Long-Term Human Immunodeficiency Virus Infection in Asymptomatic Homosexual and Bisexual Men with Normal CD4+ Lymphocyte Counts: Immunologic and Virologic Characteristics

Alan R. Lifson, Susan P. Buchbinder, Haynes W. Sheppard, Alison C. Mawle, Judith C. Wilber,* Mark Stanley, Clyde E. Hart, Nancy A. Hessol, and Scott D. Holmberg

AIDS Program and Microbiology Laboratory, Department of Public Health, San Francisco; Viral and Rickettsial Disease Laboratory, Division of Laboratories, California Department of Health Services, and Pathology Institute, Berkeley; and Divisions of Immunologic, Oncologic and Hematologic Diseases and of HIV/AIDS, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia

From a prospective cohort study, 24 asymptomatic men were identified who had been antibody positive for human immunodeficiency virus (HIV) for at least 5 years (median = 9.1) with CD4+ lymphocyte counts ≥400 cells/mm³. Of these "nonprogressors," 23 (96%) had evidence of HIV infection by either HIV culture or the polymerase chain reaction (PCR) for HIV DNA, although only 1 (4%) had a positive assay for HIV RNA (by PCR) and no one was positive for p24 antigen. Compared with 24 antibody-negative men and 14 men with AIDS, nonprogressors had higher CD8+ counts and lower natural killer cell activity. Nonprogressors had higher β_2-microglobulin levels than did seronegative controls, suggesting some degree of immune system activation. Compared with men with AIDS, nonprogressors seemed to have a stronger antibody response to six different HIV-related proteins but did not differ significantly in neutralizing antibody or antibody-dependent cellular cytotoxic activity.

Natural history studies of human immunodeficiency virus (HIV)–infected individuals indicate that duration of infection is an important determinant of developing AIDS [1–5]. For example, in our prospective cohort study of homosexual and bisexual men, the risk of developing AIDS was relatively low for the first several years, gradually increasing to 54% after 11 years [2]. Among those individuals with long-term infection who had not developed AIDS, some had AIDS-related conditions (such as oral candidiasis or the presence of prolonged constitutional symptoms); for example, 78% of men whose estimated year of seroconversion was between 1977 and 1980 had AIDS or AIDS-related conditions [2]. Others without AIDS or AIDS-related conditions may have a low CD4+ lymphocyte count; in particular, CD4+ lymphocyte counts of ≤200 cells/mm³ are associated with the greatest likelihood of progression to AIDS [3].

Whether all HIV-infected persons will eventually develop AIDS is unknown. However, some men in our study have been seropositive for HIV antibody for 6–10.5 years and remain asymptomatic with normal CD4+ cell counts. We were interested in learning more about these "nonprogressors." In particular, the goal of our study was to learn whether subjects with long-term HIV infection who remained asymptomatic with normal numbers of CD4+ cells were infected with HIV and whether they were unique with respect to either their general immune status or their specific response to HIV infection.

Methods

Study subjects. The San Francisco City Clinic Cohort consists of 6705 homosexual and bisexual men originally recruited during 1978–1980 for studies of the prevalence and incidence of hepatitis B [6]. Unused serum specimens from these original studies were frozen and stored. In our follow-up studies of AIDS and HIV infection [2, 7], we attempted to contact all of these men. We tested current and stored serum samples from those who agreed to participate for HIV antibody. Persons who were negative for HIV antibody or whose date of seroconversion could be well characterized were enrolled in our prospective studies, which include an annual interview, physical examination, and laboratory evaluation. Date of seroconversion was estimated using previously described techniques [2, 7]. We defined AIDS-related conditions as oral candidiasis; hairy leukoplakia; prolonged idiopathic fever, night sweats, or diarrhea; or involuntary weight loss of >4.5 kg in persons not otherwise meeting the revised Centers for Disease Control AIDS surveillance case definition [8].

In the laboratory used at the time this study was done, the normal range for CD4+ cells was 408–1583 cells/mm³. For the purpose of this analysis, we defined a nonprogressor as a study subject with documented seropositivity for HIV antibody for at least 5 years, absence of AIDS or AIDS-related conditions, an absolute CD4+ lym-

Received 25 June 1990; revised 4 December 1990.

Informed consent was obtained from all participants. Guidelines of the US Department of Health and Human Services and the State of California were followed.

Financial support: Centers for Disease Control (agreement U62/CCU900 523-03-5).

Reprints or correspondence (present address): Dr. Alan R. Lifson, Department of Epidemiology and Biostatistics, Box 0560, 1699 HSW, School of Medicine, University of California, San Francisco, CA 94143-0560.

* Present address: Chiron Corp., Emeryville, CA.

The Journal of Infectious Diseases 1991;163:959–965

0022-1899/91/6305-0004$01.00

phocyte count of ≥400 cells/mm^3, no history of taking antiviral drugs such as zidovudine, and absence of other serious medical conditions that may have resulted in immunodeficiency. Between May 1988 and May 1989, we enrolled 24 subjects who met these criteria; 6 were asymptomatic with only generalized lymphadenopathy and 18 had no signs or symptoms of HIV infection. We included men with generalized lymphadenopathy because many prospective studies have shown that among HIV-infected subjects, the presence or absence of lymphadenopathy is not associated with an increased likelihood of progression to AIDS [1, 3, 9].

