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MAJOR ARTICLE

A Case of Long-Term Seronegative Human Immunodeficiency Virus (HIV) Infection: The Importance of the Humoral Response to HIV

Reed A. C. Siemieniuk,[1,2] Frank van der Meer,[3] Guido van Marle,[4] and M. John Gill[4,5,6]

[1]Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, Ontario, [2]Department of Medicine, University of Toronto, Ontario, [3]Department of Ecosystems and Public Health, Faculty of Veterinary Medicine and Departments of [4]Microbiology, Immunology and Infectious Diseases, and [5]Medicine, Cumming School of Medicine, University of Calgary, Alberta, and [6]Southern Alberta HIV Clinic, Calgary, Canada

Background. Seronegative human immunodeficiency virus (HIV) infections are exceedingly rare but might inform HIV-host physiology.

Methods. We investigate the cause and consequences of a patient infected with HIV who did not mount a humoral response to HIV for 4 years.

Results. The patient was confirmed HIV-uninfected by nucleic acid testing 4 months before rapidly progressing to acquired immune deficiency syndrome. The patient's humoral deficit was specific to HIV: he mounted robust humoral responses to all challenge vaccines including influenza A(H1N1)pdm09 and all T cell-dependent and -independent serotypes in the 23-valent pneumococcal polysaccharide vaccine. The virus had similar gp120 antigenicity to HIV-positive control serum as NL4-3 and YU2 prototype strains. Two human leukocyte antigen alleles associated with rapid progression were identified (B*08 and B*35), and a cytotoxic T-lymphocyte epitope site variant was noted: E277K. Viral decay ($t_{1/2} \approx 39$ weeks) suggested that relatively long-lived cells were the source of ongoing viremia. Human immunodeficiency virus viremia was not suppressed until after the patient developed a humoral immune response, despite therapeutic antiretroviral levels. No resistance was detected by virtual phenotyping of virus obtained from serum or from gastrointestinal biopsies despite considerable antiretroviral selection pressure.

Conclusions. Ineffective antibody production may be associated with a subgroup of extremely rapid HIV progressors. Although antiretroviral therapy may be sufficient to slow propagation of infection, it appears to be ineffective for HIV viral clearance in the absence of a humoral response.

Keywords. cellular immunity; HIV; humoral immunity; seronegative; serosilent.

The cornerstone for the diagnosis of human immunodeficiency virus (HIV) infection in the 33 million individuals living with HIV is the presence of antibodies against HIV. After the introduction of serologic testing for HIV antibodies, there have been rare reports of individuals who, despite incontrovertible evidence of active HIV infection, have been found to be "seronegative". They either did not mount, or were extraordinarily slow in mounting, an antibody response to HIV (reviewed by Spivak et al [1]). Such individuals offer a glimpse into the role of the humoral immunity in the host response to HIV.

Approximately 7 days after HIV acquisition, there is usually a substantial viremia characterized by a burst of viral protein production (detectable as p24 antigenemia), followed by the appearance of antibodies against viral proteins at a median of 13 days after infection [2]. The first detectable free plasma anti-HIV antibody is typically anti-gp41 immunoglobulin (Ig) M and/or IgG [2]. Anti-gp120 antibodies, which are more effective than anti-gp41 antibodies at controlling HIV, become detectable at a median of 28 days after infection [2]. Currently, the standard algorithm for the diagnosis of HIV infection consists of a screening enzyme-linked immunosorbent assay (ELISA), which detects anti-HIV IgM, anti-HIV IgG, and, in 4th-generation assays, p24 antigen. Positive ELISA results should be confirmed, with either an antibody-based HIV Western blot, a second ELISA that differentiates HIV-1 from HIV-2 antibodies, or HIV nucleic acid testing [3]. Antibody-based HIV testing can lead to false-negative results in seronegative infections.

In most instances, short-term seronegative HIV infection can been readily explained either by antibody-based testing during the short window period between HIV acquisition and antibody formation or by technical issues such a false-negative ELISA screening of rare and atypical virus strains [4]. Seroreversion, when antibody levels fall below a threshold of detectability after many years of viral antigen suppression with antiretroviral therapy (ART), has also been reported [5].

