Title
Acute HIV-1 Seroconversion with an Unusual Plasma Biomarker Profile

Acute HIV-1 Seroconversion with an Unusual Plasma Biomarker Profile

Najib Aziz,[a] Roger Detels,[a,d] Otoniel Martinez-Maza,[c] John Oishi,[a] Beth D. Jamieson,[d] Mallory D. Witt,[d,e] Anthony W. Butch[b]

Department of Epidemiology, Fielding School of Public Health, University of California in Los Angeles, Los Angeles, California, USA[a]; Department of Pathology & Laboratory Medicine,[b] Departments of Obstetrics & Gynecology, Microbiology & Immunology, and Molecular Genetics,[c] and Department of Medicine,[d] David Geffen School of Medicine, University of California in Los Angeles, Los Angeles, California, USA; Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, USA[e]

An unusual case of acute primary HIV-1 infection in a man with a high plasma viral load, a 51-fold increase in C-reactive protein, and antibodies against only gp160 is described. Numerous serum cytokine concentrations were elevated during HIV-1 seroconversion.

CASE REPORT

A 49-year-old male subject in the Multicenter AIDS Cohort Study (MACS) reported flulike symptoms, fatigue, and drenching night sweats that began 2 to 3 weeks prior to his MACS clinic visit. A sample of the subject's blood was reactive for HIV-1 by enzyme immunoassay (Bio-Rad Genetic Systems rLAV EIA), but Western blot confirmation testing (Bio-Rad Genetic Systems) was indeterminate, with only antibodies against gp160 (Fig. 1). The CD4$^+$-to-CD8$^+$ T cell ratio was inverted at 0.56, and his plasma contained 1.5×10^7 copies/ml of HIV-1 viral RNA (Amplicor HIV-1 Monitor UltraSensitive assay) (Table 1). For comparison, a blood sample from a usual or representative case of HIV-1 infection at seroconversion (antibodies against all HIV-1 viral proteins by Western blot assay) typically contains fewer copies of HIV-1 viral RNA (3.0×10^5 copies/ml or 50-fold less) (Table 1).

At his MACS clinic visit 6 months earlier, this unusual subject gave an unremarkable medical history and denied any current infections. A physical exam failed to reveal any abnormalities, and the subject was negative for HIV-1 by enzyme immunoassay and had a CD4$^+$-to-CD8$^+$ T cell ratio of 1.96, which was within the normal reference interval. At his follow-up visit 7 months after HIV-1 seroconversion, his Western blot assay was positive for antibodies against all HIV-1 viral proteins except p24 (Fig. 1), and his plasma contained 7.4×10^4 copies/ml of HIV-1 viral RNA. Shortly after his follow-up visit, the participant received antiretroviral drug therapy with ritonavir, Truvada, and darunavir.

Various markers of immune activation, including cytokines and chemokines, were measured in plasma samples collected 6 months before seroconversion, during HIV-1 seroconversion and 7 months postconversion (Table 1). At the first HIV-1-positive visit, CD38 expression by CD8$^+$ T cells was elevated at 13,797 molecules per cell. This was determined by converting the relative fluorescence intensity of the CD38 distribution into the number of surface molecules bound per CD8$^+$ cell, as described previously (1, 2). In addition, the levels of the immune activation markers β2-microglobulin, neopterin, and C-reactive protein were all elevated at seroconversion compared to preconversion concentrations (baseline values), which were within the normal reference interval (Table 1). The spike in C-reactive protein was most dramatic, with a 51-fold increase at seroconversion. In contrast, the