For comparison, we evaluated two control groups drawn from subjects in our prospective study: men with AIDS and men who were seronegative for HIV antibody. To control for duration of infection, those with AIDS who had documented seropositivity for HIV antibody for ≥5 years were chosen; subjects whose medical condition precluded the drawing of large amounts of blood were excluded. Fourteen were enrolled; seven had a history of at least one opportunistic infection diagnostic of AIDS (usually *Pneumocystis carinii* pneumonia) and seven had Kaposi's sarcoma as their only AIDS-defining illness. The median time between AIDS diagnosis and evaluation in this study was 11 months (mean, 14; range, 3–28). All 14 reported either past or current use of at least one antiviral drug: zidovudine in 13 (in some cases in addition to other therapies) and AL-721, dextran sulfate, and interferon-β in the other. Seronegative men, who had been evaluated for HIV antibody on multiple occasions, were recruited from our study to approximately equal the number of nonprogressors; 24 such were enrolled.

Control subjects were seen during the same time period as cases. All were reevaluated with a physical examination as part of this study, after which fresh blood was drawn for the assays described below.

Laboratory evaluation. To help determine whether detectable virus was present in the nonprogressors, peripheral blood mononuclear cells (PBMC) were cultured for HIV and evaluated for HIV DNA by the polymerase chain reaction (PCR). To help detect virus-related components potentially representing replication, samples were evaluated for HIV RNA by PCR and for p24 antigen. To evaluate general immune status, both nonprogressors and all controls were evaluated for T lymphocyte subsets, β_2 microglobulin, and natural killer cell (NK) activity. To evaluate the antibody response to HIV, all seropositive subjects were evaluated for neutralizing antibody (NA), antibody-dependent cellular cytotoxicity (ADCC) activity, and antibody response to six different core-, polymerase-, and envelope-related proteins.

Antibody. Serum specimens were tested for HIV antibody by ELISA (Organon Teknika, Research Triangle Park, NC); ELISA-reactive specimens were confirmed by the immunofluorescence assay (IFA) [10].

T lymphocyte subsets. Testing was done on cells obtained by the whole blood lysis method (Q-Prep; Coulter Diagnostics, Hialeah, FL) stained using monoclonal antibodies. Percentages of $CD4^+$ and $CD8^+$ cells were determined by flow cytometric procedures using an EPICS profile (Coulter). Absolute numbers of $CD4^+$ and $CD8^+$ cells were calculated, based on total and differential white cell counts and the percentage of lymphocytes with T cell markers.

β_2-microglobulin. Serum concentrations of β_2-microglobulin were measured using a quantitative competitive enzyme immunoassay according to the manufacturer's protocol (Pharmacia Diagnostics, Piscataway, NJ).

NK activity. NK activity was measured by assaying the ability of the subject's PBMC to kill the NK-sensitive cell line K562, using a ^{51}Cr release assay [11]. Fresh PBMC were incubated with [^{51}Cr]-labeled cells at effector-to-target (E:T) ratios of 50:1, 25:1, 12:1, and 6:1. After incubation for 4 h at 37°C in 5% CO_2, 0.1 ml of supernatant fluid was removed from each well and counted in a gamma counter. Spontaneous and maximum release of ^{51}Cr was determined by incubating the medium alone or with 1% Triton X, respectively. Results were expressed as lytic units/10^7 cells, calculated according to the formula of Pross et al. [12].

HIV culture. HIV was isolated by cocultivation of 1×10^6 PBMC from study participants with 1×10^6 phytohemagglutinin (PHA)-stimulated PBMC from healthy seronegative donors. Every week, fresh PHA-stimulated donor PBMC were added to each cell pellet, and supernatant fluids were assayed for p24 antigen. Antigen-negative cultures were kept for 32 days. The presence of virus in culture fluids was confirmed by assay for reverse transcriptase (RT).

PCR for HIV DNA. As previously described, samples were tested in duplicate for HIV DNA using two sets of primer pairs, complementary to two different, conserved *gag* regions of the viral genome: SK38/39 and SK 101/145 [13]. After 30 cycles of amplification, the amplified product was detected by oligomer hybridization using one of two radiolabeled probes: SK19 (corresponding to SK38/39) and SK102 (corresponding to SK101/145).

PCR for HIV RNA. Samples were tested for HIV RNA as previously described [14]. After extraction of RNA and reverse transcription, the complementary DNA product was amplified (35 cycles) and detected using a p32 end-labeled probe (SK19) specific for the *gag* gene region SK38/39.

p24 antigen. The presence of p24 antigen was detected using a commercial sandwich-type enzyme immunoassay that used murine monoclonal antibody (anti-HIV core antigen) coated onto microwell strips (Coulter). Reactive specimens were considered positive if the reaction could be specifically neutralized by ≥50%.

