Cell

HIV-1 at 25

David D. Ho[1,*] and Paul D. Bieniasz[1]
[1]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY 10016, USA
*Correspondence: dho@adarc.org
DOI 10.1016/j.cell.2008.05.003

In 1981, the acquired immunodeficiency syndrome (AIDS) appeared insidiously and mystified doctors and scientists alike. No one could have predicted then that it would become, arguably, the worst plague in human history. Today, 33 million persons are living with infection by the human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, while another 25 million have already died of this disease.

Discovery

Early studies suggested that AIDS was a disease transmitted through sex or blood that led to the loss of $CD4^+$ T lymphocytes. Yet any description of a similar syndrome was nowhere to be found in the medical literature. AIDS was obviously new, and the race was on to find the pathogen responsible. Twenty-five years ago this month, Barré-Sinoussi et al. (1983) reported the detection of reverse transcriptase activity in a culture of lymph node cells taken from a patient with pre-AIDS syndrome. This finding, and the morphology of viral particles found in the culture and visualized by electron microscopy (Figure 1, inset A), suggested that the etiologic agent may be a retrovirus. A year later, Popovic et al. (1984) described the isolation, propagation, and characterization of a retrovirus from numerous AIDS patients. They also developed an immunoassay to show that AIDS cases had antibodies to this virus whereas healthy persons did not. Later studies would prove that the retroviruses identified by the two groups were one and the same, and that this new agent, subsequently named HIV-1, was unequivocally the cause of AIDS.

Characterization

HIV-1 has become not only the most studied virus in history but also a model system in virology as well as an important tool for probing the cellular processes manipulated by viruses. The HIV-1 life cycle is simple in concept but enormously complex in detail, in part because it is more elaborate than a typical retrovirus and possesses several auxiliary genes (Muesing et al., 1985). Years of study have only partly unraveled these details, but en route critical discoveries have enabled antiviral drug development, have illuminated AIDS pathogenesis, and have revealed new concepts in viral and cellular biology. For example, the early realization that HIV-1 binds to, and selectively infects, cells expressing CD4 (Figure 1) provided a satisfying explanation for why AIDS was characterized by a profound loss of $CD4^+$ T cells (Maddon et al., 1986). Moreover, the finding that CD4, while necessary, is insufficient to render a cell permissive for HIV-1 entry led to studies showing that the chemokine receptors CXCR4 or CCR5 were also required as coreceptors for viral entry (Feng et al., 1996).

As the study of HIV-1 has developed, it has become increasingly evident that the biology of HIV-1 is intimately linked with that of its host cell. This theme is exemplified by the unique ways in which the HIV-1 proteins Tat and Rev modulate viral gene expression. Tat recruits the major host RNA polymerase II C-terminal domain kinase to the 5′ end of the nascent viral RNA (Wei et al., 1998), thereby inducing polymerase phosphorylation and efficient elongation of the viral transcript (Figure 1). Conversely, the Rev protein binds to a second *cis*-acting viral RNA sequence and also contains the prototype CRM1-binding nuclear export signal (Fornerod et al., 1997). This links incompletely spliced viral transcripts that serve as mRNAs for several HIV-1 proteins to a host nuclear export pathway, thus enabling the expression of the complete repertoire of viral proteins. These mechanisms result in powerful regulation of viral gene expression and provide an elegant solution to the problem posed by the need to express nine proteins from numerous variably spliced viral mRNAs. Moreover, the discovery of the mechanisms by which Tat and Rev work provided major new insights into how both viral and cellular transcriptional elongation is regulated and how certain viral and cellular proteins and RNAs are moved from the nucleus to the cytoplasm.

Other instances of molecular parasitism by HIV-1 have been uncovered—a particularly striking example occurs during the release of virus particles. Normally, the host cell ESCRT proteins function on the cytoplasmic face of cellular membranes to mediate membrane fission during multivesicular body biogenesis and cytokinesis. By mimicking sequences of the host cell proteins that normally recruit the ESCRT complexes, HIV-1 diverts this machinery to sites of viral particle assembly to enable the topologically equivalent fission of cellular and nascent virion membranes (Garrus et al., 2001), leading to release of viral particles (Figure 1). Subversion of host functions is a recurring theme in HIV-1 replication, and as such there have been numerous reciprocal exchanges of insight between researchers working on HIV-1 and in other areas of molecular and cellular biology.