C-reactive protein concentration in usual or representative cases is typically within the normal reference interval (<8 mg/liter) at seroconversion (Table 1). The plasma concentrations of interleukin 1β (IL-1β), IL-2, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, gamma interferon (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) in this unusual case were elevated during HIV-1 seroconversion, and all but IL-7 declined to baseline concentrations 7 months later. IL-7 was still above baseline 7 months later (Table 1). In contrast, the plasma concentration of the chemokine IL-8 decreased at seroconversion, and no change in IL-4 and tumor necrosis factor alpha (TNF-α) was observed at seroconversion. Seven months later, IL-8 and TNF-α remained unchanged, whereas IL-4 had declined to undetectable levels (Table 1). Similar increases in IL-1β, IL-2, IL-10, IL-12, IL-13, IFN-γ, and GM-CSF concentrations are also observed for usual or representative HIV-1 cases during seroconversion (Table 1). In contrast, plasma concentrations of IL-7 are typically decreased, whereas the concentrations of IL-4, IL-8, and TNF-α are elevated in usual or representative HIV-1 cases during seroconversion (Table 1).

The MACS is one of the largest prospective studies examining the natural and treated history of HIV-1 infection in men who have sex with men. The MACS, which was started in 1984, has centers located in Baltimore, MD, Chicago, IL, Pittsburgh, PA, and Los Angeles, CA (3, 4). Blood samples are collected from participants twice per year during clinic visits, and seronegative participants are routinely screened for antibodies against HIV-1. Blood from each clinic visit is cryopreserved, providing a valuable resource for elucidating the pathogenesis of HIV infection and for developing therapeutic interventions (5, 6).

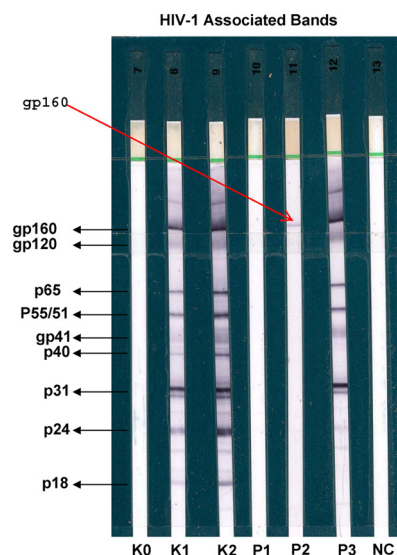The clinical symptoms associated with primary HIV-1 infec-

FIG 1 HIV-1 Western blot results at three MACS clinic visits. K0, K1, and K2 are negative, weak positive, and strong positive controls, respectively. P1, P2, and P3 are before, during (6 months later), and 7 months after seroconversion, respectively. NC is an HIV-1 negative-control plasma sample.

tion occur within days to weeks after infection and can last from a few days up to 10 weeks, although symptoms typically subside within 14 days (7). The most common symptom is fever, which is reported by nearly 75% of HIV-1-infected individuals. Other commonly reported symptoms include fatigue, headache, myalgia, and lymphadenopathy. A maculopapular skin rash, usually involving the trunk, is found in 40 to 80% of persons with symptomatic HIV-1 infection. However, not all patients present with typical symptoms, and many cases of acute HIV infection go undiagnosed or misdiagnosed, especially if a history of recent sexual activity with a high-risk person is not obtained.

This participant presented with only flulike symptoms, fatigue, and night sweats during the acute phase, which usually occurs within 6 weeks following HIV-1 infection. The presence of HIV-1 viral RNA, a reactive HIV-1 EIA, and an indeterminate Western blot pattern with antibodies against only the envelope protein gp160 suggest that the patient was infected approximately 15 to 23 days before being seen in the clinic based on the Fiebig staging system (8).

Previous studies have demonstrated that various cytokines and markers of immune activation correlate with the severity of dis-

