Detection of Infection with Human Immunodeficiency Virus Type 1 before Seroconversion: Correlation with Clinical Symptoms and Outcome

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Early (pre-seroconversion) infection with human immunodeficiency virus type 1 (HIV-1) was identified in 50 of 267 participants in the Multicenter AIDS Cohort Study. These 50 men had a positive EIA result, which detected IgM antibody (n = 35), p24 antigen, or serum HIV RNA (n = 15) at their last "seronegative" visit. At that visit, the mean CD4 lymphocyte number (890/mm³ vs. 1038/mm³) was significantly lower than in men who subsequently seroconverted but had no evidence of early infection. The decline in CD4 cells was slower and the duration of AIDS-free time longer in the 19 men who were symptomatic in comparison to the 31 asymptomatic men with early infection, but differences were not significant.

Identification and treatment of early infection due to human immunodeficiency virus type 1 (HIV-1) has been advocated as a means of reducing viral replication and favorably altering disease progression [1, 2]. As a significant number of persons with primary infection are asymptomatic [3], it has been suggested that symptomatic incident HIV-1 infection is associated with more rapid immunologic deterioration and clinical progression [4–7].

The 'seronegative' phase of primary infection generally lasts between 2 and 6 weeks [3]. Recently, an EIA capable of detecting IgM as well as IgG antibody has been shown to reduce the window period between inoculation and seroconversion by ~1 week [8]. The use of techniques to detect viral p24 antigen and RNA in serum also decreases the time between establishment and diagnosis of infection with HIV-1 [9–11]. We used assays to identify persons with early HIV infection and sought to determine whether symptomatic primary infection was associated with clinical progression.

Methods

Details of the Multicenter AIDS Cohort Study (MACS), a prospective investigation of the natural history of HIV-1 infection among homosexual and bisexual men, have been published [12]. The participants included in this analysis were the 267 men with incident infection occurring within an interval of <7 months (median, 6; range, 4–7) between the last seronegative and first seropositive visit identified using first-generation EIAs. Serum obtained at the last "seronegative" visit was retested to determine the prevalence of early infection in these 267 participants.

Symptomatic early infection was defined by self-reports of fatigue, new skin rash, fever, tender or enlarged lymph nodes, weight loss, diarrhea, and night sweats occurring during the intervisit interval. Questions relevant to symptoms persisting for 2 weeks were answered by all 267 men. Midway through the study, ques-
tions were added to ascertain if symptoms of 3 days’ duration had been present during the intervisit interval. Reports of symptoms lasting at least 3 days were recorded for 151 (56.6%) of these 267 men. The presence or absence of generalized lymphadenopathy was identified on the physical examination at the last seronegative visit, and data were available for 266 men. Lymphadenopathy was defined as enlargement of lymph nodes in two or more chains, excluding inguinal nodes.

T cell phenotyping at each visit was performed with standard flow cytometry and blood counting methods previously described [13].

Sera obtained at the last seronegative visit from 227 men in this study were retested using a direct EIA for IgG and IgM HIV-1–specific antibody (Abbott, North Chicago, IL), an assay for p24 antigen based on a monoclonal antibody sandwich EIA (Abbott), and a polymerase chain reaction (PCR)–based assay for HIV RNA [8–11]. Forty sera were tested for p24 antigen and HIV RNA only. The limit of detection for p24 antigen is 8 pg/mL and for HIV RNA, 10⁶ copies/mL. A Western blot (WB) assay was also performed on all EIA-positive sera (Cambridge Biotech, Rockville, MD). Assays were conducted in a blinded fashion, and appropriate precautions were taken to avoid carryover contamination during PCR analysis.

χ² tests of independence were used to assess whether the distribution of reported symptoms and presence of lymphadenopathy differed in symptomatic and asymptomatic men with evidence of early infection. To account for the slight skewness in CD4 and CD8 lymphocyte distributions observed at the last seronegative visit, values were logarithmically transformed, and t tests were used to evaluate differences in mean levels at that visit. Loss of CD4 cells over time and the development of AIDS [14] were used as outcomes to determine disease progression after seroconversion. CD4 cell decline per year after seroconversion was determined by fitting a linear regression model using the serial CD4 cell counts for each person. To avoid the bias due to the loss of CD4 cells associated with seroconversion, measurements within 1 year of seroconversion were not used. Only men with at least 2 subsequent measurements were included to adequately assess CD4 cell decline. t tests were used to evaluate whether mean regression slopes were different between groups.

