Autologous Stem Cell Transplantation Disrupts Adaptive Immune Responses during Rebound Simian/Human Immunodeficiency Virus Viremia

Daniel B. Reeves,a Christopher W. Peterson,b,c Hans-Peter Kiem,b,c,d Joshua T. Schiffer,a,b,c
Vaccine and Infectious Disease Divisiona and Clinical Research Division,b Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; Department of Medicinec and Department of Pathology,d University of Washington, Seattle, Washington, USA

ABSTRACT Primary HIV-1 infection induces a virus-specific adaptive/cytolytic immune response that impacts the plasma viral load set point and the rate of progression to AIDS. Combination antiretroviral therapy (cART) suppresses plasma viremia to undetectable levels that rebound upon cART treatment interruption. Following cART withdrawal, the memory component of the virus-specific adaptive immune response may improve viral control compared to primary infection. Here, using primary infection and treatment interruption data from macaques infected with simian/human immunodeficiency virus (SHIV), we observe a lower peak viral load but an unchanged viral set point during viral rebound. The addition of an autologous stem cell transplant before cART withdrawal alters viral dynamics: we found a higher rebound set point but similar peak viral loads compared to the primary infection. Mathematical modeling of the data that accounts for fundamental immune parameters achieves excellent fit to heterogeneous viral loads. Analysis of model output suggests that the rapid memory immune response following treatment interruption does not ultimately lead to better viral containment. Transplantation decreases the durability of the adaptive immune response following cART withdrawal and viral rebound. Our model’s results highlight the impact of the endogenous adaptive immune response during primary SHIV infection. Moreover, because we capture adaptive immune memory and the impact of transplantation, this model will provide insight into further studies of cure strategies inspired by the Berlin patient.

IMPORTANCE HIV patients who interrupt combination antiretroviral therapy (cART) eventually experience viral rebound, the return of viral loads to pretreatment levels. However, the “Berlin patient” remained free of HIV rebound over a decade after stopping cART. His cure is attributed to leukemia treatment that included an HIV-resistant stem cell transplant. Inspired by this case, we studied the impact of stem cell transplantation in a macaque simian/HIV (SHIV) system. Using a mechanistic mathematical model, we found that while primary infection generates an adaptive immune memory response, stem cell transplantation disrupts this learned immunity. The results have implications for HIV cure regimens based on stem cell transplantation.

KEYWORDS Berlin patient, SHIV, hematopoietic stem cell transplant, human immunodeficiency virus, macaque model, mathematical modeling

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infection (4), a rapid narrowing of observed viral sequences favoring viral escape mutants (5–12), association of protective major histocompatibility complex class I alleles with a lower HIV load set point (13), and the inverse correlation of an HIV-1-specific CD8+ T cell response with the viral load set point during primary infection (14).

The ultimate measure of the intensity and timing of the adaptive immunologic pressure on infection is a shift in viral load, which is a close surrogate of infection severity (15). During primary HIV infection, the viral load increases exponentially prior to reaching peak levels and then decreases slightly to achieve a steady state (16). This correlates temporally with expansion of HIV-specific CD8+ T cells (1–3).

Although prolonged viremia and progression to AIDS can be suppressed with combination antiretroviral therapy (cART), the cessation of cART results in rapid viral rebound in the majority of infected persons due to the persistence of latently infected cells (17–21). Whereas primary infection occurs in an immunologically naive environment, viral rebound after cART interruption, by definition, occurs in immunologically experienced patients. However, HIV-specific CD8+ T cell function declines during prolonged cART (3) and frequently targets regions that are prone to rapid escape (22). Depending on the effectiveness of the remaining immune response, it is possible that rebound viral dynamics will differ from those during primary infection, allowing quantitation of potential gains in immunity.

Here, using data from pigtailed macaques infected with simian/human immunodeficiency virus (SHIV) (23), we demonstrate that the peak viral load is lower following cART cessation than during primary infection. However, the viral load set point is unchanged, indicating that immune memory to prior SHIV exposure provides no long-term reduction in plasma viremia. When myeloablation and autologous hematopoietic cell transplantation (HSCT) are performed during cART suppression, the peak viral load is equivalent, and the viral load set point is higher relative to primary infection after cART is stopped. This result highlights the overall loss of virus-specific immune memory in the weeks following autologous transplant, as well as the importance of the endogenous response during primary infection.

Using mathematical models (24, 25), we demonstrate that there is a greater preexisting SHIV-specific cytolytic immune response following cART cessation, relative to a primary infection. However, the immune response ultimately equilibrates to levels close enough to those during primary infection to not ultimately impact viral load set point or disease progression. Autologous hematopoietic stem cell transplantation (HSCT) induces a more rapid decline of the SHIV-specific immune response relative to a primary infection, suggesting that while virus-specific immunity does not control SHIV, it does play an active role in limiting viremia in a setting of unsuppressed viral replication. On the other hand, increased levels of HIV-susceptible activated CCR5+ CD4+ T cells do not appear to persist 25 weeks after HSCT and do not drive a higher viral load set point.

The single example of an HIV cure was the “Berlin patient,” who received high-dose chemotherapy, followed by HSCT of HIV-resistant hematopoietic cells and treatment for graft-versus-host disease (26, 27). Our results suggest that the myeloablative conditioning regimen associated with the stem cell transplantation may have had deleterious effects on the SHIV-specific immune response. This highlights the essential role of HIV-resistant cells (in this case donor-derived CCR5 Δ32 cells) in enhancing virus-specific immunity and engendering a functional cure (28).

RESULTS

SHIV load dynamics during primary infection and after analytic treatment interruption are heterogeneous. Pigtailed macaques were challenged with SHIV-1157ipd3N4, observed off therapy for 25 weeks, and treated with cART for 55 weeks (29). At 80 weeks postinfection, cART was withdrawn (analytic treatment interruption [ATI]). Four macaques underwent autologous hematopoietic stem cell transplantation (HSCT) at week 55, 30 weeks after cART initiation and 25 weeks before ATI; four macaques did not undergo HSCT and served as controls. We examined primary
infection in 25 macaques (Fig. 1A). The experimental details are described in Materials and Methods.

During primary infection, plasma viral load peaked at approximately 2 weeks after virus challenge in the majority of macaques, at levels between $10^6$ and $10^8$ viral RNA copies/ml. The viral load set points were more heterogeneous ($10^2$ to $10^6$ viral copies/ml) but did not differ across intervention groups (Fig. 1B). Upon ATI, SHIV viremia recrudesced in all observed macaques with heterogeneous times to viral rebound, peak viral loads, and set points in control (Fig. 1C) and HSCT (Fig. 1D) macaques.

**SHIV rebounds to a lower peak but an equivalent viral load set point following ATI.** Given the heterogeneity noted in SHIV peak viral loads and set points during primary infection and after ATI, we normalized data to historic values, effectively allowing macaques to serve as their own controls. Normalizing to historic values is justified because the log$_{10}$ viral load peak was reasonably correlated with the log$_{10}$ viral load peak for each animal (Fig. 1E): these correlations were particularly high in animals ultimately undergoing ATI ($r = 0.74$, $P = 0.036$). Correlations were also high between time points following the peak and those 10 weeks later (Fig. 1F).

**FIG 1** Primary infection and viral rebound dynamics are qualitatively different in control and transplanted macaques. (A) Image of study animal, the pigtailed macaque (*Macaca nemestrina*) (photo courtesy of Wikipedia [CC-BY-SA 3.0]), and the experimental schematic showing the timeline for treatment in each cohort. (B) Primary infection plasma viral loads for transplant (orange/red), control (blue/green) and other experimental (gray) animals. (C and D) Respective ATI data from the control (blue/green) and transplant (orange/red) animals demonstrate qualitative differences in peak and set point viral loads. (E) Peak and average set point viral loads are well correlated. (F) Time points 10 weeks apart after week 3 of primary infection are highly correlated, justifying our use of macaques as their own historical controls.
To identify relationships between primary and ATI infection dynamics, we compared peak viral loads and set points during primary infection to those following ATI. In control macaques, we noted a 1- to 3-log decrease in peak viral load following ATI relative to primary infection (Fig. 2A and B). The relative decreases in peak viral load from primary infection to ATI were more pronounced in control ATI macaques compared to those undergoing HSCT (nonparametric rank test \( P < 0.02 \); Fig. 2B). The viral load set point did not differ from primary infection to post-ATI in control macaques (Fig. 2C and D).

**SHIV rebounds to an equivalent peak but a higher set point following HSCT and subsequent ATI.** In transplanted macaques, we noted no change in peak viral load following ATI relative to the primary infection (Fig. 2A and B). The viral load set point was 1.0 to 2.5 logs higher post-ATI relative to the primary infection in HSCT macaques (Fig. 2C and D). Increases in viral load set point from primary infection relative to ATI were more pronounced in HSCT ATI macaques compared to control ATI macaques (nonparametric rank test \( P < 0.02 \); Fig. 2D). The overall effects of ATI on viral dynamics in control and HSCT macaques are summarized in Fig. 2E.

**CD4\(^+\) and CD8\(^+\) T cell levels vary slightly between cohorts.** CD4\(^+\), CD8\(^+\), and CD4\(^+\) CCR5\(^+\) T cells (which are targets for CCR5\(^+\) tropic SHIV) differed between control and transplanted macaques at several times throughout the experimental period (Fig. 3A). In one case, an unexpected difference unrelated to an experimental intervention was identified: CD8\(^+\) T cells were lower in the HSCT arm versus controls prior to HSCT (\( P < 0.05 \) [two-sided Mann-Whitney test]).

Important temporal trends were also noted. In all macaques, average CD4\(^+\) T cell levels decreased following primary infection, whereas CD8\(^+\) T cells increased in half of the animals and decreased in the other half (Fig. 3B). Upon cART initiation, CD4\(^+\) T cell levels increased in 5 of 8 macaques, while CD8\(^+\) T cell levels were generally more stable (Fig. 3C). Transplantation led to a substantial decrease in total CD4\(^+\) T cells, with a transient increase in CD4\(^+\) CCR5\(^+\) T cells (Fig. 3D) until reequilibration around week 80 (Fig. 3A). CD4\(^+\) T cell levels dropped in the control macaques after ATI but not in macaques undergoing HSCT; however, CD4\(^+\) CCR5\(^+\) T cell levels dropped in three of
the transplanted macaques following ATI. The CD8+ T cell levels post-ATI were generally less variable across cohorts (Fig. 3E and F).

**Determinants of viral load peak and set point differ following analytic treatment interruption.** We next applied a mathematical model consisting of a set of ordinary differential equations describing the infection of susceptible CCR5+ CD4+ T cells (S) by SHIV (V). Of note, the modeled numbers of susceptible cells differ from those described in Fig. 2A because most infection occurs in the lymphatic compartment, which may exist in disequilibrium with circulating blood. Upon infection, some cells become productively (P) or unproductively (U) infected, so that only productively infected cells produce additional virus (30). All infected cells die rapidly, an assumption based on the observation of pyroptosis-induced cell death in non-productively infected cells (30, 31), as well as our prior modeling that demonstrated massive bystander depletion of CD4+ T cells during SHIV infection despite the lack of viral replication in these cells (32). The adaptive immune response is modeled with the state variable E, representing the dynamic anti-SHIV adaptive, cytolytic immune response (Fig. 4A). Importantly, this variable is intended to capture the entirety of this adaptive response, including but not limited to HIV-specific CD8+ T cells. We focused on the cytolytic immune response because the majority of observed opportunistic viral infections following HSCT in patients pertain to the loss of T cells rather than to antibody function (33).

To identify model parameters that could be responsible for changes in viral load dynamics, we inspected the multidimensional parameter space of our model with single-parameter and multiparameter sensitivity analyses (34, 35). In single-parameter variations, the following parameter changes resulted in an increase in both peak viral load and viral load set point (within biologically relevant bounds allowing persistent viremia): decreased death rate of susceptible cells (δS) and infected cell recognition (ES50) or the number of infected cells required for a half-maximal cytolytic expansion rate.
Increased killing rate by adaptive cells ($\kappa$), increased adaptive cell recruitment rate ($\omega$), and decreased elimination rate of adaptive cells ($\delta_E$) had the specific effect of lowering the viral load set point while less significantly impacting the peak viral load (Fig. 4B). The initial concentration of adaptive cells ($E_0$) correlated inversely and the susceptible cell generation rate ($\alpha_S$) correlated positively with peak viral load, while having less impact on the set point (Fig. 4B). Similar trends were noted in our multivariable parameter sensitivity analysis in which all parameters are varied simultaneously (Fig. 4C). Of note, the number of susceptible cells ($S_0$) had only a minor impact on the viral load set point in the global sensitivity analysis (Fig. 4C). Parameters pertaining to the immune response and cytolysis, such as $\kappa$, $\omega$, and $\delta_E$, affected model output intensely. This analysis shows that variability in immune parameters can affect differences in the peak viral load and viral load set point independently and/or simultaneously, depending on their combination.

**Model selection and parameter estimation from mathematical model fit to data.** We justified our model choice through a nested model analysis that focused on the inclusion or exclusion of specific biological mechanisms, including the cytolytic immune response and non-productively infected cells. We attempted to fit each model to viral load data over time, using primary infection data from a cohort of 25 SHIV-infected pigtailed macaques (Fig. 5A). These data included all animals undergoing primary SHIV infection, inclusive of the eight animals that went on to additional experimental interventions. Because of the large parameter set, the six best-known parameters from experimental studies (including those that describe viral replication,
spread, and clearance) were fixed across all animals. The eight less-studied parameters that described the intensity and persistence of the cytolytic immune response, as well as the behavior of target cells for SHIV, were allowed to vary around our best initial estimates from the literature (see the values in Tables 1 and 2 and further discussion in Materials and Methods).

We identified that accurate model fit requires the cytolytic immune response and inclusion of non-productively infected cells but does not necessitate different death rates between productive ($\delta_p$) and unproductive ($\delta_u$) infected cells. Using the log likelihood of the model given the data, the complete model in which $\delta_p \neq \delta_u$ (model

![Figure 5](https://jvi.asm.org/content/jvi/91/13/e00095-17/F5.jpg)
Fig. 4A) represented the most likely model (Fig. 6A). However, using the Akaike information criterion (AIC) (36), which also penalizes increasing numbers of free parameters in each model (maximum, 6; minimum, 3), we found that model 2 where $\delta_t = \delta_p$ was the best model and used this for all subsequent analyses. The model fit was excellent with this approach (Fig. 5A) and poorer with the simplified models. Finally, by analyzing the fitting covariance, we demonstrated that there is little substantial interplay between parameters (data not shown). Specifically, if one parameter value was perturbed to worsen the fit to the data, other parameters could not be adjusted in a compensatory fashion to achieve a better fit.

After establishing the model and best-fit parameters from the primary infection dataset, we fit our model to the two cohorts that underwent analytical treatment interruption (ATI) after prolonged ART, as in Fig. 1A: these animals included four controls and four having the additional HSCT. We used the same model and initialized the model with the animal-specific best-fit parameter sets deduced during primary infection fitting. We focused on the five most relevant parameters to the cytolytic immune response and target cell availability, assuming tighter bounds on the natural death rate of susceptible cells ($\delta_3$), the 50% immune saturation threshold ($E_{50}$), and the killing rate of cytolytic immune cells ($\kappa$). With this constrained parameter set, model fitting was also excellent (Fig. 5B).

**Immune intensity increases during early primary infection in all animals but more commonly in HSCT animals following HSCT.** The dynamics of cytolytic immune pressure are an emergent property of our model fitting. We compared these dynamics between ATI and primary infection in each macaque (Fig. 7). The SHIV-specific cytolytic response (normalized per infected cell) increased by various degrees in all eight macaques during primary infection. The control animals (top panels, blue hues) demonstrated more intense per cell cytolytic immune responses at the initiation of ATI (dotted lines), suggesting prolonged immune memory and explaining the lower peak viral load. However, the post-ATI immune response did not intensify beyond this level in three of four macaques; the exception being the single ATI control with a viral load

| Parameter | Description                                                                 | Initial value | Unit(s)       | Reference(s) |
|-----------|------------------------------------------------------------------------------|---------------|---------------|--------------|
| $B$       | Viral infectivity                                                            | $10^{-4}$     | μl/virions/day | 49, 61       |
| $T$       | Probability of cell being productively infected given viral infection        | 0.05          |               | 30           |
| $\delta_p$, $\delta_t$ | Death rate of infected cells                                                | 1.0           | Per day       | 62, 63       |
| $\pi$     | Viral production rate                                                        | $5 \times 10^4$ | Virions/cell  | 64           |
| $\gamma$  | Viral clearance rate                                                         | 23            | Per day       | 65           |
| $\alpha_e$| Production rate of adaptive immune system cells                             | 0.0001        | Cells/μl/day  | 51, 66       |

### Table 1: Initial values and references for parameters fixed in the model

| Parameter | Description                                                                 | Initial value | Unit(s)       | Reference(s) |
|-----------|------------------------------------------------------------------------------|---------------|---------------|--------------|
| $V$       | Viral infectivity                                                            | $10^{-4}$     | μl/virions/day | 49, 61       |
| $T$       | Probability of cell being productively infected given viral infection        | 0.05          |               | 30           |
| $\delta_p$, $\delta_t$ | Death rate of infected cells                                                | 1.0           | Per day       | 62, 63       |
| $\pi$     | Viral production rate                                                        | $5 \times 10^4$ | Virions/cell  | 64           |
| $\gamma$  | Viral clearance rate                                                         | 23            | Per day       | 65           |
| $\alpha_e$| Production rate of adaptive immune system cells                             | 0.0001        | Cells/μl/day  | 51, 66       |

### Table 2: Initial values and references for parameters that are allowed to vary in the model fitting around these initial estimated values

| Parameter | Description                                                                 | Initial value | Unit(s)       | Reference(s) |
|-----------|------------------------------------------------------------------------------|---------------|---------------|--------------|
| $\alpha_s$| Production rate of susceptible CD4+ T cells                                 | 100           | Cells/μl/day  | 49           |
| $\delta_s$| Death rate of susceptible CD4+ T cells                                      | 0.3           | 1/day         | 49           |
| $\kappa$  | Adaptive immune response killing rate                                       | 0.01          | μl/day/cells  | 55           |
| $\omega$  | Adaptive immune response recruitment rate                                   | 0.01          | μl/day/cells  | 54           |
| $\delta_e$| Removal rate due to death or exhaustion of adaptive immune system cells    | 0.003         | 1/day         | 54           |
| $E_{50}$  | 50% maximum value of adaptive immune cells, allows bounded growth          | 250           | Cells/μl      | 52–54        |
| $S_0$     | Initial concn of susceptible cells                                          | 330           | Cells/μl      | Calculated as $\alpha_s/\delta_s$ |
| $E_0$     | Initial concn of adaptive immune cells                                      | 1             | Cells/μl      | 51, 66       |
set point exceeding $10^4$ SHIV RNA copies (left upper panel). Moreover, the intensity of the immune response more than 10 weeks after ATI was only slightly greater after ATI versus after primary infection in three of four animals, the exception being a macaque with a viral load peak and a set point of $<10^3$ SHIV RNA copies following ATI (right upper panel).

The two HSCT animals (bottom left two panels, red hues) with viral load set points of $>10^6$ SHIV RNA copies following ATI had lower cytolytic immune responses following ATI relative to primary infection, with notable expansion of the response over time during primary infection and ATI. The two HSCT animals (bottom right two panels, red hues) with viral load set points of $<10^4$ SHIV RNA copies had equivalent or higher immune responses following ATI relative to primary infection, again with a notable expansion of the response over time during primary infection and ATI. Thus, there was a mounting SHIV-specific cytolytic immune response following all primary infections, all ATIs in HSCT animals, and in the single control following ATI with a high viral load. Determinants of absolute per cell immune intensity during ATI versus primary infection related not only to the presence or absence of HSCT but also to individual macaque characteristics governing the viral load set point.

**Prior exposure to SHIV enhances the recruitment of adaptive cells during the reactivation of virus.** The majority of solved parameter values did not significantly change between primary infection and post-ATI in control macaques. However, the recruitment rate ($\omega$) and the initial number of adaptive cells ($E_0$) increased in each

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**FIG 6** Model with immunity and unproductively infected cells provides optimal fit in nested model analysis and minimal overfitting in covariance analysis. Model selection criterion in log-likelihood (A) and Akaike information criterion (B) show that while the complete model 1 provides a slightly better fit, when penalized for extra parameters, model 2 is the optimal model. A summary of the models in terms of biological phenomena included is given in the key and is described further in Materials and Methods.

**FIG 7** Simulated SHIV-specific cytolytic immune response increases rapidly during SHIV infection and differently between control and transplant animals. SHIV-specific immunity in terms of cytolytic cells per infected cell is shown for each ATI animal. Comparisons between primary infection and ATI infection demonstrate that the SHIV-specific immune system is prepared in control animals in terms of initial value of immune cells and higher set point values. However, animals that underwent autologous stem cell transplantation had lower immune set points after ATI.
macaque (Fig. 8), which is the likely explanation for lower viral load peak but no lowering of the set point.

The most significance difference arose from the change in the clearance rate of cytolytic cells ($\delta_3$) in the HSCT animals, which provides a likely explanation for the higher viral load set point but no lowering of the viral load peak in these animals. The two macaques with an extremely high viral load set point (Fig. 5B) also had the highest cytolytic clearance rate (Fig. 8).

Notably, the initial availability of susceptible cells ($S_0$) and the rate of susceptible cell growth ($\alpha_3$) did not differ between primary and ATI infections for the control cohort. The initial value of susceptible cells was slightly lower at ATI in two HSCT animals, in rough agreement with the data analysis in Fig. 3E, which shows that the levels of CCR5$^+$ CD4$^+$ T cells were mostly recovered by ATI.

**DISCUSSION**

Our data demonstrate that primary infection with SHIV primes the macaque adaptive immune system, facilitating a more rapid engagement of the cytolytic response following ATI. However, this enhanced initial response did not have a lasting benefit, and the viral load set point was not significantly different relative to the primary infection. It is possible that virus evolution outpaces this more intense immune response, causing eventual escape and a return to previous set point viral loads.

Nevertheless, our results suggest that there is an important immune response during both primary infection and after ATI, which is inferred based on its relative absence following autologous HSCT. In a prior study, ATI was performed 6 to 10 weeks after HSCT in SHIV-infected rhesus macaques. However, the animals did not achieve the viral load set point before ART was initiated, and therefore a formal comparison of the viral dynamics pre- and post-ATI was not possible (37). Here, we showed that autologous HSCT results in a relative loss of immunity, captured most precisely in our model by a higher estimated clearance rate of the cytolytic immune response, leading to a higher viral load set point and more rapid progression to AIDS. Our prediction that the anti-SHIV cytolytic response is less durable following HSCT is consistent with the finding of disrupted CD8$^+$ and CD4$^+$ T cell homeostasis and with the increased exhaustion markers noted in the same cohort of macaques used in the present study (38).

Our model suggests that the higher viral load set point 25 weeks after HSCT is not due to greater target cell availability. Although suspected target cells of HIV (CCR5$^+$ CD4$^+$ T cells) did spike during the first several months after the transplant, these levels equilibrated by 6 months, when ATI was performed. In our prior study (23), two macaques underwent primary infection 14 weeks after HSCT and demonstrated poor viral control, presumably due to both high target cell availability and impaired cytolytic immune response at this earlier time point.

An advantage of our approach is that we used each macaque as its own control and
compared the viral load peak and the set point during primary infection and reactivation. This strategy, which we justified by showing a high correlation between the peak and set point viral loads during primary infection, allows us to bypass the previously recognized profound heterogeneity in SHIV dynamics among macaques following primary infection (28, 32) and to account for the interventions of interest. From a modeling perspective, we leveraged this approach to quantitate changes in parameters indicating mechanistic differences, as opposed to relying on absolute magnitudes of the parameters, which are imperfect estimates.

In keeping with variable viral load peaks and set points among macaques, we observed variability in CD4+ and CD8+ T cell dynamics following primary infection, cART, and ATI. While the relative changes in viral load peak and set point between primary infection and ATI also varied across macaques, statistically significant differences between the control and HSCT arms highlight predictable qualitative immunologic differences between these two groups.

Our results have relevance for HIV cure efforts. Autologous HSCT of gene-modified, HIV-resistant cells is a promising approach for achieving an overall reduction in the number of infected cells and has been associated with lower viral loads and greater CD4+ T cell protection when performed prior to primary SHIV infection (32, 39). However, the loss of HIV-specific immunity related to HSCT conditioning represents a potential barrier to efficacy. The relative decrease in overall CD4+ T cells following HSCT does not reduce the size of the latent viral reservoir (C. W. Peterson et al., unpublished data) or impair the ability of the virus to rebound to previously observed peaks, ultimately leading to a higher viral load set point. Collectively, these findings suggest that myeloablative conditioning regimens, such as the 1,020-cGy total body irradiation received by HSCT animals prior to stem cell infusion, are dispensable for HIV cure. Reduced intensity conditioning regimens that still promote stem cell engraftment should retain a greater proportion of virus-specific immune cells and increase safety for patients. We posit that genetically protected cells, analogous to the CCR5 Δ32 cells received by the Berlin patient, are the most important component of a gene/cell therapy-based HIV cure.

In HIV-infected persons treated with HSCT, cART has generally been well tolerated and has remained effective in suppressing the viral load (40, 41). This suggests that although a proportion of virus-specific immunity is lost during long-term cART, a sufficient proportion is retained to maintain effective cART-dependent suppression. Experimental CD8 depletion results in the failure of cART to suppress viremia in SIV-infected macaques (42). Similarly, we observed cART failure in previously transplanted animals that were infected during ongoing immune reconstitution (23). Interestingly, we observed continued suppression of SHIV in macaques that were infected and treated with ART prior to HSCT. Although we cannot rule out the possibility that these animals may have eventually developed SHIV rebound during continued cART, our findings suggest that these animals retained a sufficient proportion of virus-specific immunity to maintain suppression over at least 6 months following transplantation.

To the best of our knowledge, we have developed the first mechanistic model that quantifies and explains the impact of transplant biology on post-ATI HIV rebound viremia, allowing theoretical evaluation of additional interventions, including immune checkpoint inhibitors, protective gene therapy transgenes, and latency reversing strategies. Curative interventions inspired by the Berlin patient should be continually explored. Our model provides a flexible tool that enables prediction of outcomes for such costly and challenging experiments and can be modified in the setting of an HSCT-based HIV cure.

Our mathematical model also allows precise quantitation of the degree of virus-specific immune loss attributable to HSCT, which is useful for the consideration of other transplant-related infections such as cytomegalovirus, herpes simplex virus, adenovirus, and BK virus infections. The incidence of these infections is lower following autologous relative to allogeneic HSCT (43, 44). Nevertheless, viral reactivation following autolo-
ologous stem cell transplant is a significant problem that is facilitated by the loss of T cell function.

There are several limitations to our work. First, we are unable to disentangle the specific deficits in immunity induced by an autologous stem cell transplant. It is uncertain what proportion of immune loss is due to the disappearance of virus-specific cells versus a broader population of CD4<sup>+</sup> and CD8<sup>+</sup> T cells capable of inducing antiviral bystander effects (45). Our model does not consider the role of humoral immunity, which might play a role in the viral load set point following ATI in HSCT-treated animals.

Second, though we demonstrate statistical significance between control and HSCT macaques, our sample size is small, and we do observe important differences in immunity attributable to individual macaques during primary infection. It remains to be seen whether our conclusions are generalizable to all macaques undergoing treatment interruption and autologous stem cell transplantation and, more importantly, whether our findings are directly relevant to patients with HIV infection. Indeed, analogous data from HIV<sup>+</sup> patient cohorts demonstrated lower average viral load set points following ATI in patients who did not receive a transplant (46). This discrepancy might be due to differences between SHIV and HIV infection or to a longer window between ART initiation and ATI in the human ATI studies.

Third, our macaques may not have been perfectly matched by immune status since HSCT macaques coincidentally had lower CD8<sup>+</sup> T cell levels prior to HSCT relative to the controls. The reason and importance of this observation are unclear. We attempted to circumnavigate this issue by performing all of comparisons within single macaques.

Finally, while the duration between transplantation and ATI was longer than in prior studies (23), we believe that the loss of anti-SHIV immunity induced by transplantation may be transient. With an even longer delay before ATI, it is possible that the cytolytic memory compartment would repopulate and that the viral load set point would have been decreased similarly to control animals.

In conclusion, we demonstrate that primary infection engenders cytolytic immune memory to SHIV. However, further enhanced immunity does not occur with ongoing exposure to SHIV, indicating in these animals that a primed immune system is insufficient for viral control. The lack of a sustained or improved adaptive response following initial exposure to virus may be instructive in the development of effective T cell vaccines. Furthermore, the loss of the memory-driven immune compartment due to the immunosuppressive transplantation procedure is clearly demonstrated by the severe viral rebound in that cohort. This important finding highlights the fact that additional engineered SHIV resistance through gene therapy approaches will be necessary in moving toward HIV functional cure/remission techniques inspired by the Berlin patient’s transplant-mediated cure.

**MATERIALS AND METHODS**

**Nonparametric statistical analysis.** We performed a qualitative statistical analysis (shown in Fig. 2) comparing the peak viral load and the average viral load set point between primary infection and analytical treatment interruption (ATI). The peak value is the maximum of the viral load throughout infection. The average set point viral load is the geometric mean of the viral load between weeks 10 and 21. For Fig. 2B and D, we plotted the ratio of the primary infection values of each statistical quantity to the ATI values for each animal. This method provides a direct comparison within an animal and avoids the complications arising from heterogeneous viral loads. In both of these panels, we use a single-sided Mann-Whitney U test. Significance is notable because the small sample size only provides a significant result if all four animals of one group are larger or smaller than all four of the other animals. Intuitively, this result is equivalent to the probability of arranging eight elements into two groups of four, i.e., 4!4!/8! = 0.014. A similar approach is used to compare the T cell level changes in Fig. 3 and the parameter value changes in Fig. 8. In both cases, the U test compares the changed values to the null hypothesis of no change such that cases where all four animals have ratios above or below the line y = 1 are the only statistically significant results.

**Modeling SHIV infection, including adaptive immunity.** To simulate the kinetics of SHIV primary infection, we solved the model shown schematically in Fig. 4A. Many models of SIV or SHIV dynamics have been proposed based upon previous HIV models (15, 25, 32, 47, 48). These models are typically systems of ordinary differential equations tracking the time evolution of the concentrations (per µl) of cells and virus over time. To capture the SHIV infection dynamics in this study (including perturbations
due to primary infection, cART, and HSCT), we developed a model, including susceptible target cells (S), productively infected cells (P), unproductively infected cells that do not produce viable virus (U), virus (V), and an adaptive immune compartment (E). In designing the model, we built upon the basic model of HIV dynamics by including the adaptive immune response and a non-productively infected compartment of infected cells. The rates used in the model are listed in Table 1 with references given where possible. The set of ordinary differential equations can be written as follows:

\[
\begin{align*}
\dot{S} &= \alpha_S - \delta_S S - \beta SV \\
\dot{P} &= \varepsilon \beta SV - \delta_P P - \kappa PE \\
\dot{U} &= (1 - \gamma) \beta SV - \delta_U U - \kappa UE \\
\dot{E} &= \alpha_E + \omega (P + U) E / (E + E_0) - \delta_E E \\
\dot{V} &= \pi P - \gamma V - \beta SV,
\end{align*}
\]

where a derivative in time is indicated by an "overdot" notation: \( \dot{x} \). While the model is mostly analytically intractable due to nonlinear terms in multiple equations, we can derive an approximate basic reproductive number \( R_0 \) defined as the number of infected cells generated by the first infected cell at the onset of infection. First, we assume the viral dynamics are much faster than cellular dynamics so that \( \delta_U = 0 \) at all times and thus \( V = \pi \rho / \gamma \). Then, we focus on the sum of the infected cells calling \( \dot{S} + \dot{P} + \dot{U} = \dot{I} \). At the beginning of primary infection, we have the viral free equilibrium state such that \( S_0 = \alpha_S / \delta_S \) and \( E_0 = \alpha_E / \delta_E \). We therefore write the differential equation for the infected cell compartment as follows:

\[
I = \left( \frac{\tau_1 \alpha_S \pi}{\delta_S \gamma \rho} - \delta_P - \frac{\kappa \alpha_E}{\delta_E} \right) I.
\]

Dividing through by \( \delta_P \) suggests the eigenvalue equation \( I = \lambda \exp(\lambda t) \), where the eigenvalue \( \lambda \)'s sign depends on the \( \lambda \) value as follows:

\[
\lambda = \frac{\tau_1 \alpha_S \pi}{\delta_S \gamma \rho} - \frac{\kappa \alpha_E}{\delta_E} - 1.
\]

We determined the approximate basic reproductive number as follows:

\[
R_0 = \frac{\tau_1 \alpha_S \pi}{\delta_S \gamma \rho} - \frac{\kappa \alpha_E}{\delta_E},
\]

where if \( R_0 < 1 \), the infection returns exponentially to viral free equilibrium. This quantity gives us an estimate of the primary infection slope, which is known to be \( \sim 10 \) for HIV (16).

**Initial model parameter estimates obtained from the literature.** The parameters of the model define the rates of transitions among states. With the large number of parameters, we fixed seven parameters \( (\beta, \tau, \delta_S, \delta_P, \pi, \gamma, \text{and } \alpha_S) \) based on extensive modeling history or experimental findings. Notably, these parameters mostly control viral replication, spread, and clearance dynamics and do not affect our analysis of the adaptive immune system or target cell availability. Those parameters are held constant for all animals in all models. The fixed values are listed in Table 1 with the references cited. We emphasize the result of Doitsh et al., who quantified the fraction of viral infections that produce infected cells capable of generating productive virus (30). As a result of emphasizing this mechanism, the viral infectivity found by Luo et al. is notable because these authors found \( \beta = 10^{-14} = 3 \times 10^{-16} \) (49). This value should only account for productively infected cells so that our value of \( \tau_1 \varepsilon \Delta \) matches almost exactly using a commonly used value of \( \beta = 10^{-4} \) (50).

**Remaining parameters: emphasis on the effect of transplant on adaptive immune parameters.** Not all parameters for this model are as well established in the literature. To that end, we initialized at logical guesses based on literature and then used model fitting to determine the remaining six best-fit parameters for each animal. The susceptible cell rates appear to vary widely between animals; in a human treatment interruption trial, Luo et al. fit a model with the same formulation for viral-free target cell dynamics, so we chose similar values (49). Estimates from mouse models of lymphocytic choriomeningitis virus (LCMV) and some human studies can be used to estimate rates of the adaptive immune response. Initial values for fit parameters are presented in Table 2.

In particular, human data show anti-HIV cytotoxic T lymphocyte populations having precursor frequencies in the range of \( 10^{-6} \) to \( 3 \times 10^{-3} \) (51). These results provide a general idea of the initial value of \( E_0 \), because the macaques have \( 10^2 \) to \( 10^3 \) CD8\(^+\) T cells per \( \mu L \), on average. We estimated \( E_0 = 1 \) for the initial value of SHIV-specific CD8\(^+\) T cells per \( \mu L \). Several groups estimated that 20 to 70% of CD8\(^+\) T cells are virus specific at the height of the infection using LCMV mouse models (52–54), leading to our choice of \( E_{50} = 250 \) cells/\( \mu L \) (56). The number of infected cells at which the cytolytic expansion rate becomes half-maximal, again based on the CD8\(^+\) T cell concentrations measured. Halle et al. quantified the killing capabilities of CD8\(^+\) T cells using in vivo microscopy in mice, finding the rate to be \( 2 \) to \( 16 \) cells per cytotoxic T lymphocyte per day (55), leading to our initial estimate of \( \kappa \) given the typical CD8\(^+\) T cell concentration. The instantaneous recruitment rate \( \omega \) of the cytolytic response is difficult to estimate. Using the LCMV mouse model, Murali-Krishna et al. found a roughly \( 10^4 \) increase in the number of virus-specific CD8\(^+\) T cells by 8 days postinfection (54). Considering this an average rate admits \( \omega \sim \Delta E / (kE) \). Given the orders of magnitude of cytolytic and infected cells (both between \( 10^3 \) and \( 10^6 \), we...
estimate \( \omega \) to be between 1/8 and 1/800 \( \mu \text{cells/day} \). We chose the median of this range 0.01 as our initial estimate.

We emphasize that each of the above parameters is somewhat imprecisely specified for human CD8\(^+\) T cell activity whereas our model captures the entirety of the cytolytic effect in nonhuman primates including possible contributions from ADCC and CD4\(^+\) T cells. We use these estimates only to provide rough initial guesses for our model fitting.

**Local and global sensitivity analysis.** Using the dynamical system encapsulated by equation 1 and the schematic in Fig. 4A, we performed a local and global sensitivity analysis to test the impact of parameter variations on peak and set point viral loads (34, 35, 56). We initialized the local sensitivity analysis with the parameter values in Tables 1 and 2. Then, a single parameter was varied at intervals between 2 logs above and below its initial value. Because this approach was applied consistently across parameters, we refer in Fig. 4B to the “multiplier,” which ranges from 1/100 to 100 accordingly. The viral load was simulated by solving the set of ordinary differential equations numerically using odeint from the SciPy package of Python, and from that we calculated two metrics (the maximum or peak and the average viral load from weeks 10 to 20 as a surrogate for the viral set point). Those results are recorded in Fig. 4B and C. Viral set points below typical undetectable limits are displayed as 1 copy/ml.

The global sensitivity analysis was accomplished using the pyDOE package in Python. A total of 10\(^5\) Latin hypercube parameter sets were sampled from the parameter space surrounding the initial parameter values by 2 logs. Then, the value of the peak and set point viral load were correlated with each parameter based on the global variations in the parameter space. Correlations were calculated using Spearman’s \( \rho \) rank-ordered correlation coefficient.

**Computational model fitting procedure.** To perform the parameter fitting process, we used curve \_fit from the SciPy Python package. This package solves the nonlinear least-squares optimization problem based upon a Levenberg-Marquardt algorithm. The algorithm fits the log10 viral load, and the choice to use ordinary least-squares is justified by the fact that the primary infection viral load variance is approximately log-normally distributed. Therefore, maximizing the log-likelihood of the model using log-transformed data with normally distributed noise is equivalent to minimizing the \( \chi^2 \) statistic (36).

**Model selection.** Starting from the complete model Fig. 4A and equation 1, we developed five nested models by excluding various biological mechanisms. By fitting each of these models, we selected the best model by maximizing the log-likelihood (given a log-normally distributed viral load with variance 1 log) and by minimizing the Akaike information criterion (57). The model selection results, with descriptions of each model are presented in Fig. 6. The models are (1) the “complete” model shown in Fig. 4A; (2) the complete model with the simplification that the death rate of unproductively \( (U) \) and productively \( (P) \) infected cells are assumed to be identical; (3) a model in which the cytolytic immune cells do not interact with unproductively infected cells, that is, they do not kill \( U \) cells and are not recruited faster due to the presence of \( U \) cells; (4) a model in which there are no \( U \) infected cells and the adjusted infectivity of productively infected cells is \( \beta_T \); (5) a model in which there are no immune cells \( (E) \); and, finally, (6) a model in which there are no \( U \) or \( E \) cells.

**Fitting primary infection.** The initial simulation conditions were chosen such that virus was at the undetectable limit \( (V = 30 \text{ copies/ml}) \) at the time point before the first viral load detection in the data, with no infected cells, i.e., \( U = P = 0 \). Both susceptible and adaptive cells were assumed to initially be at their viral-free-equilibrium values, i.e., \( S_0 = \alpha_S/\delta_S \) and \( E_0 = \alpha_E/\delta_E \), respectively. For each primary infection data set, we fit the eight parameters starting from their initial values in Table 2. Because of the inaccurate nature of these parameters (often found from experiments in species other than nonhuman primates), the values are allowed to vary by 2 logs above and below the initial value.

We explored the parameter fit covariance matrix (data not shown) to ensure overfitting was avoided. In this case, we normalized the covariance matrix by the parameter value (i.e., we plotted cov\( (p, p)/(p, p) \)) allowing us to compare among parameters. Low values for cov\( (p, p)/(p, p) \) imply little interaction between parameters in regard to model fitting, whereas higher values imply that adjustments in a value of one parameter can still allow adequate model fit given compensatory adjustment of the paired parameter.

**Fitting analytical treatment interruption.** In fitting to ATI viral load, for a given animal, all parameters were initialized at the best-fit values found from that respective animal’s primary infection fitting procedure. We fit to viral loads beginning one time point before the first positive. A covariance analysis (described in the preceding section) indicated the model was overfit, so we further constrained the parameters by assuming that these parameters (the susceptible cell death rate \( \delta_S \), the 50% immune saturation threshold \( E_{50} \), and the killing rate of cytolytic immune cells \( \kappa_T \) should remain unaffected by ART or transplant. Numerically, we assumed these parameters could only vary by 1% of their original values.

Because the absolute values of the parameters were less important than changes in parameters based on immune experience or transplant, the results of the fitting are presented by comparison to their previous values. Specifically, we show the five unconstrained parameter ratios of each value from ATI to the respective value from primary infection in Fig. 8. This procedure normalizes for the large variation between animals.

**Study design.** Juvenile pigtail macaques were sourced from the New Iberia Research Center and SNBL-USA. Animals were entered without prior knowledge of study-relevant factors, including lymphocyte counts and response to our stem cell mobilization regimen. All animals were treated as single experimental units and were analyzed as two experimental groups of four animals each, whose size was designed to inform the impact of autologous hematopoietic stem cell transplants while accounting for potential animal-to-animal variability. Ages for the control and transplanted groups ranged from 3.9 to 5.8 years and from 3.9 to 5.5 years, respectively.
Animals received CD34+ cell doses ranging from 2.08 million to 6.45 million per kg of body weight. The conditioning regimen for each transplant consisted of a fractionated dose of 1.02 Gy of total body irradiation, as previously described (58). Data collection continued through necropsy for each animal, based on our definition of viral load (VL) rebound in tissues that could not be collected until the study endpoint.

**SHIV challenge, plasma VL assays, complete blood cell counts, and anti-SHIV antibody titers.** Intravenous inoculation with 9,500 50% tissue culture infective doses of SHIV-1157ipd3N4 (provided by Ruth Ruprecht, Texas Biomedical Research Institute), administration of cART (tenofovir [PMPA], emtricitabine [FTC], and raltegravir), and measurement of plasma VLs and absolute CD4+, CCR5+ CD4+, and CD8+ T cell counts were conducted as described previously (58–60). PMPA and FTC were gifts from Gilead Sciences, and raltegravir was a gift from Merck.

**Animal welfare.** The data used in this work were collected in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study protocol was approved by the Institutional Animal Care and Use Committees (3235-03) of the Fred Hutchinson Cancer Research Center and the University of Washington.

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**REFERENCES**

1. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, F Altin H, Ho DD. 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 68:4650–4655.
2. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol 68:5103–5110.
3. Demers KR, Makekwa G, Buggert M, Eller MA, Ratcliffe SJ, Goonetilleke N, Li CK, Eller LA, Rono K, Maganga L, Niyatayaph S, Kibuuka H, Routy JP, Sifika MK, Haynes BF, McMichael AJ, Bernard NF, Robb ML, Bettis MR. 2016. Temporal dynamics of CD8+ T cell effector responses during primary HIV infection. PLoS Pathog 12:1–24. https://doi.org/10.1371/journal.ppat.1005805.
4. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenero-Racz K, Dalesandro M, Scallon BJ, Ghrayeb J, Forman MA, Montefiori DC, Bieber EP, Letvin NL, Reimann KA. 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 283:857–860. https://doi.org/10.1126/science.283.5403.857.
5. Borrow P, Lewicki H, Wei X, Horwitz MS, Peffer N, Meyers H, Nelson JA, Gairin JE, Hahn BH, Oldstone MBA, Shaw GM. 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat Med 3:205–211. https://doi.org/10.1038/nm0297-205.
6. Price DA, Gould RJ, Klenerman P, Sewell AK, Easterbrook PJ, Troop M, Bangham CR, Phillips RE. 1997. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. Proc Natl Acad Sci U S A 94:1890–1895. https://doi.org/10.1073/pnas.94.5.1890.
7. Goonetilleke N, Liu MKP, Salazar-Gonzalez JF, Ferrari G, Giorgi E, Ganusov VV, Keefe BL, Learn GH, Turner RC, Li CK, Eller LA, Rono K, Maganga L, Nitayaphan S, Kibuuka H, Routy JP, Perelson AS, Hahn BH, McMichael AJ, McMichael J. 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. https://doi.org/10.1084/jem.20090365.
8. Fischer W, Ganusov VV, Giorgi EE, Roberth PT, Keefe BL, Leitner T, Han CS, Gleason CD, Green L, CC, Nag A, Wallstrom NC, Koo SK, McMichael AJ, Haynes BF, Hahn BH, Perelson AS, Borrow P, Shaw GM, Bhattacharya T, Korber BT. 2010. Transmission of single HIV-1 genomes and dynamics of early immune escape revealed by ultradeep sequencing. PLoS One 5:e12303. https://doi.org/10.1371/journal.pone.0012303.
9. O’Connor DH, Allen TM, Vogel TJ, Jing P, DeSouza JP, Dodds E, Dunphy EJ, Melsaeter C, Mothe B, Yamamoto H, Horton H, Wilson N, Hughes AL, Watkins DI. 2002. Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. Nat Med 8:493–499. https://doi.org/10.1038/nm0502-493.
10. Barton JP, Goonetilleke N, Butler TC, Walker BD, McMichael AJ, Chakraborty AK. 2016. Relative rate and location of intra-host HIV evolution to evade cellular immunity are predictable. Nat Commun 7:11660. https://doi.org/10.1038/ncomms11660.
11. Liu MKP, Hawkins N, Ritchie AJ, Ganusov VF, Whale V, Rackenridge S, Li H, Pavlicek JW, Cai F, Rose-Abrahams M, Treurnicht F, Hraber P, Riou C, Gray C, Ferrari G, Tanner R, Ping LH, Anderson JA, Swanson M, Cohen M, Karim SSA, Haynes B, Borrow P, Perelson AS, Shaw GM, Hahn BH, Williamson C, Korber BT, Gao F, Self S, McMichael A, Goonetilleke N. 2013. Vertical T cell immunodominance and epitope entropy determine HIV-1 escape. J Clin Invest 123:380–393. https://doi.org/10.1172/JCI69369.
12. Garcia V, Feldman MW, Regoes RR. 2016. Investigating the consequences of interference between multiple CD8+ T cell escape mutations in early HIV infection. PLoS Comput Biol 12:1–23. https://doi.org/10.1371/journal.pcbi.1004721.
13. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, Hallahan CW, Selig SM, Schwartz D, Sullivan J, Connors M. 2000. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc Natl Acad Sci U S A 97:2709–2714. https://doi.org/10.1073/pnas.0506739.
14. Streeck H, Jolin JS, Qi Y, Yassine-Diab B, Johnson RC, Kwon DS, Addo MM, Brumme C, Roup J-P, Shiver JW, Cao L, Jelinek HK, Keleher AD, Hecht FM, Sekaly R-P, Rosenberg ES, Walker BD, Carrington M, Altfeld M. 2009. Human immunodeficiency virus type 1-specific CD8+ T-cell responses during primary infection are major determinants of the viral set point and loss of CD4+ T cells. J Virol 83:7641–7648. https://doi.org/10.1128/JVI.00182-09.
15. Davenport MP, Zhang L, Shiver JW, Camasano DR, Ribeiro RM, Perelson AS. 2006. Influence of peak viral load on the extent of CD4+ T-cell depletion in simian HIV infection. J Acquir Immune Defic Syndr 41:295–295. https://doi.org/10.1097/01.qai.0000199232.31340.d3.
16. Ribeiro RM, Qin L, Chavez LL, Li D, Self SG, Perelson AS. 2010. Estimation...
of the initial viral growth rate and basic reproductive number during acute HIV-1 infection. J Virol 84:6096–6102. https://doi.org/10.1128/JVI.00127-10.

37. Hill AL, Rosen bloom DS, Fu F, Nowak MA, Siliciano RF. 2014. Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. Proc Natl Acad Sci U S A 111:15597–15597. https://doi.org/10.1073/pnas.1418561111.

38. Pinkney CH, Cromer D, Tolstrup M, Grimm AJ, Cooper D, Lewin SR, Sogaard OS, Rasmussen T, Kent SJ, Kelleher AD, Davenport MP. 2015. HIV reactivation from latency after treatment interruption occurs on average every 5 to 8 days: implications for HIV remission. PLoS Pathog 11: e1005000. https://doi.org/10.1371/journal.ppat.1005000.

39. Siliciano RF, Greene WC. 2011. HIV latency. Cold Spring Harb Perspect Med 1:a007096. https://doi.org/10.1101/cshperspect.a007096.

40. Bukrinsky M, Stanwick TL, Dempsey MP, Stevenson M. 1991. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. Science 254:423–427. https://doi.org/10.1126/science.1925601.

41. Richman DD, Margolis DM, Delaney M, Greene WC, Hazuda D, Pomer- lander AM, Greene WC. 2010. Abortive HIV infection mediates CD4 T cell double-stranded DNA virus detection after allogeneic HCT is associated with increased mortality. Blood 115:3938–3945. https://doi.org/10.1182/blood-2009-12-264924.

42. Do Boer RJ, Perelson AS. 1998. Target cell limited and immune control models of HIV infection: a comparison. J Theor Biol 190:201–214. https://doi.org/10.1006/jtbi.1997.0548.

43. Do Boer RJ. 2007. Understanding the failure of CD8+ T-cell vaccination against simian/human immunodeficiency virus. J Virol 81:2838–2848. https://doi.org/10.1128/JVI.01914-06.

44. Hütter G, Nowak D, Mossner M, Ganepola S, Mussig A, Allers K, Schneider T, Hofmann J, Kücherer C, Blau O, Blau IW, Hofmann WK, Thiel E. 2009. Long-term control of HIV by CCR5 Δ32/Δ32 stem cell transplantation. N Engl J Med 360:692–698. https://doi.org/10.1056/NEJMoa082905.

45. Allers K, Hu G, Liddendenker C, Rieger K, Thiel E, Schneider T. 2011. Evidence for the cure of HIV infection by CCR5 Δ32/Δ32 stem cell transplantation. Blood 117:2791–2799. https://doi.org/10.1182/blood-2010-09-309591.

46. Treasure GC, Aga E, Boschi J, Mullins JI, Hu S-L, Kiem H-P. 2015. Lack of viral control and development of combination antiretroviral therapy escape mutations in macaques after bone marrow transplantation. AIDS 29:1597–1606. https://doi.org/10.1097/QAD.0000000000000702.

47. Eikenhöfer IA, Nelson GE, Stosor V, Durand CM. 2014. HIV and stem cell transplantation. Curr Infect Dis Rep 16:1–10. https://doi.org/10.1007/s11918-014-0424-y.

48. Jaynes ET. 2003. Probability theory: the logic of science. Cambridge University Press, Cambridge, United Kingdom.

49. Altman JD, Moss PAH, Goulder PJR, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM. 1996. Phenotypic analysis of antigen-specific T lymphocytes as an inducible virus reservoir in HIV-1 infection. Science 234:423–427. https://doi.org/10.1126/science.1925601.

50. Perelson AS, Kirschner DE, De Boer R. 1993. Dynamics of HIV infection of CD4 T lymphocytes as an inducible virus reservoir in HIV-1 infection. Proc Natl Acad Sci USA 111:15597–15597. https://doi.org/10.1073/pnas.1418561111.

51. Altman JD, Moss PAH, Goulder PJR, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM. 1996. Phenotypic analysis of antigen-specific T lymphocytes as an inducible virus reservoir in HIV-1 infection. Proc Natl Acad Sci USA 111:15597–15597. https://doi.org/10.1073/pnas.1418561111.

52. Mavigner M, Watkins B, Lawson B, Lee ST, Chahroudi A, Kean L, Silvestri G. 2014. Persistence of virus reservoirs in ART-treated SHIV-infected rhesus macaques after autologous hematopoietic stem cell transplant. PLoS Pathog 10:e1004406. https://doi.org/10.1371/journal.ppat.1004406.

53. Peterson CW, Benne C, Polacino P, Kaur J, McAllister CE, Filali-Mouhim A, Obenza W, Pecor TA, Huang M-L, Baldessari A, Murnane RD, Wolfoeff AE, Jerome KR, Hu S-L, Klett NR, Desosa S, Sekaly RP, Kiem H-P. 2017. Loss of immune homeostasis dictates SHIV rebound after stem-cell transplantation. JCI Insight 2:e91230. https://doi.org/10.1172/jci.insight.91230.

54. Younan PM, Polacino P, Kowalski JP, Peterson CW, Nicholas J, Williams NP, Ho O, Trobridge GD, Von Laer D, Prlic M, Brian C, Derosa S, Hu S, Kiem H, Maurice NJ, Beard BC. 2014. Safety and efficacy of combination antiretroviral therapy in human immunodeficiency virus-infected adults undergoing autologous or allo- geneic hematopoietic cell transplantation for hematologic malignancies. Biol Blood Marrow Transplant 22:149–156. https://doi.org/10.1016/j.bbmt.2015.08.006.

55. Echenique IA, Nelson GE, Stosor V, Durand CM. 2014. HIV and stem cell transplantation. Curr Infect Dis Rep 16:1–10. https://doi.org/10.1007/s11918-014-0424-y.

56. Jaynes ET. 2003. Probability theory: the logic of science. Cambridge University Press, Cambridge, United Kingdom.

57. Altman JD, Moss PAH, Goulder PJR, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM. 1996. Phenotypic analysis of antigen-specific T lymphocytes as an inducible virus reservoir in HIV-1 infection. Proc Natl Acad Sci USA 111:15597–15597. https://doi.org/10.1073/pnas.1418561111.

58. Mavigner M, Watkins B, Lawson B, Lee ST, Chahroudi A, Kean L, Silvestri G. 2014. Persistence of virus reservoirs in ART-treated SHIV-infected rhesus macaques after autologous hematopoietic stem cell transplant. PLoS Pathog 10:e1004406. https://doi.org/10.1371/journal.ppat.1004406.

59. Peterson CW, Benne C, Polacino P, Kaur J, McAllister CE, Filali-Mouhim A, Obenza W, Pecor TA, Huang M-L, Baldessari A, Murnane RD, Wolfoeff AE, Jerome KR, Hu S-L, Klett NR, Desosa S, Sekaly RP, Kiem H-P. 2017. Loss of immune homeostasis dictates SHIV rebound after stem-cell transplantation. JCI Insight 2:e91230. https://doi.org/10.1172/jci.insight.91230.

60. Younan PM, Polacino P, Kowalski JP, Peterson CW, Nicholas J, Williams NP, Ho O, Trobridge GD, Von Laer D, Prlic M, Brian C, Derosa S, Hu S, Kiem H, Maurice NJ, Beard BC. 2014. Safety and efficacy of combination antiretroviral therapy in human immunodeficiency virus-infected adults undergoing autologous or allo- geneic hematopoietic cell transplantation for hematologic malignancies. Biol Blood Marrow Transplant 22:149–156. https://doi.org/10.1016/j.bbmt.2015.08.006.

61. Echenique IA, Nelson GE, Stosor V, Durand CM. 2014. HIV and stem cell transplantation. Curr Infect Dis Rep 16:1–10. https://doi.org/10.1007/s11918-014-0424-y.

62. Jaynes ET. 2003. Probability theory: the logic of science. Cambridge University Press, Cambridge, United Kingdom.
Kourilsky P, Rosenberg WMC, Moss PAH, Bell JI, Hall MA, Lanchbury JS, Nanki T, Kohsaka H, Miyasaki N, Wagner UE, Kocht K, Weyand CM, Goronzky JJ, Dudley EC, Petrie HT, Shah LM, Owen MJ, Hayday A, von Boehm H, Fehling HJ, Benit C, Lucas B, Vasseur F, Rodewald H-M, Fehling HJ, Gapin L, Trigueros C, Borgulya P, Kishi H, Uematsu Y, von Boehm H, MacMahan CJ, Fink PJ, Padovan E, Nossal GJV, Altman JD, Busch DH, Pamer EG, Butz EA, Bevan MJ, Argaet VP, Lehner PJ, Moss PAH, Tanchot C, Lemonnier FA, Perarnau B, Freitas AA, Rocha B, Tanchot C, Rocha B, Bersins SP, Boyd RL, Miller JFAP, Douek DC. 1999. A direct estimate of the human αβ T cell receptor diversity. Science 286:958–961. https://doi.org/10.1126/science.286.5441.958.

53. Butz EA, Bevan MJ. 1998. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. Immunity 8:167–175. https://doi.org/10.1016/S1074-7613(00)80469-0.

54. Murali-Krishna K, Altman JD, Suresh M, Sourdive DJD, Zajac AJ, Miller JD, Slansky J, Ahmed R. 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity 8:177–187. https://doi.org/10.1016/S1074-7613(00)80470-7.

55. Halle S, Keyser KA, Stahl FR, Busche A, Marquardt A, Zheng X, Galla M, Heissmeyer V, Heller K, Boelter J, Wagner K, Bischoff Y, Martens R, Braun A, Werth K, Uvarovskii A, Kempf H, Meyer-Hermann M, Arens R, Dembo M. 2005. Sensitivity analysis for chemical models. Chem Rev 105:2811–2827. https://doi.org/10.1021/cr040659d.

56. De Boer RJ, Mohri H, Ho DD, Perelson AS. 2003. Turnover rates of B cells, T cells, and NK cells in simian immunodeficiency virus-infected and uninfected rhesus macaques. J Immunol 170:2479–2487. https://doi.org/10.4049/jimmunol.170.5.2479.

57. Markowitz M, Louie M, Hurley A, Sun E, Di Mascio M, Perelson AS, Ho DD. 2003. A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo. J Virol 77:5037–5038. https://doi.org/10.1128/JVI.77.8.5037-5038.2003.

58. Blattman JN, Antia R, Sourdive DJD, Wang X, Kaech SM, Murali-Krishna K, Altman JD, Ahmed R. 2002. Estimating the frequency of naive antigen-specific CD8 T cells. J Exp Med 195:657–664. https://doi.org/10.1084/jem.20001021.