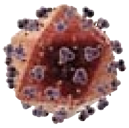

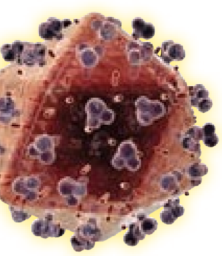

# CAN HIV BE CURED?

Eliminating HIV from the body would require flushing the virus out of its hiding places and preventing those reservoirs from being refilled. A tall order but perhaps not impossible

By Mario Stevenson

## KEY CONCEPTS

- Current drug regimens can dramatically suppress HIV in patients, but none of these agents can completely eliminate the virus.
- To eradicate HIV from infected individuals, researchers must figure out where the virus hides and how to hit it in those places.
- Recent findings have exposed some of HIV's refuges, suggesting new therapeutic targets.

*—The Editors*

In contrast to the failed attempts at developing a vaccine against HIV, efforts to provide drug therapies stand as a great success. More than 25 agents have been approved thus far, and the right combinations can suppress replication of the virus, often keeping blood levels so low as to be undetectable by standard tests. These powerful drug cocktails, collectively termed highly active antiretroviral therapy, or HAART, have prolonged life and health in countless infected individuals. Yet vexingly, today's treatments cannot actually cure the infection. If for any reason therapy is interrupted, the virus rapidly rebounds.

Figuring out how HIV manages to hang around in the company of these potent drugs is one of the most important tasks currently facing researchers. Over the past decade investigators have gleaned key insights into this mystery. The answers, we hope, will ultimately reveal whether complete eradication of the virus in a patient is feasible.

Understanding the nature of HIV's hiding places, or reservoirs, and what it will take to eradicate them requires some insight into how HIV typically behaves in the body. Like all viruses, HIV needs to get into the body's cells to replicate. There the invader exploits the cells' machinery to make copies of its own genome and to translate viral genes into proteins. It thus generates new viral copies, called virions, which spread to other cells. But unlike most human viruses, HIV actually inserts its genome into that of the cell. Every time the cell reproduces, the viral genes get copied and passed down to the daughter cells, thereby ensuring that the virus persists for as long as the cell and its progeny survive in the body.

The immune system typically manages to eliminate viruses by knocking out infected cells. It identifies such cells readily by the bits of viral proteins, or antigens, they display on their surface to flag the presence of interlopers within. In the case of HIV, the immune system has a hard time eradicating infected cells on its own in part because the virus attacks components of the immune system itself. The body does manage for a while to counterattack, generating healthy new immune cells able to recognize the virus and other infectious agents. In untreated individuals, however, the virus gains the upper hand over time, leading to AIDS.

Today's powerful drug combinations protect the immune system because they suppress HIV replication and limit the spread of virus to new cells. In theory, these treatments should permit

ZYGOTE MEDIA GROUP, INC. (*all 3-D computer renderings of HIV*)

LYING IN WAIT: Even after therapy forces HIV in the blood down to undetectable levels, the virus still lurks elsewhere—ready to storm back if given the chance.

MARK HOOPER (*human silhouette*); COURTESY OF MARIO STEVENSON (*author*)

the still healthy parts of the immune system to clear out any remaining infected cells and cure the disease. So why is the drug-protected immune system failing to do that job?

## Keeping a Low Profile

A big component of the answer appears to be the persistence of cells that are genetically able to make new virions but that do not produce any and thus do not inform the immune system of their presence. As David I. Watkins notes in "The Vaccine Search Goes On," starting on page 69, HIV preferentially infects immune cells called helper T lymphocytes, which mostly reside in the lymph nodes and connective tissue of the gastrointestinal tract but also occupy other lymph nodes and circulate in the blood.

In the course of fighting most kinds of viral infections, the bulk of helper T cells involved in the fight die off when they are no longer needed. A subset, however, survives as long-lived memory T cells, ready to multiply and call in the reserves when they encounter signs of reinfection. It is these memory T cells that appear to produce the most virus in HIV-infected patients. As they prepare to divide to fight remembered pathogens, they both duplicate their own DNA and proteins and churn out new HIV virions. Most of the infected memory cells die from the virus itself or the immune attack against them, but some return to a dormant state. At that point, HIV exists only as viral DNA sitting quietly in

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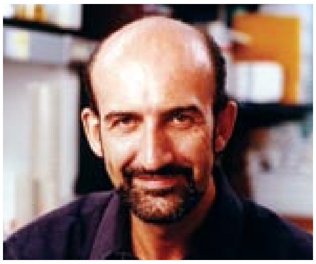

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the cells' genome. This viral DNA does not get copied and does not give rise to viral proteins, so no protein bits get displayed on the surface. Consequently, anti-HIV drugs have no effect on the cells, and the immune system remains blind to them.