Kaplan-Meier survival estimates were used to ascertain the time to AIDS by group. Men without a diagnostic event were considered alive without AIDS if they were contacted as of 1 July 1994, that is, 1 year prior to the date of analysis. Otherwise, a subject was censored at the time of last contact or at the date of death. Log-rank statistics were used to determine whether there were significant differences in AIDS-free survival. Since the hazard functions were not proportional, we did not use multivariate proportional hazard models; subgroup differences were assessed using stratification.

Results

Fifty of the 267 seroconverters were identified as infected at the last seronegative visit (figure 1). Thirty-seven (16.3%) of 227 sera assayed for IgM and IgG antibody, but negative for antibody using a first-generation EIA, were reactive. To confirm HIV infection, sera were sequentially assayed by WB, sandwich EIA for p24 antigen, and PCR for HIV RNA. Ten of these 37 sera had a positive WB, with two or more positive bands: p24, gp41, or gp120/160. Of the remaining 27, 10 were positive for p24. Fifteen of the remaining 17 with negative or indeterminate WB results and negative for p24 antigen had positive HIV-1 RNA assays. Thus, among men who were EIA-positive (n = 37), being positive on WB (n = 10), positive for p24 (n = 10), or positive for HIV-1 RNA (n = 15) was sufficient to confirm early infection. Of the 190 sera negative by direct EIA, 14 had detectable p24 antigen. Eleven of these 14 were positive for HIV-1 RNA. Thus, 15 of these 190 men were positive for HIV RNA and were considered to have primary HIV infection. None of the 40 sera not assayed by EIA but tested for p24 or HIV RNA were positive.

Ten (20%) of the 50 participants with early infection had at least one symptom persisting ≥2 weeks, compared with 44 (20.3%) of 217 men without evidence of infection at the last seronegative visit. The most common HIV-1–related symptoms were fatigue (6.4%), a new skin rash (5.9%), night sweats (4.5%), and lymph node tenderness or enlargement (4.1%). In the smaller subset (n = 151) of participants who answered questions related to symptoms persisting for 3 days, the most frequent complaints were fatigue (20.0%), rash (18.0%), nodal enlargement or tenderness (18.3%), and night sweats (13.3%). Only 9.2% of the men reported fever. Among the men with evidence of early infection, fatigue, rash, nodal enlargement, fever, and night sweats were noted for 3 days by 38.1%, 26.3%, 55.0%, 40.0%, and 36.4%, respectively. Fourteen of these 19 had two or more symptoms. Overall, 19 (38%) of 50 men with early infection and 58 (27%) of 217 men with no evidence of infection at the last seronegative visit reported at least one symptom persisting for 3 days or 2 weeks (P = .11). Eight (16.0%) of the 50 with primary infection and 39 (18.1%) of the 216 participants without evidence of infection at that visit had lymphadenopathy.

The mean CD4 lymphocyte count was significantly (P = .03) lower among the 50 infected men (890 ± 437.6/mm³) than among the 217 seroconverters without evidence of infection (1038 ± 435.4/mm³) at the last “seronegative” visit. The mean CD4 cell number for these 217 men did not differ significantly.
Figure 2. Kaplan-Meier survival curves delineating time from seroconversion to clinical AIDS according to symptom status at last seronegative visit among HIV-1 seroconverters with evidence of infection at that visit. Dotted line, those reporting one or more symptoms; dashed line, asymptomatic subjects. Vertical hatches represent censoring times for seroconverters without AIDS diagnosis.

(P = .42) from the mean value of the CD4 lymphocyte number for the persistently uninfected cohort in the MACS (1008 ± 388.6/mm³). The mean CD8 lymphocyte numbers at the last seronegative visit were not significantly (P = .3) different between the seroconverting groups.

Following the estimated date of seroconversion, defined as the midpoint between the last seronegative and first seropositive visits, the mean follow-up time to AIDS or date of analysis (1 July 1995) among the 267 seroconverters was 8.5 years. The mean CD4 cell count slope (±SE) for the 19 infected symptomatic men was −56.5 ± 128.1/mm³ and for the 31 infected asymptomatic men was −71.1 ± 119.5/mm³ (P > .5). Men with symptomatic primary infection tended to progress to AIDS more slowly than asymptomatic infected men during the follow-up period (figure 2), but the difference was not significant (P = .07). Participants with two or more symptoms also were free of AIDS for longer than the remaining 36 men with primary infection who were asymptomatic or who had only one symptom.

