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HIV Antibody Characterization as a Method to Quantify Reservoir Size During Curative Interventions

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Quantitative humoral profiling of recent samples from a human immunodeficiency virus (HIV)–infected adult who was cured following a delta32/delta32 CCR5 stem cell transplant in 2007 revealed no antibodies against p24, matrix, nucleocapsid, integrase, protease, and gp120, but low levels of antibodies against reverse transcriptase, tat, and gp41. Antibody levels to these HIV proteins persisted at high and stable levels in most noncontrollers, elite controllers, and antiretroviral-treated subjects, but a rare subset of controllers had low levels of antibodies against matrix, reverse transcriptase, integrase, and/or protease. Comprehensive HIV antibody profiles may prove useful for monitoring curative interventions.

Keywords. antibodies; elite controllers; HIV-1; HIV-1 persistence; serology; viral reservoirs.

Long-term antiretroviral therapy (ART) routinely suppresses human immunodeficiency virus (HIV) RNA levels to very low or undetectable levels [1]. Cell-associated HIV DNA levels and the frequency of cells containing virus that can replicate ex vivo are also very low. Although several mechanisms likely contribute to HIV persistence during ART, a primary mechanism is the establishment and maintenance of a long-lived population of infected resting memory $CD4^+$ T cells [2–5]. The frequency of cells that harbor replication-competent HIV during long-term ART is exceedingly small, making it challenging to quantify HIV persistence in the growing number of studies aimed at accelerating the decay of the reservoir [1].

A subset of infected individuals is able to naturally control HIV replication in the absence of therapy ("elite" controllers) [6]. The vast majority of controllers have low but detectable levels of viremia, with the source of virus likely from a combination of ongoing cycles of replication and persistent production from a stable reservoir. A rare subset of controllers exhibit exceptional levels of viral suppression, with persistently undetectable plasma HIV RNA levels and very low levels of cell-associated HIV DNA. Using luciferase immunoprecipitation systems (LIPS), we recently demonstrated that some exceptional controllers had no detectable antibodies against the reverse transcriptase and integrase, and low antibody responses against p24, matrix, and gp41, likely reflecting the very low level of HIV replication present in these subjects [7].

In 2007, an HIV-infected adult received an allogenic delta32/delta32 CCR5 stem cell transplant for a hematologic malignancy and subsequently has remained free of detectable HIV RNA in the absence of ART for over 5 years [8, 9]. Detailed studies of this individual are consistent with a cure, with replacement of HIV-infected host cells with donor-derived uninfected $CD4^+$ cells [9]. Using standard assays, HIV antibodies were noted to wane with time, and Western blot showed partial reversion [8]. HIV-specific T-cell responses were also similar to HIV-uninfected adults [10].

Because most routine assays of HIV persistence have sensitivities at or near the level of HIV in most treated adults [11], there is intense interest in developing novel methods to characterize viral reservoir during therapy [1]. Such measurements might be used to develop curative interventions, or to identify individuals whose reservoir size is so low that they might be able to stop therapy. In order to further develop the antibody approach, we analyzed anti-HIV antibody profiles in samples from the Berlin Patient (who represents the cured state) and compared these results to uninfected, controllers, and HIV-infected individuals from before and after ART-induced virologic control.

METHODS

Serum samples were collected under institutional review board–approved protocols from uninfected blood donors

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enrolled in studies at the National Institutes of Health Clinical Center or HIV-infected subjects enrolled in SCOPE [10]. Samples were collected at a single time point from uninfected donors (n = 10) and from untreated HIV-seropositive adults with undetectable HIV RNA levels (<50 copies RNA/mL; "elite" controllers; n = 10). Yearly samples were evaluated from 9 HIV-infected subjects before and during 4–5 years of ART. Prior to ART, the 9 HIV-infected subjects had a median viral load of 22 400 copies/mL (interquartile range [IQR] of 4335–225 311) and after 1 year of therapy had a median level of 50 copies/mL (IQR, 10–75), which remained stable thereafter. The median CD4 counts prior to ART was 333 (IQR, 225–432) and after 1 year of ART was 455 (IQR, 398–588). Details of the "Berlin Patient" have been described [8–10]. Five serial serum samples collected 51–67 months after the February 2007 transplant were analyzed. During this same time period, HIV RNA and DNA analysis found no consistent evidence of infection [9] and the median CD4 count was 811 (IQR, 697–845).

LIPS utilizes recombinant proteins that are chimeras of light producing *Renilla* luciferase and pathogen-specific antigens for quantitative detection of antibodies [13]. In the current study, previously described HIV constructs for p24, matrix, nucleocapsid, reverse transcriptase, integrase, protease, tat, gp41, and gp120 were employed [13]. LIPS was performed with a master plate of serum samples and light units (LUs) were averaged from at least 2 separate experiments. GraphPad Prism (San Diego, CA) was used for statistical analyses. Antibody levels are reported as median levels with interquartile range (IRQ). The nonparametric Mann–Whitney *U* test was used for comparison of the different groups. Principal component analysis (PCA) of the antibody responses against the 9 HIV proteins was analyzed using RapidMiner (www.rapidminer.com).

Heatmap was employed for visualization of the anti-HIV antibody responses. For construction of the heatmap, the uninfected blood donors were used as a reference group. The level of each antibody above the mean plus 3 standard deviations of the uninfected controls was calculated as a *Z* score value for each subject and then color-coded.

Integrated HIV DNA was measured by PCR in $CD4^+$ T cells isolated from peripheral blood mononuclear cells from blinded samples obtained from the controllers and those on ART (6 of 9 available samples), as previously described [12].

RESULTS AND DISCUSSION

Using the LIPS assay, antibody responses against 9 HIV proteins were evaluated in uninfected donors (n = 10), elite controllers (n = 10), HIV-infected adult subjects from before and after several years of ART-induced virologic control (n = 9) and the Berlin Patient.

The loss of antibodies to p24 seemed to be uniquely associated with the cured state. The median anti-p24 level in the Berlin Patient was 11 290 LUs (IQR, 10 444–17 140); this level was similar ($P = .37$) to the median level of 10 850 LUs (IQR, 3023–13 790) in the uninfected blood donor controls (Figure 1*A*). In contrast, much higher levels of anti-p24 antibodies were detected in all of the HIV-seropositive individuals, including the controllers (median of 1 529 000 LUs; IQR, 583 700–2 254 000), the HIV-infected subjects before ART (median, 2 366 000 LUs; IQR, 1 767 000–2 804 000) and the HIV-infected subjects after 4–5 years of ART (median, 2 626 000 LUs; IQR, 2 202 000–5 978 000) (Figure 1*A*).

The Berlin Patient also lacked antibodies against the matrix, integrase, protease (Figure 1*B*–*D*), and nucleocapsid (data not shown). Interestingly, we identified a subset of HIV-seropositive individuals who also lacked responses to some of these proteins. This was particularly true for the controllers, a few of whom appeared to be comparable to the Berlin Patient (but distinct from the noncontrollers before and after ART) in having low levels of antibodies against integrase, protease, and/or reverse transcriptase (Figure 1*B*–*E*). The responses to protease and gp120 were variable in the controllers, pre-ART noncontrollers, and ART-suppressed groups, with no apparent differences in spectrum of responses within these groups (Figure 1*D* and 1*F*). It is possible that the LIPS assay may underestimate the level of anti-gp120 antibodies because of the heterogeneity in gp120 sequences found in different HIV-infected individuals.

Although antibody responses to p24, matrix, integrase, protease, and nucleocapsid were essentially indistinguishable from that in uninfected adults, the Berlin Patient had detectable antibody levels against several HIV proteins. As shown in Figure 1*E*, the median level of anti-reverse transcriptase antibodies in the Berlin Patient (107 800 LUs; IQR, 87 730–139 900) was significantly higher than uninfected controls (21 800 LUs; IQR, 14 960–31 940; $P = .0007$), but 10- and 20-fold lower than in the elite controllers and ART-treated HIV-infected adults, respectively. Similarly, the median level of antibodies against gp41 were significantly higher in the Berlin Patient compared to uninfected controls (250 600 LUs (IQR, 223 500–312 700) versus 14 510 LUs (IQR, 12 650–18 660); $P = .0007$) and were 3- and 8-fold lower compared to the elite controllers and HIV patients after ART, respectively (Figure 1*G*). The Berlin Patient also had higher antibodies against tat as compared to the uninfected blood donors ($P = .0007$) (Figure 1*H*). Across all proteins, antibody responses against gp41 tended to show the most consistent group-to-group differences, with increasing responses across the uninfected, the Berlin Patient, the controllers, and the treated group readily apparent (Figure 1).

Analysis of the noncontrollers before and after 4–5 years of ART-induced virologic control demonstrated that the levels of antibodies against the p24, matrix, integrase, protease, and reverse transcriptase did not significantly change over time despite the dramatic decline in viral load (Figure 1*A*–*H*). Six of

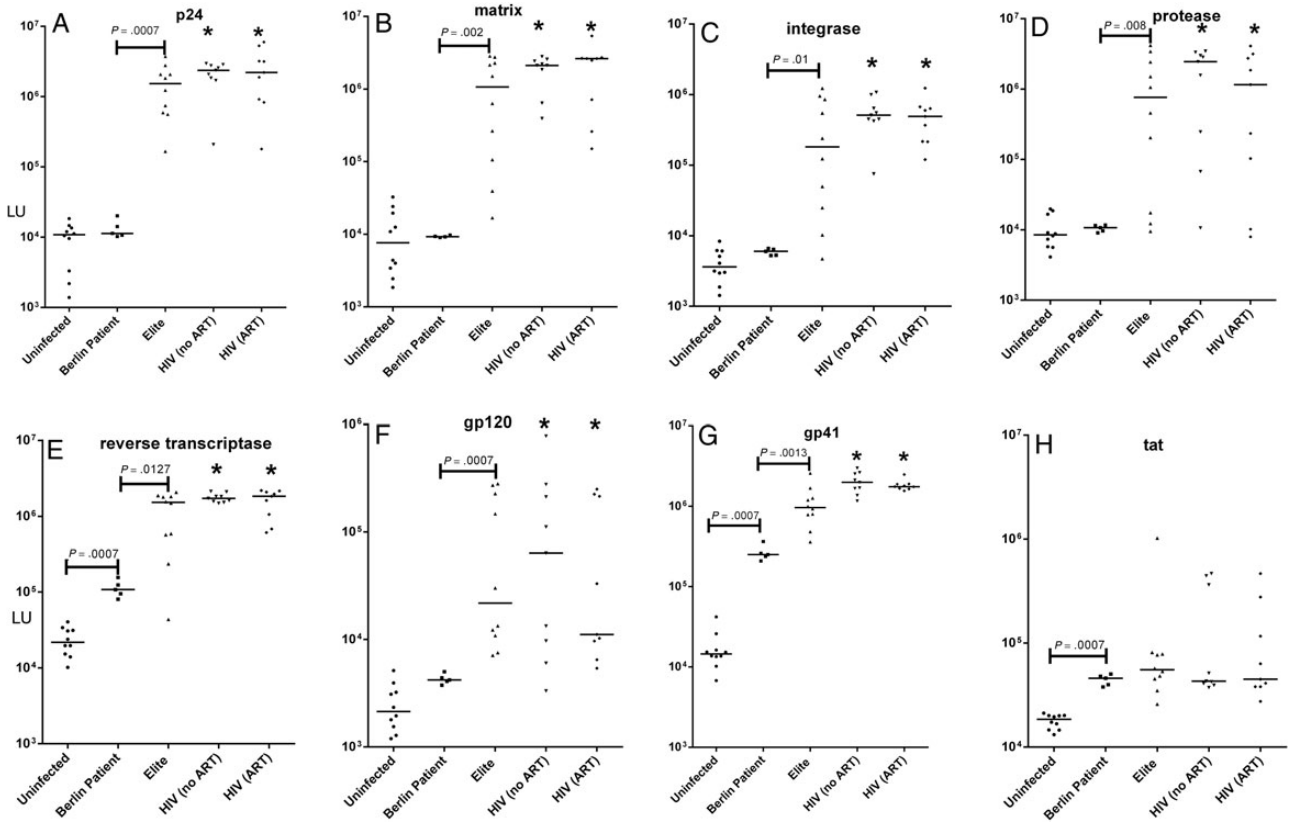

Figure 1. Antibody profiles against HIV antigens in the Berlin Patient and other HIV-infected subjects. The antibody levels against each of the 9 HIV proteins were determined in the uninfected subjects (n = 10), the 5 serial samples from the Berlin Patient, elite controllers (n = 10), and 9 HIV subjects (n = 9) from before (no ART) and after ART. The antibody levels are plotted on the *y*-axis using a $\log_{10}$ scale and the median value in each group is shown by a solid horizontal line. Only statistically significant *P* values between the Berlin Patient and other groups are shown and were calculated using the Mann–Whitney *U* test. For comparison between the Berlin Patient and HIV (no ART) and HIV (ART) groups, *P* values less than .008 are denoted by an asterisk. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus.

the 9 ART-treated subjects showed stable or slightly higher antibodies over time, while 3 showed a noticeable drop in antibody levels against several HIV proteins, including p24, MA, integrase, and/or protease.

We next analyzed the total antibody responses against all 9 proteins. As expected, the Berlin Patient had very low antibody responses that were just above the uninfected subjects (Figure 2*A*). Five of the 10 controllers and 3 of the 9 ART-treated subjects had low total antibodies (relative to the untreated noncontrollers). Additional PCA modeling of the antibody data revealed that the uninfected subjects and Berlin Patient clustered together and were in close proximity to the 5 controllers with low antibody levels (Figure 2*B*). Moreover, 3 HIV subjects who had a decrease in HIV antibodies following ART had a corresponding shift in their PCA profile closer to the uninfected controls (Figure 2*B*; labeled 1, 2, and 3). Further heatmap visualization of the antibody profiles in each subject revealed that the Berlin Patient had 3 low anti-HIV antibody responses that were distinct from the uninfected population (Figure 2*C*). The heatmap also highlights the unexplained heterogeneity of antibody responses in the controllers, whose patterns varied from one close to that observed in the cured state to that which was indistinguishable from those on ART (Figure 2*C*). Heterogeneity was also observed among those on effective ART.

We next measured the level of integrated HIV DNA in the aviremic subsets (controllers and those on ART) to determine if the degree of HIV persistence predicted the antibody response. As expected, the frequency of $CD4^+$ T cells harboring integrated HIV DNA was significantly lower in controllers when compared to ART-treated subjects ($P = .038$). The 5 controllers with consistently low antibody responses had 0, 0, 0, 12, and 21 integrated HIV DNA copies per 10^6 CD4 T cells, while the 5 controllers with higher antibody levels had 0, 2, 2, 2, and 4 integrated copies, suggesting in this small sample that reservoir size was not a direct cause or consequence of the antibody response. These observations are consistent with our previous study [7].

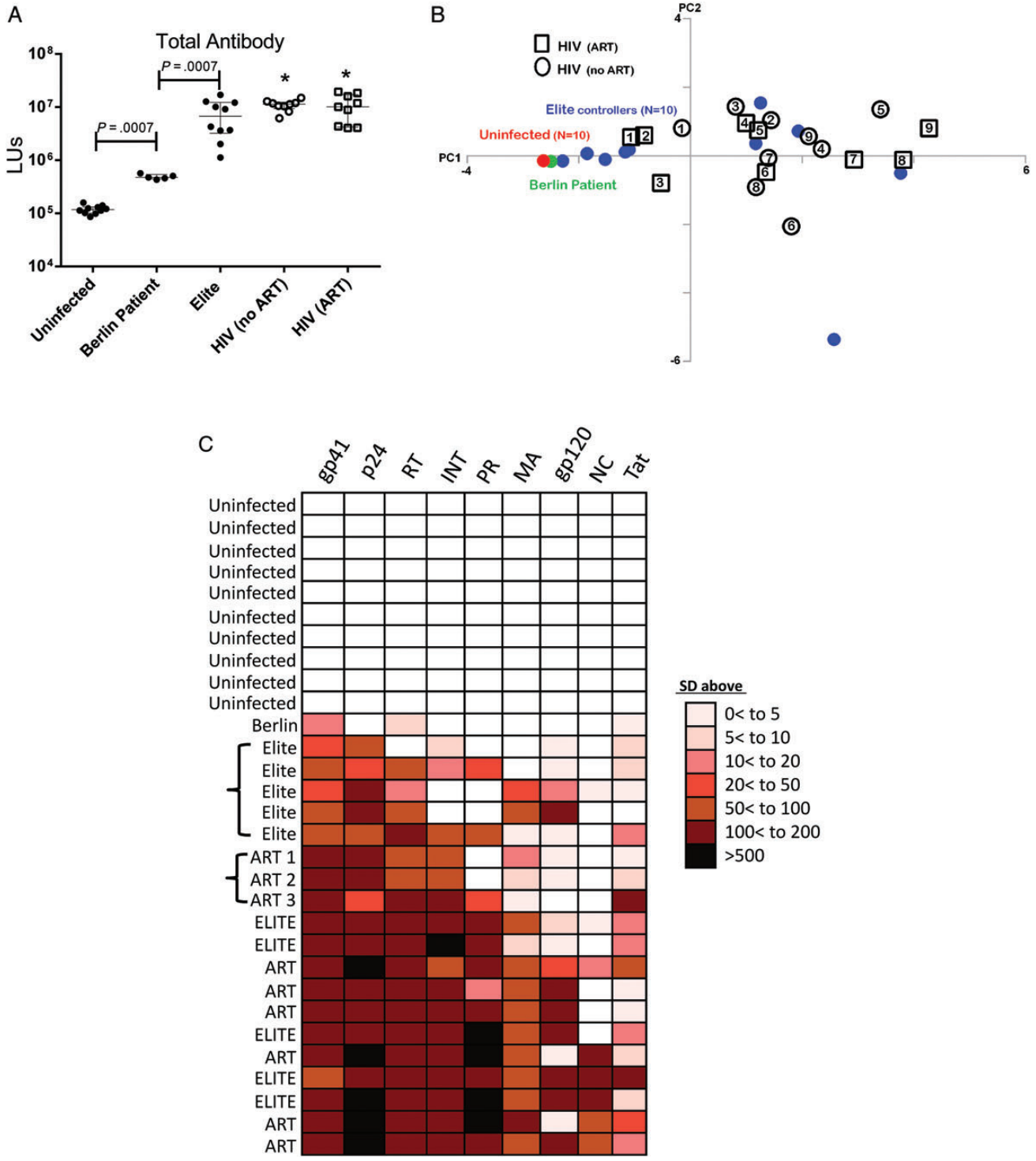

Figure 2. The anti-HIV antibody landscape in the Berlin Patient, elite controllers, and HIV-ART subjects. *A*, Total antibody levels against all 9 proteins is shown. For comparison between the Berlin Patient and HIV (no ART) and HIV (ART) groups, *P* values less than .001 are denoted by an asterisk. *B*, Principal component analysis was used to model the anti-HIV antibody data. A biplot representing PC1 versus PC2 derived from the antibody levels against the 9 HIV proteins are shown for 10 uninfected controls (red; for all 10), the Berlin Patient (green), elite controller (blue), and HIV subjects before (open circles) and after ART (open squares). Each HIV patient from before and after ART is individually numbered. Three HIV-treated subjects (labeled, 1, 2, and 3) showed a shift in their profile to the left after treatment. *C*, Heatmap analysis shows limited anti-HIV antibodies in the Berlin Patient. As described in the Methods, each subject was color-coded using the *Z*-score scale on the right and reflects antibody levels greater than the mean plus 3 SD values of the reference uninfected controls. The rank order of the subjects was based on PCA analysis shown in Figure 2*B* and the 3 HIV-ART subjects with associated decline in anti-HIV antibodies are labeled 1, 2 and 3. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; PCA, principal component analysis.

Integrated DNA was also determined in 6 of the ART-treated subject who had sufficient samples. The 3 subjects with higher HIV antibody levels had 0, 7, and 13 integrated HIV DNA copies per 10^6 CD4 T cells. Interestingly, the 3 ART-treated subjects with low HIV antibody levels had surprisingly the highest reservoir size (80, 143, and 632 integrated HIV DNA copies per 10^6 CD4 T cells).

Our study has several limitations. Although our sample size was sufficient to demonstrate clear differences among the groups in terms of antibody responses, a larger study using more comprehensive measurements will be needed to untangle the association between antibody responses and size and distribution of the reservoir. Also, we focused only on the chronic phase of the infection. It has been established that individuals who start therapy during acute infection have low antibodies and indeed occasionally serorevert using standard antibody tests [14].

In summary, we found that an HIV cure was associated with the complete loss of antibodies against p24, and low but detectable responses to gp41. No HIV-infected person exhibited these patterns. Monitoring the response to p24 and gp41 may prove useful in curative studies. The responses to matrix, protease, reverse transcriptase, and integrase were low or undetectable in the Berlin Patient, but a number of other individuals—particularly controllers—exhibited some aspects of these patterns. While it is known that antibody levels after immunization wane at different rates [15], the low levels of sustained antibody responses against reverse transcriptase, tat, and gp41 in the Berlin Patient may involve the unique stability of these proteins or their higher human leukocyte antigen–binding activities. The substantial heterogeneity in antibody responses to HIV proteins in controllers and those on ART remains unexplained, and will be the focus of future studies.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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