One of the more exciting recent developments in AIDS research comes from the realization that HIV-1 replicates in an intrinsically hostile environment. Evolution has endowed cells with factors that can directly inhibit retrovirus replication (Figure 1), presumably because retroviruses imparted recurrent selec-

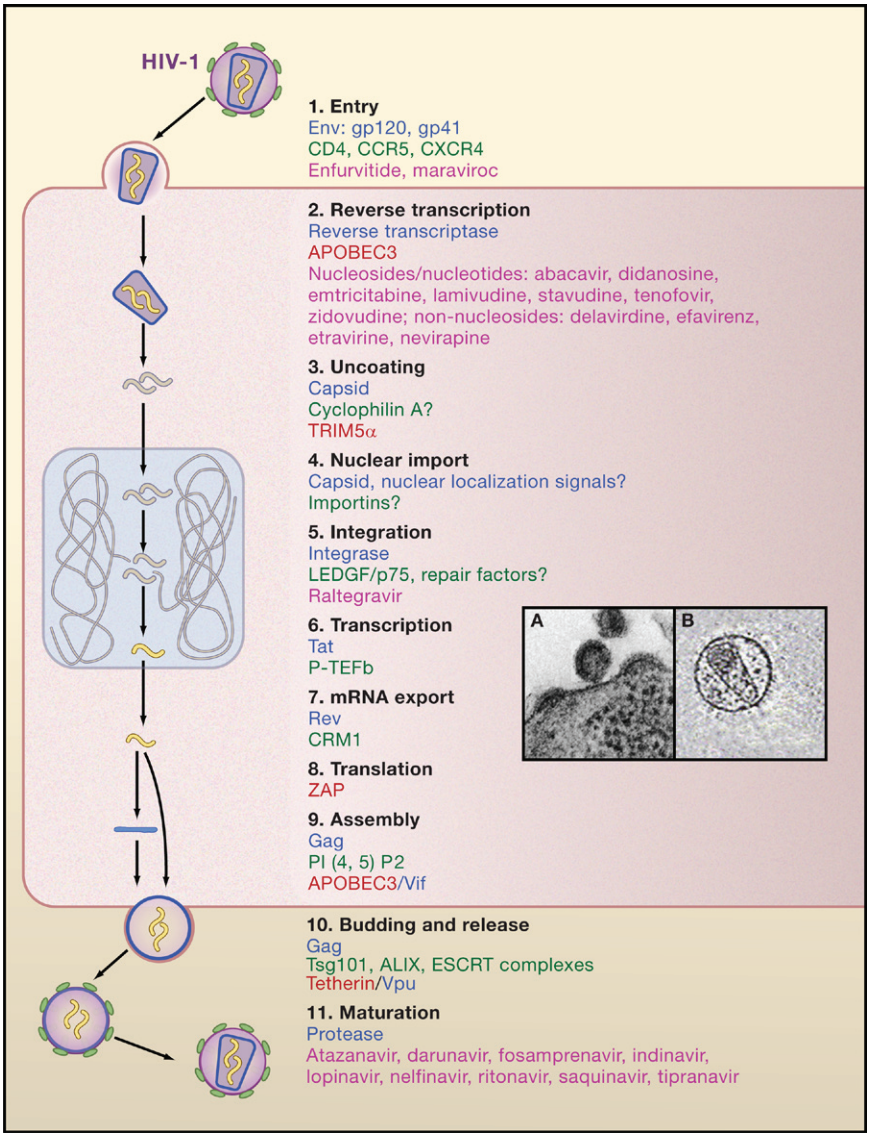

Figure 1. Key Steps in the HIV-1 Life Cycle

Viral proteins involved in each step are shown in blue, whereas cellular cofactors of host cells are shown in green. Host cell inhibitors of retrovirus replication are shown in red, and approved antiretroviral drugs targeting each step in the replication cycle are shown in pink. (Inset) Electron micrograph showing budding and immature HIV-1 particles (A), and a photomicrograph of a mature HIV-1 particle (B). (A, from Barré-Sinoussi et al., 1983; B, courtesy of J.A. Briggs and S.D. Fuller.)