TABLE 1 Laboratory test results at MACS clinic visits

	Value					
	Preconversion		Seroconversion		Postconversion	
Cytokine, chemokine, or marker	Subject	Usual case[a]	Subject	Usual case[a]	Subject	Usual case[a]
Cell surface markers[b]						
CD3$^+$ (cells/μl)	1,906	1,572	1,953	1,555	2,745	1,624
CD4$^+$ (cells/μl)	1,246	993	686	689	1,021	717
CD8$^+$ (cells/μl)	635	579	1,214	905	1,596	928
CD4$^+$/CD8$^+$ ratio	1.96	1.71	0.56	0.76	0.64	0.77
CD38 expression on CD8$^+$ cells (molecules per cell)	315	369	13,797	3,130	7,469	4,752
HIV-1 viral load (copies/ml)[c]	<50	<50	1.5×10^7	3.0×10^5	7.4×10^4	7.5×10^4
Activation markers[d]						
β2 microglobulin (mg/liters)	1.1	1.9	2.4	3.3	2.1	3.5
Neopterin (nmol/liters)	6.3	6.4	20.3	14.0	12.5	17.3
C-reactive protein (mg/liters)	3.3	5.2	170.0	2.9	1.4	5.0
Cytokines and chemokines (pg/ml)[e]						
IL-1β	<0.1	0.1	1.4	5.5	<0.1	<0.1
IL-2	1.6	0.2	9.4	6.8	0.1	0.7
IL-4	2.3	<0.1	1.8	2.0	<0.1	<0.1
IL-5	0.3	0.1	1.1	0.1	0.1	0.1
IL-6	0.2	1.8	6.6	2.6	0.4	2.0
IL-7	1.2	4.2	24.4	<0.1	9.0	4.2
IL-8	24.4	8.4	5.7	14.9	6.5	11.7
IL-10	6.4	9.7	50.6	30.7	0.4	26.0
IL-12	0.1	1.6	8.2	43.6	0.1	1.5
IL-13	<1.1	<1.1	5.8	60.1	<1.1	<1.1
IFN-γ	<0.1	<0.1	4.5	1.0	<0.1	<0.1
GM-CSF	0.6	2.5	5.8	12.4	0.1	1.6
TNF-α	7.0	9.9	7.9	27.4	9.4	21.8

[a] An individual with antibodies against all the HIV-1 Western blot viral proteins at the time of seroconversion is considered a usual or typical case.

[b] Cell phenotypes were determined using a FACSCalibur flow cytometer.

[c] The limit of detection is 50 copies/ml.

[d] β2 microglobulin, neopterin, and C-reactive protein were measured by an Abbott IMx immunoassay analyzer, a BRAHMS competitive enzyme immunoassay, and an Immunodiagnostics ELISA kit, respectively.

[e] The lower limit of detection was 1.1 pg/ml for IL-13 and 0.1 pg/ml for all other cytokines and chemokines. IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor. Cytokines and chemokines were measured by Milliplex magnetic high-sensitivity assays using the Luminex 200 multiplexing instrument with Milliplex Analyst 3.1 software.

ease and/or the clinical outcome following HIV-1 infection (9–14). Furthermore, the expressions of cytokines, chemokines, and activation markers shortly after infection are critical factors for determining the disease course (15). For example, elevated plasma concentrations of IL-1β, IL-10, IFN-γ, and TNF-α during the acute phase of HIV-1 infection have been shown to correlate with viral replication (15, 16).

In the case presented herein, we detected elevated plasma concentrations of IL-1β, IL-6, IL-10, and IFN-γ during seroconversion that declined to preconversion levels 7 months later. Interestingly, we did not observe changes in the proinflammatory cytokine TNF-α throughout the monitoring period. A surge in proinflammatory cytokines (IL-1β and IL-6) can result in increased production of acute phase proteins by the liver, which in turn can play a role in inhibiting viral replication (17). Our inability to detect changes in TNF-α during acute HIV-1 infection might be related to the time period when the blood sample was collected for analysis. For instance, at least one other study failed to detect increases in TNF-α during acute HIV infection (18). It is noteworthy that in usual or representative cases of acute HIV-1 infection, the plasma concentrations of TNF-α are elevated at seroconversion and at follow-up MACS clinic visits (Table 1).

Although we found increased levels of IL-2, IL-5, IL-7, IL-12, and IL-13, indicating a broad cytokine response for this subject during seroconversion, IL-5 and IL-7 are not typically increased in usual or representative cases of acute HIV-1 infection. IL-4 was not increased over baseline for this subject, a finding observed in another study (18). Contrary to levels reported in other studies, the IL-2 level was elevated (18, 19) and the IL-8 level was decreased during seroconversion for the subject described here (16). Discrepancies in plasma cytokine concentrations among studies may reflect differences in study design, timing of sample collection, and variability in individual viral immune responses during the acute phase of HIV-1 infection.

