monoclonal antibody. *J Virol* 1991;65:489-93.
- Moore JP, Cao Y, Qin L, et al. Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies. *J Virol* (in press).
- Walker CM, Moody DJ, Stites DP, Levy JA. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. *Science* 1986;234:1563-6.
- Walker CM, Thomson-Honniebier GA, Hsueh FC, Erickson AL, Pan L-Z, Levy JA. CD8+ T cells from HIV-1-infected individuals inhibit acute infection by human and primate immunodeficiency viruses. *Cell Immunol* 1991;137:420-8.

24. Walker CM, Erickson AL, Hsueh FC, Levy JA. Inhibition of human immunodeficiency virus replication in acutely infected CD4+ cells by CD8+ cells involves a noncytotoxic mechanism. *J Virol* 1991;65:5921-7.
25. Kannagi M, Chalifoux LV, Lord CI, Letvin NL. Suppression of simian immunodeficiency virus replication in vitro by CD8+ lymphocytes. *J Immunol* 1988;140:2237-42.
26. Michael NL, Vahey M, Burke DS, Redfield RR. Viral DNA and mRNA expression correlate with the stage of human immunodeficiency virus (HIV) type 1 infection in humans: evidence for viral replication in all stages of HIV disease. *J Virol* 1992;66:310-6.
27. Bagasra O, Hauptman SP, Lischner HW, Sachs M, Pomerantz RJ. Detection of human immunodeficiency virus type 1 provirus in mononuclear cells by in situ polymerase chain reaction. *N Engl J Med* 1992;326:1385-91.
28. Patterson BK, Till M, Otto P, et al. Detection of HIV-1 DNA and messenger RNA in individual cells by PCR-driven in situ hybridization and flow cytometry. *Science* 1993;260:976-9.
29. Cohen J. Jitters jeopardize AIDS vaccine trials. *Science* 1993;262:980-1.
30. Pantaleo G, Menzo S, Vaccarezza M, et al. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. *N Engl J Med* 1995;332:209-16.
31. Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 1993;362:355-8.
32. Embretson J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* 1993;362:359-62.
33. Piatak M Jr, Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* 1993;259:1749-54.
34. Spira AI, Ho DD. Effect of different donor cells on human immunodeficiency virus type 1 replication and selection in vitro. *J Virol* 1995;69:422-9.
35. Williams LM, Cloyd MW. Polymorphic human gene(s) determines differential susceptibility of CD4 lymphocytes to infection by certain HIV-1 isolates. *Virology* 1991;184:723-8.
36. Lifson AR, Buchbinder SP, Sheppard HW, et al. Long-term human immunodeficiency virus infection in asymptomatic homosexual and bisexual men with normal CD4+ lymphocyte counts: immunologic and virologic characteristics. *J Infect Dis* 1991;163:959-65.
37. Jassoy C, Harrer T, Rosenthal T, et al. Human immunodeficiency virus type 1-specific cytotoxic T lymphocytes release gamma interferon, tumor necrosis factor alpha (TNF- α), and TNF- β when they encounter their target antigens. *J Virol* 1993;67:2844-52.
38. Huang Y, Zhang L, Ho DD. Characterization of *nef* sequences in long-term survivors of human immunodeficiency virus type 1 infection. *J Virol* 1995;69:93-100.
39. Kirchhoff F, Greenough TC, Brettler DB, Sullivan JL, Desrosiers RC. Absence of intact *nef* sequences in a long-term survivor with nonprogressive HIV-1 infection. *N Engl J Med* 1995;332:228-32.
40. Kestler HW III, Ringler DJ, Mori K, et al. Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. *Cell* 1991;65:651-62.

Massachusetts Medical Society
Registry on Continuing Medical Education

To obtain information on continuing medical education courses in the New England area, call between 9:00 a.m. and 12:00 noon, Monday through Friday, (617) 893-4610 or in Massachusetts 1-800-322-2303, ext. 1342.