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Evaluating Immune Responses to Therapeutic Vaccination and Correlates of Post-Treatment Control in SIV^{+} Mauritian Cynomolgus Macaques

By

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Chapter 5
An introduction to post-treatment control of HIV for a broader audience

I am grateful for the Wisconsin Initiative for Science Literacy's initiative to include a chapter in my thesis to convey the work I have done to a non-specialist audience. This chapter will guide the reader through some of the broad questions that underpin human immunodeficiency virus (HIV) infection, as well as the major goals and findings of my research. I hope that by the end of this chapter, the reader will come away with a sense of 1) why HIV remains a global health concern, 2) how we attempted to develop a therapeutic strategy to induce viral remission, and 3) how our identification of consistent post-treatment control in a cohort of Mauritian cynomolgus macaques can set the stage for future HIV remission strategies.

5.1 Is HIV even still a problem?

I recently had a discussion with a colleague who is not in the STEM field, and she pointed out to me that she wasn't aware that HIV was really still an issue. After all, you rarely hear anything on the news or social media about HIV^+ individuals getting sick or dying in large numbers. This position seems to be a common one among people in the US, but not one I had really considered because, as someone who studies HIV, I literally think about it every day. So I wanted to start by giving a brief background of HIV and the current state of the global HIV/AIDS epidemic.

"You have HIV." 40 years ago, those words would have been equated with a death sentence, a nightmare diagnosis. Acquired immunodeficiency syndrome (AIDS) began presenting in the early 1980s in populations of men who have sex with men and intravenous drug users. A mysterious infectious agent was decimating the immune systems of infected individuals and allowing opportunistic infections and cancers to run rampant. When it was discovered in 1983-1984 that HIV causes AIDS, most people believed that a vaccine would be quickly created and promptly implemented. In the US, over 100,000 people died of AIDS-related illnesses by 1990, with nearly one-third of those deaths occurring in 1990 alone. This number rose constantly to a peak in the early 2000s, with approximately two million people dying of HIV/AIDS in 2004. Needless to say, vaccines and treatments were, unfortunately, not readily forthcoming.

So, how did we go from millions of people dying each year to the current state of HIV not appearing to pose a significant threat in the U.S. in just 40 years? The advent of antiretroviral therapy, or ART, has completely changed the way we think of HIV/AIDS as well as the treatment, prognosis, quality of life, and stigma.

AZT was the first antiretroviral drug approved to treat HIV in 1987. Throughout the late 1980s, multiple other ART drugs were approved for HIV treatment, and while these drugs helped initially, all proved highly toxic. These drugs were also very burdensome for HIV^+ individuals; some regimens required taking three capsules every eight hours. Indefinitely. Additionally, because all of these early ART drugs functioned very similarly, patients rapidly developed resistance. Researchers then began developing ART drugs of different classes that target different stages of the HIV life cycle, and the first drug of a second class was approved in 1995. Since then, more than 30 drugs spanning 6 different classes have been developed. Starting in the late 1990s, it was also proposed that combining multiple drugs spanning multiple classes might help improve viral suppression and prevent resistance. This turned out to be a very successful strategy. Combination ART regimens have evolved and improved over the last ~20 years and currently consist of at least three ART drugs spanning two or more different classes.

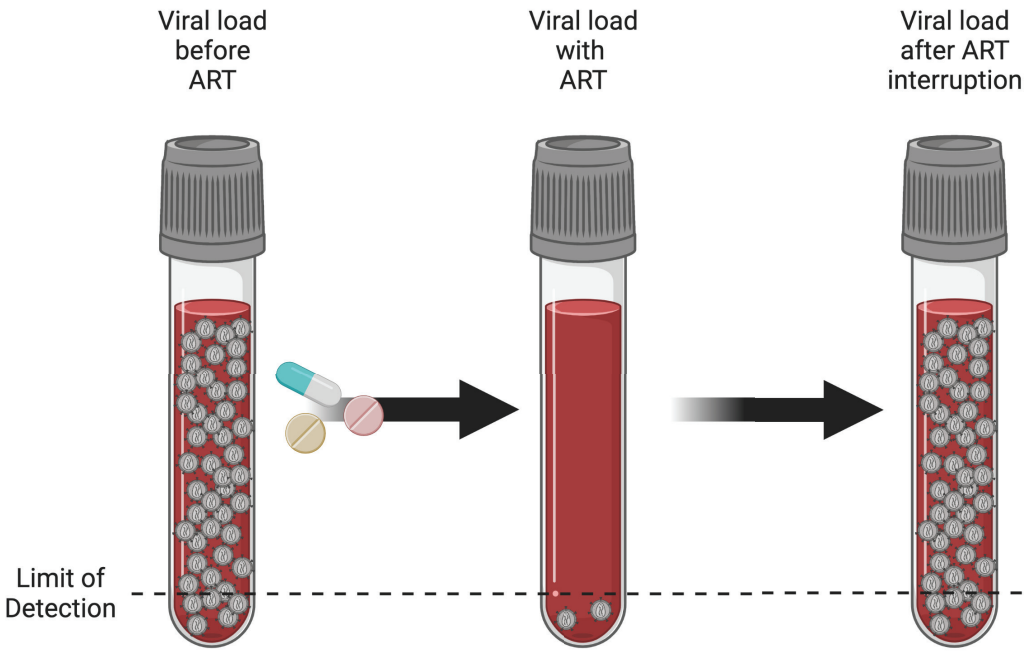

Figure 5.1 Typical viral load kinetics of HIV-infected individuals.

