A Case of Seronegative HIV-1 Infection

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Patients infected with human immunodeficiency virus type 1 (HIV-1) typically seroconvert within weeks of primary infection. In rare cases, patients do not develop antibodies against HIV-1 despite demonstrable infection. We describe here a human leukocyte antigen (HLA)–B*5802–positive individual who presented with acquired immune deficiency syndrome despite repeatedly negative HIV-1 antibody screening test results. Phylogenetic analysis of env clones revealed little sequence diversity, and weak HIV-1–specific CD8+ T cell responses were present to Gag epitopes. The patient seroconverted after immune reconstitution during receipt of highly active antiretroviral therapy. Lack of an antibody response to HIV-1 is rare and appears to be due to a defect in HIV-1–specific immunity rather than infection with attenuated virus.

Acute infection with human immunodeficiency virus type 1 (HIV-1) is characterized by high viral loads and a rapid decrease in CD4+ T cells [1]. A humoral response with antibodies that target multiple viral epitopes classically follows acute infection by 3 weeks [2] and is the mainstay of clinical diagnosis [3]. In rare cases, patients do not develop humoral immunity to HIV-1 [4–6]. We describe the clinical, immunologic, and virologic features of one such patient.

Case report. In January 2007, a 59-year-old man presented to Johns Hopkins Hospital with complaints of chest pain, shortness of breath, and cough, all of which had worsened over a 3-week period. He reported a negative HIV test result 3 months earlier. The patient was admitted to the hospital with a diagnosis of community-acquired pneumonia. A screening HIV-1 enzyme-linked immunosorbent assay (ELISA) result was negative. He was found to have pancytopenia, which prompted concern for malignancy or occult infection. Serum and urine protein electrophoresis test results were normal. A peripheral blood smear test revealed a normochromic, normocytic anemia, and leukocyte flow cytometry results were negative for leukemia or lymphoma markers. Quantitative plasma immunoglobulin tests revealed immunoglobulin G (IgG) and immunoglobulin A (IgA) levels that were slightly above the reference range; immunoglobulin M (IgM) levels were normal. Test results for human T-lymphotropic virus 1 and 2 (HTLV-1 and 2) were negative.

The patient remained persistently hypoxic despite antibiotic therapy and underwent bronchoscopy and bronchoalveolar lavage, which revealed Pneumocystis jiroveci. His CD4+ T cell count was 90 cells/μL, and his HIV-1 RNA level was >100,000 copies/mL. Although repeat HIV-1 ELISA and Western blot results were again negative (Figure 1), the patient was given a diagnosis of HIV-1 infection and AIDS. HIV-1 genotyping revealed clade B pansensitive virus, with minor mutations in reverse transcriptase and protease coding regions. The patient denied illicit drug use but had engaged in unprotected sex with a commercial sex worker 2 months before hospital admission. He improved clinically and began receiving combination antiretroviral therapy (ART) with tenofovir, emtricitabine, and efavirenz. He experienced a steady rise of his CD4+ T cell count to >500 cells/μL, and his viral load fell to 57 copies/mL. Four months after the initiation of ART, HIV serologic tests were repeated, and the results were strongly positive (Figure 1).

Methods. A portion of the data presented in the Results section was obtained as part of the diagnostic work-up, including the patient’s history, initial HIV-1 antibody test results, HIV-1 genotypic resistance profiling and clade determination, serum immunoglobulin assays (including of IgG to common antigens), CD4+ T cell counts, and viral loads. The patient’s initial negative Western blot result was confirmed with a commercial kit. The Health Insurance Portability and Accountability Act (HIPAA) confidentiality regulations were followed in compiling this information from the patient’s medical records. After informed consent was obtained, the patient donated...
and p24 are identified (bands consistent with the presence of antibodies to gp120, p55/51, p40, p65). Thus, the results of these blots are both negative. However, these bands are not as intense as the corresponding low standard bands. Therefore, the results of these blots are both negative. Three months after initiating HAART, the patient seroconverted, with positive ELISPOT and standard bands. The results of these blots are both negative. For a patient’s Western blot result to be considered positive, at least 2 of the major bands (gp160, gp120, gp41, and p24) must be present. In panel A, there are faint bands consistent with the presence of antibodies to p24, p31, p40, and p65 (red arrows). These bands are not as intense as the corresponding low standard bands. Thus, the results of these blots are both negative. Three months after initiating HAART, the patient seroconverted, with positive enzyme-linked immunosorbent assay and Western blot results [8]. Positive bands consistent with the presence of antibodies to gp120, p55/51, p40, and p24 are identified (red arrows).

Blood for laboratory evaluation on 2 separate occasions. Phlebotomy analysis was performed in accordance with a protocol approved by the Johns Hopkins Institutional Review Board. Plasma and peripheral blood mononuclear cells (PBMCs) were obtained via ficoll gradient centrifugation. The patient’s PBMCs were tested by an interferon enzyme-linked immunospot (ELISPOT) assay, using antibodies obtained from Mabtech. Duplicate wells containing 10⁵ PBMCs were stimulated with overlapping peptides representing the entire Gag proteome, which were obtained from the AIDS Research and Reference Reagent Program. All plates were evaluated with an automated ELISPOT reader system (Carl Zeiss MicroImaging) and KS4.8 software by an independent scientist in a blinded fashion (Zellnet Consulting). A positive response was defined as more than a mean of 5 positive cells/well. Negative controls routinely had fewer than a mean of 1.5 positive cells/well. HLA genotyping was performed by evaluation of the patient’s genomic DNA obtained from PBMCs [7].

Replication-competent virus was cultured from the patient’s resting CD4+ T cells, and gag and pol sequencing were performed [8]. The C2-V4 region from env was amplified, cloned, and sequenced from plasma from the first time point, and phylogenetic analysis was performed on these sequences as described elsewhere [9]. Sequences have been submitted to GenBank (accession nos. GQ465542–GQ465590). Virus from the patient’s plasma was grown in MT-2 cells (obtained from the AIDS Research and Reference Reagent Program) and in healthy donor CD4+ lymphoblasts for 5 days, and p24 quantitation (PerkinElmer) was performed according to manufacturer’s instructions on days 0, 3, and 5 to determine viral tropism.

**Results.** We performed quantitative immunoglobulin testing to rule out a global defect in antibody production. Our patient had slightly elevated levels of IgG and IgA and normal levels of IgM. In addition, IgG antibodies to cytomegalovirus, hepatitis A virus, and herpes simplex virus types 1 and 2 were present. He had no evidence of other known chronic viral infections, including hepatitis C virus, HIV-2, or HTLV-1 and -2. To estimate how long the patient had been infected, we performed phylogenetic analysis of plasma env C2-V4 sequences. The analysis of 52 independent clones revealed minimal sequence diversity (Figure 2A). Genetic homogeneity among HIV-1 quasispecies is highly suggestive of recent infection, because the virus does not have time to diversify [10]. However, we cannot rule out the possibility that this observed genetic homogeneity is a reflection of the absence of selective pressure mediated by neutralizing antibodies. We measured viral coreceptor tropism by a viral culture assay in which the patient’s virus, isolated from plasma, was grown in both primary CD4+ lymphoblast and MT-2 cell line cultures. CD4+ lymphoblasts express both CCR5 and CXCR4, and MT-2 cells express CXCR4 only. The patient’s virus grew in CD4+ lymphoblasts but not in MT-2 cells, as measured by p24 quantification (PerkinElmer). The cell cultures were also infected with 2 viral strains with known coreceptor tropism, IIb (X4) and Ba-L (R5), to serve as controls. Ba-L virus grew in CD4+ lymphoblast cultures, whereas IIb virus grew in MT-2 cells. The patient’s virus is CCR5 tropic by this assay (Figure 2B).

ELISPOT assays evaluating interferon γ responses to Gag epitopes were performed at 2 different time points to assess the HIV-1–specific cellular immune response of this patient, the first during the patient’s seronegative period and the second after immune reconstitution and seroconversion. At both early and late time points, he had weak responses to 2 Gag epitopes. In response to the EKAFSPEVIPMFSALSEGA epitope (Gag 165–179), 50 spot-forming cells (SFCs)/10⁶ PBMCs was detected at the early time point and 95 SFCs/10⁶ PBMCs at the late time point. In response to the SPEVIPMFSEQA epitope (Gag 161–175), 50 SFCs/10⁶ PBMCs was detected at the early time point and 75 SFCs/10⁶ PBMCs at the late time point. Although the
Characterization of \textit{env} sequences. The phylogenetic tree of plasma \textit{env} sequences sampled when the patient was seronegative is shown on the left. The tree shows a striking lack of diversity. Roughly half of these 52 independent sequences are identical (inset), with the remainder differing by a small number of nucleotides. On the right, p24 levels from infected primary CD4$^+$ lymphoblast and MT-2 cell line cultures over a 5-day period were used to determine the tropism of the patient’s virus. CD4$^+$ lymphoblasts, which express both CCR5 and CXCR4, and MT-2 cells, which express CXCR4 only, were infected with 3 strains of virus: IIIb (X4), Ba-L (R5), and the patient’s virus. Ba-L virus and the patient’s isolate grew in CD4$^+$ lymphoblast cells, whereas only IIIb virus grew in MT-2 cells. The patient’s virus is CCR5 tropic by this assay.

First Gag epitope contains the HLA-B*57/5801–restricted epitope KF11 (162–172), no response to this optimal epitope was seen (data not shown). His cellular immune response to HIV-1 Gag appears to have been present throughout infection but did not expand with immune reconstitution despite the emergence of a humoral response to HIV-1 antigens (Figure 1).