Additionally, in this case, several biomarkers of immune activation were elevated during acute infection. Most notably, a 51-fold increase in the plasma concentration of C-reactive protein was detected at seroconversion. At follow-up 7 months later, the C-reactive protein concentration was surprisingly within the normal reference interval of <8 mg/liter. C-reactive protein is not increased in usual cases of HIV-1 infection at seroconversion or during the follow-up visit. Neopterin and β2 microglobulin levels were also elevated during seroconversion (neopterin reference interval, <9.7 nmol/liter; β2 microglobulin reference interval, 1.0 to 2.1 mg/liter) for this subject and might be indicative of future disease progression (12, 13, 19). Interestingly, neopterin and β2 microglobulin are also elevated in usual or representative cases of HIV-1 infection during and after seroconversion. The number of CD38 molecules per $CD8^+$ T cell was also increased 44-fold over baseline for the subject at seroconversion and continued to be elevated (24-fold over baseline) 7 months later. CD38 expression on $CD8^+$ T cells serves as a marker of cellular activation and can be predictive of disease progression in untreated HIV-1-infected individuals (20).

In summary, we report an unusual HIV-1 case identified during seroconversion that presented with an indeterminate Western blot assay based on antibodies against only the gp160 viral protein. Seven months later, the subject was still negative for antibodies against the P24 viral protein. A large spike in C-reactive protein was observed at seroconversion, and the plasma HIV-1 viral load was very high at 1.5×10^7 copies/ml. This dramatically contrasts with usual cases of acute HIV-1 infection at seroconversion that have considerably lower HIV-1 viral loads and normal concentrations of C-reactive protein. There was also a surge in numerous cytokines and chemokines at seroconversion for this subject, including IL-5, IL-6, and IL-7, levels of which typically remain unchanged or decrease in usual cases of HIV-1 infection during seroconversion. Biomarkers of immune activation are typically produced by T cells, macrophages, and dendritic cells and mediate host responses to infection and inflammatory stimuli (21). An understanding of immune responses shortly after infection can provide valuable insight into the pathogenesis of HIV-1 infection. In addition, changes in blood concentrations of immune activation markers following HIV-1 infection may provide valuable clues for developing and assessing vaccines for protection against HIV-1 infection since particular cytokine responses can contribute to viral replication and immunopathology. It will be important to continue to follow this subject to determine if his cytokine and activation marker response patterns are predictive of outcome and/or response to therapy.

ACKNOWLEDGMENTS

REFERENCES

1. **Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, Giorgi JV.** 1997. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **16:**83–92.

2. **Hultin L, Matud JL, Giorgi JV.** 1998. Quantitation of CD38 activation antigen expression on CD8+ T cells in HIV-1 infection using CD4 expression on CD4+ T lymphocytes as a biological calibrator. Cytometry **33:**123–132.

3. **Chmiel JS, Detels R, Kaslow RA, Van Raden M, Kingsley LA, Brookmeyer R.** 1987. Factors associated with prevalent human immunodeficiency virus (HIV) infection in the Multicenter AIDS Cohort Study. Am. J. Epidemiol. **126:**568–577.

4. **Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR, Jr.** 1987. The Multicenter AIDS Cohort Study: rationale, organization and selected characteristics of the participants. Am. J. Epidemiol. **126:**310–318.

5. **Chattopadhyay PK, Douek DC, Gange SJ, Chadwick KR, Hellerstein M, Margolick JB.** 2006. Longitudinal assessment of de novo T cell production in relation to HIV-associated T cell homeostasis failure. AIDS Res. Hum. Retroviruses **6:**501–507.