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Correspondence: M. J. Gill, Medical Director, Southern Alberta HIV Clinic, #3223 1213 4th St SW, Calgary, Alberta, Canada T2R 0X7 (john.gill@albertahealthservices.ca).

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In a review of 25 reported seronegative HIV infections [1], clinical presentations were severe and mortality often occurred soon after infection, from opportunistic infections or HIV itself. HIV diagnosis was most often based on high plasma HIV viral load and severely depressed CD4 count (always <250 cells/mm^3). The presence of any overt underlying humoral immune deficiency such as hypogammaglobulinemia or common variable immunodeficiency was seldom found. Moreover, an unrestricted ability to mount an appropriate antibody response to other pathogens was also common. No unique subtype of virus or unifying host human leukocyte antigen (HLA) genotype was identifiable. In almost all of the surviving cases, seroconversion occurred within 4 months of initiating ART.

Persistently seronegative HIV infections (ie, beyond 4 months of infection) are an even rarer subgroup of seronegative HIV-infected patients. Understanding such outliers is important because they may reveal clues to the nature of the immune response to HIV infection, which is of interest for those developing antibody-dependent vaccines [6] and antibody-based therapies for patients infected with HIV [7, 8]. We report a case of acute HIV infection presenting as acquired immune deficiency syndrome (AIDS) in a patient who failed to develop an antibody response for 4 years despite ART and CD4 cell recovery.

Case

This case involves acute HIV infection in a healthy 35-year-old gay male with a lifestyle at high-risk for HIV acquisition; the patient also underwent routine HIV screening every 6 months (AxSYM HIV-1/HIV-2 gO 3rd-generation immunoassay; Abbott, Saint-Laurent, Quebec). One month before his initial presentation, he was confirmed to be HIV-uninfected by ELISA and HIV polymerase chain reaction (PCR) RNA testing (performed retrospectively; Table 1). He was subsequently seen in an emergency department for fever and malaise, and a work-up including mononucleosis antibody screen was negative. Within 1 week, he developed diffuse watery diarrhea, a generalized desquamating erythematous rash with sparing of his palms and soles, as well as hepatitis, pancreatitis, and thrombocytopenia. Extensive viral testing (including HIV with a 3rd-generation ELISA for antibodies to HIV) was negative. He was prescribed prednisone 40 mg daily for 7 days followed by a 2-week taper, leading to some symptomatic relief and resolution of the rash. However, the diarrhea persisted and his weight declined 16 kg: a *Giardia lamblia* enteric infection was successfully treated with antimicrobials.

Over the next 4 months, he was hospitalized twice with AIDS-defining opportunistic infections. He first presented with shortness of breath and hypoxia: *Pneumocystis pneumonia*, diagnosed via bronchoscopy, was treated with trimethoprim/sulfamethoxazole and prednisone [9]. Again, HIV 3rd-generation ELISA was negative (Table 1). Two weeks later, he was admitted with cytomegalovirus pneumonitis and retinitis as well as Kaposi's sarcoma. Multiple ELISA serologic tests for HIV were negative (AxSYM HIV-1/HIV-2 gO 3rd-generation immunoassay). Human immunodeficiency virus-1 diagnosis was confirmed through detection of proviral DNA by PCR, presence of HIV-1 p24 antigen, and a plasma RNA viral load >100 000 copies/mL (Roche UltraSensitive Amplicor assay, version 1.5; Roche Diagnostic Systems Inc, Somerville, NJ).