To further characterize the patient’s immunologic response to infection, we isolated genomic DNA obtained from his PBMCs and performed HLA genotyping, which identified the following alleles and allele groups: HLA-A*02, HLA-A*26, HLA-B*35, and HLA-B*5802. HLA-B*5802 has been associated with rapid disease progression [11]. The presence of this allele, along with his clinical history of a recent high-risk sexual exposure and the evidence of minimal quasispecies diversity by phylogenetic analysis, suggests that our patient experienced an accelerated disease course.

\textbf{Discussion.} The patient had clinical and laboratory evidence of AIDS despite HIV-1 antibody test results that remained persistently negative until after he had achieved immune reconstitution with highly active ART (HAART). False-negative HIV-1 screening test results are rare and are usually attributed to testing within several weeks of primary infection, before a detectable humoral response develops [12]. Little evidence exists to support the phenomenon of “seroreversion,” in which patients with HIV-1 infection lose their antibody response over time [13]. The patient described here, who presented with symptomatic AIDS and was persistently seronegative over a 5-month period, does not fall into either of these categories and was given a diagnosis of seronegative HIV-1 infection. Making this diagnosis often involves a combination of repeatedly negative serum HIV-1 ELISA and Western blot results without prior positive results, clinical evidence of AIDS or HIV-related symptoms, and evidence of HIV infection on the basis of p24 antigen positivity, HIV-1 DNA or RNA positivity by polymerase chain reaction, or HIV-1 culture positivity. Previously described cases of seronegative HIV-1 infection have not documented spontaneous seroconversion; patients who were not treated for HIV-1 with ART either died or were lost to follow-up, whereas several of
those treated with HAART, including our patient, developed HIV-1 antibodies after immune reconstitution [4–6].

In sequencing gag, pol, and env genes from this patient’s virus, we were unable to identify any large deletions that could have explained the lack of an HIV-specific antibody response. Both clinical evidence and viral culture assays suggested that he was infected with a replication-competent virus. This is in keeping with prior descriptions of seronegative HIV-1 infection [4–6], in which a host immune defect appears to be responsible for seronegativity rather than infection with an attenuated or mutated virus.

The role played by antibodies in the control of untreated HIV-1 infection is not well understood. Several lines of evidence suggest that neutralizing antibodies do not play a major role in controlling viral replication. A recent report demonstrated minimal titers of neutralizing antibodies to autologous virus in elite suppressors—those HIV-1–infected patients with stable CD4+ cell counts who maintain viral loads <50 copies/mL without ART—despite their lack of disease progression; conversely, untreated patients with progressive disease were found to have the highest titers of antibodies to autologous virus [14]. Given a lack of evidence that HIV-1 antibodies can slow the progression of disease, it seems unlikely that seronegativity alone is responsible for what appears to be the rapid disease progression seen in the patient described here.

In contrast to neutralizing antibodies, cytotoxic T cells (CTLs) appear to be central to host defense against HIV-1 infection. This is evident in simian immunodeficiency virus models in which CD8+ cell depletion leads to increased viral infection. Both clinical evidence and viral culture assays suggested that he was infected with a replication-competent virus. This is in keeping with prior descriptions of seronegative HIV-1 infection [4–6], in which a host immune defect appears to be responsible for seronegativity rather than infection with an attenuated or mutated virus.

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In contrast to neutralizing antibodies, cytotoxic T cells (CTLs) appear to be central to host defense against HIV-1 infection. This is evident in simian immunodeficiency virus models in which CD8+ cell depletion leads to increased viral loads that are suppressed after recovery of these cells [15]. It is intriguing that one of the HLA alleles present in this patient, HLA-B*5802, is associated with rapid progression. This allele differs by only 3 amino acids from HLA-B*5801, an allele associated with low viral loads and good disease prognosis [11].

Our patient did have a weak but reproducible T cell response to 2 Gag epitopes while he was seronegative, something that has not been demonstrated previously in these patients. The only other case report to describe HIV-1–specific T cell immunity in seronegative patients did not find T cell responses to the entire HIV-1 proteome in either of 2 patients [6]. Interestingly, once our patient achieved immune reconstitution, his antibody response expanded dramatically (Figure 1), but his T cell responses remained unchanged.

We cannot draw definitive conclusions regarding the pathophysiology of seronegative HIV-1 infection from a single case report; however, some intriguing clues are present. It has been previously demonstrated that CTLs usually appear within weeks of primary infection and are largely responsible for controlling both viremia and the tempo of disease progression. One explanation of the pathophysiology of seronegative HIV-1 infection is that the transmitted virus, replicating without significant immune pressure because of an absent [6] or narrow CTL response such as the one seen in the patient described here, leads to overwhelming immune suppression and symptomatic AIDS. Thus, the hallmark of seronegative HIV-1 infection—lack of detectable HIV-1 antibodies—may be a marker for deficient HIV-specific cellular immunity in these patients, which in turn may be the underlying etiology of their unique and dramatic clinical course. This hypothesis needs to be tested in a larger number of patients to better understand the interaction between cellular and humoral immunity in the control of HIV-1 infection, which could have major implications for HIV-1 vaccine design.

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