Seroreversion in Subjects Receiving Antiretroviral Therapy during Acute/Early HIV Infection

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Background. We assessed human immunodeficiency virus (HIV) antibody seroreversion among individuals initiating antiretroviral therapy (ART) during acute/early HIV infection and determined whether seroreversion was associated with loss of cytotoxic T lymphocyte responses.

Methods. Subjects in a cohort with acute/early HIV infection (<12 months into infection) who initiated ART within 28 days after study entry and maintained HIV type 1 ribonucleic acid levels of ≤500 copies/mL for ≥24 weeks were selected. Two clinically available second-generation enzyme immunoassays (EIAs) and a confirmatory Western blot were used to screen subjects for antibody reversion. Those with negative screening test results underwent additional antibody testing, including a third-generation EIA, and were assessed for cytotoxic T lymphocyte responses.

Results. Of 87 subjects identified, 12 (14%) had negative antibody test results at the start of ART; all 12 had seroconversion, although 1 had seroconversion only on a third-generation EIA. Of the 87 subjects, 6 (7%) had seroreversion on at least 1 EIA antibody assay while receiving ART during a median follow-up of 90 weeks. The only clinical predictor of seroreversion was a low baseline “detuned” (less sensitive) antibody. Cytotoxic T lymphocyte responses to HIV Gag peptides were detected in 4 of 5 subjects with seroreversion who could be tested. All 5 who had seroreversion who stopped ART experienced virologic rebound and antibody evolution.

Conclusions. HIV antibody seroconversion on second-generation EIA antibody tests may fail to occur when ART is initiated early. Seroreversion was not uncommon among subjects treated early, although cytotoxic T lymphocyte responses to HIV antigens remained detectable in most subjects. Antibody seroreversion did not indicate viral eradication. A third-generation EIA was the most sensitive test for HIV antibodies.

Although not universally accepted, treatment of acute/early HIV infection is common in clinical practice. The rationale for the use of antiretroviral therapy (ART) during acute/early HIV infection includes preservation of HIV-specific immune responses [1–5], which correlate with lower HIV load [5–8], thereby potentially decreasing the rate of disease progression [9, 10]. Early treatment may also restrict viral evolution [11] and limit the development of the latent reservoir of nonreplicating HIV in CD4+ T cells, one of the major challenges to HIV eradication [12, 13]. Additionally, reductions in high HIV loads observed during acute infection may decrease the potential for secondary transmission [14–17]. On the other hand, concerns about the use of ART during acute/early HIV infection include drug-related toxicities with reduction in quality of life, greater cumulative drug exposure, and earlier development of drug resistance that may limit future ART options.

Cell-mediated immune responses may wane when HIV infection is treated early [8, 18–20], and low levels of HIV-specific antibody and even antibody reversion have been reported [8, 21]. The present study was undertaken to evaluate further the effects of early ART initiation on humoral and cellular antibody responses.
Methods

Subject Selection

Subjects were identified from the Options Project cohort with acute/early HIV infection at the University of California, San Francisco (UCSF). The Options Project enrolls subjects within the first 12 months of HIV infection as defined by a history compatible with recent HIV infection, plus at least one of the following criteria: (1) a negative/indeterminate HIV-1 antibody test result with an HIV-1 RNA level of $\geq 3000$ copies/mL on 2 occasions; (2) a reactive standard HIV-1 antibody test result but a nonreactive “detuned,” or less-sensitive, EIA (LS-EIA) result [22]; or (3) a positive HIV-1 antibody test result and a documented negative HIV-1 antibody test result within the previous 12 months. Subjects were considered to be acutely infected if they had a negative/indeterminate antibody test (criterion 1, above) and to be in early infection if they had a reactive antibody test (criterion 2 or 3, above).