NA. NA titers were evaluated using monolayers of an HIV-sensitive MT-2 cell line and a standard HTLV-III$_B$ strain in individual wells on a microtiter plate [15]. Plates were incubated for 5–6 days, with formation of HIV-induced microplaques. Propidium iodide–stained microplaques were visualized by placing the plate on an ultraviolet light box and counted through a stereomicroscope. Results were expressed as the dilution of serum at which 50% of the plaque-forming units were neutralized (i.e., 50% reduction in plaque count relative to the average count in control wells). Results were interpolated using a curve fit to the data points by nonlinear least squares regression analysis.

Subunit-specific HIV antibodies. Serum specimens were assayed for antibodies to six recombinant DNA–derived antigens. Two proteins (p24 and p55) corresponded to the *gag* region of the viral genome, one (p31/66) to the *pol* region, and three (gp41, NH_2-terminal component of gp120, and COOH-terminal component of gp120) to the *env* region. Testing was done at a serum dilution of 1:100 using an enzyme immunoassay (Antibody Subunit Quantitation; Beckman Instruments, Diagnostic Systems Group, Brea, CA) with a goat anti-human IgG conjugated to alkaline phosphatase [16]. Results were expressed as optical density (OD) readings.

ADCC activity. As previously described, ADCC activity was evaluated using heat-inactivated sera incubated with a ^{51}Cr-labeled cell line (C5D7) chronically infected with HIV [17]. Sera from each donor were assayed at a final concentration of 1:10 and 1:100. Fresh PBMC from healthy volunteers were used as effector cells at E:T

Table 1. T lymphocyte subsets, β_2-microglobulin, and natural killer cell (NK) activity for nonprogressors, men with AIDS, and seronegative controls.

Parameter	Nonprogressors (n = 24)	AIDS (n = 14)	Seronegatives (n = 24)	P, nonprogressors vs. AIDS	P, nonprogressors vs. seronegative
$CD4^+$ count, cells/mm^3					
Median (mean)	641 (746)	58 (97)	962 (960)	<.001	.01
Range	422–1427	11–516	549–1666		
$CD8^+$ count, cells/mm^3					
Median (mean)	1036 (1114)	584 (676)	552 (616)	.009	<.001
Range	706–2137	98–1594	319–1181		
$CD4^+$:$CD8^+$ ratio					
Median (mean)	0.62 (0.72)	0.07 (0.14)	1.60 (1.65)	<.001	<.001
Range	0.29–1.51	0.03–0.52	0.91–3.05		
β_2-microglobulin, mg/l					
Median (mean)	2.47 (2.73)	3.81 (4.10)	1.47 (1.61)	.002	<.001
Range	1.46–5.17	2.44–9.01	1.00–4.82		
NK, lytic units/10^7 cells					
Median (mean)	27 (46.8)	87 (119.0)	89 (102.2)	.03	.02
Range	3–150	18–556	10–556		

ratios of 50:1, 25:1, 12:1, and 6:1. Cells were incubated and harvested as for NK activity. In all assays, a control without serum was run to determine the level of nonspecific killing of the target cell. ADCC was defined as the percentage of antibody-specific killing minus nonspecific killing. The percentage killing was defined as [cpm(exper) − cpm(spon)] × 100/[cpm(max) − cpm(spon)], where cpm(exper) was the experimental counts per minute, cpm(spon) was the spontaneous release, and cpm(max) was the maximum release.

Statistics. Normally distributed continuous variables were compared between two groups using Student's t test and between three groups using one-way analysis of variance. Continuous variables not normally distributed were compared using the Wilcoxon rank sum test. Discrete variables were compared using either the χ^2 or Fisher's exact test. Correlation between two continuous variables was evaluated using the Pearson correlation coefficient. Missing data were excluded from analyses.

Results

Study participants. Similar to the entire cohort, nonprogressors had a mean age of 36 years (range, 30–46) and were predominantly (96%) white. Nonprogressors were younger than seronegative men (mean, 41 years; $P < .001$) and did not significantly differ with respect to race/ethnicity; nonprogressors and men with AIDS did not significantly differ with respect to either age or race/ethnicity. Nonprogressors had a mean of 8.7 years (median, 9.1; range, 6.3–10.5) between their estimated date of HIV seroconversion and evaluation in this study. This time did not significantly differ from that of men with AIDS (mean, 9.1 years [$P > .10$]; median, 8.9; range, 7.4–10.5).

Viral studies. Of the 24 nonprogressors, 23 (96%) had a positive PCR assay for HIV DNA, and 18 (75%) had a positive HIV culture. The subject negative for HIV DNA by PCR also had a negative HIV culture. None of the nonprogressors had a positive assay for p24 antigen. Of 23 evaluated for HIV RNA by PCR, only 1 (4%) had a positive assay; results were indeterminate for another.

Measures of general immune status. Results of testing for $CD4^+$ lymphocyte subsets, $CD8^+$ lymphocyte subsets, $CD4^+$:$CD8^+$ ratios, β_2-microglobulin, and NK activity are summarized in table 1. Overall, the three groups were significantly different with respect to all five assays ($P < .01$ by Wilcoxon rank sum test).

