Association of Human Immunodeficiency Virus (HIV) p24 Antigenemia with Decrease in CD4+ Lymphocytes and Onset of Acquired Immunodeficiency Syndrome during the Early Phase of HIV Infection

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Human immunodeficiency virus (HIV) p24 antigenemia was assessed in a longitudinal study of 52 homosexual men who developed serum antibody to HIV. Antibody seroconversion to HIV as defined by a positive HIV enzyme immunoassay (EIA) confirmed by Western (immuno-) blot was associated with three major patterns of HIV antigenemia. In the first pattern, a transient antigenemia was noted 6 (six subjects) and 12 (one subject) months prior to detection of antibody by HIV EIA and Western blot in 7 (13.5%) of the 52 men. Use of an EIA employing a recombinant envelope protein (ENV9) was able to detect antibody in four of these seven men at the time of this early antigenemia. In the second pattern, HIV p24 antigenemia occurred in 8 (15.4%) of the 52 subjects within the first 12 months after HIV antibody seroconversion. No p24 antigen was detected in the 37 (71.1%) remaining subjects. CD4+ cell numbers were lower in antigen-positive men before and after antibody seroconversion. Development of acquired immunodeficiency syndrome (AIDS) or AIDS-related complex was strongly associated with evidence of persistent p24 antigenemia during the early, postseroconversion period. HIV p24 antigenemia may be of value in determining appropriate cohorts for drug therapy trials for subjects with early-phase HIV infection.

MATERIALS AND METHODS

Subjects. The study cohort consisted of 52 homosexual and bisexual men enrolled in the Pitt Men’s Study, the Pittsburgh site of the Multicenter AIDS Cohort Study (11). They were among 842 men seronegative for HIV as determined by EIA and Western blot on entry into the study from April 1984 through March 1985. The subjects were examined at approximately 6-month intervals for a total of seven study visits over 3 years. The clinic visits included a physical exam for symptoms of AIDS, an epidemiologic questionnaire on medical, sexual, and drug history, and samplings of blood and body fluids. At a subsequent, semiannual study visit, all 52 subjects were documented as having become infected with HIV. The men were assumed to have become infected with HIV between the two study visits in which a change in EIA and Western blot antibody status occurred. During the time of this study, the men were not being treated with antiviral chemotherapy (zidovudine) unless indicated.

Blood specimens. Blood was obtained by venipuncture and placed into vacuum tubes for serum collection or into vacuum tubes containing 7 U of preservative-free heparin per ml of blood for plasma collection (Terumo Medical Corp., Elkton, Md.). Clotted and anticoagulated blood was centrifuged at 750 × g for 10 min; serum and plasma were divided into aliquots and frozen at −70°C.

Specimens for serologic testing were thawed, blind coded, and shipped on dry ice to the Du Pont Glasgow Research Laboratory (Wilmington, Del.) for testing of HIV p24 antigen and antibody to HIV as measured by EIA with viral lysate or a recombinant envelope protein (ENV9) (12). Testing of specimens for HIV antibodies by Western blot as

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HIV serology. Antibody to HIV was assayed with two commercial EIA kits that utilize whole-virus-lysate antigen (LAV-EIA [Genetic Systems, Seattle, Wash.] and HIV-ELISA [Du Pont Co., Wilmington, Del.]). Western blot assay was done with a commercial kit (Novapath Immunoblot Assay, Bio-Rad Laboratories, Hercules, Calif.). This assay uses nitrocellulose strips impregnated with inactivated, whole-virus proteins. Viral protein bands were visually scored as negative (0) or +1, +2, and +3 on the basis of increasing intensity; detectable bands were p15, p24, p31, gp41, p53, p55, p64, gp120, and gp160. EIA and Western blot assays done in the Pittsburgh laboratory were performed by the same technician. Antibody seroconversion to HIV was defined as a positive HIV EIA and a summed positive Western blot score of greater than or equal to +3 (19).

Antibody to HIV envelope glycoproteins, gp41 and gp120, was assayed in plasma samples by using the Du Pont Recombinant HIV ELISA. This assay utilizes recombinant envelope protein (ENV9)-coated wells and the same reagents as the Du Pont HIV EIA for the detection of HIV antibodies. A positive result is defined as the ratio of absorbance of the sample to the positive control; this value must be greater than or equal to 0.5.