tive pressures during primate evolution. Moreover, a role for some HIV-1 auxiliary gene products in counteracting host inhibitors has been uncovered. A case in point, APOBEC3 cytidine deaminase of the host cell infiltrates retroviral particles during assembly and induces massive, lethal hypermutation and destabilization of the viral genome during subsequent reverse transcription (Sheehy et al., 2002). Remarkably, the HIV-1 accessory protein Vif recruits APOBEC3 proteins to a destructive ubiquitin ligase complex, thereby denuding the cell of this critical antiretroviral defense. Additionally, an inducible inhibitor of HIV-1 particle release, termed tetherin, whose activity is neutralized by the HIV-1 accessory protein Vpu, has recently been discovered (Neil et al., 2008). Divergence in intrinsic antiretroviral molecules limits cross-species transmission of HIV-1 and other retroviruses; this characteristic has hampered the development of animal models of AIDS. Indeed, the inability of HIV-1 to infect most nonhuman primate cells is determined partly by species-specific variation in TRIM5α proteins that recognize and inactivate incoming retroviral capsids (Stremlau et al., 2004).

Our understanding of several aspects of HIV-1 replication has become highly evolved, and in some cases, structural and biochemical studies of HIV-1 proteins have provided atomic resolution detail. For example, high-resolution imaging and structural studies of HIV-1 Gag have revealed how viral particles assemble, and how the conical core in mature virions is formed (Figure 1, inset B). Structural and biochemical studies of the viral proteins reverse transcriptase, protease, integrase, and envelope have facilitated the development of antiretroviral drugs and provided mechanistic explanations for how certain viral mutations confer drug resistance. Nonetheless, despite the highly detailed understanding of some aspects of HIV-1 biology that have accrued, much remains to be learned. For example, our understanding of the events that occur during and between viral entry into the host cell and integration of the viral DNA into the host cell genome remains somewhat rudimentary. In addition, the precise role of HIV-1 Vpr and Nef continues to be elusive, and structures of viral and relevant host proteins remain to be solved. These are major gaps that need to be filled, if a complete understanding of HIV-1 replication is to be achieved.

Pathogenesis

Much is known about HIV-1 pathogenesis in vivo. There is an acute burst of virus replication 2–3 weeks after transmission, when the infected person typically suffers a flu-like illness. As this acute syndrome resolves spontaneously, concurrent with the onset of specific immune responses, the level of viremia is partially down-modulated, reaching a steady state or setpoint within a few months. Variable setpoints are observed among infected individuals, which determine the long-term prognosis of the infected person (Mellors et al., 1996).

The HIV-1 setpoint is maintained by a dynamic equilibrium between virus production and virus clearance (Wei et al., 1995; Ho et al., 1995). Half of the virus

particles in blood are cleared within ~30 min, whereas half of the productively infected T cells die in ~0.7 days. To maintain the steady state, these parameters must be equally matched by newly produced virions and newly infected T cells. Such findings reveal extraordinary levels of HIV-1 replication in vivo that are continuous not only for days to months but for years to decades. Therefore, it is not surprising that such a remarkable level of virus replication has serious consequences for the infected person. One adverse outcome is the loss of $CD4^+$ T cells over time because virus replication is tightly coupled to a lymphocyte turnover rate that is heightened several-fold (Mohri et al., 2001). Both $CD4^+$ and $CD8^+$ T cell populations are activated, and the magnitude of this activation is proportional to the viral load in plasma. It should also be noted that most of the T cells are turning over as the result of a generalized lymphocyte activation (that is, apoptosis that follows cellular proliferation) and not as the sequela of direct HIV-1 infection.

Another outcome of HIV-1 dynamics is the plasticity of viral sequences. High replication rates, in conjunction with the high error rate of reverse transcription, result in the creation of a massive number of new viral variants each day. Consequently, swarms of diverse yet related viruses, known as quasispecies, appear within each infected person, and distinct HIV-1 subtypes or clades emerge within the global pandemic. This unprecedented degree of viral heterogeneity poses significant problems for immune recognition, antiretroviral therapy, and vaccine development.

