Absence of Detectable Antibody in a Patient Infected with Human Immunodeficiency Virus

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Infection with human immunodeficiency virus (HIV) is routinely and easily diagnosed with use of enzyme immunoassay (EIA) test kits. We describe an unusual patient who developed AIDS despite testing negative for antibodies to HIV 35 times over a 4-year period. HIV infection was confirmed by the results of p24-antigen assays and polymerase chain reaction amplification of proviral DNA. Sequence analysis of the virus demonstrated that it was closely related to a strain obtained from the patient’s sexual partner. The explanation for this patient’s persistently negative EIA results is unclear. However, this case does suggest that physicians who treat patients with AIDS-defining conditions but for whom standard HIV antibody testing is negative should consider the possibility that HIV infection is present and may be identified by additional testing procedures.

Infection with HIV has been reliably detected in infected individuals by using EIAs approved by the U.S. Food and Drug Administration (FDA) in 1985 [1, 2]. The main limitation of EIA has been its inability to detect infection in patients during the ‘‘window period’’ from initial virus acquisition to the time when antibodies become detectable. Otherwise, the screening EIAs and confirmatory western blot tests have been believed to be of sufficient sensitivity and specificity to be considered diagnostic of HIV infection [2–6].

We describe a patient with the clinical manifestations of AIDS for whom multiple EIAs were negative far beyond the usual window period; additional nonroutine testing conclusively demonstrated infection with HIV. (This case was previously reported in part [7].)

See editorial response by Morens on pages 101–3.

Case Report

A previously healthy 36-year-old male developed fatigue in early 1995. In April 1995, an EIA was negative for antibodies to HIV. He was admitted to the hospital in June 1995 for evaluation of a 27-lb weight loss, diarrhea, shortness of breath that worsened with exertion, nonproductive cough, fever, chills, headaches, myalgias, and right-upper-quadrant pain. He denied any history of liver, renal, or immunosuppressive disease or treatment with immunosuppressive drugs. He had traveled to Europe in the early 1980s, but had remained in the southwestern United States since then. He had been employed as a construction worker and painter until his illness. Routine laboratory tests, a chest radiograph, and another EIA for HIV were all negative. The patient was discharged without a diagnosis.

He was readmitted in August 1995 for evaluation of bilateral pulmonary infiltrates. The results of routine sputum cultures and culture of a sample obtained by bronchoalveolar lavage were nondiagnostic. Gomori’s methenamine silver stain of an open lung biopsy yielded Pneumocystis carinii. The CD4+ lymphocyte count was 129/mm3, but an EIA and western blot assay for antibodies to HIV, which were performed by the Utah Health Department Laboratory (Salt Lake City), were again negative. Two months after this illness, the patient was readmitted to the hospital with bacterial pneumonia and empyema. His CD4+ lymphocyte count had decreased to 69/mm3.

The patient was married and reported sexual contact without condom use with his wife between 1989 and 1993; the couple separated in 1993 and had no further sexual contact. The wife was interviewed and reported sexual contact with a known HIV-infected partner between 1984 and 1989. She reported that this partner had died of AIDS in 1994. She developed P. carinii pneumonia in 1994 and was found to be seropositive for HIV at that time.

Our patient was unaware of her diagnosis until after his hospitalization in August 1995. His only identified risk factor for HIV infection was sexual contact with his HIV-infected wife. He denied homosexual contact or receipt of a transfusion. He had used multiple nonparenteral drugs but denied injection drug use.

The patient had also been tested by EIA for antibodies to HIV while donating plasma multiple times between August
1990 and April 1994 at a plasmapheresis center. The records at the plasmapheresis center indicated that the patient had donated plasma 33 times during this interval, and an EIA was negative for antibodies to HIV each time.

Because of the strong clinical evidence of immunosuppression and the patient’s history of sexual exposure to an HIV-infected individual, additional testing of samples obtained in October 1995 and December 1995 was arranged via the Utah State Health Department and performed at the Centers for Disease Control and Prevention.

These samples were weakly reactive (signal/cutoff ratio, <2.2) with use of an HIV-EIA test kit (Abbott Laboratories, North Chicago, IL). HIV-EIA assays with use of test kits from Organon Teknika (Durham, NC), and Genetics Systems (Seattle) were completely nonreactive (signal/cutoff ratio, <1.0). Western blot assays showed a weak gag band (p17), which was considered an indeterminate result. Assays for p24 antigen were weakly reactive, but because the reactivity was not neutralizable, the assay results were not clearly supportive of HIV infection.

When subjected to base dissociation to disrupt immune complexes, the p24-antigen results became strongly reactive and neutralizable. HIV proviral DNA was also found in peripheral blood mononuclear cells from the patient and his wife in a nested PCR (DNA-PCR). Sequencing showed that the isolates from the patient and his wife differed by 7.4% over 345 nucleotides of the C2V3 region of the env gene and by 3.1% over 383 nucleotides of the p17 region of the gag gene. Construction of a phylogenetic tree demonstrated the close relationship between the HIV isolates from the patient and his wife, with bootstrap support of 98% and 100%, respectively, for each gene region.

Additional information was obtained to better define the patient’s ability to mount an immune response. At the time that testing for antibodies to HIV was negative, an EIA for antibodies to cytomegalovirus (CMV) was positive (signal/cutoff ratio, 2.33), and serology for antibodies to Epstein Barr nuclear antigen was positive (titer, 1:80). Total levels of IgA, IgG, and IgM were 393 mg/dL, 1,000 mg/dL, and 166 mg/dL, respectively—all within normal limits in the testing laboratory. Preinoculation titers and postinoculation titers (at 1 month) for antibodies to Corynebacterium diphtheriae and Clostridium tetani were as follows: preinoculation, 1:320 and 1:2,560; and postinoculation, 1:2,560 and 1:20,480, respectively.

With respect to the possible implications for recipients of our patient’s blood products, we determined that he had only donated plasma at a collection facility regulated by the FDA. All of the plasma was subsequently treated with viral inactivation techniques to ensure that the plasma products were HIV inactivated. Workers at the FDA reviewed the processing records of the plasmapheresis center and determined that none of the blood products from the patient had been transfused without being subjected to an inactivation process. A retrospective investigation of persons who may have received blood products from the patient was not conducted.

**Discussion**

In this report we document the case of an HIV-infected man with 35 negative EIAs for HIV. Because the time when the patient actually became infected cannot be determined, the true number of false-negative tests and the actual time line of his infection are unknown. However, these negative results clearly persisted through a period of asymptomatic infection that evolved into advanced HIV infection, with the occurrence of two separate AIDS-defining opportunistic infections and with CD4⁺ lymphocyte counts indicative of severe immunosuppression.

The persistently seronegative status of our patient is not associated with previously recognized causes. The most common cause of negative results is testing during the window period when seroconversion is occurring [2, 8]; in this case, the patient was well past a window period and had advanced infection while he was still HIV seronegative. Isolated case reports have suggested that patients with late-stage infection may lose antibodies because of overwhelming failure of the immune system (seroreversion). However, seroreversion seems unlikely in our patient because of the prolonged duration of testing before he evidenced advanced disease. On the basis of antibody responses to immunization, he was clearly capable of producing antibodies and should have been capable of displaying an antibody response to HIV. Moreover, investigators who have studied large numbers of HIV-infected patients have failed to identify any with true seroreversions [9, 10].

There has been recent concern about the divergence of HIV strains, which may make detection of the virus with use of the current kits impossible [11]. For example, group O strains of HIV are not consistently detected by EIAs designed to detect HIV-1 and HIV-2 in the United States [12]. The first group O infection was identified in the United States in July 1996 [13]. Genetic analysis of our patient’s virus showed a group M, subtype B strain typical of those usually found in the United States. This strain was practically identical genetically to that causing a typical, HIV-antibody-positive infection in his wife.

A small number of persistently seronegative HIV-infected patients have been described, and this phenomenon cannot be explained [14–16]. In these cases, disease progression was rapid, and samples for testing were obtained only after the patients became ill. Our patient has not had a rapidly progressive clinical course and remains stable despite his episodes of P. carinii infection and CMV infection 6 months after his initial presentation.

Without a definite explanation for our patient’s negative antibody status, it is difficult to determine the likelihood of additional similar cases although reports of such cases are rare. The results of viral culture, p24-antigen detection, and proviral DNA amplification techniques have rarely demonstrated infection in patients with known high-risk exposures and negative antibody tests [17–20]. In the present report, we describe a patient for whom standard laboratory screening failed to detect HIV infection but for whom p24 antigen detection and DNA-PCR established a diagnosis.
The most important implication of false-negative test results is the effect it may have on the blood supply. The blood supply in the United States is screened through deferral of donors with a history of exposure risks and by laboratory testing of all donors [21]. Since 1985, a total of 35 cases of AIDS have been associated with receipt of presumed window-period donations [22]. The sensitivity of routine screening has improved over time. In addition, the FDA has recently issued guidelines requiring HIV-1 p24-antigen testing, primarily as a way of decreasing the window period [23]. The recent commercial availability of this test may have the additional benefit of identifying other patients similar to ours.

While antibody-seronegative HIV infection is likely a rare occurrence, physicians who treat patients with clinical signs suggestive of immunosuppressive disease and negative EIAs should consider requesting specialized laboratory assistance from their state or local health departments in evaluating for seronegative HIV infection. It is important to recognize that a patient may be reportable as an AIDS case even in the absence of a positive test for antibodies to HIV. For example, a definitive diagnosis of P. carinii pneumonia, regardless of the CD4+ lymphocyte count or a diagnosis of other opportunistic illnesses, in association with a CD4+ lymphocyte count of <400/ mm³, defines AIDS [24, 25]. Laboratory procedures such as standard antigen testing, antigen testing after immune complex dissociation, and nucleic acid detection assays may be helpful in defining the HIV status of such patients.

References