After initiating ART (efavirenz, tenofovir, and emtricitabine), there was a reduction in plasma viral load to 47 000 copies/mL

Table 1. Human Immunodeficiency Virus Testing Results

	ELISA (S/C)[a]			
Days From Diagnosis	AxSYM 3rd gen	Bio-Rad 3rd Gen	Confirmatory Testing	Viral Load (Copies/μL)
–158 (Asymptomatic)	Neg		Neg NAAT	
–120 (Acute HIV)	Neg (0.42)		Neg WB	
0 (AIDS)	Neg (0.43)		Pos p24 Neg WB	250 000
107	Neg (0.32)			69 000
211	Neg (0.41)			8900
252	Neg (0.43)			>200 000
552	Neg (0.34)			39 000
1008	Neg (0.42)	Neg (0.33)	Neg WB	1456
	Architect 4th gen	Bio-Rad 4th gen		
1336	Pos (2.43)	Pos (>20)	Indeterminate WB[b]	517
1447	Pos (9.10)	Pos (>20)	Pos WB[c]	898
1691	Pos (48.70)	Pos (>20)		681
1855	Pos (48.89)			

Abbreviations: AIDS, approximately; ELISA, enzyme-linked immunosorbent assay; gen, generation; HIV, human immunodeficiency virus; NAAT, nucleic acid amplification test; Neg, negative; Pos, positive; p24, p24 antigen; S/C, signal/cutoff ratio; WB, Western blot.

[a] ELISA is positive if signal/cutoff >1.

[b] Very weak band at p24. No bands detected at p31, gp41, p51, p55, p66, or gp120/160.

[c] Very weak bands detected at p24, gp41, and gp120/160. No bands detected at p31, p51, p55, or p66.