Treatment

Our accrued understanding of HIV-1 replication has led to the development of 25 approved antiretroviral drugs (Figure 1) and 5 fixed-dose combinations. Shortly after the discovery of the virus, a number of nucleoside analogs, previously developed for cancer or antibacterial chemotherapy, were screened for inhibitory activity against HIV-1 in vitro. Zidovudine became, in 1987, the first to be approved as an AIDS drug (Figure 1). Didanosine and several more followed, including a nucleotide analog, tenofovir. Essentially, the phosphorylated forms of these drugs serve to terminate DNA elongation during reverse transcription. Beginning in the late 1980s, a class of non-nucleoside inhibitors of reverse transcriptase emerged (Figure 1). Although structurally different, each of these compounds binds to a common site on the reverse transcriptase to block its activity. By the early 1990s, and guided by a crystal structure, potent inhibitors of the HIV-1 protease began to appear. Such small molecules insert into the catalytic site and block polypeptide processing, thereby preventing virion maturation (Figure 1). Nevertheless, prior to 1995, all of the available protease and reverse transcriptase inhibitors were used individually or as dual-therapy, with only limited benefit to patients.

Antiretroviral therapy took on new life after the unraveling of HIV-1 dynamics (Wei et al., 1995; Ho et al., 1995). This knowledge led to calculations that showed the rate of formation and accumulation of mutations was so great that monotherapy and dual-therapy were doomed to fail because of the rapid emergence of drug resistance. On the other hand, the math also predicted that it was highly improbable ($<10^{-8}$ per day) for HIV-1 to mutate in $\geq$3 positions, simultaneously, in a single genome. This realization led clinical investigators to pursue the use of three or more drugs in combination, starting in early 1995. By the time of the International AIDS Conference in Vancouver in the summer of 1996, it was clear that such combination antiretroviral therapy resulted in durable control of HIV-1 replication (Perelson et al., 1997). Viremia was reduced to below the level of detection for more than a year, and significant immunological and clinical recovery was observed.

Thus, 1996 marked a turning point in the AIDS pandemic. AIDS-associated mortality has since dropped by 80%–90% in the US and Europe and, conservatively, more than 3 million person-years of life have been saved. HIV-1 infection is no longer an automatic death sentence; it is now a manageable disease. The therapeutic arsenal continues to improve with the advent of new reverse transcriptase and protease inhibitors, as well as two new classes of drugs targeting viral entry and integration (Figure 1). This remarkable success story has become the model for drug development against other viral pathogens.

Several daunting challenges in antiretroviral therapy remain, however. Although current combination therapy allows viral replication to be controlled, HIV-1 is not eradicated. It persists latently in resting memory $CD4^+$ lymphocytes, and a cure is not possible until this reservoir is purged. In addition, there is a larger global problem of social injustice that must be addressed. About 90% of the infected population reside in developing countries where antiretroviral drugs are generally not available. Nascent efforts are now underway, led by the United Nations and the US government, to deliver treatment to poor regions of the world. But much more is needed and, sadly, apathy is a major obstacle to many more lives being saved.

Prevention

The HIV-1 epidemic continues to spread at the alarming rate of 2.5 million new cases per year despite educational efforts worldwide to modify behaviors at risk for infection. The use of topical microbicides to block viral transmission has been met with repeated disappointments. Most distressing, however, has been the inability of the scientific community to come up with a preventive vaccine. Approaches using whole inactivated virus or envelope subunit protein have failed. Live attenuated forms of the simian immunodeficiency virus (SIV) have protected monkeys from a genetically matched SIV challenge (Daniel et al., 1992), but such an approach in humans is generally deemed infeasible for safety reasons. Recently, a recombinant adenoviral vector vaccine, designed to elicit cell-mediated immunity to the viral Gag, Pol, and Nef proteins, has also failed to demonstrate any hint of protection. HIV-1 vaccine development has now stalled and is desperately seeking new directions.

In retrospect, it is apparent that the field rushed forward with vaccine development, bypassing a number of warning signs. It is well known that HIV-1 is rarely controlled by immune responses during the natural course of infection, an observation that does not bode well for a protective vaccine. Likewise, patients with superinfection have been well documented, showing that immu-

