CONCISE REPORT

Lack of Evidence of Prolonged Human Immunodeficiency Virus Infection Before Antibody Seroconversion

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Recently, considerable concern has been raised regarding the possibility that antibody-based screening tests for the human immunodeficiency virus (HIV) may fail to detect certain high-risk individuals for prolonged periods of time. It has been proposed that testing for HIV-related antigen may be a necessary procedure to detect such individuals. To address this issue, we longitudinally studied two groups of homosexual men: direct sexual partners of acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) patients and individuals who ultimately seroconverted. There was no evidence of prolonged infection with HIV in the absence of detectable antibody in these two groups. It appears at this time that, even among subjects at very high risk for HIV infection, currently available antibody-based assays are sufficient to identify infected individuals.

MATERIALS AND METHODS

Subjects. Homosexual or bisexual men have been followed in the Boston area as part of two epidemiological studies over the past 3 years.2,3 Fifteen HIV enzyme-linked immunosorbent assay (ELISA) antibody seroconverters and 37 seronegative partners of AIDS or ARC patients were selected for more detailed serological and virologic analysis. The cohort studies have been approved by the institutional review boards of the participating institutions.

HIV antigen assay. Sera that had been stored for up to 2 years at −70°C were thawed and tested in duplicate for HIV p24 antigen by using the Abbott antigen capture assay (Abbott Laboratories, Chicago) according to previously published techniques.6,7 A standard curve was established by using antigen standards, and the amount of antigen present in the serum was extrapolated from this curve. Control sera from previously characterized HIV antigen-positive and antigen-negative subjects were run in these assays.

HIV culture. Peripheral blood mononuclear cells were obtained by Ficoll-Hypaque density centrifugation and cultured in the presence of phytohemagglutinin and interleukin-2 with the periodic addition of allogeneic peripheral blood mononuclear cells as previously described.1 The supernatant was tested for reverse transcriptase activity. A culture was termed positive if reverse transcriptase activity with a poly r A template was at least 1,000 cpm greater than with a poly dA template on two or more assay days.

HIV antibody. All sera were tested for antibody to HIV by using a commercial ELISA (Electronucleons, Columbia, MD) and Western blot. HIV antigen for Western blot was prepared by using standard techniques, and antibody-positive control sera were used to ensure the presence of envelope (gp120, gp41), core (p24 p55), and polymerase (p53, p65) antigens. Known antibody-positive and -negative sera were used as appropriate controls in each Western blot assay.

RESULTS

Sera were available for study for up to 24 months at intervals of 6 months in both epidemiological groups. None of 15 antibody seroconverters was found to have HIV antigen present 6 months or earlier from the time of seroconversion. Similarly, none of the 37 antibody-seronegative sexual contacts had HIV antigen detected. The limit of resolution of the antigen assay is positive at values of ≥70 pg/mL (National Institutes of Health AIDS treatment evaluation units (ATEU) Virology Subcommittee), and all antigen values for
the study subjects were well below this value (2.67 ± 2.99 and 5.89 ± 4.56 pg/mL, mean ± SD for seroconverters and partners, respectively). There was no evidence of intermit-
tent antigen positivity in any of the sequential samples.

Western blot analysis confirmed the antibody negativity by ELISA. There was no evidence of single reactive bands to structural core–associated HIV proteins among the 37 anti-
body-seronegative sexual partners. Similarly, Western blot analysis was negative 6 months or more before ELISA reactivity among the seroconverters.

Sixteen of the 37 antibody-negative sexual partners had cultures performed on one or more occasions for HIV. No virus was detected in any of these cultures. These results are summarized in Table 1.

## DISCUSSION

Our study of a large number of antibody seroconverters and direct sexual partners of AIDS or ARC patients has failed to demonstrate a state of prolonged HIV carriage (eg, >6 months) preceding antibody seroconversion. In marked contrast to the recent study from Finland1 that described both significant HIV antigenemia and the presence of anti-
odies to only core and/or sor, tat, and 3'orf antigens of HIV, our subjects were nonreactive to antigens in the high/low molecular weight regions of the core by Western blot. Although recombinant antigens were used in some of the antibody assays in the Finnish study, the patients who reacted to recombinant antigen also had p24 antigen detected by the Abbott antigen assay or antibody to native structural (eg, core) antigens on Western blot. In our study, the absence of antigen was confirmed by the lack of viremia by culture of peripheral blood mononuclear cells.

The reasons for the discrepancy between our study and the recent work from Finland are unclear. We used an identical HIV p24 antigen assay and a similar Western blot technique. The sensitivity and type of ELISA used to measure serum antibody in the Finnish study were not specified, so their results might be attributed to an insensitive assay. Another theoretical explanation includes different antibody responses to HIV dependent on immune response genes that are not common to ethnically homogeneous Finns v a genetically diverse cohort of Americans. Furthermore, it is possible that our performance of the antigen assay was somehow flawed and failed to detect circulating HIV core proteins. Our study confirms previous work from our group indicating that a state of HIV antibody negativity with virus cultured from the peripheral blood is rare even among individuals at very high risk for HIV infection.1,2 Our study is also reassuring with respect to the possibility that significant numbers of HIV-infected individuals could donate blood and not be detected by using the current ELISA antibody screening tests. Although it is clear that antibody seroconversion may take several weeks to be detected after acute HIV infection, during which time p24 antigen may be found within plasma,4 such a period appears to be less than 6 months' duration for the subjects in our cohort. The study from Finland suggested that high-risk homosexual men could manifest HIV antigenemia over a period of greater than 1 year without development of antibody and that this antigenemia reached high levels and was intermittent. Although it might be argued that intermittent antigenemia could be missed by our study of sequential serum samples obtained every 6 months, we would have expected to find at least one or several individuals in our study population with positive antigen presence. It would be of interest to apply the recently reported technique of detect-
ing rare HIV DNA sequences in peripheral blood cells by using an automated polymerase chain reaction to the sub-
jects from Finland with reported latent infection and inter-
mittent antigenemia.5

In conclusion, we have failed to confirm the data that currently used tests for antibody are inadequate in diagnosing HIV infection among high-risk individuals who either ultimately seroconvert or are epidemiologically repeatedly exposed to virus by sexual contact. Until further data from other groups can be generated, it appears that HIV antigenemia is relatively short lived before the development of antibody and rarely if ever persists without the appearance of antibody.

## REFERENCES


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