6. **Rinaldo CR, Jr, Beltz LA, Huang XL, Gupta P, Fan Z, Torpey DJ, III.** 1995. Anti-HIV type 1 cytotoxic T lymphocyte effector activity and disease progression in the first 8 years of HIV type 1 infection of homosexual men. AIDS Res. Hum. Retroviruses **11:**481–489.

7. **Schacker T, Collier AC, Hughes J, Shea T, Corey L.** 1996. Clinical and epidemiological features of primary HIV infection. Ann. Intern. Med. **125:**257–264.

8. **Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, Heldebrant C, Smith R, Conrad A, Kleinman SH, Busch MP.** 2003. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS **17:**1871–1879.

9. **Fahey JL, Taylor JM, Manna B, Nishanian P, Aziz N, Giorgi JV, Detels R.** 1998. Prognostic significance of plasma markers of immune activation, HIV viral load and CD4 T-cell measurements. AIDS **12:**1581–1590.

10. **Aziz N, Nishanian P, Fahey JL.** 1998. Levels of cytokines and immune activation markers in plasma in human immunodeficiency virus infection: quality control procedures. Clin. Diagn. Lab. Immunol. **5:**755–761.

11. **Aziz N, Nishanian P, Taylor JM, Mitsuyasu RT, Jacobson JM, Dezube BJ, Lederman MM, Detels R, Fahey JL.** 1999. Stability of plasma levels of cytokines and soluble activation markers in patients with human immunodeficiency virus infection. J. Infect. Dis. **179:**843–848.

12. **Shi M, Taylor JM, Fahey JL, Hoover DR, Muñoz A, Kingsley LA.** 1997. Early levels of CD4, neopterin, and beta 2-microglobulin indicate future disease progression. J. Clin. Immunol. **17:**43–52.

13. **Hofmann B, Wang YX, Cumberland WG, Detels R, Bozorgmehri M, Fahey JL.** 1990. Serum beta 2-microglobulin level increases in HIV infection: relation to seroconversion, CD4 T-cell fall and prognosis. AIDS **4:**207–214.

14. **Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, Nishanian P, Giorgi JV.** 1990. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N. Engl. J. Med. **322:**166–172.

15. **McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes BF.** 2010. The immune response during acute HIV-1 infection: clues for vaccine development. Nat. Rev. Immunol. **10:**11–23.

16. **Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, Lebedeva M, DeCamp A, Li D, Grove D, Self SG, Borrow P.** 2009. Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. J. Virol. **83:**3719–3733.

17. **Misse D, Yssel H, Trabattoni D, Oblet C, Lo Caputo S, Mazzotta F, Pene J, Gonzalez P, Clerici M, Veas F.** 2007. IL-22 participates in an innate anti-HIV-1 host-resistance network through acute-phase protein induction. J. Immunol. **178:**407–415.

18. **Biglino A, Sinicco A, Forno B, Pollono AM, Sciandra M, Martini C, Pich P, Gioannini P.** 1996. Serum cytokine profiles in acute primary HIV-1 infection and in infectious mononucleosis. Clin. Immuno. Immunopath. **78:**61–69.

19. **Sinicco A, Biglino A, Sciandra M, Forno B, Pollono AM, Raiteri R, Gioannini P.** 1993. Cytokine network and acute primary HIV-1 infection. AIDS **7:**1167–1172.

20. **Giorgi JV, Liu Z, Hultin LE, Cumberland WG, Hennessey K, Detels R.** 1993. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow up. The Los Angeles Center, Multicenter AIDS Cohort Study. J. Acquir. Immune Defic. Syndr. **6:**904–912.

21. **Goonetilleke N, Liu MK, Salazar-Gonzalez JF, Ferrari G, Giorgi E, Ganusov VV, Keele BF, Learn GH, Turnbull EL, Salazar MG, Weinhold KJ, Moore S, Clinical Core CHAVI B, Letvin N, Haynes BF, Cohen MS, Hraber P, Bhattacharya T, Borrow P, Perelson AS, Hahn BH, Shaw GM, Korber BT, McMichael AJ.** 2009. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J. Exp. Med. **206:**1253–1272.