ART treatment *can* prevent HIV acquisition if delivered prophylactically as pre-exposure prophylaxis (PrEP) or within 72 hours of HIV exposure as post-exposure prophylaxis (PEP). ART can also suppress virus replication in the blood to undetectable levels, prevent viral transmission, and preserve immune function in HIV⁺ individuals (Figure 5.1, center). ART is a very effective therapeutic option and has benefitted millions of people living with HIV globally. Unfortunately, ART is not a cure and must be taken every day in order to maintain efficacy (described in more detail below). If an HIV⁺ person stops taking their ART medications for any reason, viremia returns (also called viral rebound), and the disease can progress to AIDS (Figure 5.1, right). ART is also expensive and can be difficult to acquire, particularly outside of the U.S., so not everyone has consistent access to these life-saving medications. Moreover, ART supply and access dramatically declined during the COVID-19 pandemic, interrupting treatment adherence and virus suppression for many HIV⁺ individuals.

Even though you probably don't hear much about HIV today, it is still a major global health concern. In 2021 alone, over 38 million people were living with HIV, ~1.5 million new HIV infections were acquired, and ~650,000 people died of AIDS-related illnesses worldwide. Hopefully, I've convinced you that HIV is, indeed, still a problem, and we should still care about improving therapeutic options for HIV⁺ individuals.

5.2 Why isn't there a cure for HIV? Isn't ART good enough?

HIV is a lifelong disease because HIV persists in viral reservoirs in specific cells in the body. To briefly introduce you to two of the major types of immune cells that are important in the context of HIV infection,

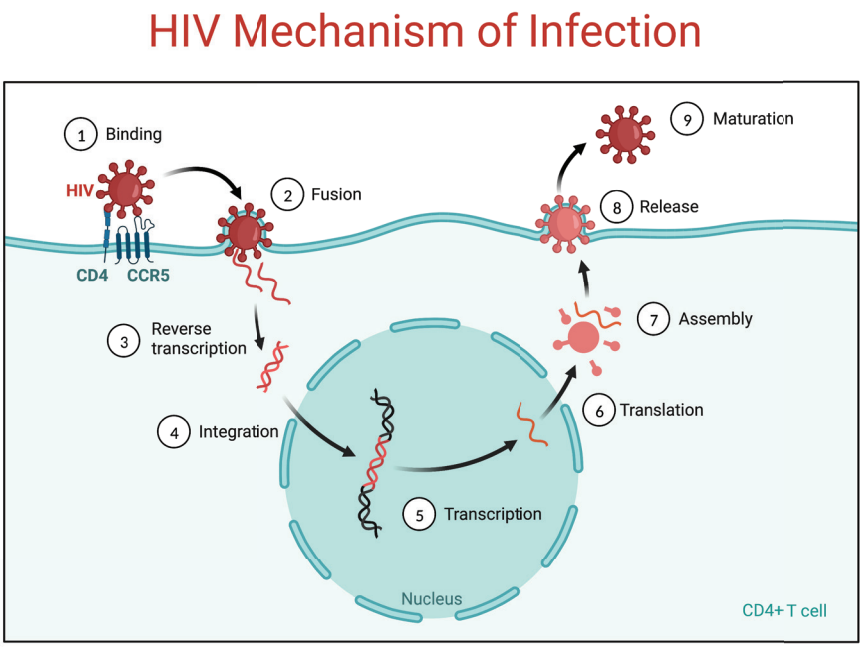

Figure 5.2 The HIV lifecycle.

I want to highlight the main features of $CD4^+$ T cells and $CD8^+$ T cells. Very broadly, $CD8^+$ T cells (cytotoxic T cells) surveil, recognize, and kill damaged or infected cells, while $CD4^+$ T cells (helper T cells) interact with and provide help to other immune cells, including $CD8^+$ T cells. HIV preferentially infects $CD4^+$ T cells, where it integrates its genome into the host DNA (Figure 5.2). Because HIV is incorporated into the host cell's DNA, every time that cell divides, HIV is also present in both new cells.

Additionally, if the infected $CD4^+$ T cells are long-lived, HIV can linger in those cells indefinitely, forming what is called the latent viral reservoir. This reservoir in $CD4^+$ T cells can be compared to a volcano. Just as a volcano can lay dormant for extended periods of time before erupting and releasing a surge of lava and ash, latently infected $CD4^+$ T cells can remain in a resting state for a long time before suddenly activating, proliferating, and releasing a wave of new viral particles (Figure 5.3). This productive infection, also called latent reactivation, can cause a surge in viral load and subsequent disease progression. Unfortunately, the viral reservoir persists even if the infected individual is taking ART. For these reasons, a sterilizing cure, that is, the individual no longer being infected with HIV, may not be possible. Therefore, the best outcome we can hope for is immune suppression of virus replication in the absence of treatment, also called viral remission.