Detection of HIV p24 antigen in plasma was done with a commercial kit (HIV p24 Core Antigen ELISA; Du Pont) which utilizes monospecific polyclonal antibody to HIV p24 antigen for capture and detection of antigens in the patient’s sample. A positive result is determined by comparison of the absorbance of the patient’s sample with the reactive threshold value (0.100 added to the mean of the negative control). The sample must be equal to or greater than the reactive threshold value to be considered reactive. All reactive specimens were confirmed as positive by repeat testing and neutralization of specific signals with human antibodies to HIV (Du Pont); confirmed positive samples showed a reduction in absorbance with the addition of confirmatory reagent of at least 50%. This assay can quantitate 6 pg (0.03 ng/ml) or more of HIV p24 antigen in plasma or serum. In addition, specimens were considered reactive if they had less than 0.03 ng/ml but were greater than the negative control. Of the 26 antigen-positive specimens, 8 fell within this lower range of reactivity. Seven of these eight specimens were confirmed as positive by a second p24 antigen test (Abbott Laboratories, North Chicago, Ill.). One of these eight samples was confirmed positive by one assay (Du Pont) and was negative by the other (Abbott) (Table 1; sample T2, subject no. 6).

T-cell phenotyping. The number of T lymphocytes was determined by staining of peripheral blood mononuclear cells with fluorescent-conjugated antibodies to CD3 (Leu 4; Becton Dickinson, Mountain View, Calif.), CD4 (Leu 3a), and CD8 (Leu 2a) by established methods (15). The total T-cell numbers were obtained by multiplying the percentage of T cells by the number of lymphocytes as determined in a commercial laboratory (MedCheck Laboratory, Pittsburgh, Pa.).

RESULTS

Antibody seroconversion to HIV. The majority of the men (36 of 52 [69.2%]) seroconverted during the first 12 months of the study (visits 1 to 2, or 2 to 3); the remaining men seroconverted at later study visits. A total of 38 (73%) of the 52 men were studied for at least 12 months after antibody seroconversion, whereas 48 (92%) of the 52 were studied for at least 6 months after seroconversion; the remaining four participants were studied up to their first HIV seropositive visit.

Results for antibody to HIV with two conventional EIA tests and a second-generation recombinant HIV envelope assay (ENV9-EIA) agreed in 48 of the 52 men. However, in 1 of these 49 subjects (subject 1), the EIA was not confirmed by the Western blot (sample T2, summed score, +2) (Table 1). In three of the other four men, blood samples were positive for antibody to ENV9 by EIA at the study visit 6 months before that which was first positive by both of the conventional HIV-EIAs and confirmed by Western blot. The Western blot summed scores of these HIV EIA-negative, ENV9 EIA-positive samples were 0 for subject 3, +4 for subject 5 (p24, +2; gp41, +1; p55, +1) and +1 for subject 7 (gp160, +1). In subject 4 (no. 16), blood samples were positive by ENV9-EIA and negative for all other serologic tests at sequential visits for 29 months prior to the time of Western blot-confirmed seroconversion. All subjects had multiple bands positive by Western blot at visit T0 and thereafter (data not shown).

Early transient HIV p24 antigenemia. Circulating HIV p24 antigen was detected during the very early, preseroconver-
sion period of infection in 7 (13.5%) of the 52 men (group 1; Table 1). The antigenemia was present 6 months before the first sample demonstrating HIV antibodies as measured by HIV EIA, which utilizes whole-virus lysate, in five of these seven subjects (subjects 2 to 5 and 7) and at 12 months prior to seroconversion in one study subject (subject 6). In study subject 1, EIA was positive but Western blot was negative by our criteria when p24 antigen was detected. Antibody to HIV was detectable in the HIV EIA-negative samples by ENV9 EIA in three of seven cases as described above. Western blot assay also revealed the presence of envelope (gp41 and gp160) and core (p55) bands in some of these three HIV EIA-negative samples (see above).

Blood specimens obtained from group 1 men after seroconversion as determined by HIV EIA were negative for p24 antigen except in one of these subjects (subject 6) at one visit (Table 1). Levels of antibody to p24 were relatively high after seroconversion in six of these seven men, with Western blot densities of +2 and +3.