Subjects were retrospectively identified who enrolled in the Options Project cohort between June 1996 and June 2003 and who met the following three criteria: (1) initiated ART within 28 days after Options Project entry, (2) had undetectable HIV-1 RNA level while receiving ART, and (3) maintained an undetectable HIV-1 RNA level while receiving ART for at least 24 weeks while receiving continuous ART. An undetectable HIV-1 RNA level was defined as $< 500$ copies/mL (Bayer bDNA, version 2.0 prior to 1 Jan. 2002 and version 3.0 after 1 Jan. 2002). All participants gave written, informed consent, obtained using protocols approved by the Committee on Human Research, UCSF.

Humoral Immune Responses

Antibody assays. Cryopreserved plasma specimens (at $-70^\circ$C) were used to perform the following assays.

Screening EIA. Organon Technika Vironostika (OTV) HIV-1 Microelisa System EIA (bioMeriex) [23] is an in vitro ELISA intended for use in the detection of IgG and IgM antibodies to HIV-1 in human serum or plasma. It is a second-generation EIA based on HIV-1 viral lysate and is US Food and Drug Administration (FDA) licensed for diagnostic testing but not donor screening.

Bio-Rad/Genetic Systems (BR) rLAV EIA (for HIV-1) (Bio-Rad Laboratories) [24] is an in vitro EIA for the detection of IgG and IgM antibodies to HIV-1 in human serum or plasma. It is a second-generation EIA and is FDA licensed for diagnostic testing.

BR HIV-1/HIV-2 Peptide EIA (Bio-Rad Laboratories) [25] is a second-generation EIA for the in vitro detection of IgG antibodies to HIV-1 and/or HIV-2 in human serum and plasma. It is based on a combination of HIV-1 and HIV-2 peptides and is FDA licensed for diagnostic testing and screening of blood, plasma, organ, and tissue donors.

BR HIV-1/HIV-2 PLUS O EIA (Bio-Rad Laboratories) [26] is an in vitro EIA for the detection of antibodies to HIV-1 and/or HIV-2. It is FDA approved for screening blood donor serum and plasma and cadaveric serum specimens. This assay has a third-generation antigen-sandwich EIA assay format, capable of sensitively detecting both IgM and IgG antibodies, whereas second-generation tests primarily detect IgG antibodies.

Supplemental (confirmatory) immunoblot assay. Chiron Recombinant Immunoblot Assay (RIBA) HIV-1/HIV-2 Strip Immunoblot Assay (SIA) (Chiron) [27] is an in vitro semi-quantitative immunoassay for the detection of antibodies in human serum or plasma that bind to 4 discrete recombinant HIV-1 and HIV-2 antigens arrayed in an immunoblot format. Although approved and used for anti-HIV antibody confirmation in other countries, the assay is not licensed in the United States.

Cambridge Biotech HIV-1 Western Blot (Ortho Clinical Diagnostics) [28] is an in vitro qualitative Western blot assay for the detection and identification of antibodies to HIV-1 in human serum, plasma, or urine. It is licensed by the FDA for use as a confirmatory test on specimens found to be reactive by use of a screening procedure, such as EIA testing.

BR HIV-1 Western Blot (Bio-Rad Laboratories) [29] is an in vitro qualitative Western blot assay for the detection and identification of antibodies to HIV-1 in serum, plasma, or dried blood spot specimens. It is FDA licensed for use as a confirmatory test on specimens found to be repeatedly reactive by use of a screening procedure, such as EIA testing.

These assays were selected to represent antibody tests commonly used in clinical practice and routine blood screening as well as assays with improved sensitivity that are currently used primarily in research settings.

Antibody test screening. Anti-HIV antibody testing was performed on all subjects at 2 time points: (1) a fixed time point, week 48 after ART initiation, and (2) a variable time point, corresponding to each subject’s longest duration of undetectable HIV-1 RNA (LDU). The following antibody assays were performed on each subject at both time points: BR HIV-1/HIV-2 Peptide EIA, OTV HIV-1 EIA, Chiron HIV-1/HIV-2 RIBA, and Cambridge HIV-1 Western Blot. Results were interpreted according to manufacturer’s instructions [23, 25, 27, 28] and Centers for Disease Control and Prevention (CDC) guidelines [30]. Subjects were considered to have HIV antibody seroreversion if assay results at either of the time points were negative or indeterminate.