When data were analyzed for all cases and controls, $CD4^+$ cell count was inversely correlated with β_2-microglobulin levels ($r = -.571$, $P < .001$), and $CD8^+$ cell counts were inversely correlated with NK activity ($r = -.352$, $P = .009$). $CD4^+$ cell count was not significantly correlated with $CD8^+$ cell count or NK activity; β_2-microglobulin levels were not significantly correlated with $CD8^+$ cell count or NK activity ($P > .10$). When data were analyzed for just nonprogressors, β_2-microglobulin levels were significantly associated with $CD8^+$ cell count ($r = .481$, $P = .017$); the correlation between β_2-microglobulin and $CD4^+$ cell count ($r = -.355$, $P = .089$) or NK activity ($r = -.427$, $P = .061$) was of borderline significance.

Nonprogressors seemed relatively stable with respect to $CD4^+$ cell count. As noted above, the analysis reported in table 1 was conducted between mid-1988 and mid-1989. Between 1988 and 1990, each nonprogressor had at least three $CD4^+$ lymphocyte determinations (median, 5), including at least one count after the testing reported in table 1. The mean $CD4^+$ cell count for nonprogressors in 1988 (714 cells/mm^3) did not significantly differ from that in 1990 (734 cells/mm^3; $P > .10$).

The seven subjects with a history of at least one AIDS-

defining opportunistic infection had a lower $CD4^+$ lymphocyte count than the seven with only Kaposi's sarcoma (median, 90 vs. 41 cells/mm^3), although these differences were not statistically different ($P > .10$) in part because of small numbers and limited power.

Virus-specific antibody studies. Fifty percent plaque neutralization occurred at a median dilution of 1:990 for nonprogressors and 1:618 for men with AIDS ($P > .10$, Wilcoxon rank sum test). With respect to ADCC activity, nonprogressors and those with AIDS did not significantly differ at any E:T ratio with a serum dilution of either 1:10 or 1:100 ($P > .10$, Wilcoxon rank sum test). For example, median ADCC results for nonprogressors at a serum dilution of 1:10 (expressed as percentage lysis) were 18% at an E:T ratio of 50:1, 19.5% at 25:1, 24% at 12:1, and 20% at 6:1; corresponding results for men with AIDS were 19.5% at an E:T of 50:1, 24% at 25:1, 20% at 12:1, and 14% at 6:1.

As shown in table 2, for six different antibodies to the *gag*, *pol*, and *env* regions of the viral genome, the OD reading was significantly higher for nonprogressors than for men with AIDS. OD readings for the p24, p31/66, p55, gp41, and COOH-terminal region of gp120 were highly correlated ($r > .70$ for all comparisons, $P < .001$); OD readings for the NH_2-terminal region of gp120 were less correlated with results for other antibodies ($.39 \leq r \leq .47$), although these correlations were still highly significant ($P \leq .002$).

Although the lower limit of normal for $CD4^+$ cells at the time of this study was ~400 cells/mm^3, we repeated our analysis for 19 nonprogressors with ≥500 cells/mm^3. Compared with subjects with AIDS, nonprogressors still had higher $CD4^+$ and $CD8^+$ cell counts, as well as a higher $CD4^+$:$CD8^+$ ratio, a lower β_2-microglobulin level, and higher OD readings for all six antibodies ($P \leq .05$ for all comparisons); the groups did not differ with respect to NK activity, NA titers, or ADCC activity.

Table 2. Subunit-specific antibodies for nonprogressors and men with AIDS.

Antibody	Nonprogressors ($n = 24$)	AIDS ($n = 14$)	P
p24			
Median (mean)	2.9 (2.7)	0.4 (1.1)	<.001
Range	0.8–3.8	0.05–3.4	
p31/66			
Median (mean)	3.2 (2.7)	1.4 (1.5)	.001
Range	0.5–4.0	0.1–3.2	
p41			
Median (mean)	3.4 (3.4)	3.2 (3.1)	.02
Range	2.8–3.7	1.6–3.7	
p55			
Median (mean)	3.4 (3.3)	1.9 (1.8)	<.001
Range	1.5–4.0	0.1–3.5	
gp120, NH_2-terminal			
Median (mean)	0.4 (0.6)	0.2 (0.2)	.001
Range	0.1–3.1	0.03–0.4	
gp120, COOH-terminal			
Median (mean)	1.9 (1.8)	0.5 (0.7)	.001
Range	0.3–3.1	0.03–2.3	

NOTE. Data are optical density readings.

Discussion

The purpose of this study was to characterize the immune system and response to HIV in a group of men who were seropositive for up to 10.5 years without developing AIDS, AIDS-related conditions, or low $CD4^+$ cell counts. In many other studies of HIV-infected subjects, date of seroconversion to HIV was unknown. Since asymptomatic subjects in these other studies may have been infected for less time than subjects with AIDS, differences between the groups could reflect duration of infection. In this study, nonprogressors and subjects with AIDS had very similar lengths of time from seroconversion to evaluation.

Our results indicate that asymptomatic men seropositive for a median of 9 years were still infected with HIV. Most nonprogressors had a positive HIV culture, and all but one had HIV DNA detected by PCR. Previous studies from our cohort using HIV culture also have identified persistent infection in apparently healthy men [18]; additional studies using PCR for HIV DNA have identified viral sequences in asymptomatic subjects seropositive for ≥7 years [13].

After initial infection with HIV, provirus may integrate with establishment of latency [19–21]. Alternatively, viral replication may result that is either low-level or more productive; immunodeficiency may be more likely to occur in the setting of productive viral infection. Because they reflect viral replication and expression, we evaluated samples for p24 antigen and HIV RNA. Only one nonprogressor had a positive PCR result for HIV RNA, and none had a positive test for p24 antigen.

β_2-microglobulin is a protein present as a subunit of the class I major histocompatibility complex on the surface of many somatic cells, including T and B lymphocytes and macrophages [3, 22]; increased levels of β_2-microglobulin have been associated with stimulation of lymphoid cells [23] and have been found to be a predictor of progression to AIDS [3]. Our finding of higher levels of β_2-microglobulin in nonprogressors compared with seronegative controls suggests some degree of immune system activation in those HIV-infected but asymptomatic. Whether this activation, if present, represents a protective or harmful process is uncertain. Activation may reflect an immunologic mechanism helping to inhibit viral replication. On the other hand, mild elevations of β_2-microglobulin may reflect low-level viral replication and stimulation of the immune system. According to one hypothesis, chronic immune system activation (possibly through the interaction of the HIV envelope glycoprotein gp120 with the $CD4^+$ receptor) may actually promote $CD4^+$ cell depletion and immunodeficiency [24, 25].

A unique feature of nonprogressors compared with both seronegative controls and men with AIDS was a significantly

higher $CD8^+$ cell count. Studies evaluating the prognostic significance of the $CD8^+$ cell count have suggested different conclusions. For example, one study found an elevated $CD8^+$ cell count to be significantly associated with subsequent development of AIDS and a steeper decline in $CD4^+$ cells [9, 26], while other studies have not suggested such an association [27]. The different findings of our study may in part reflect the fact that HIV-positive individuals in other studies have a range of $CD4^+$ cell counts and have been infected for various periods of time.

Other studies of healthy HIV-infected homosexual men have demonstrated the presence of $CD8^+$ lymphocytes that inhibit HIV replication, possibly through production of a lymphokine [28, 29]. Preliminary studies of our nonprogressors suggest that these subjects had a greater suppressor cell activity than did those with AIDS [30]. Another analysis using participants from our cohort study also found that asymptomatic HIV-infected men had higher numbers of $CD8^+$ cells than did seronegative controls [31]; using monoclonal antibodies to define $CD8^+$ cell subsets, seropositives had higher levels of $Leu2^+$ 15^- (cytotoxic T cells) [32]. Additional preliminary data from our nonprogressors also identified an elevated cytotoxic lymphocyte response to p24 core and gp120 envelope antigens over that in subjects with AIDS [30, 33]. Our findings and these other data suggest that the $CD8^+$ suppressor or cytotoxic cell response may play an important role in helping control viral replication and the development of immunodeficiency.

Another unique feature of nonprogressors compared with both seronegative controls and men with AIDS was a lower NK activity. In contrast to our findings, several other studies have suggested that NK activity is lower in those with AIDS than in those HIV-infected but asymptomatic [34, 35]. Additional preliminary data from this study suggest that the lower NK activity in our nonprogressors cannot be explained by a smaller number of cells bearing NK-associated cell surface markers, such CD16(Leu11b) [33]; other studies also have found that a decrease in NK activity did not correlate with altered numbers of cells bearing CD16 (NK) markers [34]. Although NK activity has been shown to decrease with increasing length of infection [34], the fact that nonprogressors and men with AIDS had a similar duration of seropositivity makes this a less likely explanation for our results.

Since we identified an inverse correlation between NK activity and $CD8^+$ cell count, a possible explanation for our findings is that the lower NK activity in our nonprogressors is related to an increased $CD8^+$ cell-mediated suppressor activity. A second hypothesis is that NK activity in our subjects with AIDS was higher than expected due to the benefits of antiviral therapy. Both hypotheses deserve additional investigation. In any event, our analysis did not identify a beneficial role for increased NK activity in nonprogressors. Although NK cells are important in immune surveillance [36], they may be less helpful in controlling HIV disease progression once chronic HIV infection has occurred.

Studies correlating HIV-specific ADCC activity with clinical status have reached different conclusions [37–39]. For example, one study found a significantly higher ADCC activity in healthy HIV-seropositive men than in subjects with AIDS [37]; another study, however, found that within several months of seroconversion, serum ADCC activity stabilized and was not related to HIV disease stage or progression [38]. Our analysis also does not support a beneficial role for ADCC activity in preventing HIV disease progression. Additional prospective studies may help further define the association of ADCC activity with duration of infection and HIV disease progression.