Onset or persistence (or both) of HIV p24 antigenemia after HIV antibody conversion. A second pattern of antigenemia was observed in 8 (15.4%) of the 52 men (group 2; Table 2). Antigen was detected in five of these eight men by 4 to 12 months after the first HIV-EIA-positive visit (subjects 8, 10, 11, 14, and 15). If one assumes that HIV infection occurred at the midpoint between the last antibody-negative and the first antibody-positive study visits, detection of p24 antigenemia in these five men was a median of 7.5 months after infection. In subjects 9, 12, and 13, p24 antigen was first noted 6 months prior to seroconversion (subject 13) and at the first HIV-EIA-positive study visit (subjects 9 and 12). Antigenemia was still evident in most of these men at later study visits. Certain subjects were not evaluable at visits beyond T6 months due to AIDS illness (subject 8) or failure to complete later study visits (subjects 9 and 14). The levels of HIV antigen did not correlate with time of occurrence or individual study subject (data not shown). Antibody to p24 as determined by Western blot was low (0 and +1 densities) in four of the eight group 2 men (subjects 10 to 12 and 15).

Absence of detectable HIV p24 antigenemia. In 37 (71.1%) of the 52 men, HIV serum antigen was not detectable before or after onset of antibody production (group 3). All of the 37 men had antibody to p24 by Western blot assay. Of these 37, 5 had low levels (+1) of p24 antibody, whereas the other 32 had antibody densities of +2 and +3.

Relationship of antigenemia with clinical course and T-cell levels. To date, group 1 and group 3 men with early, transient antigenemia or no detectable antigenemia have remained asymptomatic. However, three of the eight group 2 men, who had antigenemia after antibody conversion, developed AIDS (two Pneumocystis carinii pneumonia and one Kaposis sarcoma) within 13, 29, and 31 months of antibody conversion. These three men died within 6 months of diagnosis of disease, regardless of treatment with zidovudine in all cases. Two of the other eight group 2 men developed AIDS-related complex (ARC) (i.e., weight loss, oral thrush, and lymphadenopathy), whereas the remaining three were asymptomatic. Thus, a strong association was observed between the development of AIDS or ARC in the group 2 men (5 of 8) compared with group 1 men (0 of 7) (P < 0.02, Fisher’s exact test). This relationship was more significant if the group 1 and 3 men were combined (0 of 44) and compared with those in group 2 (5 of 8) (P < 0.001).

Table 2 provides the mean ± standard error of CD4+ cell counts in groups 1, 2, and 3 at observed time points before and after HIV seroconversion. Reductions in CD4+ lymphocytes were observed for all groups during the time of this study. Groups 1 and 2 exhibited reduced mean CD4+ cell numbers before antibody seroconversion and lower mean numbers over time compared with the group 3 men. These differences were significant at 6-month (T12) and 12-month (T24) intervals after seroconversion for groups 1 and 2 compared with group 3 (P < 0.05, Duncan’s new multiple range test).

Similar analysis showed that there was an increase in CD8+ cell numbers over time in all three groups of men. The cell numbers displayed a temporal, sharp rise at 6 months preconversion in group 1 (P < 0.05 compared with group 2) (Fig. 2).
DISCUSSION

The present longitudinal study indicated that development of HIV p24 antigenemia during the first 12 months after antibody seroconversion to HIV (group 2 homosexual men) is associated with decreased numbers of CD4+ helper T cells and development of AIDS and ARC. These results confirm and extend previous reports that the presence of HIV p24 antigenemia is a significant risk factor for lower CD4+ cell numbers and development of AIDS in HIV antibody-positive homosexual men (5, 7, 16, 17) and hemophilia patients (1). Of greater interest is that our data suggest that p24 antigenemia is a marker for risk of AIDS and ARC in homosexual men during the earliest, post-antibody conversion phase of HIV infection.

Three patterns of HIV p24 antigenemia were observed in the early period of HIV infection. A transient, temporal antigenemia was detected in 13.5% (7 of 52) of the study subjects prior to seroconversion (group 1). This concurs with other studies that have shown a short-term presence of p24 antigen in the blood of homosexual men and hemophilia patients prior to onset of HIV antibody as detected by EIA and Western blot (2, 9, 13, 18, 20, 21). This antigenemia “window” disappears within a few weeks in most subjects, during the period in which antibody to p24 appears in the circulation. It is probable that this transient antigenemia was missed in most of the 52 men because of the 6-month sampling schedule.