This understanding has been informed by studies published in 1997. Teams led independently by Robert F. Siliciano of Johns Hopkins University, Anthony S. Fauci of the National Institutes of Health and Douglas D. Richman of the University of California, San Diego, found that inactive T lymphocytes isolated from HIV-infected individuals do not manufacture HIV. When those cells were roused, however, the previously dormant virus began replicating anew. HIV is not the only virus to exhibit such latency. An array of viruses can enter into similarly quiet states. In fact, some, such as the herpesviruses, make proteins that actually encourage the virus to become latent. Estimates based on the life span of memory T cells suggest it would take in excess of five decades for the reservoir of cells infected with latent HIV to naturally die out.

Researchers are also beginning to comprehend that it is not only latent helper T cells that bring HIV back after therapy stops. It seems that despite the absence of virus in the blood, some helper T cells and other cells keep on making new virus at a low level even when therapy seems to be working beautifully. This activity falls under the radar of tests, because the virus either hides successfully in the cells or, when released, stays trapped in tissues and does not find its way into the blood. In the past year, for instance, research has revealed that helper T lymphocytes in the gut get depleted within weeks of the individual contracting HIV and even before the virus is detected in the blood. It is therefore possible that during treatment the virus can continue to replicate in tissues such as those of the gut—activity that could go unnoticed for quite some time until the virus spills over into the blood.

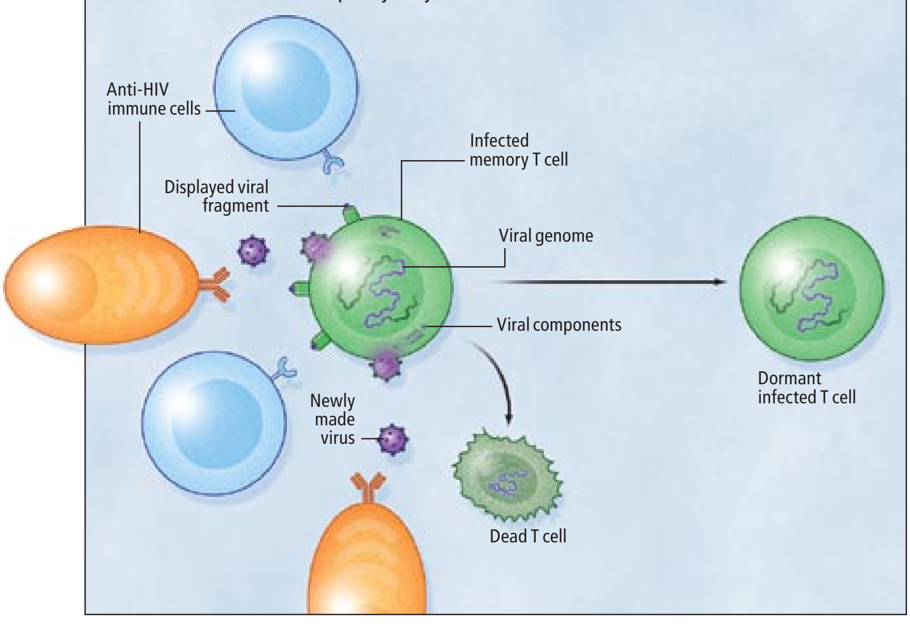
## FAST FACTS

- In 2007 an estimated **33 million** people worldwide were living with HIV.
- Every day some **6,000** people die from HIV and another **6,800** contract the virus.
- **Less than a third** of people who need HIV treatment have access to it.
- Highly active antiretroviral therapy (HAART) increases patient survival by **13.3 years** on average.

[OBSTACLE TO A CURE]

## THE MAKING OF A HIDEOUT

Most HIV in the blood appears to come from immune cells known as memory T lymphocytes that have been infected by the virus. These cells, which display bits of HIV on their surface, usually die from the infection itself or from an immune attack targeted to the displayed bits. But some survive and enter a dormant state (*far right*). In this condition they harbor the HIV genome in their DNA and can make new copies of the virus if reactivated but tend to sit quietly for years.