Detailed analysis of subjects with seroreversion. Specimens from subjects who demonstrated seroreversion were tested at the following time points after ART initiation: weeks 0, 4, 8, 12, and 24 and every 24 weeks for the duration of undetectable HIV-1 RNA. At each time point, the following assays were...
Table 1. Characteristics of 87 subjects receiving antiretroviral therapy (ART) with and without HIV antibody seroreversion.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects (n = 87)</th>
<th>Seroreversiona (n = 6)</th>
<th>Nonreversionb (n = 81)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years</td>
<td>35.8</td>
<td>38.2</td>
<td>35.6</td>
<td>.13</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>92</td>
<td>100</td>
<td>91</td>
<td>1.0</td>
</tr>
<tr>
<td>Baseline CD4+ cell count, mean cells/μL</td>
<td>541</td>
<td>608</td>
<td>536</td>
<td>.6</td>
</tr>
<tr>
<td>Baseline HIV-1 RNA level, mean log₁₀ copies/mL</td>
<td>4.63</td>
<td>4.17</td>
<td>4.67</td>
<td>.4</td>
</tr>
<tr>
<td>Subjects treated during acute HIV infection, %</td>
<td>21</td>
<td>33</td>
<td>20</td>
<td>.6</td>
</tr>
<tr>
<td>Baseline detuned EIA, mean optical density</td>
<td>0.16</td>
<td>0.006</td>
<td>0.18</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Time to initiation of ART, mean days after study enrollment</td>
<td>9</td>
<td>7</td>
<td>9</td>
<td>.8</td>
</tr>
<tr>
<td>ART regimen, no. of patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRTIs only</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>.6</td>
</tr>
<tr>
<td>NRTIs and PIs</td>
<td>68</td>
<td>3</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>NRTIs and NNRTIs</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>NRTIs, PIs, and NNRTIs</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Time from entry to first HIV-1 RNA level of &lt;500 copies/mL, mean weeks</td>
<td>8.7</td>
<td>10.7</td>
<td>8.5</td>
<td>.6</td>
</tr>
<tr>
<td>LDU, median weeks (IQR)</td>
<td>105 (72–161)</td>
<td>140 (72–204)</td>
<td>105 (72–162)</td>
<td>.7</td>
</tr>
</tbody>
</table>

**NOTE.** IQR, interquartile range; LDU, longest duration of undetectable HIV RNA while receiving ART; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

a Subjects with negative HIV antibody testing result at either week 48 or LDU time point.

b Subjects with positive HIV antibody testing result throughout follow-up.

performed: BR HIV-1/HIV-2 PLUS O EIA, BR HIV-1/HIV-2 Peptide EIA, BR rLAV HIV-1 EIA, OTV HIV-1 EIA, and BR HIV-1 Western Blot. Results were interpreted according to manufacturer’s instructions [23–26, 29] and CDC guidelines [30].

**Statistical analysis.** Differences between subjects with and subjects without seroreversion were compared in univariate analyses using Mann-Whitney U test for continuous variables and Fisher’s exact test for categorical variables for the following predictors: age, sex, baseline CD4+ cell count, baseline HIV-1 RNA level, acute versus early HIV infection, baseline LS-EIA value, time to initiation of ART (after study enrollment), type of ART initiated (i.e., whether regimen contained a protease inhibitor [PI], nonnucleoside reverse-transcriptase inhibitor [NNRTI], both, or neither), time from start of ART to first HIV-1 RNA level of <500 copies/mL, and LDU time point.

**Cellular Immune Responses**

Cellular responses were determined among those with seroreversion by use of cryopreserved PBMCs.

**Antigens.** Peptide pools consisting of 15–amino acid peptides with 11–amino acid overlaps were used corresponding to HIV-1 Gag P55 (BD Biosciences), HIV-1 Nef, HIV-1 Tat, HIV-1 Vif, CEF Control Peptide Pool (National Institutes of Health AIDS Research and Reference Reagent Program), and cytomegalovirus (BD Biosciences). The CEF peptide pool is made up of 32 peptides corresponding to major histocompatibility complex class I restricted CD8+ T cell epitopes from cytomegalovirus, Epstein-Barr virus, and influenza virus.

**Amplispot assay.** Production of IFN-γ by antigen-specific CD4+ and CD8+ T cells was detected using the enhanced ELISPOT assay (Amplispot), as described elsewhere [31]. In brief, 96-well microtiter plates (Millipore) were coated for 1 h at 4°C with 5 μg/mL of the anti–IFN-γ monoclonal antibody (1-D1K; Mabtech). PBMCs were plated in duplicate at a concentration of 2 × 10^5 cells/well, along with peptide pools at a final concentration of 2 μg/mL for each peptide, and were incubated overnight. Spots were counted using the AID ELISPOT Reader System (Cell Technology). The results were expressed as spot-forming cells per 10^6 PBMCs. For a sample to be considered positive, a response of at least 2-fold higher than the background signal was required.

**RESULTS**

**Subjects**

Eighty-seven subjects meeting entry criteria were identified: 12 with acute HIV infection and 75 with early HIV infection. The median length of follow-up for all subjects was 90 weeks (interquartile range, 61–163 weeks). Subject characteristics are summarized in table 1.
Humoral Immune Responses

**Antibody status while receiving ART.** All 12 subjects who initiated ART prior to antibody conversion developed positive EIA test results for HIV antibodies while receiving ART; however, 1 subject (OP-568) did not develop a positive HIV antibody test result using any second-generation EIA assay. OP-568 had seroconversion only on a third-generation EIA antibody test (BR HIV-1/HIV-2 PLUS O EIA), and test results remained positive through week 60 of ART (table 2). He developed a positive Western blot result only at 16 weeks, which reverted to indeterminate by week 24 (tables 2 and 3). This individual would have been considered HIV antibody negative by use of standard diagnostic testing. Convincing evidence that he was, in fact, infected with HIV—in addition to multiple positive third-generation EIA antibody test results and conversion of the Western blot—including initial HIV-1 RNA levels that were >500,000 copies/mL on samples independently tested in different laboratories using a Bayer bDNA 3.0 assay (Bayer Diagnostics) and a Roche Amplicor HIV-1 Monitor PCR test (Roche Molecular Systems). Subsequent samples also had detectable HIV-1 RNA at weeks 3 and 4 of ART (6000 copies/mL and 854 copies/mL, respectively), by use of the Roche PCR test.

Of the 75 subjects with positive EIA antibody test results at the start of ART, 5 subsequently developed a negative result on at least 1 EIA assay while receiving ART. Thus, a total of 6 (7%) of the 87 subjects demonstrated negative HIV antibody results while receiving ART on ≥1 diagnostic tests, which would have been interpreted as indication that they were uninfected with HIV (tables 2 and 3), and were considered to have seroreversion. Two of the 6 with seroreversion demonstrated negative EIA antibody results at week 48, and 5 of the 6 at the LDU time point (1 subject tested negative at both time points). The only subject characteristic that was found to be associated with seroreversion was a low value on the initial LS-EIA (table 1).

**Antibody assay performance.** The sensitivity of each of the 4 screening assays performed on all subjects is shown in table 2. In general, the BR HIV-1/HIV-2 Peptide EIA, the OTV HIV-1 EIA, and the Cambridge HIV-1 Western Blot demonstrated comparable sensitivity. The Chiron HIV-1/HIV-2 RIBA proved less sensitive at both time points in this patient population.

**Detailed analysis of subjects with negative antibody testing.** Seroconversion during ART occurred at varying times in the 6 subjects, depending on the antibody assay (tables 2 and 3). The third-generation EIA test, BR HIV-1/HIV-2 PLUS O EIA, was more sensitive than the other EIA assays, reverting to negative in only 1 subject (OP-568) (table 2). The OTV HIV-1 EIA also remained reactive in all subjects except OP-568, for whom it never showed conversion. BR HIV-1/HIV-2 Peptide EIA and BR rLAV HIV EIA performed in a similar manner, with nonreactive results in 5 of 6 subjects, although only 3 subjects developed negative results on both of these assays. There was poor correlation of reversion between EIA and Western blot tests in individual subjects (table 2). With use of the BR HIV-1 Western Blot, antibodies to gp160 and, to a lesser extent, p24 persisted over time, whereas antibodies to other HIV antigens, including gp120, were lost (table 3). Visual and graphic demonstrations of antibody loss are shown for representative subjects in figure 1.