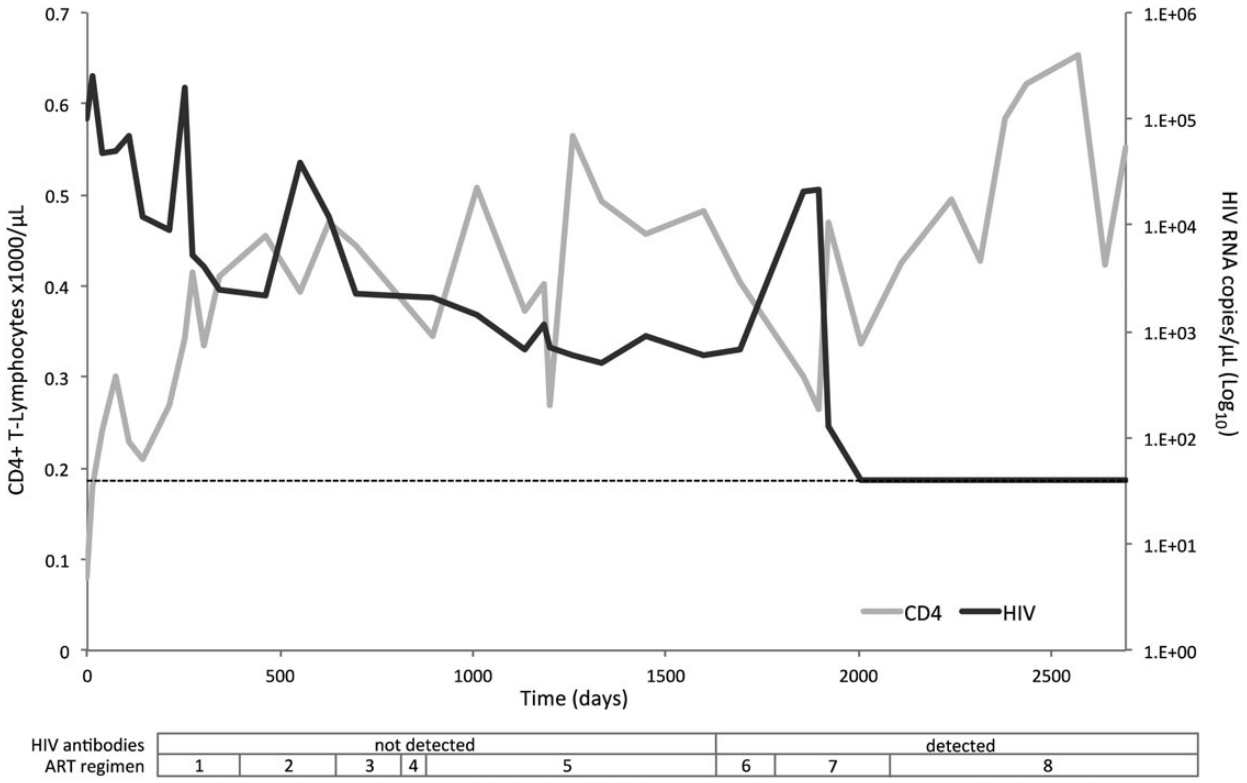

Figure 1. Timeline of human immunodeficiency virus (HIV) parameters. The patient did not achieve viral suppression until 5 years and 7 months after initiating antiretroviral therapy, which only occurred after the development of anti-HIV antibodies, first detected at 4 years 0 months (day 1447). The longitudinal representation of the HIV viral load (in RNA copies/µL) and CD4 cell counts during the 7.5-year observation period (day 0 is the time of HIV diagnosis). The detection limit of the polymerase chain reaction for the RNA copies was 40 RNA copies/µL indicated with dashed line (-----). The antiretroviral therapy (ART) regimens were as follows: 1. EFV/TDF/FTC; 2. AZT/3TC/LPV/r; 3. AZT/3TC/DRV/r; 4. AZT/3TC/LPV/r; 5.TDF/FTC/LPV/r; 6. ABC/3TC/LPV/r; 7. TDF/FTC/RAL; 8. ETR/DRV/r. Abbreviations: ABC, abacavir; AZT, azidothymidine; DRV, darunavir; EFV, efavirenz; FTC, emtricitabine; LPV, lopinavir; RAL, raltegravir; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine.

at 5 weeks (Nuclisens EasyQ, version 1.1; bioMéieux Inc, St. Laurent, Quebec) and a CD4 recovery from a nadir of 80 cells/mm^3 (12%) to 242 cells/mm^3 (33%). Over next 33 months, multiple ART regimens were used for a pan-sensitive virus; however, the patient remained persistently viremic. There was moderate CD4 cell recovery (maximum CD4 count, 565 cells/mm^3; maximum percentage, 37); however, antibodies to HIV-1 were not detectable until 49 months after infection (Figure 1). Of practical interest, following the appearance of antibodies, the persistent viremia resolved. Overall, the patient remained well clinically and without evidence of progressive HIV/AIDS disease while adherent to ART. He did experience adverse effects from ART, including diarrhea while prescribed lopinavir and reversible renal toxicity while prescribed tenofovir disoproxil fumarate.

We confirm that the patient did not mount an antibody response (to rule out laboratory error) and document extremely rapid progression to AIDS in this HIV-infected, seronegative patient. We also investigate the viral and host factors that might explain the prolonged seronegativity. Finally, we report on the co-occurrence of persistent HIV viremia despite ART and speculate on its relationship with an absence of humoral immune response.

METHODS

We present results obtained through clinical diagnostic workup and disease monitoring, including the patient's history, physical exam findings, HIV antibody screening, viral RNA resistance profiling, and routine blood monitoring. Standardized commercial kits were used in all cases unless otherwise specified. The patient gave informed consent to have clinical data published in anonymity.

Human Immunodeficiency Virus Testing

We used an IgM/IgG ELISA assay (AxSYM HIV-1/HIV-2 gO 3rd-generation immunoassay) for HIV testing until 48 months after infection, when a 4th-generation ELISA assay was introduced as standard of care in our region (4th-generation assays include p24 antigen in addition to IgM/IgG). To rule out assay error as a cause of seronegativity, results were confirmed with a second assay (Bio-Rad 3rd-generation immunoassay; Bio-Rad Laboratories, Hercules, CA) and by Western blot. To confirm that the

infection was recent upon presentation, nucleic acid amplification testing for HIV was performed on a 0.7 mL plasma sample, which was stored at −70°C at the time of the patient's last negative routine screening test (1 month before initial symptoms).