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### Another Unwitting Accomplice

Most AIDS research has focused on helper T cells because they circulate in the blood, which can easily be drawn for study. Recently, however, investigators have come to realize that other immune cells infected by HIV—macrophages and dendritic cells—may also contribute to resurgence of the virus after HIV therapy is halted or after the virus becomes resistant to it. Less is known about macrophages and dendritic cells because they are located strictly in tissues, but recent findings suggest that drug therapy may not totally stop HIV reproduction in these cells. The level may be too low to result in the virus reaching the blood in detectable amounts. It may, however, be high enough to reach nearby T lymphocytes and to continually restock the reservoir of dormant infected memory T cells. Also, some infected macrophages seem to evade being killed by the virus inside them or by other components of the immune system. Macrophages, then, may sit ready to pump up replication when drug therapy stops.

In 2001, for instance, Malcolm A. Martin of the NIH and his colleagues reported that although monkeys infected with simian immunodeficiency virus (SIV)—a close relative of HIV—lost most of their helper T lymphocytes within a few weeks of being infected, copious quantities of virus were still being produced. Macrophages, it turned out, were generating the virus. Subsequent treatment of the monkeys with a drug that inhibits viral replication—and thus prevents infection of new cells—failed to significantly lower the amount of virus in the animals' blood. This finding meant that the macrophages were not dying in the process of spewing out new copies of the virus.

HIV also seems to replicate somewhat differently in macrophages as compared with T cells—in a way that may be additionally advantageous to the virus. Whereas in T cells the virus components assemble close to, and subsequently detach from, the cell surface, in macrophages some viral particles appear to be deposited into compartments within the cells called vacuoles. Eventually the vacuoles may migrate up to the cell surface to release the stored virus particles. The packing of the virus into walled-off compartments might help HIV dodge immune detection by preventing the display of antigens on the cell surface that tip the immune system off to the presence of an intruder.

Finally, studies suggest that higher drug concentrations are needed to suppress viral replication in macrophages than in T cells. Exactly why this should be the case is uncertain. Yet we do know that some cellular proteins whose normal function is to excrete biological substances from the cell can interfere with drug therapy by hindering the uptake and retention of drugs. Perhaps, then, in macrophages these cellular proteins are particularly active and so prevent the drugs from being efficiently retained inside the cells. The same thing may occur in dendritic cells, although so far very little is known about how these cells respond to HIV.

## Anatomical Refuges

It is not only the inherent properties of helper T cells and macrophages that allow HIV to persist in the face of intensive therapy. Certain of these cells also sit in anatomical compartments that may shelter them from various drugs or immune defenses, or both. Ridding the body of HIV would necessitate reaching it in those places.

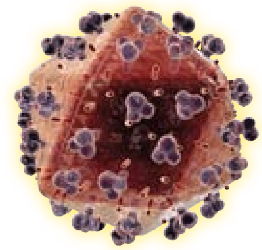
The central nervous system (CNS) is one such compartment. Researchers have long known that the CNS is susceptible to HIV infection. The neurological problems that arise in late-stage AIDS stem largely from the production of neurotoxins released from infected macrophages in the brain. To enter the brain, any molecule or cell must cross the blood-brain barrier, essentially a selectively permeable membrane that regulates the traffic of cells and other substances from the blood to the CNS. Macrophages that become infected with HIV in the tissues outside the CNS can apparently cross the blood-brain barrier and settle down in the CNS, where the virus may go on to infect specialized macrophages known as microglia, which reside permanently within the CNS.

Evidence suggests that infection of cells in the CNS would afford the virus some degree of protection from drugs because certain of them—notably protease inhibitors important to the proper processing of new viral proteins—do not efficiently cross the blood-brain barrier. Further, most other circulating immune cells stay out of the brain. No one knows whether infected cells in the brain can send HIV out to other parts of the body, but if the virus-infected macrophages can cross the blood-brain barrier into the CNS, they can probably filter back out as well.

Other sites that seem difficult for some drugs to penetrate include the walls of the gastrointestinal tract and the genital tract. Semen often contains HIV RNA even in people whose blood seems to be clear of the virus.

## New Plans of Attack

At a minimum, thoroughly clearing HIV from an infected individual would require removal of all latently infected T cells. One way that researchers are currently exploring to address

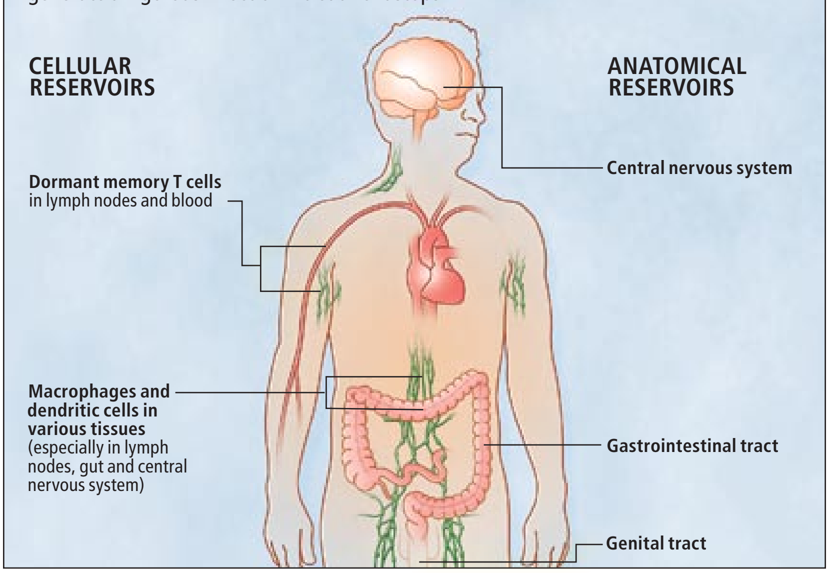


At a minimum, thoroughly clearing HIV from an infected individual would require removal of all latently infected T cells.

[WHERE THE VIRUS HIDES]

## HIV'S MANY RESERVOIRS

Beyond lying in wait in dormant memory T cells, HIV may reproduce at a low rate in certain other immune system cells—particularly macrophages and dendritic cells that seem inherently able to ward off immune defenses and anti-HIV drugs to some extent. Further, HIV-infected cells in a few parts of the body may be physically shielded to a degree from the immune system and certain drugs. HIV made in cellular and anatomical reservoirs does not reach the blood readily in aggressively treated patients but might generate a vigorous infection if treatment stops.

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the latent reservoirs is treating patients with compounds that stimulate dormant infected T lymphocytes to divide, in the hopes that the cells will make virus and thus become vulnerable to antiretroviral therapy. A couple of limited human trials have tested this approach using drugs previously approved to treat other conditions. They have yielded mixed results, however.

The ideal agents would tickle the T cells enough to rekindle the production of the viral proteins that get displayed on the cell surface but not so much as to trigger the cells to make new copies of the virus. To that end, researchers are currently exploring the potential of drugs that would induce the synthesis of HIV proteins by altering the organization of chromatin (complexes of DNA and protein that compose chromosomes) in dormant infected T cells. Yet even these so-called chromatin remodelers would be of limited use if they worked only in T cells and the virus were also present in macrophages.

A second prong of attack for clearing HIV from the body would involve blocking all viral replication, so that HIV disappears not only from the blood but from all tissues and from all cell types that harbor it. Drugs currently in use typically interfere with one of two enzymes: reverse transcriptase, which converts the virus's genetic material from RNA to DNA for insertion into the cellular genome, or protease, which helps nascent viral particles to mature. Within weeks after a person starts standard therapy, the level of virus in the individual's blood drops

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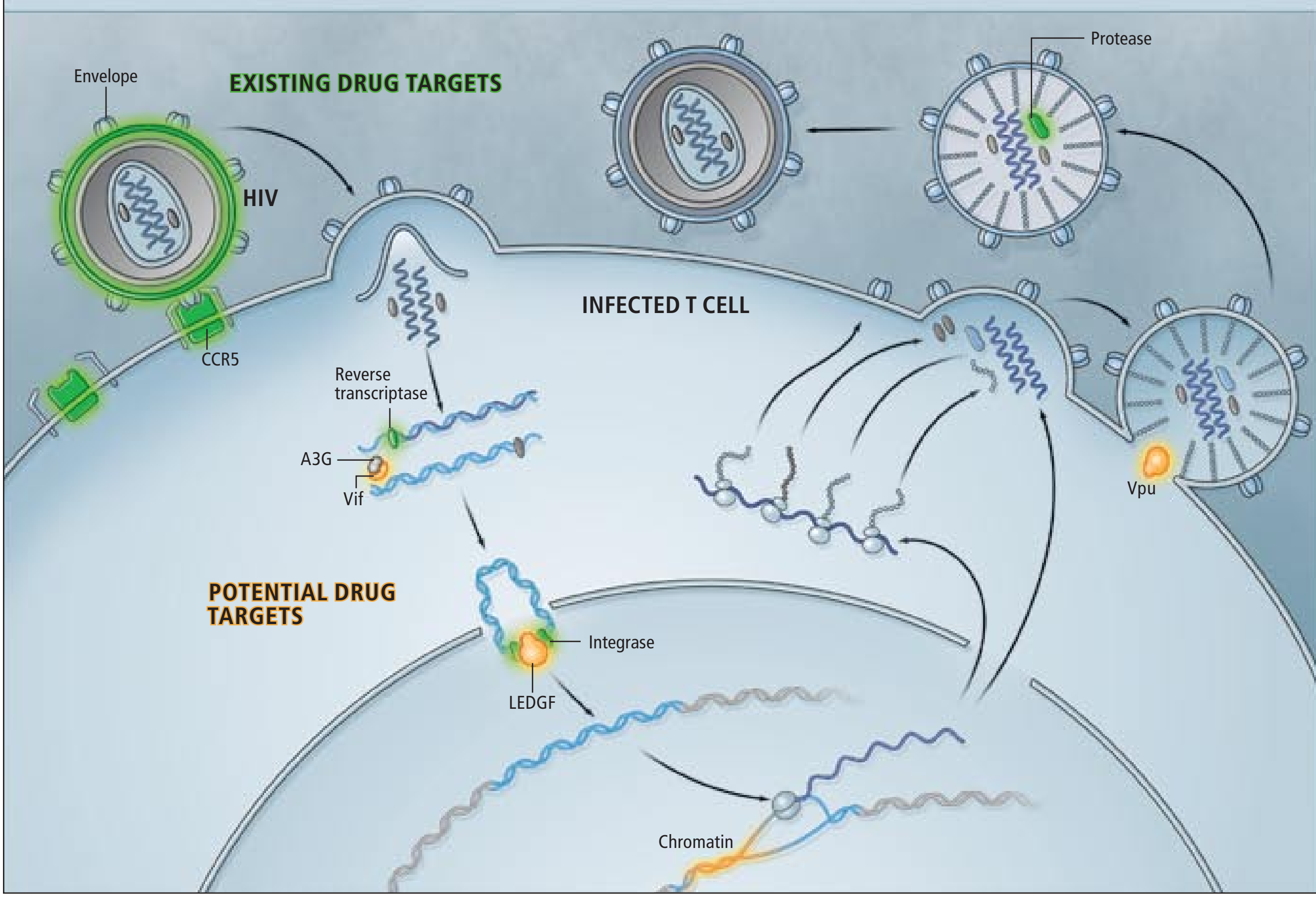
[NEW IDEAS]

## PROMISING TREATMENT APPROACHES

At a minimum, erasing HIV from the body would require inducing infected dormant T cells to make new virus or viral proteins—actions that would invite attack by drugs or the immune system. Such treatments would be given together with standard drugs that block cell-to-cell spread of the virus. New evidence suggests that intensifying control of HIV replication—by hitting new viral or cellular targets—could be helpful as well.

Some potential therapeutic targets for achieving these aims (*orange*) are described at the right. Drugs already on the market take aim at the virus's envelope protein and the T cell's CCR5 receptor (to block viral entry into cells) and try to inhibit HIV's reverse transcriptase, integrase and protease enzymes (to halt, respectively, the copying of HIV's genome, its insertion into the cells' DNA and the maturation of HIV proteins).



to undetectable levels. The slope of decay is fairly consistent from patient to patient, which researchers have taken to mean that the therapies thoroughly forestall viral replication. Yet recent studies have shown that intensifying existing drug regimens with raltegravir, a new drug that targets a viral enzyme not hit by earlier agents (the viral integrase enzyme, which stitches HIV DNA into the cells' own DNA), actually accelerates the viral decay. This success suggests that infected cells can probably be hit faster and more effectively than is now the case. If that surmise is correct, the work also implies that intensifying HIV therapy even further might limit the size of the original latent reservoir, block its later restocking and—dare we hope—lower replication so much that the immune system really can wipe out any virus-making reservoirs left over when latent infected memory cells are eliminated.

In the past year several new drugs that interfere with previously untargeted steps in viral replication have entered into clinical trials. In addition to the integrase inhibitor, another drug blocks infection by interfering with the ability of the virus to attach to a molecular receptor known as CCR5 that sits on the cell surface. Research also suggests that certain cellular proteins may be good therapeutic targets. Whereas HIV commandeers some of these proteins to aid its replication (CCR5, for example), it is now apparent that other cellular proteins—or cellular restrictions, as they are termed—actually antagonize viral replication.

Six years ago Michael H. Malim of King's College London and his research group identified the first of these cellular restrictions, called A3G. This protein is abundant in macrophages and in lymphocytes. Unfortunately, the virus has evolved a countermeasure to A3G: it makes a protein called Vif that induces the degradation of A3G. The good news is that both A3G and the viral Vif protein represent promising targets for therapy. Drugs that inhibit Vif or otherwise protect A3G from degradation would theoretically render human cells resistant to HIV infection.

Just this year Paul D. Bieniasz of the Aaron Diamond AIDS Research Center in New York City and John C. Guatelli of U.C.S.D. and their teams independently identified a second cellular restriction, named tetherin, that prevents the release of new copies of the virus from infected cells. The virus has evolved a defense against tetherin, too—in this case, the viral Vpu protein. Drugs that stymie Vpu could prevent HIV from spreading to new cells.

Basic research will probably continue to reveal novel therapeutic targets, which could lead to the development of new antiviral agents that hit HIV in multiple ways. If we can design drugs that complement and intensify the effects of existing therapies, we may finally be able to deplete the all-important latent reservoir and eradicate the virus. To that end, larger studies exploring the impact of long-term therapy intensification on the virus are currently under way, with results expected within the next two years. Those findings should tell us whether the eradication of HIV from an infected individual is a realistic goal. We wait with great anticipation. ■

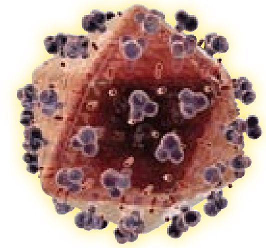
## POTENTIAL DRUG TARGETS

**Vif (viral infectivity factor)**
A cellular protein called A3G undercuts HIV's viability by dramatically mutating its genes. But HIV's Vif protein interferes. Inhibition of Vif or some other way of shielding A3G should allow A3G to carry out its antiviral tasks.

**LEDGF (lens epithelium-derived growth factor)**
In HIV-infected cells, LEDGF, a cellular protein, helps integrase to splice HIV DNA into the cell's genome. Some findings indicate that LEDGF inhibition reduces HIV replication.

**CHROMATIN (complexed DNA and protein that composes chromosomes)**
Drugs called chromatin remodelers would alter the organization of chromatin in dormant infected T cells in a way that activates synthesis of HIV proteins—a step that would render the cells visible to the immune system and susceptible to attack.

**Vpu (viral protein U)**
HIV-infected cells tether newly made virus to the surface, but HIV's Vpu protein sets it free. A Vpu inhibitor should keep the virus from spreading to other cells.



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## MORE TO EXPLORE

**Macrophages Are the Principal Reservoir and Sustain High Virus Loads in Rhesus Macaques after the Depletion of CD4+ T Cells by a Highly Pathogenic Simian Immunodeficiency Virus/HIV Type 1 Chimera (SHIV): Implications for HIV-1 Infections of Humans.** T. Igarashi et al. in *Proceedings of the National Academy of Sciences USA*, Vol. 98, No. 2, pages 658–663; January 16, 2001.

**Isolation of a Human Gene That Inhibits HIV-1 Infection and Is Suppressed by the Viral Vif Protein.** A. M. Sheehy et al. in *Nature*, Vol. 418, pages 646–650; August 8, 2002.

**Antiretroviral Therapy with the Integrase Inhibitor Raltegravir Alters Decay Kinetics of HIV, Significantly Reducing the Second Phase.** J. M. Murray et al. in *AIDS*, Vol. 21, No. 17, pages 2315–2321; November 12, 2007.

**Tetherin Inhibits Retrovirus Release and Is Antagonized by HIV-1 Vpu.** S.J.D. Neil et al. in *Nature*, Vol. 451, pages 425–430; January 24, 2008.