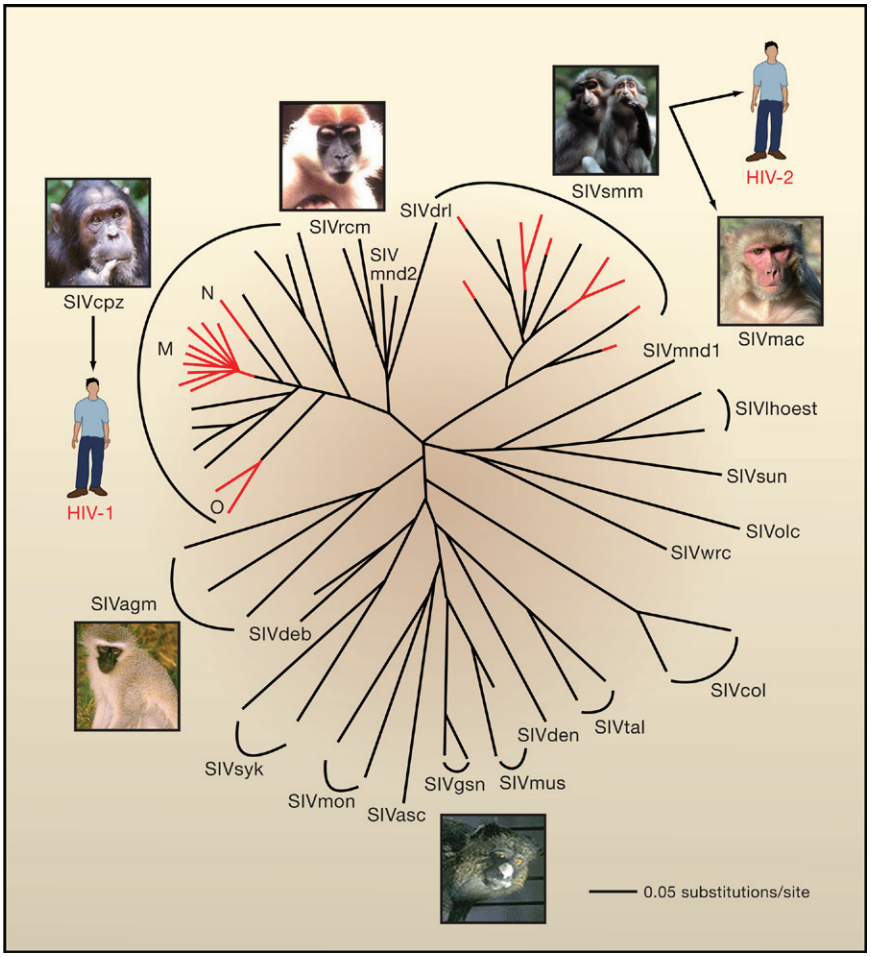

Figure 2. Relationship of Primate Immunodeficiency Retroviruses
Shown are the phylogenetic relationships of primate immunodeficiency retroviruses (black lines), including HIV-1 and HIV-2 in humans (red lines). The precursor of HIV-1 is a virus found in chimpanzees, SIVcpz, which is in turn the product of a recombination between SIVrcm (in red-capped mangabeys) and SIVgsn (in greater spot-nosed monkeys). (Phylogenetic relationships are based on a figure by M. Worobey; photos of nonhuman primates are courtesy of B. Hahn.)

nity generated against one viral strain is often insufficient to block the transmission of another. Lastly, it has long been appreciated that HIV-1 is relatively resistant to antibody neutralization, the basis of nearly all successful viral vaccines to date. Notwithstanding the tremendous gains in understanding the structure of the viral envelope glycoprotein (Kwong et al., 1998), the scientific community has struggled to come up with any antigen design that will consistently raise antibodies capable of penetrating the protective shield on the envelope glycoprotein created by variable loops, extensive glycosylation, and entropic forces. A protective HIV-1 vaccine will likely remain elusive until this fundamental problem is solved.

In the meantime, it should be kept in mind that HIV-1/AIDS is completely preventable. For example, the regular use of a condom during sexual intercourse would go a long way to stem the spread of this epidemic. Redoubling of a worldwide effort to disseminate risk-reduction messages is absolutely essential.

Origin

It would serve us well to understand the origin of this devastating epidemic. HIV-1 belongs to a large family of primate retroviruses naturally found only in African nonhuman primates (Figure 2). The pandemic strains of HIV-1 (group M) are, in all likelihood, derived from a single transmission event from a chimpanzee carrying SIVcpz to one human, probably in the vicinity of southern Cameroon (Keele et al., 2006). Molecular dating studies suggest that such a zoonosis took place some 60 to 80 years ago (Korber et al., 2000). One could only speculate what led to the initial chimp-human transmission, and how its spread then became explosive decades later in Africa and beyond. Yet this example of zoonotic transmission is not alone among primate immunodeficiency viruses. