Low HIV-1 proviral DNA burden detected by negative polymerase chain reaction in seropositive individuals correlates with slower disease progression

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During 1989, 316 members of a cohort of homosexual men were tested for HIV-specific DNA by the polymerase chain reaction (PCR) using a pair of *gag*-region primers. Of 125 HIV-seronegative subjects, 123 (98.4%) were PCR-negative while 158 (82.7%) of 191 HIV-seropositive subjects were PCR-positive. Fewer of the 33 subjects who were seropositive and PCR-negative were at Centers for Disease Control (CDC) stage IV than the seropositive, PCR-positive subjects (6 versus 25%; $P = 0.030$). The seropositive, PCR-negative group had higher median CD4 counts (640 versus 490 $\times 10^6$ cells/l; $P = 0.006$), higher CD4 : CD8 ratios (0.92 versus 0.64; $P = 0.004$), lower immunoglobulin (Ig) G levels (1290 versus 1645 mg/dl; $P = 0.002$), lower IgA levels (168 versus 251 mg/dl; $P < 0.001$), and lower C1q binding activity (8 versus 14%; $P = 0.010$) than the seropositive, PCR-positive subjects. The median rate of CD4 cell decline in the 3 years preceding the PCR sample was less marked in the seropositive, PCR-negative group than the seropositive, PCR-positive group (-58 versus -77 $\times 10^6$ cells/l per year; $P = 0.028$). To control for duration of infection, we restricted the analysis to the subgroups of 11 seropositive, PCR-negative subjects and 34 seropositive, PCR-positive subjects who had seroconverted earlier in the cohort study. Both subgroups had similar durations of infection, yet the same pattern of differences persisted. Moreover, despite a median of 51 months since seroconversion, the seropositive, PCR-negative subgroup had CD4 counts and antecedent rates of CD4 cell decline that were similar to those of the 123 seronegative, PCR-negative homosexual controls who were not infected with HIV. We conclude that PCR can identify a group of HIV-infected people with very low levels of proviral DNA who demonstrate slower progression of the effects of HIV.

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Introduction

The polymerase chain reaction (PCR) is a powerful tool for the detection of low numbers of nucleic acid targets of interest [1]. The method has been used recently to detect proviral DNA in HIV-1 infection [2]. PCR has proved useful in ascertaining HIV infection prior to seroconversion [3]; determining the HIV infection status of newborns [4]; differentiating active infection from seroconversion due to administration of HIV-contaminated blood products [5], and monitoring HIV viral load during antiviral therapy [6]. PCR appears to be a better marker

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of disease progression than the detection of p24 antigen in HIV-seropositive patients [7]. Although HIV may be isolated from peripheral-blood lymphocytes (PBL) and plasma in 97% of antibody-positive individuals [8] with virus titers dependent upon disease stage [9], virus isolation is expensive and cumbersome, requiring large blood volumes and taking up to 28 days or more.

The natural history of HIV infection has been characterized into three phases [10]: a short-term, acute phase; a chronic, latent phase; and the final crisis phase corresponding to severe immune dysfunction and AIDS. The factors responsible for the maintenance and duration of the chronic phase remain the subject of considerable interest. A number of markers have been shown to identify subjects with a more rapid rate of progression through the chronic phase to AIDS [11]. We have carried out PCR and immunological testing on 316 subjects within a long-standing cohort of homosexual men. We report here on a subgroup of HIV-antibody-positive, PCR-negative homosexual men who show evidence of a decreased susceptibility to the effects of HIV infection.

Subjects and methods

Subjects

As previously described [12], the Vancouver Lymphadenopathy–AIDS Study (VLAS) is an ongoing prospective study of homosexual men. During the period November 1982 to February 1984, a total of 729 subjects were recruited from six general practices in central Vancouver. Two more practices were later added and 271 subjects were enrolled from October 1986 to December 1987. Initially, follow-up visits occurred approximately every 6 months; since October 1986, however, subjects have been seen on an annual basis. At each visit, subjects completed a questionnaire, underwent a complete physical examination and were classified using the Centers of Disease Control (CDC) staging criteria [13]. At the same time, blood samples were drawn for laboratory and HIV-antibody testing. Laboratory tests included a complete blood cell count, differential, and immunoglobulin levels. Immune complex activity was determined by iodine-125-labelled C1q binding according to the method of Zubler *et al.* [14]. Lymphocyte subset analyses were carried out using a Coulter EPICS-C flow cytometer (Coulter Electronics Inc., Haileah, Florida, USA). According to the study protocol, subjects are followed only until they develop an AIDS-defining illness.

Subjects in the cohort are termed seroprevalent (SP) if they were already seropositive at the time of their enrolment visit, and seroincident (SI) if they seroconverted under study. For the SI group, the estimated point of seroconversion is taken to be the mid-point between the last negative and first positive HIV-antibody test.

Because of logistical considerations involving the shipping and testing of specimens, the present investigation involving PCR was restricted to subjects in the cohort who visited the laboratory (on Tuesdays or Wednesdays) during the period February through May and July through December 1989. For convenience, this is referred to as the PCR visit.

Polymerase chain reaction

At the PCR visit, a whole blood sample was collected in EDTA. A 2 ml aliquot was shipped via an overnight courier service at ambient temperature to the Federal Centre for AIDS laboratory in Ottawa for PCR analyses. Lymphocytes were separated by Ficoll-Hypaque gradient and adjusted to 6×10^6 cells/ml prior to lysis (by a buffer containing 10 mmol/1 tris-HCl, pH 8.3, 6 mmol/l $MgC1_2$, 50 mmol/l KCl, 0.45% NP40, 0.45% Tween 20, 1 mg/ml gelatin and 60 µg/ml proteinase K) [15]. This DNA preparation was incubated for 1 h at 56°C then at 95°C for 10 min. PCR was carried out on 25 µl of this cell lysate (corresponding to 1 µg of genomic DNA) as previously described [4]. Briefly, target DNA was subjected to 35 cycles of denaturation, primer annealing and primer extension in the presence of 3.0 units of AmpliTaq (Perkin Elmer Cetus, Norwalk, Connecticut, USA), 200 µmol/l of each of the four nucleotide triphosphates, and 50 pmol of each of two primer pairs. Primer pairs used were SK38/39 and SK68/69 (Synthetic Genetics, San Diego, California, USA), corresponding to a conserved region of the *gag* (coding for p24) and *env* (coding for the gp41 transmembrane protein) genes, respectively. Subsequently, solution hybridizations were performed with ^{32}P-5′-end-labelling on SK19 or SK70 oligonucleotide probes for the SK38/39 and SK68/69 amplification products. Confirmation of the specificity of the amplification reaction was verified by the use of restriction enzyme digestion of the target-probe hybridization products yielding specific diagnostic fragments. Samples were tested in duplicate with each primer/probe combination. Parallel amplifications and hybridizations were performed with DQα primers and probes (Synthetic Genetics) to ensure that integrity of the original DNA preparation was adequate for HIV PCR analysis [16]. Positive controls included serially diluted plasmid DNA (200 to 6 molecules), a gift from Dr Chin-Yiu Ou (Centers for Disease Control, Atlanta, Georgia, USA), and lymphocyte lysate from a symptomatic HIV-antibody-positive person. Negative controls consisted of DNA from an asymptomatic seronegative donor and DNA-free reaction mixture. Positive PCRs were those giving intensities on radiographs greater than or equal to the 25-molecule standard, negative PCRs were those showing no probe–target duplexes (or bands less than the six molecule standard) with both primer/probe sets, and indeterminates were those yielding intensities near the cut-off. People performing PCR were blinded from the immunological, clinical and HIV-antibody status of individuals in the cohort.

HIV serology testing

Antibody status of sera was ascertained at the Federal Center for AIDS laboratory in Ottawa by enzyme immunoassay (EIA) using the Vironostika anti-HTLV-III kit (Organon Teknika Inc., Scarborough, Ontario, Canada) and/or Recombigen-HIV EIA ENV + GAG kit (Cambridge

BioScience Corp., Worcester, Massachusetts, USA). Positive EIA tests were confirmed by the HIV Western Blot kit (Du Pont Co., Wilmington, Delaware, USA). Western blot positivity required reactivity to two *env* bands with or without reactivity to *pol* gene products.

Statistical analysis

Coded PCR results were returned to the data centre for comparison with HIV serology and other study variables. Statistical analyses of the data were carried out using both parametric and distribution-free methods. For non-parametric analyses, medians and interquartile ranges (25th, 75th percentiles) are provided. All reported *P* values are two-sided. In order to study changes in CD4 counts, we identified for each subject an earlier visit from our prospective study to obtain a reference CD4 count. For seronegative and seroprevalent subjects, this was the visit with the first available CD4 result. For seroincident subjects, this was the visit with the first available CD4 count after seroconversion. This earlier visit is referred to as the reference visit. Mean CD4 cell loss per year for each subject was calculated by subtracting the CD4 count at the reference visit from the CD4 count at the PCR visit and dividing by the elapsed time (in years).

Results

A total of 378 men were sampled for PCR testing during the study period. Of these, 41 samples (11%) were lost or delayed in transit and could not be tested. Of the remaining 337 samples, 21 results (6%) were excluded from the analysis as PCR-indeterminate. Thus, a total of 316 samples were included, of which 160 (51%) were PCR-positive (denoted P+), and 156 (49%) were PCR-negative (denoted P−). None of the 316 subjects had an AIDS-defining illness.

Of the 316 samples, 191 (60.4%) were seropositive (denoted S+), and 125 (39.6%) were seronegative (denoted S−). Of the 191 seropositive specimens, 158 (82.7%) were PCR-positive (S+/P+). There were 33 specimens which were seropositive but negative by PCR (S+/P−). Of the 125 seronegative samples, 123 (98.4%) were PCR-negative (S−/P−) and two (1.6%) were PCR-positive (S−/P+). PCR was repeated on the 33 S+/P− specimens and the two S−/P+ specimens with no change in the results. Thus, a total of 281 of 316 specimens (88.9%) had complete agreement between PCR and HIV serology. Taking HIV serology as the gold standard, PCR demonstrated a sensitivity of 82.7% [95% confidence limits (CL): 76.6, 87.8] and a specificity of 98.4% (95% CL: 94.3, 99.8).

Among the 21 subjects for whom PCR results were indeterminate, 14 (67%) were antibody-positive. This was similar to the seropositivity rate in the 316 subjects for whom PCR results were determined, of whom 191 (60.4%) were antibody-positive ($P = 0.74$).

We investigated the characteristics of the 33 subjects who were seropositive but PCR-negative (S+/P−). At the PCR visit, only two of this group (6%) were classified as CDC stage IV, the remaining subjects being in stages II and III. These two subjects both had oral hairy leukoplakia (stage IVC-2) with CD4 counts of 210 and 350 × 10^6/l, respectively. The latter subject was taking zidovudine at the time of the PCR visit while the former was not. Only two other subjects in the S +/P− group reported use of zidovudine at the PCR visit. In contrast, 39 (25%) of the S+/P+ group were classified as CDC stage IV at the PCR visit. This difference in the proportion of subjects in CDC stage IV was significant (6 versus 25%; $P = 0.030$ by Fisher's exact test). Alternatively, of the 148 seropositive subjects in CDC stages II or III, PCR was positive in 119 (80.4%), while of 41 subjects in CDC stage IV, PCR was positive in 39 (95.1%).

Table 1 presents a comparison between the S+/P− and S+/P+ groups with respect to laboratory parameters at the PCR visit. The S+/P− group had significantly higher CD4 counts (640 versus 490 × 10^6/l; $P = 0.006$) and CD4 : CD8 ratios (0.92 versus 0.64; $P = 0.004$) than the S+/P+ group. Furthermore, the S+/P− group exhibited significantly lower IgG levels, lower IgA levels and lower C1q binding activity than the S+/P+ group. IgM levels were also lower in the S+/P− group but this failed to attain conventional significance. Significant differences were not observed with respect to CD8 cell count, hemoglobin, white blood cell count, lymphocyte count, neutrophil count, or platelet count.

Table 2 presents an analysis of the rate of CD4 cell decline between the reference visit and the PCR visit in the S+/P+ and S+/P− groups. In addition, the seronega-

Table 1. Comparison of HIV-seropositive/polymerase chain reaction (PCR) -negative (S + /P−) and HIV-seropositive/PCR-positive (S + /P +) groups with respect to laboratory variables at the PCR visit.

Variable	Median (interquartile range†) S + /P− group (n = 33)	S + /P + group (n = 158)	*P* value*
CD4 (× 10^6/l)	640 (430–925)	490 (330–680)	0.006
CD8 (× 10^6/l)	660 (555–1000)	750 (530–1060)	0.488
CD4 : CD8 ratio	0.92 (0.50–1.31)	0.64 (0.40–0.85)	0.004
IgG (mg/dl)	1290 (994–1850)	1645 (1330–1970)	0.002
IgA (mg/dl)	168 (125–251)	251 (177–362)	< 0.001
IgM (mg/dl)	112 (94–180)	149 (111–193)	0.069
C1q binding (%)	8 (6–14)	14 (8–23)	0.010

Ig, immunoglobulin. *Based on Wilcoxon rank sum test; †interquartile range = (25th − 75th percentile).

tive, PCR-negative (S−/P−) subjects are considered as a group of uninfected homosexual controls. The median rate of antecedent CD4 cell decline in the S+/P+ group was significantly greater than that of the S+/P− group (−77 versus −58 $\times 10^6$ cells/l per year; $P = 0.028$) which was in turn significantly greater than that of the uninfected S−/P− group (−58 versus −21 $\times 10^6$ cells/l per year; $P = 0.016$).

Because laboratory and clinical parameters may be related to duration of infection, we controlled for this by restricting the analysis to those individuals in both the S+/P− and S+/P+ groups who were seroincident (SI), that is, who seroconverted while under study and whose duration of infection could therefore be estimated. A total of 11 subjects (33.3%) in the S+/P− group and 34 (21.5%)in the S+/P+ group were seroincident ($P = 0.22$). Duration of HIV infection was similar in the two seroincident subgroups. The S+/P− and S+/P+ seroincident subjects had seroconverted a median of 51 months (range 17–69) and 57 months (range 7–77) prior to the PCR visit ($P = 0.61$). A total of six (17.6%) of the 34 S+/P+ seroincident subjects were classified as CDC stage IV at the PCR visit, whereas none of the 11 S+/P− subjects had progressed to stage IV. The six S+/P+ seroincident subjects were classified as CDC stage IV at 39, 43, 57, 61, 62, and 65 months following seroconversion, respectively.

Table 3 (columns A and B) presents a comparison of the two seroincident subgroups with respect to laboratory parameters at the PCR visit. The S+/P− seroincident subgroup had significantly higher CD4 counts (870 versus 485 $\times 10^6$/l; $P < 0.001$) and CD4 : CD8 ratios (1.05 versus 0.63; $P < 0.001$) than the S+/P+ seroincident subgroup. Furthermore, the S+/P− seroincident subgroup exhibited significantly lower IgA levels and lower C1q binding than the S+/P+ group, as well as lower IgG levels which just failed to attain conventional significance. Significant differences were not observed with respect to CD8 cell count, IgM level, hemoglobin, white blood cell count, lymphocyte count, neutrophil count, or platelet count.

We compared the S+/P− seroincident subgroup with the S−/P− uninfected controls as seen in Table 3 (columns B and C). At the PCR visit, the S+/P− seroincident subgroup had significantly lower CD4 : CD8 ratios (1.05 versus 1.64; $P = 0.008$) but this was attributable to significantly higher CD8 counts (640 versus 500 $\times 10^6$/l; $P = 0.006$) and not to any difference in CD4 counts (870 versus 790 $\times 10^6$/l; $P = 0.436$). In addition, the S+/P− seroincident subgroup exhibited higher IgG levels than the S−/P− group (1230 versus 1000 mg/dl; $P = 0.042$) but was similar with respect to IgA levels, IgM levels, C1q binding, hemoglobin, white blood cell count, lymphocyte count, neutrophil count, and platelet count.

Table 4 presents an analysis of the rate of CD4 count decline between the reference visit and the PCR visit in the S+/P+ and S+/P− seroincident subgroups and the S−/P− uninfected controls. The reference visit occurred a median of 7 and 6 months following

Table 2. Change in CD4 count in HIV-seropositive/polymerase chain reaction (PCR) -positive (S + /P +) subjects, S + /P− subjects, and S−/P− subjects between the reference visit and the PCR visit.

Group	Mean time between visits (years)	CD4 count [Median (interquartile range†)] Reference visit	PCR visit	Median decline per year*
(A) S+/P+	3.2	745 (559–920)	490 (330–680)	−77 [−147 to (−23)]
(B) S+/P−	3.9	820 (586–1125)	640 (430–925)	−58 [−97 to (−2)]
(C) S−/P−	4.4	890 (670–1098)	790 (620–1010)	−21 [−58 to (+19)]

*(A) versus (B): $P = 0.028$ (Wilcoxon rank sum test); (B) versus (C): $P = 0.016$ (Wilcoxon rank sum test); †interquartile range = (25th−75th percentile).

Table 3. Comparison of the HIV-seropositive/polymerase chain reaction (PCR) -positive (S+/P+) and HIV-seropositive/PCR-negative (S+/P−) *seroincident* subgroups and seronegative/PCR-negative (S−/P−) men with respect to laboratory variables at the PCR visit.

Variable	Median (interquartile range‡) (A) S+/P+ seroincident group (n = 34)	(B) S+/P− seroincident group (n = 11)	(C) S−/P− group (n = 123)	*P* value*	*P* value†
CD4+ (× 10^6/l)	485 (328–670)	870 (720–1030)	790 (620–1010)	<0.001	0.436
CD8 (× 10^6/l)	695 (528–1028)	640 (570–1030)	500 (380–650)	0.895	0.006
CD4 : CD8 ratio	0.63 (0.43–0.88)	1.05 (0.95–1.48)	1.64 (1.28–2.14)	<0.001	0.008
IgG (mg/dl)	1580 (1368–1813)	1230 (913–1740)	1000 (840–1130)	0.065	0.042
IgA (mg/dl)	274 (196–465)	168 (139–242)	189 (148–237)	0.012	0.890
IgM (mg/dl)	126 (105–161)	108 (83–177)	122 (80–156)	0.267	0.903
C1q binding (%)	15 (10–22)	6 (4–12)	6 (5–8)	0.002	0.895

*(A) versus (B), based on Wilcoxon rank sum test; †(B) versus (C), based on Wilcoxon rank sum test; ‡interquartile range = (25th − 75th percentile).

the estimated date of seroconversion in the S+/P+ and S+/P− seroincident subgroups, respectively (P = 0.76). At the reference visit, the S+/P+ group demonstrated lower CD4 cell counts than the S+/P− group of marginal statistical significance (740 versus 860 $\times 10^6$/l; P = 0.086). The median rate of antecedent CD4 cell decline in the S+/P+ seroincident group was significantly greater than that of the S+/P− seroincident group (−79 versus −13 $\times 10^6$cells/l per year; P = 0.042). However, rates of CD4 cell decline were similar in the S+/P− seroincident group and in the S−/P− uninfected controls (−13 versus −21 $\times 10^6$ cells/l per year; P = 0.824). The analyses in Tables 1–4 were repeated using CD4 proportions instead of absolute CD4 counts and a virtually identical pattern of results was obtained.

In the 8–19 months (median: 13) between the PCR visit and August 1990, 10 of the 158 S+/P+ subjects have developed AIDS compared with none of the 33 S+/P− subjects. The two subjects who were seronegative and PCR-positive were asymptomatic, free of lymphadenopathy, and had no recent history of an acute retroviral-like illness. Both subjects had normal laboratory profiles, including CD4 counts of 1680 and 820 $\times 10^6$/l and CD4 : CD8 ratios of 1.31 and 1.46, respectively.

Discussion

The sensitivity of the PCR in this study (83%) using two primer pairs compares favorably with the 86% reported by Stoeckl *et al.* [7] using three different pairs of primers. Similarly, Horsburgh *et al,* [17] found, using two primer pairs, that four (17%) of 23 homosexual men who had recently developed HIV antibody were PCR-negative. Even in studies using techniques which may improve detection of low levels of proviral DNA, such as additional *env* primers [15] or a double amplification procedure [18], seropositive PCR-negative subjects have been observed. Our PCR results were completely concordant between SK38/39 *gag* and SK68/69 *env* primers, as has been observed in other studies [17,19]. Nevertheless, we readily acknowledge that the conditions for HIV PCR may not have been optimal in the present study in that all seropositives were not positive by PCR. It appears, however, that PCR has served as a semi-quantitative tool providing evidence of an association between proviral HIV load and rate of disease progression. Indeed, a PCR technique which cannot differentiate between high and low numbers of HIV DNA molecules, although highly sensitive when compared with serology, would fail to detect such an association with rate of disease progression. Recently, Schnittman *et al.* [20] quantified HIV-1 DNA by performing PCR on serial 10-fold dilutions of sort-purified CD4 cells and found a correlation between the frequency of CD4 cells with proviral DNA and disease progression in a small series of subjects.

Failure of PCR to detect proviral DNA in our seropositive subjects is clearly not a random phenomenon and in fact appears to be differentiating a different host–agent interaction. The 33 individuals whose HIV serology was positive but who showed no evidence of proviral DNA by PCR were significantly different from those whose PCR and HIV serology were both positive. Thirty-one of these 33 people were in less advanced clinical stages of HIV disease (CDC stages II and III). Furthermore, the seropositive, PCR-negative group had significantly higher absolute CD4 counts and CD4 : CD8 ratios, slower rates of CD4 cell decline, and lower levels of IgG, IgA and immune complex formation than did the seropositive, PCR-positive subjects. Taken together, these data present a profile of a group of subjects with a less advanced level of immune dysfunction than that found in their seropositive, PCR-positive counterparts.

In any analysis involving seroprevalent subjects, it is possible that a difference in disease progression might merely be a function of different durations of HIV infection. We therefore restricted the analysis to seroincident subjects; the same pattern of differences in disease progression persisted in subgroups whose length of HIV infection was similar. This demonstrates that the relative health of the seropositive, PCR-negative group is not attributable to more recent infection, but to other factors that appear to have allowed them to cope better with the infection thus far. Indeed, one of the most striking results was that, despite a median of 51 months elapsed since seroconversion, the 11 seroincident, PCR-negative subjects had CD4 counts and antecedent rates of CD4 cell decline that were similar to those of homosexual controls who were not infected with HIV.

In a cross-sectional study, it is impossible to determine how long proviral DNA had not been detectable in

Table 4. Change in CD4 count in HIV-seropositive, PCR-positive (S+/P+) seroincident subjects, S+/P− seroincident subjects and S−/P− subjects between the reference visit and the PCR visit.

Group	Mean time between visits (years)	CD4 count × 10^6/l Median (interquartile range†) Reference visit	PCR visit	Median decline per year*
(A) S+/P+ seroincident	3.6	740 (559–920)	485 (320–670)	−79 [−127 to (−31)
(B) S+/P− seroincident	3.4	860 (820–1200)	870 (720–1030)	−13 [−64 to (+5)]
(C) S−/P−	4.4	890 (670–1098)	790 (620–1010)	−21 [−58 to (+19)]

PCR, polymerase chain reaction. *(A) versus (B): P = 0.042 (Wilcoxon rank sum test); (B) versus (C): P = 0.824 (Wilcoxon rank sum test); †interquartile range = (25th, 75th percentile).

the seropositive, PCR-negative group prior to the PCR sample. That the seroincident, PCR-negative group had marginally higher CD4 counts than the seroincident, PCR-positive group at the reference visit a median of only 7 months after seroconversion suggests that a differential response to HIV may have been operative early in the natural history of the infection [21]. At the very least, the lower rate of antecedent CD4 cell decline in the seropositive, PCR-negative group suggests that they have experienced less HIV-induced CD4 cell loss for a considerable time prior to the PCR sample. In addition to these data, further supportive evidence is found in the observation that in the 8–19 months following the PCR sample, none of the 33 seropositive, PCR-negative subjects developed AIDS whereas 10 cases arose in the 158 seropositive, PCR-positive subjects.