We also found that nonprogressors did not have stronger NA titers to a standard $HTLV\text{-}III_B$ strain than did subjects with AIDS. Our results are consistent with other studies showing that NA titers to standard strains of virus have correlated poorly with clinical status [40]. In addition, an HIV-infected subject's neutralizing activity may vary against different HIV strains [41–43]. Although HIV-infected subjects may develop isolate-specific NA to autologous strains, one study of HIV-infected subjects followed over time identified the emergence of viral variants with markedly reduced sensitivity to neutralization by the host's own sera [41].

Finally, we measured antibodies to six different HIV-related core, polymerase, and envelope proteins. Although we did not quantify antibody titers for these proteins, we did evaluate the ELISA OD as a reflection of the strength of antibody response. Absorbance values may not be linearly proportional to antibody titers, particularly with high antibody activity; however, studies of the ELISA used in other settings have indicated that absorbance assays at a single serum dilution may provide a semiquantitative assessment of antibody activity [44]. In studies evaluating enzyme immunoassays to HIV, the degree of ELISA reactivity (expressed as the ratio of the test absorbance value to the cutoff) was significantly associated with the likelihood of obtaining a positive result on other diagnostic tests for HIV, such as culture and Western blot [45].

For all six antibodies we measured, the OD reading was stronger for nonprogressors than for men with AIDS. Whether antibody to particular components of HIV confers protection against either HIV infection or progression of HIV disease remains uncertain. Declining titers of antibody to p24 may be associated with an increased progression to AIDS, but this may be secondary to a rise in p24 antigen and formation of antigen-antibody complexes [46, 47]. A major question raised by our findings is whether the lower OD readings for those with AIDS reflect a determinant or consequence of immunodeficiency. The lower OD reading for all proteins is more consistent with a general immune impairment seen in persons with AIDS. However, we cannot exclude the possibility that some or all of these antibodies were initially depressed

in people who subsequently developed immunodeficiency. To investigate this possibility, we plan to test specimens obtained before the development of AIDS and prospectively evaluate nonprogressors with serial antibody determinations. Recent studies have also suggested that targets for neutralizing antibodies may be related to specific epitopes of gp120 or gp41 [48], such as the principal neutralizing epitope of HIV, situated within the third hypervariable region, the V3 loop of gp120 [49, 50]. Additional studies of our nonprogressors using antibodies directed against specific epitopes may also help to further clarify these issues.

Our findings concerning men with AIDS may differ from previous studies for two reasons. First, almost all men with AIDS reported past or current use of zidovudine. Whether zidovudine may have altered the immune response by suppressing viral replication or through some other mechanism cannot be answered in this study and is best addressed by prospectively evaluating individuals entering clinical trials or initiating therapy for medical indications. Second, those with AIDS in this study had a range of clinical manifestations and $CD4^+$ cell counts. This allowed us to evaluate men with varying degrees of immunodeficiency and clinical presentations rather than only those with the most severe immunologic derangements.

In this analysis, we primarily focused on immunologic characteristics and the host response to HIV infection. These factors may be only some of the many determinants of HIV disease progression. Other relevant factors may include genetic background (especially human leukocyte antigen type) [51, 52], age [5], and characteristics of the viral strain or strains infecting an individual (including cellular host range and pathogenicity) [21, 53, 54]; laboratory studies have also suggested that other infectious agents may serve as cofactors [55, 56]. Most likely multiple factors, acting in a complex interrelationship, modulate the likelihood of developing specific manifestations of HIV disease.

Whether these asymptomatic men with long-term HIV infection and normal $CD4^+$ cell counts will ultimately develop AIDS or other evidence of immunodeficiency is unknown. Our results suggest that certain immunologic parameters, such an elevated $CD8^+$ cell count, may be associated with delayed progression to AIDS, while other responses, such as NK and ADCC activity, do not seem protective. Additional prospective and retrospective studies will help further define the role of $CD8^+$ cells, antibody response to specific components of the virus, and other immunologic and host-specific parameters in suppressing viral replication and preventing HIV disease progression. The study of HIV-infected subjects who have not developed low $CD4^+$ cell counts or evidence of HIV disease is important not only for understanding the pathogenesis of HIV infection and determining prognosis but also for the development of effective vaccines and therapies.

Acknowledgment

We thank the following for their assistance: Lowell Barnhart, Lyn Cannon, Carl Hanson, Bob Kono, Brian Louie, Paul O'Malley, Michael Piccini, Mitchell Ridgeway, George Rutherford, and Robert Wade.