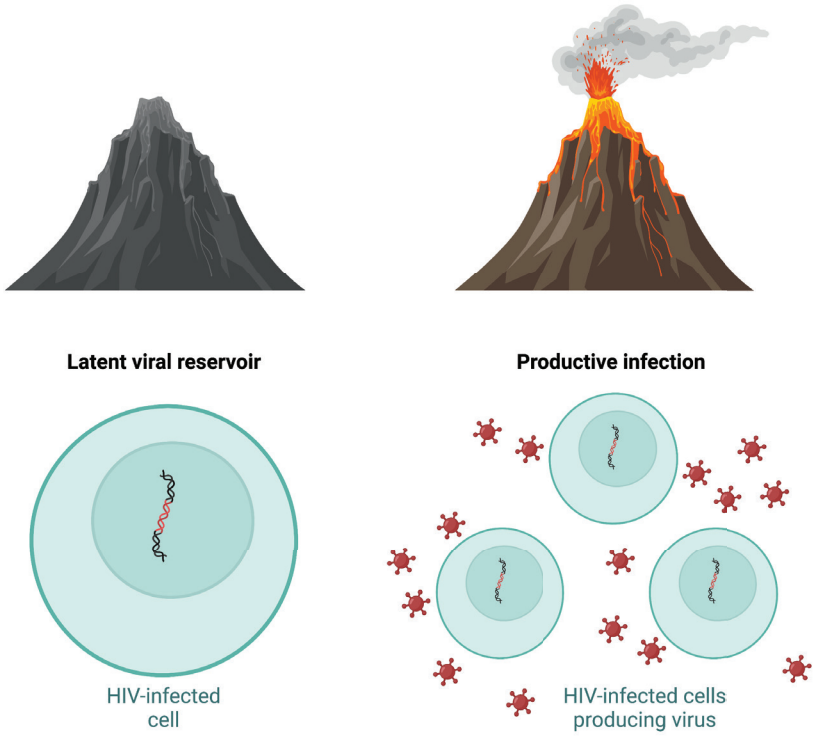

Figure 5.3 The latent viral reservoir (left) compared to productive HIV infection (right).

Viral remission is important because it indicates that even though HIV^+ individuals are still infected, the disease is not progressing to AIDS, and these individuals are likely not transmitting

HIV. The ultimate goal of HIV therapeutic development is to enable HIV⁺ individuals to stop taking daily lifelong antiviral therapeutics (ART) and remain in viral remission, an outcome called post-treatment control (Figure 5.4). Post-treatment control is challenging to study because it is extremely rare in humans. Thus, there are currently no therapeutics that lead to post-treatment control.

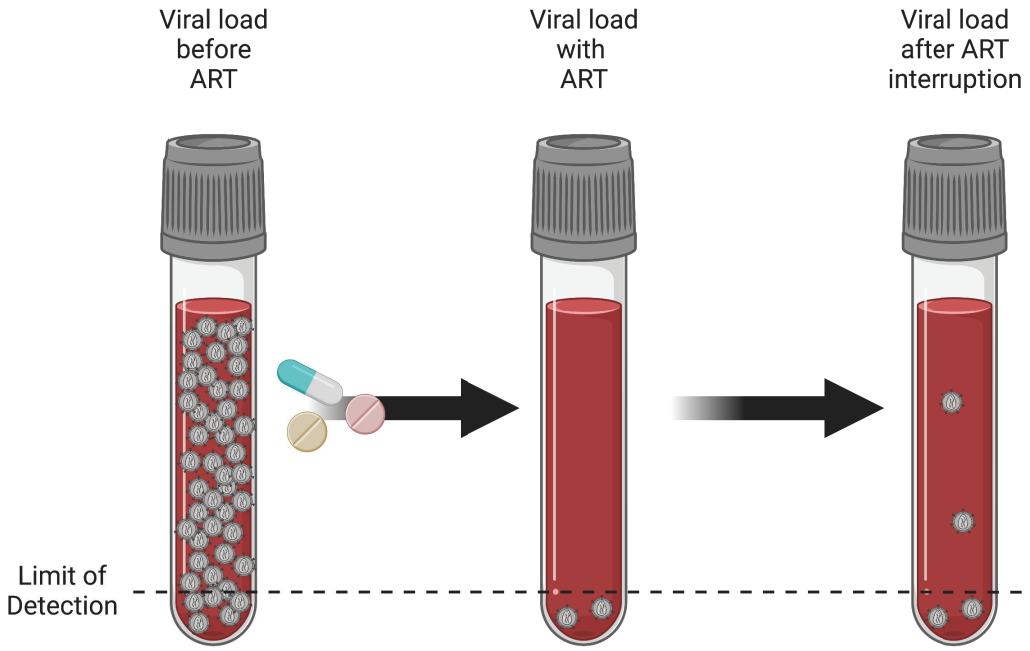

Figure 5.4 Viral load kinetics of post-treatment control.

One important topic to mention in this section discussing why there isn't a cure for HIV is that there have actually been a few individuals who have been cured. These individuals received stem cell transplants (Figure 5.5) consisting of essentially brand-new immune systems with cells that are not susceptible to HIV infection. If you've heard of the famous cases of individuals who were cured of HIV (the Berlin Patient [Timothy Ray Brown], the London Patient [Adam Castillejo], and the New York Patient [identity unknown]), you might be wondering why we can't just cure everyone in that same way. While the success of curing these individuals has generated excitement, and researchers are evaluating similar treatment strategies, stem cell transplantation is not a feasible cure effort for the majority of HIV⁺ individuals. It is not scalable and is highly toxic, with a high risk of morbidity and death. It also requires a stem cell transplant from an HLA-matched individual (the chance of finding an exact match with an unrelated donor is approximately one in 100,000).

This individual also must carry two copies of the HIV resistance mutation present in just ~1-3% of Caucasians of European descent. So you can imagine the odds of finding a matched donor carrying HIV-resistant cells are incredibly slim. For these reasons, other treatment options, including vaccination and immunotherapy, are also being developed to help a greater number of HIV$^+$ individuals, particularly those without consistent access to ART.

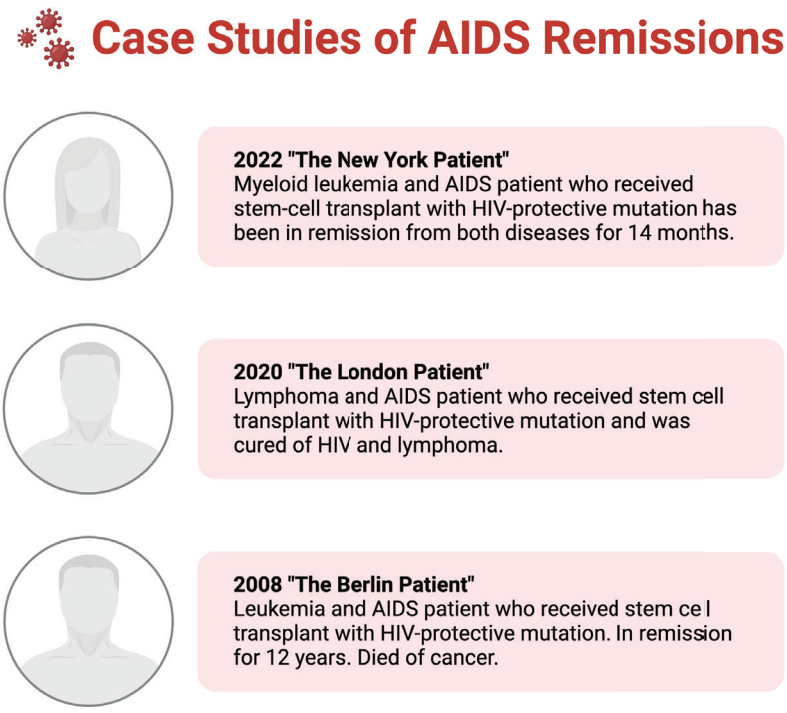

Figure 5.5 Three HIV cure cases.

5.3 How can you test interventions for HIV preclinically?

Before vaccines or drugs can be tested in humans, they first need to be tested in the lab setting. Any HIV medications that end up in clinical trials in humans were first tested in animals. There are many different animals that can be used to model various diseases, but because HIV specifically infects immune cells and causes immune pathologies, any animal model for HIV must possess a similar immune system to humans. Fortunately, monkeys have a very similar immune composition to humans, making them a potentially useful model.

However, one important question we must ask is: *which* monkeys can and should be used to study HIV? The origins of HIV give us some clues. Simian immunodeficiency virus (SIV) is nearly ubiquitous among many monkey species and is not pathogenic in these natural hosts. In other words, many monkey species, like chimpanzees, that are infected with SIV have high amounts of the virus detectable in their blood (viremia) but do not get sick with AIDS or other AIDS-related illnesses. SIV transmissions from natural SIV hosts like chimpanzees, gorillas, and sooty mangabeys to humans led to the emergence of HIV infections, which are pathogenic in

humans (Figure 5.6). Similarly, SIV transmission from sooty mangabeys to macaque monkeys (not natural SIV hosts) resulted in pathogenic SIV infections in macaques, leading SIV^+ macaques to be the most common model in which we preclinically evaluate HIV vaccines and therapeutics. ART also works in monkeys similarly to humans, allowing treatment regimens in macaques to closely resemble treatment regimens in humans.

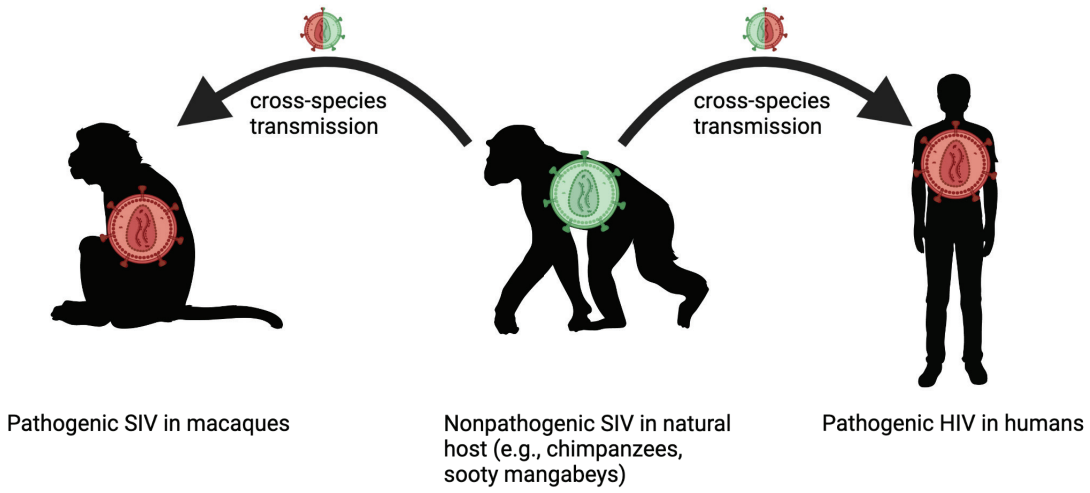

Figure 5.6 Cross-species transmissions of SIV from natural hosts (center) led to pathogenic SIV in macaques (left) and HIV in humans (right).

The two types of macaques that are important to introduce in order to understand my research are Indian-origin rhesus macaques and Mauritian cynomolgus macaques. Indian-origin rhesus macaques are by far the most widely used model for HIV; their consistent course of SIV pathogenesis and rapid disease progression to AIDS (≤2 years) make them an ideal model for HIV/AIDS. Our lab, however, also uses Mauritian cynomolgus macaques. Like Indian-origin rhesus macaques, Mauritian cynomolgus macaques are capable of developing pathogenic SIV infections. But because Mauritian cynomolgus macaques descended from a geographically isolated population on the island of Mauritius, these animals have very limited genetic diversity, specifically in their major histocompatibility complex (MHC) genetics. This limited genetic diversity is important for two reasons. First, we can match cohorts of animals based on their MHC genetics, allowing us to limit animal-to-animal variability due to genetic differences. Second, because some immune responses are determined by an animal's (or a human's!) genetic composition, it is much easier to evaluate those immune responses when we know what the MHC genetics are and

those genetics are shared among animals. For these reasons, we used Mauritian cynomolgus macaques for this project.

5.4 HIV/immune system hide and seek: the original interventions we set out to test

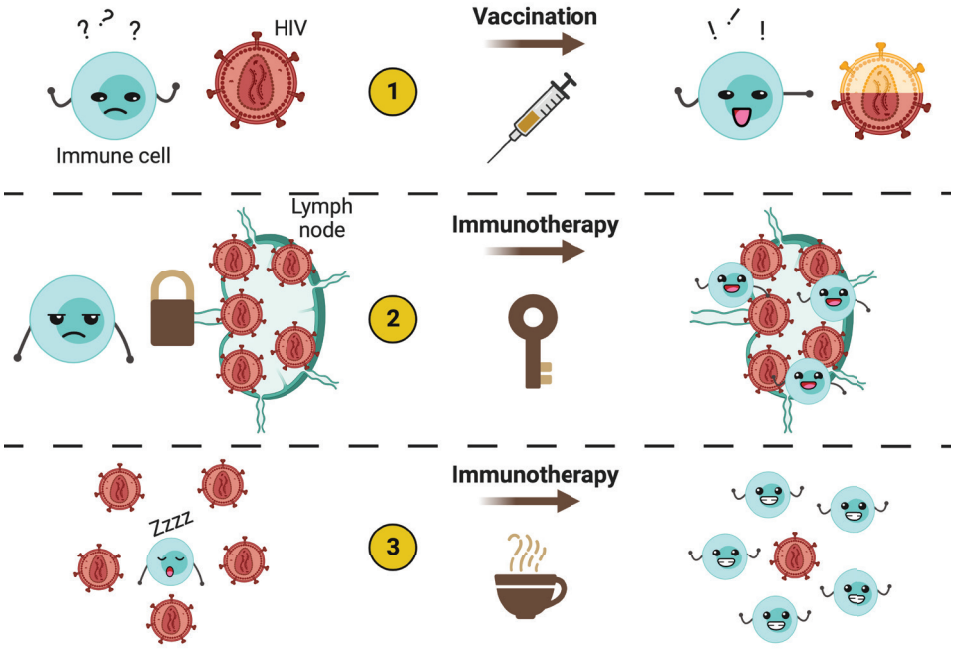

Figure 5.7 Three major HIV immune evasion strategies and the goal of each therapeutic intervention to counter each strategy.

The dynamics of HIV and the immune system are much like hide and seek: HIV hides, and the job of the immune system is to seek and destroy. HIV avoids immune destruction in three major ways: 1) HIV mutates rapidly, changing itself to be unrecognizable to immune cells, 2) HIV hides in lymph nodes, which are places that not all immune cells have access to, and 3) because HIV infection is lifelong, the immune system eventually becomes exhausted and can no longer effectively fight HIV and other pathogens. I initially set out to test a new treatment regimen combining vaccination during ART and immunotherapy after ART interruption to combat the three described immune evasion strategies used by HIV/SIV (Figure 5.7). The ultimate goal of this therapeutic regimen was to enable the immune system to control virus replication after ART interruption in SIV^{+} Mauritian cynomolgus macaques (Figure 5.8, bottom). In this section, I will explain why we selected the therapeutic regimen that we used to accomplish that specific goal, the purpose of each intervention, and the outcomes.

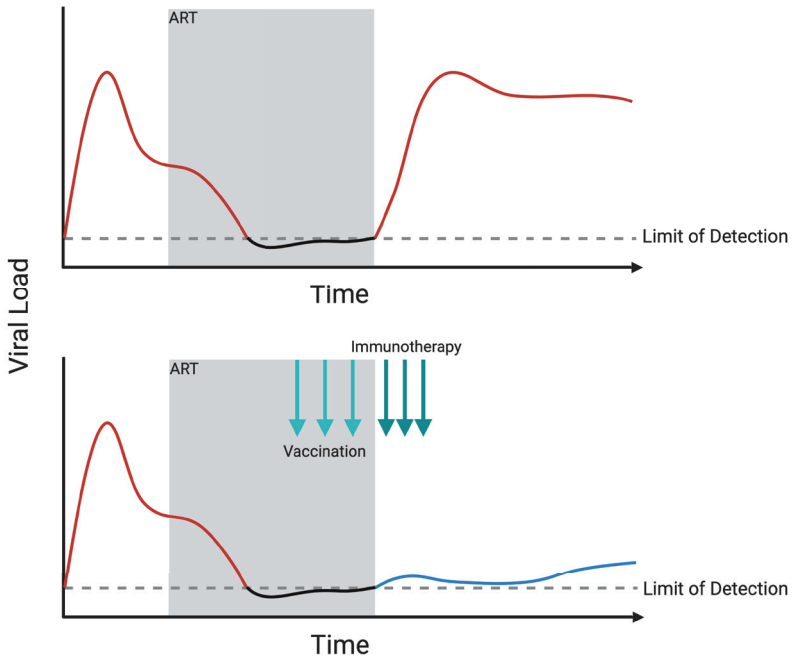

Figure 5.8 Sample graphs depicting the hypothesized viral loads for the unvaccinated animals (top) and vaccinated animals (bottom).

The vaccine regimen consisted of three separate injections, each delivering SIV Gag, which is a part of the SIV virus. As mentioned earlier, HIV and SIV mutate rapidly, which is one reason they are very good at avoiding immune pressure. Immune cells are constantly surveilling for pathogens or intruders, and they are very good at recognizing pathogens they have encountered before, but only if they resemble the previously-encountered pathogen. For example, if a man broke into your house and you didn't get a good look at his face, but he had a mustache, you would definitely remember the mustache. But if you saw that same man on the street and he was clean-shaven, you couldn't be sure it was the same person. You would need to be able to recognize his face to be sure since he can't change his face. This is similar to how you can think about the Gag protein of HIV/SIV. Gag is a structural protein in HIV/SIV, and mutations in Gag are usually bad for the virus, which means that Gag usually exists relatively unchanged from virus to virus. For this reason, it makes a good target for immune cells to recognize. Like plastering the streets with wanted posters of the intruder's face for all to study and recognize: the vaccines deliver Gag, and it circulates through the blood, giving many of the T cells in the body a good look at Gag so they can recognize it the next time they see it.

And as a point of clarification, we delivered the vaccines to animals that were already infected with SIV and were also receiving daily ART drugs. So while the animals were infected, there was no detectable virus in the blood at the time of vaccination. Therefore, we were essentially training the immune system to recognize Gag better and suppress virus replication *later* after stopping ART.

We also wanted to give the immune system the best possible chance of suppressing virus replication, so we delivered three doses of an immunotherapy agent called N-803 to the animals just after ART interruption. N-803 is a drug that tells T cells to proliferate, become activated, and survive. Clinical trials including N-803 as an immunotherapy component for novel cancer treatment regimens are currently underway, and because of its effects on boosting $CD8^+$ T cells, HIV researchers thought N-803 might also be an effective therapeutic option for HIV. Importantly, N-803 has also been shown to cause $CD8^+$ T cells to migrate to lymph nodes in monkeys. This is a key component of HIV immunotherapy because HIV-infected $CD4^+$ T cells tend to hide in lymph nodes, and $CD8^+$ T cells generally do not have access to the part of the lymph nodes where HIV-infected $CD4^+$ T cells reside. Therefore, we hypothesized that N-803 might be able to facilitate $CD8^+$ T cell access to lymph nodes to encounter and kill infected $CD4^+$ T cells. The idea of delivering N-803 after stopping ART was to recall and boost the cells that were elicited by vaccination, direct these cells to lymph nodes, and enable them to suppress virus replication as it began to rebound.

To summarize briefly, this set of interventions was designed to enable SIV^+ Mauritian cynomolgus macaques to be able to maintain post-treatment control after stopping ART. We also included a group of animals that did not receive any vaccines or N-803 as a control group, and we expected virus replication to return after stopping ART in these animals, as it does in humans that stop taking ART (Figure 5.8, top).

We measured the immune responses to each of these interventions, and we detected a high magnitude of vaccine-elicited T cells in the blood that recognized Gag. These cells were proliferative and expanded after each vaccine and each dose of N-803, but the expansion was not long-lived, and the frequency of cells declined to the baseline pre-vaccination amount before the next intervention. Unfortunately, N-803 treatment also did not direct the vaccine-elicited

cells to the lymph nodes, so the effects of these interventions on immune populations were not as considerable as we had hoped. But you might ask: what about viral rebound after stopping ART? Did detectable virus return in all the animals, and did they all progress to AIDS? You'll have to read on to find out…

5.5 Surprises, mysteries, and really cool science

A previous mentor had a saying that I think aptly sums up this transition point of my project: "If research worked the first time, it would just be called 'search.'" - Dr. Kyle McQuade.

As it turns out, three of the four animals that received vaccines and N-803 did not have rebound viremia after stopping ART. There was one vaccinated animal that did rebound, and it took over nine weeks, which is very slow (average ≈ two weeks). Now, I know what you're thinking: "Olivia, didn't you *just* tell me that the goal of the therapeutic regimen was to make the animals post-treatment controllers, and three of the four vaccinated animals became post-treatment controllers? Doesn't that mean 'success?'" Well, yes. But also no. It also turns out that *all four* of the *unvaccinated* animals also became post-treatment controllers (Figure 5.9).

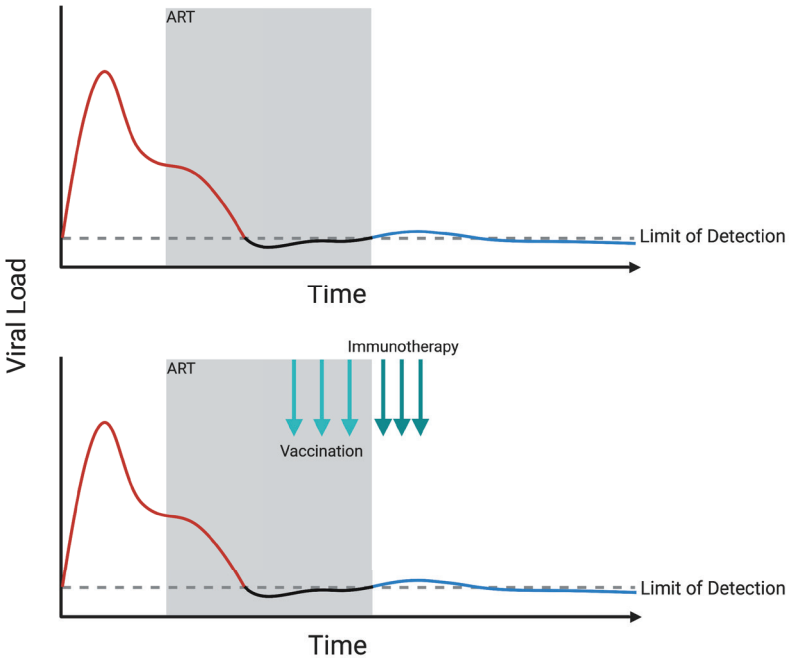

Figure 5.9 Sample graphs depicting the viral loads observed in the unvaccinated animals (top) and vaccinated animals (bottom).

Imagine, for a moment, that HIV⁺ individuals or SIV⁺ monkeys are dormant volcanoes when ART is present to prevent virus replication (or preventing volcanic eruptions, Figure 5.10, left). Just as a dormant volcano can suddenly erupt and cause significant damage, if ART is stopped, almost all SIV⁺ Indian-origin rhesus macaques and HIV⁺ humans experience viral rebound; the virus reactivates and starts replicating again, causing disease progression and transmission to others (Figure 5.10, top right). Originally, we hypothesized that the therapeutic regimen we were testing would prevent viral rebound (prevent eruption), but based on decades of observations in humans and Indian-origin rhesus macaques, we expected the unvaccinated animals would exhibit viral rebound (eruption, Figure 5.10, center right). Shockingly, seven of the eight Mauritian cynomolgus macaques, including all four unvaccinated animals, did not rebound (Figure 5.10, bottom right). Furthermore, the one animal that did rebound did so slower and with a lower viral load than is typically observed in Indian-origin rhesus macaques with similar infection histories. This surprisingly large number of post-treatment controllers identified in this cohort of Mauritian

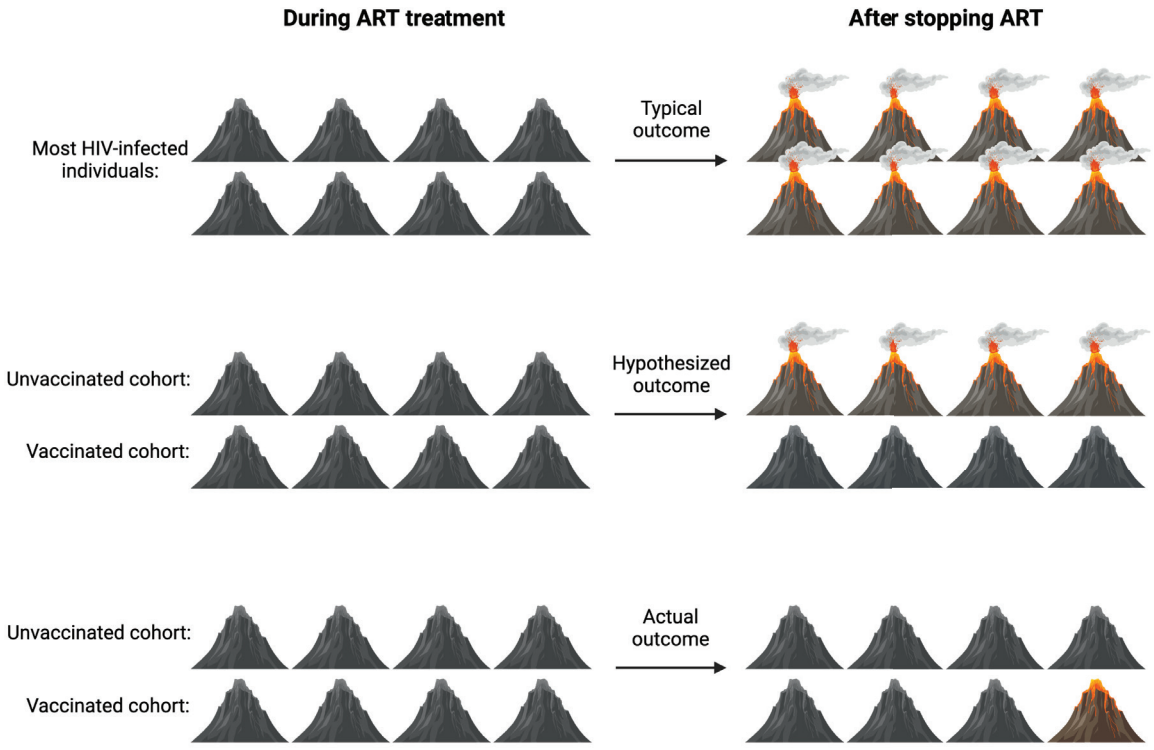

Figure 5.10 Viral reservoir kinetics during ART treatment (left) and after stopping ART (right) for typical humans or rhesus macaques (top panel). Hypothesized viral reservoir kinetics for the unvaccinated and vaccinated Mauritian cynomolgus macaque cohorts are shown in the center panel. Observed viral reservoir kinetics in the Mauritian cynomolgus macaques in are depicted in the bottom panel.

cynomolgus macaques stands in stark contrast to Indian-origin rhesus macaque cohorts, where less than 4% typically become post-treatment controllers.

It is unclear whether the vaccine regimen elicited immune responses that were sufficient to enable post-treatment control because the unvaccinated animals did not rebound. However, in comparing the immune responses we measured to those elicited by similar studies in the literature, it is unlikely that they would have been sufficient to suppress virus replication. Post-treatment control is very rare in both humans and Indian-origin rhesus macaques, which begs the question: why did seven of the eight Mauritian cynomolgus macaques become post-treatment controllers, regardless of whether they received the therapeutic regimen? That was exactly the question we needed to answer next, and we sought to answer it by first addressing three secondary questions:

Question 1) Were the animals still infected, or did they somehow clear the virus?

Answer: The animals were still infected. We measured the amount of virus that could be activated from the reservoir, even though it was undetectable in the blood. We could induce virus replication in cells from at least six of the eight animals, indicating that the virus was still present and was being suppressed by some unknown mechanism.

Question 2) Even though we stopped giving the animals ART, were the ART drugs still in the system and suppressing virus replication?

Answer: We measured the concentrations of the ART drugs in animals before and after we stopped giving them ART and the ART drugs were completely absent within a few months of stopping ART, so the post-treatment control was not due to ART lingering.

Question 3) Was there some mechanism of immune-mediated post-treatment control in these animals?

Answer: $CD8^+$ T cells are usually the most potent cell mediators of virus suppression, so we wondered whether $CD8^+$ T cells were suppressing virus replication. To test this hypothesis, we depleted all $CD8^+$ cells (which includes $CD8^+$ T cells) from all the animals so we could determine whether virus replication resumed once $CD8^+$ T cells were gone. SIV rapidly rebounded immediately after the $CD8^+$ T cells were depleted, indicating that the presence of these cells was required for post-treatment control.

What we observed in these animals was $CD8^+$ T cell-mediated post-treatment control that was somehow generated in all the animals and was not due to the vaccine regimen. Yet, it was difficult to determine what was unique about the $CD8^+$ T cells from these animals without a comparator group. For this reason, we included a second group of SIV^+ Mauritian cynomolgus macaques that did exhibit viral rebound after stopping ART in order to ask the question: what is different about the Mauritian cynomolgus macaques that became post-treatment controllers and the ones that did not? We measured multiple parameters of the $CD8^+$ T cells to see if there were any differences between post-treatment controllers and animals that had viral rebound after stopping ART, and we found that $CD8^+$ T cells from the post-treatment controllers showed reduced exhaustion. Exhausted T cells express specific markers, so we can measure the frequency of cells expressing these different markers to evaluate the levels of immune exhaustion at different times throughout the study (e.g., during ART treatment or after stopping ART). Since immune exhaustion means the immune system is less effective at suppressing virus replication, it makes sense that less exhausted immune cells would be more likely to contribute to post-treatment control.

To further understand why the original cohort of Mauritian cynomolgus macaques was predisposed to becoming post-treatment controllers, we also measured the size of the viral reservoir and found that Mauritian cynomolgus macaques form unusually small viral reservoirs compared to Indian-origin rhesus macaques. It stands to reason that having a smaller amount of virus in the reservoir also contributes to enabling post-treatment control. To bring back the hide-and-seek illustration, if you were an immune cell and you were seeking viruses hiding in the reservoir, it would logically be easier to keep track of fewer viruses. We don't know yet *why*

Mauritian cynomolgus macaques form smaller viral reservoirs, but that is one question we are trying to answer moving forward.

To summarize, we found three major factors that were associated with post-treatment control in Mauritian cynomolgus macaques: 1) the presence of $CD8^+$ T cells capable of suppressing virus replication, 2) a reduction in T cell exhaustion, and 3) small viral reservoirs. While the results of the initial vaccine strategy were not particularly exciting, what we actually discovered was a novel potential model of post-treatment control.

5.6 How do these results impact the HIV field moving forward?

We discovered that SIV^+ Mauritian cynomolgus macaques that were initiated on ART two weeks after SIV infection were predisposed to become post-treatment controllers. This is exciting because post-treatment control is the ideal outcome for HIV^+ individuals, but humans rarely become post-treatment controllers, and there was previously no known animal model of post-treatment control. But you might wonder how this could help HIV^+ individuals.

Studying post-treatment control in HIV^+ individuals is extremely challenging due to its rarity. Having a consistent and reproducible animal model of post-treatment control could greatly enhance our understanding of how it occurs and facilitate the development of therapeutic interventions to elicit it. By identifying the immune responses necessary for post-treatment control in Mauritian cynomolgus macaques, we could generate effective therapies for individuals living with HIV. A few immediate next steps to work toward this goal are:

1. We will repeat the process of infecting Mauritian cynomolgus macaques with SIV, initiating ART two weeks later, and then stopping ART to confirm that this is a reproducible animal model of post-treatment control.
2. We will also include a cohort of Indian-origin rhesus macaques in the study outlined in point one to directly compare Indian-origin rhesus macaques (who we do not expect to become post-treatment controllers) and Mauritian cynomolgus macaques. It would be valuable to have these side-by-side cohorts so we can answer questions like: “Why do Mauritian cynomolgus macaques become post-treatment controllers while Indian-origin rhesus macaques do not?”

3. On a more granular level, we also plan to evaluate different cell populations in the lab to mechanistically understand how and why Mauritian cynomolgus macaques maintain post-treatment control. Some of the questions we plan to answer are: a) *How* are the $CD8^+$ T cells suppressing virus replication? Are they directly killing infected target cells, or are they functioning by some other mechanism like preventing virus production from infected cells? b) Are Mauritian cynomolgus macaques' $CD4^+$ T cells somehow less susceptible to virus infection, and that is why these animals form smaller viral reservoirs?

This possible model of post-treatment control could have potentially significant impacts on public health and HIV treatment strategies. Moving forward, the ultimate goal of answering these questions is to be able to use this model to identify therapeutic targets for inducing durable HIV remission, ultimately leading to a functional HIV cure in humans. Stepping back even further, if a therapeutic intervention could be developed and scaled up to make HIV^+ individuals post-treatment controllers, this would alleviate many of the burdens (including health-related side effects and financial difficulties) of having to access daily lifelong ART.

Acknowledgment: All figures were created with BioRender.com