An important feature of a cross-sectional sample within a cohort is that those individuals with the most rapid progression who developed AIDS prior to the present study will have been missed. We might therefore have selectively studied a group with an inherently slower and more homogeneous rate of disease progression. The net effect is that this could inflate the estimate of the rate of PCR negativity among seropositives; on the other hand, it would also tend to attenuate any differences between the groups which serves to strengthen our conclusions regarding differences in the apparent effects of HIV.

Although many questions about the course of HIV infection remain unanswered, a clearer picture of its natural history is beginning to emerge. Mathematical models of progression based on transfusion-associated infection and seroincident cohorts suggest that the median incubation period between infection and AIDS is approximately 10 years [22–24]. A corollary is that a significant proportion of infected individuals will have incubation periods much longer than this; indeed, those in the upper tail of the incubation distribution could progress over a period of 20 years or more. The identification of such subjects who manage to delay (or perhaps even avoid) progression to AIDS as well as the factors associated with this more favorable response to HIV are matters of critical importance. If such subjects could be identified early in HIV infection, we suggest that they would demonstrate many of the characteristics found in our seropositive, PCR-negative subjects. We postulate, therefore, that PCR may have served to identify a group demonstrating relative resistance to the effects of HIV. The seroincident comparisons suggest that in the early stages of infection seropositive, PCR-negative subjects have immune profiles similar to those of uninfected controls and clearly different from those of PCR-positive subjects. That the resistance to HIV-induced effects is relative and not absolute, however, is suggested by the comparisons including seroprevalent subjects who have longer durations of infection than the seroincident subjects. In these overall comparisons, the seropositive, PCR-negative group showed some of the characteristic changes associated with HIV infection when compared with uninfected controls, though to a far lesser degree than observed in the seropositive, PCR-positive group. That a smaller number of the seropositive, PCR-negative group have progressed to CDC stage IV-C2 illness is further evidence that they are not afforded complete protection from HIV-induced immunodeficiency.

People with less advanced HIV disease have lower virus titers [9], higher levels of neutralizing antibodies [25], and a lower burden of proviral HIV DNA as determined by PCR [7]. This study indicates that subjects who are HIV-seropositive but negative by PCR are clinically healthier with more favorable immunologic profiles. One might speculate that with viral replication limited by neutralizing antibody and/or other host or agent factors, the HIV proviral DNA, as detected by PCR, may not be able to expand. As mutations arise in the envelope region, antigenically altered viruses could escape immune surveillance and proliferate in other susceptible CD4-bearing cells until the proviral DNA load reaches the threshold of PCR detectability. Virus can be isolated from plasma at all stages of disease [9], suggesting that circulating neutralizing antibody alone may not control pathogenesis. Cell-mediated immunity may play an important role in maintaining the chronic phase of disease [10]. Progression to the chronic phase from the acute stage of infection parallels an increase in cytotoxic T lymphocytes (CTL). HIV-specific CTL inhibit viral replication in PBL and may be important in containing virus replication in HIV-infected people [26]. Evidence of strongly activated CTL, neutralizing antibodies, and low or undetectable proviral DNA may be important prognostic markers of infection and could indicate optimal times for antiviral intervention strategies.

In summary, we have identified a group of subjects with positive serology for HIV in whom the PCR, using two primer pairs corresponding to a conserved region of the *gag* and *env* genes, was negative. These subjects appear to have decreased susceptibility to the effects of HIV infection as demonstrated by lesser clinical progression, even after adjustment for time since seroconversion. The host, agent, and/or environmental factors responsible for this decreased susceptibility remain to be established.

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