References

1. Lifson AR, Rutherford GW, Jaffe HW. The natural history of human immunodeficiency virus infection. J Infect Dis **1988**;158:1360–7.
2. Rutherford GW, Lifson AR, Hessol 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.
3. Moss AR, Bacchetti P, Osmond D, et al. Seropositivity for HIV and the development of AIDS or AIDS-related condition: three-year follow-up of the San Francisco General Hospital cohort. BMJ **1988**;296: 745–50.
4. Ward JW, Bush TJ, Perkins HA, et al. The natural history of transfusion-associated infection with human immunodeficiency virus: factors influencing the rate of progression to disease. N Engl J Med **1989**; 321:947–52.
5. Goedert JJ, Kessler CM, Aledort LM, et al. A prospective study of human immunodeficiency virus type 1 infection and the development of AIDS in subjects with hemophilia. N Engl J Med **1989**;321:1141–8.
6. Hadler SC, Francis DP, Maynard JE, et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med **1986**;315:209–14.
7. Hessol NA, Lifson AR, O'Malley PM, Doll LS, Jaffe HW, Rutherford GW. Prevalence, incidence and progression of human immunodeficiency virus infection in homosexual and bisexual men in hepatitis B vaccine trials, 1978–1988. Am J Epidemiol **1989**;130:1167–75.
8. Centers for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR **1987**;36(suppl 1S):1S–15.
9. Polk BF, Fox R, Brookmeyer R, et al. Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men. N Engl J Med **1987**;316:61–6.
10. Gallo D, Diggs JL, Shell GR, Dailey PJ, Hoffman MN, Riggs JL. Comparison of detection of antibody to the acquired immune deficiency syndrome virus by enzyme immunoassay, immunofluorescence, and western blot methods. J Clin Microbiol **1986**;23:1049–51.
11. Timonen T, Ortaldo JR, Herberman RB. Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. J Exp Med **1981**;153:569–82.
12. Pross HF, Baines MG, Rubin P, Shragge P, Patterson MS. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. J Clin Immunol **1981**;1:51–63.
13. Lifson AR, Stanley M, Pane J, et al. Detection of human immunodeficiency virus DNA using the polymerase chain reaction in a well-characterized group of homosexual and bisexual men. J Infect Dis **1990**;161:436–9.
14. Hart C, Schochetman G, Spira T, et al. Direct detection of HIV RNA expression in seropositive persons. Lancet **1988**;2:596–9.
15. Hanson CV, Crawford-Miksza L, Sheppard HW. Application of a rapid microplaque assay for determination of human immunodeficiency virus neutralizing antibody titers. J Clin Microbiol **1990**;28:2030–4.
16. Ng VL, Chiang CS, Debouck C, McGrath MS, Grove T, Mills J. Reliable confirmation of antibodies to human immunodeficiency virus type 1 (HIV-1) with an enzyme-linked immunoassay using recombinant

antigens derived from the HIV-1 *gag*, *pol* and *env* genes. J Clin Microbiol **1989**;27:977–82.
17. Scheppler JA, Mawle AC, McDougal JS. Evaluation of target cells for HIV-1 specific antibody-dependent cellular cytotoxicity assays. J Immunol Methods **1988**;115:247–53.
18. Jaffe HW, Feorino PM, Darrow WW, et al. Persistent infection with human T-lymphotropic virus type III/lymphadenopathy-associated virus in apparently healthy homosexual men. Ann Intern Med **1985**; 102:627–8.
19. Folks T, Powell DM, Lightfoote MM, Benn S, Martin MA, Fauci AS. Induction of HTLV-III/LAV from a non-virus-producing T-cell line: implications for latency. Science **1986**;231:600–2.
20. Fauci AS. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science **1988**;239:617–22.
21. Levy JA. Human immunodeficiency viruses and the pathogenesis of AIDS. JAMA **1989**;261:2997–3006.
22. Cresswell P, Springer T, Strominger JL, Turner MJ, Grey HM, Kubo RT. Immunological identity of the small subunit of HLA-A antigens and beta2-microglobulin and its turnover on the cell membrane. Proc Natl Acad Sci USA **1974**;71:2123–7.
23. Kin K, Kasahara T, Itoh Y, Sakurabayashi I, Kawai T, Morita M. Beta2-microglobulin production by highly purified human T and B lymphocytes in cell culture stimulated with various mitogens. Immunology **1979**;36:47–54.
24. Ascher MS, Sheppard HW. AIDS as immune system activation: a model for pathogenesis. Clin Exp Immunol **1988**;73:165–7.
25. Ascher MS, Sheppard HW. AIDS as immune system activation. II. The panergic imnesia hypothesis. J Acquir Immune Defic Syndr **1990**; 3:177–91.
26. Munoz A, Carey V, Saah AJ, et al. Predictors of decline in CD4 lymphocytes in a cohort of homosexual men infected with human immunodeficiency virus. J AIDS **1988**;1:396–404.
27. Goedert JJ, Biggar RJ, Melbye M, et al. Effect of T4 count and cofactors on the incidence of AIDS in homosexual men infected with human immunodeficiency virus. JAMA **1987**;257:331–4.
28. 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.
29. Walker CM, Levy JA. A diffusible lymphokine produced by $CD8^+$ T lymphocytes suppresses HIV replication. Immunology **1989**;66: 628–30.
30. Buchbinder SP, Mawle AC, Mackewicz CE, et al. Cellular antiviral responses may influence non-progression in HIV infected individuals [abstract 304]. In: Program and abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy (Atlanta). Washington, DC: American Society for Microbiology, **1990**.
31. Nicholson JKA, McDougal JS, Jaffe HW, et al. Exposure to human T-lymphotropic virus type III/lymphadenopathy-associated virus and immunologic abnormalities in asymptomatic homosexual men. Ann Intern Med **1985**;103:37–42.
32. Nicholson JKA, Echenberg DF, Jones BM, Jaffe HW, Feorino PM, McDougal JS. T-cytotoxic/suppressor cell phenotypes in a group of asymptomatic homosexual men with and without exposure to HTLV-III/LAV. Clin Immunol Immunopathol **1986**;40:505–14.
33. Mawle AC, Ridgeway MR, Kieny MP, Lifson AR. CTL response to p24(*gag*) in long-term asymptomatic seropositive individuals infected with HIV-1 [abstract S.A.254]. In: Final program and abstracts, VI International Conference on AIDS (San Francisco). **1990**.
34. Cai Q, Huang XL, Rappocciolo G, Rinaldo CR. Natural killer cell responses in homosexual men with early HIV infection. J Acquir Immune Defic Syndr **1990**;3:669–76.
35. Voth R, Rossol S, Graff E, et al. Natural killer cell activity as a prognostic parameter in the progression to AIDS. J Infect Dis **1988**; 157:851–2.
36. Ritz J. The role of natural killer cells in immune surveillance. N Engl J Med **1989**;320:1748–9.
37. Rook AH, Lane HC, Folks T, McCoy S, Alter H, Fauci AS. Sera from HLTV-III/LAV antibody-positive individuals mediate antibody-dependent cellular cytotoxicity against HTLV-III/LAV infected T-cells. J Immunol **1987**;138:1064–7.
38. Ojo-Amaize E, Nishanian PG, Heitjan DF, et al. Serum and effector-cell antibody-dependent cellular cytotoxicity (ADCC) activity remains high during human immunodeficiency virus (HIV) disease progression. J Clin Immunol **1989**;9:454–61.
39. Ljunggren K, Bottiger B, Biberfeld G, Karlson A, Fenyo E, Jondal M. Antibody-dependent cellular cytotoxicity inducing antibodies against human immunodeficiency virus; presence at different clinical stages. J Immunol **1987**;139:2263–7.
40. Prince AM, Pascual D, Kosolapov LB, Kurokawa D, Baker L, Rubinstein P. Prevalence, clinical significance and strain specificity of neutralizing antibody to the human immunodeficiency virus. J Infect Dis **1987**;156:268–72.
41. Albert J, Abrahamsson B, Nagy K, et al. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. AIDS **1990**;4:107–12.
42. Looney DJ, Fisher AG, Putney SD, et al. Type-restricted neutralization of molecular clones of human immunodeficiency virus. Science **1988**;241:357–9.
43. Tremblay M, Wainberg MA. Neutralization of multiple HIV-1 isolates from a single subject by autologous sequential sera. J Infect Dis **1990**;162:735–7.
44. De Savigny D, Voller A. The communication of ELISA data from laboratory to clinician. J Immunoassay **1980**;1:105–28.
45. Ward JW, Grindon AJ, Feorino PM, Schable C, Parvin M, Allen JR. Laboratory and epidemiologic evaluation of an enzyme immunoassay for antibodies to HTLV-III. JAMA **1986**;256:357–61.
46. Pedersen C, Nielsen CM, Vestergaard BF, Gerstoft J, Krogsgaard K, Nielsen JO. Temporal relation of antigenaemia and loss of antibodies to core antigens to development of clinical disease in HIV infection. BMJ **1987**;295:567–9.
47. Lange J, Goudsmit J. Decline of antibody reactivity to HIV core protein secondary to increased production of HIV antigen. Lancet **1987**;1:448.
48. Bolognesi DP. HIV antibodies and vaccine design. AIDS **1989**;3(suppl 1): S111–8.
49. LaRosa GJ, Davide JP, Weinhold K, et al. Conserved sequence and structural elements in the HIV-1 principal neutralizing determinant. Science **1990**;249:932–5.
50. Goudsmit J, Kuiken CL, Nara PL. Linear versus conformational variation of V3 neutralization domains of HIV-1 during experimental and natural infection. AIDS **1989**;3(suppl 1):S119–23.
51. Jeannet M, Sztajzel R, Carpentier N, Hirschel B, Tiercy JM. HLA antigens are risk factors for development of AIDS. J Acquir Immune Defic Syndr **1989**;2:28–32.
52. Kaslow RA, Duquesnoy R, VanRaden M, et al. A1,Cw7, B8,DR3 HLA antigen combination associated with rapid decline of T-helper lymphocytes in HIV-1 infection. Lancet **1990**;335:927–30.
53. Cheng-Mayer C, Seto D, Tateno M, Levy JA. Biologic features of HIV-1 that correlate with virulence in the host. Science **1988**;240:80–2.
54. Tersmette M, Lange JMA, DeGoede REY, et al. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet **1989**;1:983–5.
55. Laurence J. Molecular interactions among herpesvirus and human immunodeficiency viruses. J Infect Dis **1990**;162:338–46.
56. Wright K. Mycoplasmas in the AIDS spotlight. Science **1990**;248:682–3.