Discussion

Fifty men with incident HIV-1 infection in the MACS had laboratory evidence of early HIV-1 infection at their last ‘seronegative’ visit. Thirty-five men were seropositive when tested using a direct EIA that detects IgM and IgG HIV-specific antibody, and infection was confirmed by a positive WB, a p24 antigen assay, or assay of serum for HIV RNA. The remaining 15 subjects were positive for virus markers but had not developed serologic evidence of an immune response at this visit.

If infection occurred uniformly across the intervisit interval (median, 6 months), one-sixth of the participants with incident infection would have been expected to be in the 2- to 6-week ‘pre-seroconversion’ window and have evidence of infection at their last ‘seronegative’ visit. The proportion (18.7%) of participants in this study with laboratory evidence of infection at this visit is consistent with this postulate. These results illustrate that assays sensitive for the presence of antibody and virus can identify persons with early HIV-1 infection.

A greater proportion of participants with evidence of primary infection than of those without such findings reported symptoms persisting for 3 days; however, data were available for only a limited number (56.6%) of the men. Lymphadenopathy was uncommon in participants both with and without laboratory evidence of primary infection. However, utilization of self-reported symptoms or presence of lymphadenopathy or both to identify persons with early HIV-1 infection in this study would have falsely excluded a large number of men with incident infection. Strategies to identify persons with early HIV-1 infection will require periodic laboratory testing of high-risk persons in addition to a screening history and physical examination.

Studies of early infection from the MACS, Amsterdam, and Vancouver cohorts avoid selection and diagnostic biases potentially present in investigations dependent on referral of patients from clinical practices. In the other cohort studies, symptomatic primary HIV-1 infection has been reported to be associated with more rapid progression [4, 5], but this association could
not be confirmed in our evaluation of men with early HIV-1 infection. The subsequent decline in CD4 lymphocytes did not differ significantly, and AIDS-free survival time was, if anything, longer in men reporting symptoms than in participants with asymptomatic early infection. The reasons for the lack of prognostic differences between symptomatic and asymptomatic men with early infection in this study are not immediately apparent. The identification of men with incident infection in this cohort, by use of more sensitive methods of detecting infection, could have resulted in selection of a unique group of persons with early infection or in more complete identification of persons with early HIV-1 infection. Alternatively, an interval history covering 6 months may misclassify participants because of underreporting as a result of poor recall or overreporting of nonspecific symptoms.

Application of more sensitive techniques to detect HIV-1 infection, as demonstrated in this study, offers a means of identifying early HIV-1 infection that allows for investigation of early immunologic and virologic events and evaluation of responses to therapy during early infection. Potentially, a major public health benefit could result if early administration of antiretroviral therapy reduced sexual transmission or led to earlier and effective counseling regarding safer sex practices and needle sharing.

Investigators in the Multicenter AIDS Cohort Study

The Multicenter AIDS Cohort Study (MACS) includes the following: Johns Hopkins University School of Hygiene and Public Health, Baltimore: Alfred J. Saah (Principal Investigator), Haroutune Armenian, Homayoon Farzadegan, Neil Graham, Nancy Kass, Joseph Margolick, Justin MacArthur, Ellen Taylor; Howard Brown Health Center-Northwestern University Medical School, Chicago: John P. Phair (Principal Investigator), Joan S. Chmiel, Bruce Cohen, Maurice O’Gorman, Daina Variakojis, Jerry Wesch, Steven M. Wolinsky; University of California, Los Angeles, Schools of Public Health and Medicine: Roger Detels (Principal Investigator), Barbara R. Visscher, Janice P. Dudley, John L. Fahey, Janis V. Giorgi, Andrew Kaplan, Oto Martinez-Maza, Eric N. Miller, Hal Morgenstem, Parunag Nishanian, John Oishi, Jeremy Taylor, Jerome Zack; University of Pittsburgh Graduate School of Public Health: Charles R. Rinaldo (Principal Investigator), Lawrence Kingsley, James T. Becker, Phalguni Gupta, John Mellors, Anthony Silvestre, Roger Anderson, Sharon Zuconi; Data Coordinating Center, Johns Hopkins School of Hygiene and Public Health: Alvaro Muñoz (Principal Investigator), Cheryl Enger, Stephen Gange, Donald R. Hoover, Lisa P. Jacobson, Clara Chu, Steven Piatadosi, Sol Su; National Institute of Allergy and Infectious Diseases: Lewis Schrager (Project Officer); National Cancer Institute: Daniela Seminara.

References