Acute HIV-1 seroconversion with an unusual plasma biomarker profile

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ABSTRACT
An unusual case of acute primary HIV-1 infection in a man with a high plasma viral load, a 5124 fold increase in C-reactive protein, and antibodies against only the 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 flu-like symptoms, fatigue and drenching night sweats that began two to three 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 (Figure 1). The CD4+ to CD8+ T cell ratio was inverted at 0.56 and his plasma contained 1.5 x 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 on Western blot) typically contains fewer copies of HIV-1 viral RNA (3.0 x 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. 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 seven months after HIV-1 seroconversion, his Western blot was positive for antibodies against all HIV-1 viral proteins except p24 (Figure 1) and his plasma contained 7.4 x 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 chemokine were measured in plasma samples collected 6 months before sero-conversion, during HIV-1 sero-conversion and 7 months post-conversion (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 (1, 2). In addition, the immune activation markers β2-microglobulin, neopterin, and C-reactive protein were all elevated at sero-conversion, when compared to pre-conversion concentrations (baseline values) that were within the normal reference interval (Table 1). The spike in C-reactive protein was most dramatic with a 51-fold increase at sero-conversion. In contrast, the C-reactive protein concentration in usual or representative cases are typically within the normal reference interval (<8 mg/L) at sero-conversion (Table 1). The plasma concentrations of IL-1β, IL-2, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, interferon-γ, and GM-CSF in this unusual case were elevated during HIV-1 sero-conversion 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 sero-conversion and no change in IL-4 and TNF-α was observed at sero-conversion. 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, interferon-γ and GM-CSF concentrations are also observed for usual or representative HIV-1 cases during sero-conversion (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 sero-conversion (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 sero-negative 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 infection 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 maculo-papular skin rash, usually involving the trunk, is found in 40–80% 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 flu-like symptoms, fatigue and night sweats during the acute phase, which usually occurs within six 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 disease and/or the clinical outcome following HIV-1 infection (9-15). Furthermore, the expression of cytokines, chemokines and activation markers shortly after infection are critical factors for determining the disease course (16). For example, elevated plasma concentrations of IL-1β, IL-10, interferon-γ, and TNF-α during the acute phase of HIV-1 infection have been shown to correlate with viral replication (16, 17).

In the case presented herein, we detected elevated plasma concentrations of IL-1β, IL-6, IL-10, and interferon-γ during sero-conversion that declined to pre-conversion levels seven months later. Interestingly, we did not observe changes in the pro-inflammatory cytokine TNF-α throughout the monitoring period. A surge in pro-inflammatory 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 (18). 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 (19). It is noteworthy that in usual or representative cases of acute HIV-1 infection the plasma concentrations of TNF-α are elevated at sero-conversion 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 sero-conversion, 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 (19). Contrary to other studies, IL-2
was elevated (19, 20) and IL-8 was decreased during sero-conversion for the subject described here (17). 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 sero-conversion. At follow-up seven months later the C-reactive protein concentration was surprisingly within the normal reference interval of <8 mg/L. C-reactive protein is not increased in usual cases of HIV-1 infection at sero-conversion or during the follow-up visit. Neopterin and β2 microglobulin levels were also elevated during sero-conversion (neopterin reference interval, <9.7 nmol/L; β2 microglobulin reference interval, 1.0 – 2.1 mg/L) for this subject and might be indicative of future disease progression (12, 13, 20).

Interestingly, neopterin and β2 microglobulin are also elevated in usual or representative cases of HIV-1 infection during and after sero-conversion. The number of CD38 molecules per CD8+ T cell was also increased 44-fold over baseline for the subject at sero-conversion 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 (21).

In summary, we report an unusual HIV-1 case identified during sero-conversion that presented with an indeterminate Western blot 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 sero-conversion and the plasma HIV-1 viral load was very high at 1.5 x 10^7 copies/mL. This dramatically contrasts with usual
cases of acute HIV-1 infection at sero-conversion 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 sero-conversion for this subject that included IL-5, IL-6 and IL-7, which typically remains unchanged or decreases in usual cases of HIV-1 infection during sero-conversion. Biomarkers of immune activation are typically produced by T cells, macrophages and dendritic cells, and mediate host responses to infection and inflammatory stimuli (22). 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 pattern is predictive of outcome and/or response to therapy.