Viral Testing

We tested the patient's virus for antigenicity. Peripheral blood mononuclear cells ([PBMCs] 5×10^6) were isolated through Ficoll gradient centrifugation and then cultured in Roswell Park Memorial Institute medium 1640 with 10% fetal bovine serum. Cultures were stimulated with recombinant interleukin-2 and phytohemagglutinin as previously described [10]. Media containing the patient's virus was harvested at days 3, 5, 7, and 10. Virus was then precipitated overnight with PEG8000 and concentrated by centrifugation at 13 000 rpm in a microcentrifuge. Virus pellets were resuspended in Laemmli sample buffer [11] and separated with 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. A Western blot was performed with the patient's virus: filters were incubated with serum from a different HIV-infected person with high neutralizing antibody titers [12]. Infectious virus particles (1×10^5) were loaded into each lane, and the blotted sera were diluted 1:100 for the seronegative patient and 1:500 for the seropositive control.

We used PCR to amplify and clone *nef* genes, using previous protocols [13], to identify any known changes associated with altered disease progression compared with consensus *nef* sequences from the prototype strains HIV-1 NL4-3 [14] and YU-2 [15].

Host Factors

We examined potential host factors that might explain the lack of humoral response by testing for innate immunodeficiencies (compliment and quantitative Igs) and by quantifying the humoral response to challenge antigens, including influenza A (H1N1)pdm09, tetanus antitoxin, and polysaccharide pneumococcal antigens.

Persistent Viremia

We examined the lack of ART resistance despite prolonged exposure to substantial selection pressure from ART: we analyzed genetic evolution in the reverse-transcriptase (RT) and *nef* genes with sequences obtained from cDNA and proviral DNA from PBMCs obtained from serum samples at 0 and 3 years after infection. The methods are reported elsewhere [13, 16].

We also evaluated the cellular immune response: to rule out the emergence of cytotoxic T-lymphocyte (CTL) escape mutations as an explanation for the persistent viremia, we performed in silico analyses 4, 10, and 18 months after the initiation of ART. We cross-referenced the patient's *pol* and *nef* amino acid sequences with the Cytotoxic T-Lymphocyte T-Cell Epitope Variant and Escape Mutation Database as well as the epitope binding prediction tool at the Immune Epitope DataBase provided by the Los Alamos HIV molecular immunology database (available at: http://www.hiv.lanl.gov/).

The gut-associated lymphoid tissue is an important reservoir for HIV replication and drug resistance [16–18]. The patient had routine gastrointestinal biopsies as part of regular colon cancer screening. Nonnucleoside RT inhibitors (NNRTIs) and nucleoside RT inhibitors (NRTIs) drug resistance profiles were confirmed by clonal sequencing using nested PCR products, from these gastrointestinal biopsies with methods previously described [13, 16].

The rate of decay of viremia is thought to be a function of the longevity of newly infected cells: therefore, we assessed the half-life of the persistent viremia [19]. We excluded measurements when the patient was known to be nonadherent or when the viral load increased from the previous measurement.

RESULTS

The serum from our seropositive control had similar immunoreactivity to the gp120 from the case patient's virus as it did to the prototype strains NL4-3 [14] and YU2 [15]. However, serum from the case patient did not have immunoreactivity to gp120 from the prototype HIV strains or to the case patient's virus, suggesting that a host rather than viral factor was responsible for the lack of humoral immune response (Figure 2).

Prolonged Seronegativity

Multiple ELISA (AxSYM HIV-1/HIV-2 gO 3rd-generation immunoassay) and Western Blot assays were negative (Table 1). Ten HIV-1/2 ELISA tests were negative (signal-to-cutoff ratio range, 0.32–0.43 [>1.0 is the cutoff for a positive test]), over 3 years from initial presentation. All ELISA results were confirmed with a second assay (Bio-Rad 3rd-generation immunoassay). After 45 months of infection, the patient tested weakly positive with a 4th-generation ELISA (signal-to-cutoff ratio, 2.3) and indeterminate by Western Blot (weak band at p24, no antibodies detected).

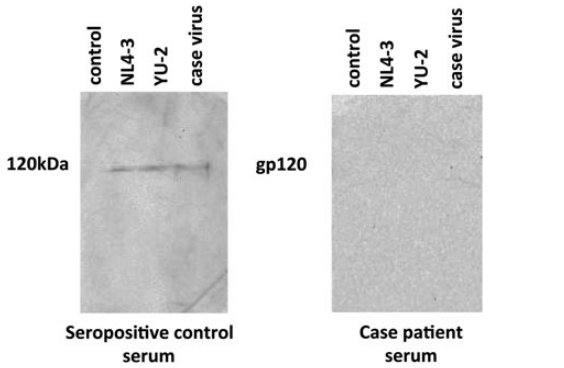

Figure 2. Western blots of serum from seronegative case patient and the seropositive control patient. The seropositive control (left) mounted a robust response to gp120 from virus isolated from the seronegative case patient and to prototype human immunodeficiency virus strains (NL4-3 and YU-2). The case patient's serum did not mount any detectable humoral response to the case virus or the prototype viruses, suggesting that host rather than viral factors explains the lack of humoral immune response. Abbreviations: gp120, envelope glycoprotein 120; kDa, kilodalton.

Antibodies were first detected 49 months from initial presentation (ELISA signal-to-cutoff ratio, 9.1), very weak bands were detected at gp41 and gp120/160. Subsequent testing has remained positive with a 4th-generation immunoassay (4th-generation ARCHITECT HIV Ag/Ab Combo; Abbott, Chicago, IL).

Host Factors

The patient did not have any evidence of immunodeficiency to explain the prolonged seroconversion period. Quantitative Ig (IgA, IgM, and IgG) and complement testing (C4, C4, and CH50) at the time of presentation did not show any evidence of immunodeficiency. Human leukocyte antigen typing was HLA-A*11,*24; B*08, *35; C*07, *04; DRB1*03, *07; DRB3*01; DRB4*Present; and DQB1*02:01, 02.

The patient also mounted a robust immune response to several challenge vaccines, including influenza A(H1N1)pdm09 (prevaccine titer, 1:10; postvaccine titer, >1:1280) and tetanus antitoxin (postvaccine titer, 0.26 IU/mL, reference >0.1 IU/mL), despite the patient remaining seronegative to HIV. The patient had an adequate serological response to all serotypes of the 23-valent pneumococcal polysaccharide vaccine, including T cell-dependent and -independent antigens, which were administered 5 months after HIV diagnosis, while still seronegative to HIV (Appendix Table 1).

Viral Factors

The virus was HIV type 1, group M, subtype B by sequence analysis of the RT gene. Genotypic resistance testing of plasma RNA (Vircotype HIV-1; Janssen Diagnostics, Beerse, Belgium) at 3, 10, 18, 44, and 61 months showed the virus was pansensitive to NNRTIs, NRTIs, and protease inhibitors (PIs). The virus was CCR5 tropic by virtual phenotyping (Trofile Co-receptor Tropism Assay; Monogram Biosciences, San Francisco, CA).

There were no insertions, deletions, or amino acid mutations compared with consensus *nef* sequences from the prototype strains. As mentioned above, virus obtained from the patient by culturing PBMCs, reacted with serum from other patients in Western blot analysis (gp120 positive; Figure 2) similar to the prototype viruses, suggesting no unique antigenicity of the viral envelope.

Persistent Viremia

Despite appropriate ART and use of different antiretroviral agents active at various stages of the HIV lifecycle, low-level HIV viremia persisted for 5 years. No NNRTI or NRTI resistance was detected by clonal sequencing of generated PCR products.

We decided to expand the amount of clones for the 3-year samples because our initial genetic evaluation of RT indicated some nucleotide changes. One clone had an E138G mutation, which is associated with low-level resistance to NNRTIs. This was the only mutation associated with NRTI or NNRTI resistance detected in 12 clones. Some of the other clones derived from 3-year material had 1 or 2 amino acid changes when compared with HXB2, but none of those were indicative of a resistance development to either NRTI or NNRTI (S163G, K43R, K46R, L34F, F130S, K104E, and E204G). The mean distance between sequences over 3 years was 0.0057 ± 0.0028. Overall, no significant drug resistance mutations were found at either time point, including in any of the gastrointestinal samples, despite 3 years of continuous ART and persistent viremia.

We also evaluated the typically conserved *nef* gene at 3 years and demonstrated an insertion (nucleic acid position 8880: GCCAGCAGCAGT) compared with prototype strains HXB2 and NL4-3. This resultant amino acid changes for HXB2 are AEPAADRVGAA to AEPAAEPAAVGVGAA, which are similar to insertions found in the prototype HIV-1 YU-2, and are also found in other isolates [13, 20]. To our knowledge, these do not appear to be linked to altered pathogenesis.

We did not identify any CTL escape mutations after the initiation of ART. A CTL epitope variant at HXB2 *pol*(273–282) resulting in the amino acid change of VPLDEDFRKY to VPLDkDFRKY had been identified in a previous report of 2 patients with HLA B*35-Px who had faster than expected progression [21]. No *nef* epitope mutations or variants were identified that would explain the inability to produce a humoral response.

We detected 2 distinct phases of viral decay: the average half-life for the first 20 weeks was 14 days. For the next 5 years, the average calculated half-life was 270 days (39 weeks).

Host Factors

Because there was no evidence of meaningful drug resistance mutations or other identifiable viral factors explaining the persistent viremia, we decided to rule out subtherapeutic ART levels as an explanation of the persistent viremia. Quantitative trough serum testing for lopinavir and ritonavir were performed retrospectively, without the patient having advanced notice. Lopinavir was well above the trough 95% effective concentration (EC95): minimum lopinavir level = 3640 ng/mL, trough EC95 = 926 ng/mL [22]. Two other samples were consistent with the trough levels: one 2 hours after ingestion and 1 random level, respectively (lopinavir = 9970 ng/mL and 12 400 ng/mL; ritonavir = 1270 ng/mL and 1210 ng/mL). Moreover, the patient reported >95% ART adherence, which was corroborated by pharmacy refill data.

Switching ART (including multiple different classes) also failed to achieve viral suppression while the patient remained seronegative (Figure 1). Furthermore, secondary evidence of compliance included an expected increase in mean corpuscular volume when prescribed zidovudine (all measurements >100 fL/cell while prescribed zidovudine and returned to normal range when not prescribed zidovudine, usually approximately 90 fL/cell). The patient also consistently described persistent watery stools when prescribed ritonavir-boosted lopinavir, which resolved while not prescribed lopinavir.

DISCUSSION

To our knowledge, the patient in this study has the longest reported duration of seronegative HIV infection, with a delay of 49 months between infection and seroconversion [23].

The severe presentation of acute HIV infection seen in our patient, similar to presentations reported in other seronegative HIV patients, suggests that the humoral immune system in response to acute HIV may be vital in delaying progression to AIDS. The patient was confirmed to be HIV negative with nucleic acid amplification test of a stored sample only 1 month before his presentation with symptoms most consistent with acute HIV infection. The patient then had an extremely rapid progression to AIDS-defining illnesses, only 4 months after his acute HIV illness. The term "rapid progressor" is used to define a group of patients with progression to AIDS within 5 years of infection [24, 25]. To our knowledge, this patient progressed to AIDS more rapidly than any other documented case. Analogous to the subgroup of "elite controllers", there may be a subgroup of rapid progressors, "extremely rapid progressors", who develop AIDS within 6 months of infection. Our case adds strength to the possibility that an inadequate humoral immune response might contribute to rapid HIV progression.

The patient did not develop either HIV-specific antibodies or achieve viral suppression in plasma viral load for 4 years, despite appropriate ART and adequate CD4 recovery. Similar to other reports of seronegative HIV patients, we document an apparently otherwise normal immune function [1]. One intriguing aspect of his care is that he received high doses (both daily doses and cumulative dose) of prednisone soon after infection. Although corticosteroids are widely used for their immunosuppressive effects [26], often in the context of common infections [27], they are not known to suppress antibody production [28]. It also seems improbable that a corticosteroid effect would be so prolonged after discontinuation of therapy. The patient's CTL-epitope variant (HXB2 E277K) and HLA alleles (B*08 and B*35) may be associated with rapid HIV progression through suboptimal cellular immune responses [21, 29, 30]. It is possible that the interaction of the HLA alleles, CTL-epitope variant, and corticosteroids had synergistic effects leading to rapid progression and inability to mount a humoral immune response, although this is highly speculative.

An intriguing feature of this case is the persistent viremia. Although high viral loads appear to be a consistent feature of the few reported cases of seronegative HIV [1], the handful of reported surviving cases rapidly seroconverted after immune reconstitution occurred with ART. In this case, viral resistance did not explain failure to suppress the serum viral load; moreover, excellent self-reported compliance was supported by plasma ART levels well above therapeutic concentrations and by secondary markers of compliance such as an increased red blood cell mean cell volume with zidovudine. Multiple viral resistance tests, including sequencing of viral sequences obtained from serum and gut mucosa, proved that the virus remained sensitive to ART. Despite this, suppression of viral replication did not occur with multiple different antiretroviral classes, including PIs, NRTIs, NNRTIs, and integrase inhibitors. Although transient low-level viremia occasionally occurs while on ART, persistent viremia is not known to occur unless there is viral resistance [31].

This case adds to mounting evidence for the vital role of the humoral immune system in HIV viral suppression. Current ART therapy is limited in that although it can inhibit multiple steps from viral entry to integration, it neither reduces production of virus by already infected cells nor can it increase viral clearance [32]. This case adds evidence to the recent success of monoclonal antibody therapy, where transient viral control can be achieved with the antibodies alone [7, 8]. The source of our patient's persistent viremia may have been from already infected CD4 cells and persistent HIV reservoir activation. Viremia decay is likely a function of the longevity of the infected cells. For the first 20 weeks of ART, the half-life was approximately 2 weeks, consistent with decay phase II (thought to be from infected macrophages) [19]. Afterwards, the half-life was considerably longer, approximately 39 weeks, consistent with phase III decay [19]. Therefore, the cells producing the virus are relatively longer lived—more likely to be representative of the HIV reservoir. Antiretroviral therapy in itself may be insufficient to control HIV replication: the humoral immune response may play a vital role in controlling HIV replication and clearance with or without ART.

CONCLUSIONS

We present a case of seronegative HIV infection in a patient who progressed extremely rapidly to AIDS and did not achieve viral suppression until seroconversion after 49 months. Such cases present a rare but important challenge to programs that still exclusively use HIV antibody detection (3rd-generation ELISA) to safeguard the donor blood supply. This case demonstrates the need for expeditious and widespread adoption of 4th-generation testing, which includes viral antigen detection.

There is some practical advice for clinicians that can be drawn from this case. Patients with seronegative HIV infection should be monitored closely for rapid progression to AIDS. In any patient who fails to suppress viral replication despite adequate ART and reports of excellent adherence, serum ART levels can rule out an absorptive problem. In these exceedingly rare cases of persistent viremia and absence of ART resistance, ART should be continued because the patient's disease is at high risk of rapidly progressing.

The sequence data and absence of a serological response were not due a non-antigenic viral variant. The mechanism underlying the failure to produce antibodies appeared to be host-related

and specific to HIV in the absence of other detectable immunodeficiencies; however, the cause was unclear. An absent humoral response to HIV may explain 2 curious findings in our patient: (1) extremely rapid progression to AIDS and (2) an inability to suppress HIV viremia despite appropriate ART.

The limited ongoing viremia despite adequate ART and CD4 cell recovery offers a clue to the importance of the immune response in clearing viremia. This case adds support for antibody-based vaccine and therapy development, especially in the search for a functional HIV cure. These pathophysiologic insights suggest that antibody-based therapy might be necessary to control chronic HIV infection.

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Author contributions. M. J. G. conceived the idea for the study. R. A. C. S. drafted the manuscript. F. v. d. M. and G. v. M. performed critical experiments. All authors provided critical revisions to the manuscript.

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APPENDIX

Table A1. Antibody Responses to 23-Valent Pneumococcal Polysaccharide Vaccine

Serotype	Immunoglobulin Response (μg/mL)
1	0.26
3	0.31
4	0.18
6B	0.19
7	0.27
9V	0.17
11	0.30
12	0.12
14	1.89
15	0.33
18C	0.34
19F	0.52
23F	0.29
33	0.71