**Cellular Immune Responses**

Antigen-specific IFN-γ production by T cells was evaluated using the Amplispot assay on specimens obtained within 4 weeks after ART initiation and from the LDU among 3 subjects who demonstrated seroreversion. Specimens at the LDU time point only were evaluated in 2 additional subjects. Lack of

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**Table 2. Time point of first nonreactive EIA or indeterminate Western blot after antiretroviral therapy (ART) initiation in subjects with subsequent negative antibody test results.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>BR HIV-1/HIV-2 PLUS O EIA</th>
<th>BR HIV-1/HIV-2 Peptide EIA</th>
<th>BR rLAV HIV-1 EIA</th>
<th>OTV HIV-1 EIA</th>
<th>BR HIV-1 Western blot</th>
<th>LDU, weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP-36</td>
<td>+</td>
<td>8</td>
<td>24</td>
<td>+</td>
<td>+</td>
<td>139</td>
</tr>
<tr>
<td>OP-255</td>
<td>+</td>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>81</td>
</tr>
<tr>
<td>OP-264</td>
<td>+</td>
<td>+</td>
<td>48</td>
<td>+</td>
<td>+</td>
<td>144 250</td>
</tr>
<tr>
<td>OP-336</td>
<td>+</td>
<td>72</td>
<td>48</td>
<td>+</td>
<td>+</td>
<td>192</td>
</tr>
<tr>
<td>OP-568</td>
<td>60</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>OP-685</td>
<td>+</td>
<td>4</td>
<td>12</td>
<td>+</td>
<td>12 62</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Numbers indicate the earliest time point, in weeks after ART initiation, of reversion to a nonreactive or indeterminate test result. Results of the tests were positive from the initiation of ART until the week indicated, except for the BR HIV-1 Western blot result for subject OP-568, which was initially indeterminate, converted to positive at week 16, and reverted to indeterminate from week 24 onward. See Methods section for description of antibody tests. BR, Bio-Rad/Genetic Systems; LDU, longest duration of undetectable HIV RNA; NR, nonreactive at ART initiation and throughout follow-up; OTV, Organon Tecnika Vironostika; +, reactive EIA or positive Western blot results throughout follow-up while receiving ART.
<table>
<thead>
<tr>
<th>Subject and time point</th>
<th>gp160</th>
<th>gp120</th>
<th>p65</th>
<th>p55/p51</th>
<th>gp41</th>
<th>p40</th>
<th>p31</th>
<th>p24</th>
<th>p18</th>
<th>Interpretation</th>
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<tr>
<td>OP-36</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Baseline</td>
<td>1+</td>
<td>–</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>Positive</td>
</tr>
<tr>
<td>Week 48</td>
<td>1+</td>
<td>–</td>
<td>+/–</td>
<td>2+</td>
<td>+/–</td>
<td>2+</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
<td>Positive</td>
</tr>
<tr>
<td>LDU (120 weeks after ART initiation)</td>
<td>1+</td>
<td>–</td>
<td>+/–</td>
<td>2+</td>
<td>1+</td>
<td>2+</td>
<td>+/–</td>
<td>2+</td>
<td>+/–</td>
<td>Positive</td>
</tr>
<tr>
<td>OP-255</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>+/–</td>
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<td>2+</td>
<td>Positive</td>
</tr>
<tr>
<td>Week 48</td>
<td>2+</td>
<td>+/–</td>
<td>–</td>
<td>2+</td>
<td>+/–</td>
<td>2+</td>
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<td>2+</td>
<td>+/–</td>
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<td>LDU (80 weeks after ART initiation)</td>
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<td>–</td>
<td>1+</td>
<td>+/–</td>
<td>1+</td>
<td>–</td>
<td>2+</td>
<td>+/–</td>
<td>Positive</td>
</tr>
<tr>
<td>OP-264</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Baseline</td>
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<td>–</td>
<td>1+</td>
<td>–</td>
<td>2+</td>
<td>–</td>
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<td>LDU (250 weeks after ART initiation)</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>OP-336</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>2+</td>
<td>+/–</td>
<td>1+</td>
<td>2+</td>
<td>+/–</td>
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<td>–</td>
<td>Positive</td>
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<tr>
<td>Week 48</td>
<td>1+</td>
<td>–</td>
<td>+/–</td>
<td>+/–</td>
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<td>Indeterminate</td>
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<tr>
<td>Baseline</td>
<td>+/-</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/–</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>Week 48</td>
<td>+/-</td>
<td>–</td>
<td>+/–</td>
<td>–</td>
<td>+/–</td>
<td>1+</td>
<td>+/–</td>
<td>+/–</td>
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<td>Indeterminate</td>
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<tr>
<td>LDU (72 weeks after ART initiation)</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
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<td>Indeterminate</td>
</tr>
<tr>
<td>OP-685</td>
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<td></td>
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<td></td>
<td></td>
<td>Indeterminate</td>
</tr>
<tr>
<td>Baseline</td>
<td>1+</td>
<td>–</td>
<td>+/-</td>
<td>1+</td>
<td>–</td>
<td>2+</td>
<td>+/–</td>
<td>2+</td>
<td>–</td>
<td>Positive</td>
</tr>
<tr>
<td>Week 48</td>
<td>2+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/–</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>LDU (62 weeks after ART initiation)</td>
<td>2+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
<td>Indeterminate</td>
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**NOTE.** ART, antiretroviral therapy; LDU, longest duration of undetectable HIV RNA; –, negative; +/–, equivocal; 1+, weakly positive; 2+, strongly positive.

sufficient PBMC specimens precluded longitudinal evaluation of other subjects.

The results obtained in response to the CEF, cytomegalovirus, HIV-1 Gag, and HIV-1 Nef peptide pools are illustrated in figure 2. All evaluable samples had a detectable response to the CEF control peptide pool that remained stable over time ($P = 1.00$, by Mann-Whitney $U$ test). In response to the cytomegalovirus peptide pool, a weakly positive trend was observed between baseline and LDU ($P = .40$, by Mann-Whitney $U$ test). Two of the 3 subjects evaluated with longitudinal specimens had detectable HIV-specific responses against the Gag peptide pool, which did not change during the follow-up period ($P = 1.00$, by Mann-Whitney $U$ test), as did 2 additional subjects tested only at LDU. Only 2 subjects had a detectable response against the HIV-1 Nef peptide pool, and no responses were detected against the HIV-1 Tat and HIV-1 Vif peptide pools (data not shown). One subject, OP-568, did not demonstrate any HIV-1–specific response against Gag, Nef, Tat, or Vif at either baseline or LDU. As noted above, this subject also demonstrated decreased HIV antibody responses.

**Clinical Observations**

Of the 6 subjects with seroreversion, 1 continued to receive ART with undetectable HIV load at last evaluation (OP-568 at week 60). Each of the 5 subjects who elected to discontinue ART developed detectable HIV-1 RNA and experienced evolution of antibody responses with positive EIA and Western blot test results (data not shown). Three of the 5 subjects who discontinued ART resumed therapy after 4–12 weeks and again achieved undetectable HIV-1 RNA, with persistent seroreactivity through subsequent follow-up. The other 2 subjects continued to not receive ART, with HIV-1 RNA levels of <5000 copies/mL.

**DISCUSSION**

We found that HIV-1 antibody seroreversion on standard EIA assays was common among individuals for whom ART during acute/early HIV infection was effective. In current HIV clinical-testing algorithms, samples with negative EIA antibody test results are considered uninfected, whereas those with reactive EIA antibody tests undergo confirmatory testing with either
Table 4. Sensitivity of antibody assays in 87 subjects who initiated antiretroviral therapy during acute/early HIV infection.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity at week 48 (95% CI)</th>
<th>Sensitivity at LDU (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR HIV-1/HIV-2 Peptide EIA</td>
<td>96.6 (90.3–99.3)</td>
<td>97.7 (91.9–99.7)</td>
</tr>
<tr>
<td>OTV HIV-1 EIA</td>
<td>98.9 (93.8–99.9)</td>
<td>98.9 (93.8–99.9)</td>
</tr>
<tr>
<td>Chiron HIV-1/HIV-2 RIBA</td>
<td>82.8 (73.2–90.0)</td>
<td>73.6 (63.0–82.4)</td>
</tr>
<tr>
<td>Cambridge HIV-1 WB</td>
<td>97.7 (91.9–99.7)</td>
<td>94.3 (87.1–98.1)</td>
</tr>
</tbody>
</table>

NOTE. BR, Bio-Rad; LDU, longest duration of undetectable HIV RNA; OTV, Organon Tecnika Vironostika; RIBA, recombinant immunoblot assay; WB, Western blot.

Western blot or, less commonly, immunofluorescent antibody testing [30]. In our evaluation, 6 (7%) of 87 HIV-infected subjects receiving ART tested HIV negative by this algorithm. Although not statistically significant, more individuals tested antibody negative with longer periods of virologic suppression, which suggests that seroreversion may become more frequent with longer duration of ART. The only subject characteristic that we found to be associated with seroreversion was a low value on the initial LS-EIA, or “detuned” antibody test. Unexpectedly, initiation of ART prior to HIV antibody seroconversion was not associated with a greater frequency of subsequent negative antibody test results, compared with initiation of ART after seroconversion. Our limited sample size, however, precludes making strong conclusions about the effects of timing of ART initiation in early HIV infection on antibody reactivity over time.

Our observations support the findings of Kassutto et al. [21], who identified reduced HIV antibody responses in 3 (2%) of 150 subjects who initiated ART during early HIV infection. A single case of antibody reversion was also described in a Dutch patient treated during acute HIV infection with 5 antiretroviral medications plus mycophenolate mofetil [32]. By selecting subjects who not only initiated ART in acute/early infection but also maintained undetectable HIV-1 RNA for at least 24 weeks,

Figure 1. Changes in EIA and Western blot results over time in representative subjects (OP-685 and OP-264). Top panels, Antibody responses to the Bio-Rad/Genetic Systems (BR) HIV-1/HIV-2 PLUS O EIA, the BR HIV-1/HIV-2 Peptide EIA, the BR rLAV HIV EIA, and the Organon Tecnika Vironostika (OTV) HIV EIA. Bottom panels, Results of BR HIV-1 Western blot. The first 2 Western blot strips are strongly positive and weakly positive controls, the third strip is a negative control, and subsequent strips correspond to the time points in the graphs (weeks receiving antiretroviral therapy [ART]) located immediately above each strip. OD, optical density.
we enriched our study population with individuals likely to demonstrate seroreversion.

Reports of seroreversion in the absence of ART are rare in the medical literature. A review of the US Army HIV Data System from 1985–1992 identified no seroreversion among 4911 HIV-seropositive individuals [33]. Antibody loss in chronic HIV infection has been observed late in disease when CD4+ cell counts are extremely low [34]. Additionally, rare case reports exist of persistently seronegative HIV/AIDS [35–37], but explanations for such cases are unclear. Furthermore, ART initiation in chronic HIV infection has little effect on antibody responses [38].

The sensitivity of antibody detection varies among assays. In our subjects, the Chiron RIBA was the least sensitive assay, whereas the third-generation BR HIV-1/HIV-2 PLUS O EIA was the most sensitive and thus least likely to demonstrate seroreversion. EIA and Western blot results may be discordant among those with seroreversion, an important observation for clinical HIV antibody testing in cases in which the EIA may revert, triggering a negative result, but the Western blot may remain reactive. Our results also demonstrate that, although uncommon, complete failure to seroconvert on second-generation EIA assays, which are typically used in clinical testing, may occur if ART is initiated while the patient tests antibody negative.

We postulate that the mechanism of HIV antibody loss among our subjects is reduction in antigenic stimulus as a result of effective virologic control with ART. In contrast to humoral immune responses, cytotoxic T lymphocyte responses to HIV Gag and Nef peptide pools appeared to be better maintained in our subjects. This observation suggests that either active viral replication occurred below the level of detection in peripheral blood or replication in nonplasma compartments, such as lymphoid tissue, may have been adequate to maintain cellular but not humoral immune responses.

Although HIV antibody testing is not routinely performed on patients receiving treatment, such a situation may arise if an individual changes HIV care providers, where routine practice may include serologic testing on all patients entering a clinic. Clinicians and patients need to understand that, despite reversion of HIV antibody tests, individuals such as those we studied do have persistent infection, as evidenced by viral rebound after ART discontinuation. Furthermore, negative antibody test results do not imply decreased risk of HIV trans-

![Figure 2. Cellular immune responses among individuals with negative HIV antibody test results. Magnitude of antigen-specific IFN-γ production by T cells was measured using the Amplispot assay in response to CEF, cytomegalovirus (CMV), HIV-1 Gag P55, and HIV-1 Nef peptide pools. The results are expressed as spot-forming cells (SFC) per 10^6 PBMCs. Antigen-specific responses were measured at baseline in specimens obtained within 4 weeks after initiation of antiretroviral therapy (from subjects OP-336, OP-568, and OP-885) (squares) and in a second sample obtained close to the longest duration of undetectable HIV RNA level (LDU) (triangles). A dashed line links paired samples. In addition, results for subjects OP-036 and OP-264, from whom a baseline PBMC specimen was not available, are included for a specimen at the LDU time point, but without a line linking them to a baseline result. Values considered to be insignificant are denoted by open symbols. P values reflect change in the IFN-γ response, determined by Mann-Whitney U test.](https://academic.oup.com/cid/article/42/5/700/317336)
mission, beyond the possible reduction of infectivity associated with lower viral loads [15, 16].

These results have implications for the safety of blood product and organ and tissue donation. Theoretically, an HIV-infected individual who has an undetectable viral load while receiving ART and negative antibody test results may be misidentified as being HIV negative by current laboratory screening methods. However, such a situation could only occur with deliberate intent of an individual who does not reveal the HIV infection during the donor interview process. The potential for such an occurrence is greater, although still remote, in the case of cadaveric organ and tissue donation, for which a history from the donor is not possible [39], with reliance instead on information from relatives who may not be privy to a donor’s full medical history. This possibility does emphasize the importance of using the most sensitive donor-screening techniques, such as third-generation HIV-1/HIV-2 EIAs, as well as sensitive testing for HIV-1 RNA with nucleic acid amplification technology [40–42].

Acknowledgments

We gratefully acknowledge the skillful assistance of Gerald Spotts (Positive Health Program, University of California, San Francisco) in data management, Lea Liou (Positive Health Program, University of California, San Francisco) for statistical support, and Joan Chapman (Gladdist Institute of Virology and Immunology, University of California, San Francisco) for help with the Amplispot assays.

Financial support. Support for this work was provided by National Institutes of Health grant U01 AI41531. Additional support for laboratory assays was provided by Bio-Rad Laboratories (Redmond, WA), Ortho Clinical Diagnostics (Raritan, NJ), and Chiron Corporation (Emeryville, CA).

Potential conflicts of interest. B.H.P. is an employee of Chiron Corporation. S.S.A. is an employee of Ortho Clinical Diagnostics. C.B. is an employee of Bio-Rad Laboratories. Employees of companies involved in the manufacturing of diagnostic tests used in this manuscript (B.H.P., S.S.A., and C.B.) assisted in providing assays. They were not involved in analysis of data, interpretation of data, or development of discussion or conclusions stated in the article. All other authors: no conflicts.

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