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References


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

<table>
<thead>
<tr>
<th>Cell surface markers</th>
<th>Pre-conversion</th>
<th>Sero-conversion</th>
<th>Post-conversion</th>
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<tbody>
<tr>
<td></td>
<td>Subject</td>
<td>Usual case</td>
<td>Subject</td>
</tr>
<tr>
<td>CD3⁺ (cells/uL)</td>
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<td>1,572</td>
<td>1,953</td>
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<td>CD4⁺ (cells/uL)</td>
<td>1,246</td>
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<td>CD8⁺ (cells/uL)</td>
<td>635</td>
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<td>1,214</td>
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<td>CD4⁺/CD8⁺ ratio</td>
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<td>1.71</td>
<td>0.56</td>
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<tr>
<td>CD38 expression on CD8⁺ cells (molecules per cell)</td>
<td>315</td>
<td>369</td>
<td>13,797</td>
</tr>
</tbody>
</table>

HIV-1 viral load:<br>Pre-conversion: <50<br>Sero-conversion: 1.5 x 10⁷<br>Post-conversion: 7.4 x 10⁴<br>

Activation markers:<br>β2 microglobulin (mg/L):<br>Pre-conversion: 1.1<br>Sero-conversion: 2.4<br>Post-conversion: 2.1<br>
Neopterin (nmol/L):<br>Pre-conversion: 6.3<br>Sero-conversion: 20.3<br>Post-conversion: 12.5<br>C-reactive protein (mg/L):<br>Pre-conversion: 3.3<br>Sero-conversion: 170.0<br>Post-conversion: 6.5

Cytokines & chemokines:<br>IL-1β:<br>Pre-conversion: <0.1<br>Sero-conversion: 1.4<br>Post-conversion: 5.5<br>IL-2:<br>Pre-conversion: 1.6<br>Sero-conversion: 9.4<br>Post-conversion: 6.8<br>IL-4:<br>Pre-conversion: 2.3<br>Sero-conversion: 1.8<br>Post-conversion: 2.0<br>IL-5:<br>Pre-conversion: 0.3<br>Sero-conversion: 1.1<br>Post-conversion: 0.1<br>IL-6:<br>Pre-conversion: 0.2<br>Sero-conversion: 6.6<br>Post-conversion: 2.6<br>IL-7:<br>Pre-conversion: 1.2<br>Sero-conversion: 24.4<br>Post-conversion: <0.1<br>IL-8:<br>Pre-conversion: 24.4<br>Sero-conversion: 8.4<br>Post-conversion: 5.7<br>IL-10:<br>Pre-conversion: 6.4<br>Sero-conversion: 50.6<br>Post-conversion: 30.7<br>IL-12:<br>Pre-conversion: 0.1<br>Sero-conversion: 1.6<br>Post-conversion: 8.2<br>IL-13:<br>Pre-conversion: <1.1<br>Sero-conversion: <1.1<br>Post-conversion: <1.1<br>Interferon-γ:<br>Pre-conversion: <0.1<br>Sero-conversion: 4.5<br>Post-conversion: <0.1<br>GM-CSF:<br>Pre-conversion: 0.6<br>Sero-conversion: 5.8<br>Post-conversion: 12.4<br>TNF-α:<br>Pre-conversion: 7.0<br>Sero-conversion: 9.9<br>Post-conversion: 7.9

*aAn individual with antibodies against all the HIV-1 Western blot viral proteins at the time of sero-

conversion is considered as a usual or typical case.

bCell phenotypes were determined using a FACScalibur flow cytometer.
The limit of detection is 50 copies/mL.

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

The lower limit of detection was 1.1 pg/mL for IL-13 and 0.1 pg/mL for all other cytokines and chemokines. Units are pg/mL; IL, interleukin; GM-CSF, granulocyte macrophage-colony stimulating factor; TNF, tumor necrosis factor. Cytokines and chemokines were measured by Milliplex magnetic high sensitive assays using the Luminex 200® Multiplexing Instrument with MILLIPLEX Analyst 3.1 software.