Group 1 men, who had the transient, p24 antigenemia, had lower CD4+ cell numbers at the time of the antigenemia compared with men who were not antigenemic. One possible explanation is that these men were infected earlier than groups 2 and 3 and therefore had reduced CD4+ cell counts. If so, one would presume that they would be at greater risk for development of symptomatic disease. However, this was not observed. Alternatively, other host factors associated with lower CD4+ cell numbers might be related to the transient appearance of p24 antigenemia. The temporarily enhanced CD8+ cell numbers seen at 6 months preconversion may have been a host response to the HIV infection. Group 1 men have remained asymptomatic during the follow-up period since their antibody seroconversion to HIV.

The second and more important pattern of antigenemia was a persistent or late-onset (or both) form that was observed in 16.7% (8 of 48) of the men who have been studied for at least 6 months after HIV antibody conversion (group 2). Antigen was first detected in these men within 12 months of the first EIA antibody-positive sample. Levels of antibody to p24 as assessed by Western blot were low in four of these eight men. This correlation of p24 antigenemia with lower levels of antibody to HIV p24 has been noted in several other studies (1, 7, 14) and may be related to either complexing of antigen and antibody or lower production of antibody to p24. However, others have recently reported that serum p24 antigenemia is not linked to antigen-antibody complexes (21).

Antigenemia in the group 2 men was associated with lower CD4+ cell counts before and after HIV antibody conversion compared with group 3 men, who remained asymptomatic after seroconversion and were not antigenemic. This suggests that men who develop antigenemia before seroconversion (group 1) and early after HIV antibody seroconversion (group 2) could have depressed immunity prior to HIV infection as measured by CD4+ cell numbers. Other factors, such as strain of HIV and coinfections, could have a role in this effect. Of importance is the fact that group 2 men appeared to be more susceptible to the immunosuppressive effects of HIV infection than group 1 and 3 men, who did not have antigenemia during the early postconversion phase of infection. Further evidence for this is that the first three cases of AIDS in our 52 subjects who seroconverted to HIV occurred in group 2 men by 13, 29, and 31 months after the first HIV EIA antibody-positive specimen. Two other group 2 men developed ARC. These findings are in contrast with those for the 44 men in groups 1 and 3 who have not had detectable p24 antigenemia after seroconversion, have relatively higher CD4+ cell numbers, and have remained asymptomatic during this early follow-up period. This association of severe disease with persistent antigenemia was not simply due to a poor follow-up record in the group 3 men. In fact, 70.3% (26 of 37) of group 3 men and 62.5% (5 of 8) of group 2 men were studied for 12 months or more after seroconversion, and 37.8 (14 of 37) and 37.5% (3 of 8), respectively, were observed for 24 months after seroconversion.

Testing for HIV p24 antigen has been considered for
screening of blood donors in order to identify potentially infectious blood products which are negative by conventional HIV-EIA or in which a positive EIA is not confirmed by Western blot. However, screening of blood donors from the general population in Western Germany for HIV antigenemia has not been significant (3). Such testing is also quite costly in supplies and labor. The present results show that use of a second-generation EIA that employs a recombinant HIV envelope protein was able to detect HIV antibody in four of the seven antigen-positive, preconversion subjects. Such improved testing for HIV antibody combined with voluntary blood donor deferral by individuals at high risk for HIV infection appears to be the most efficient method for maintaining a safe blood supply.

This study suggests that HIV antigenemia is an early marker for severity of HIV infection, measured both by reduced CD4⁺ cell counts and clinical outcome. This could be of importance in development of appropriate protocols for anti-HIV drug therapy. Sharp decreases in levels of p24 antigen in blood have been reported for AIDS patients receiving zidovudine (6, 10). Monitoring of HIV p24 antigenemia may be of greater importance as an early marker for efficacy of drug therapy in asymptomatic HIV-seropositive men, for whom effects on clinical parameters of infection may require longer-term follow-up.

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LITERATURE CITED


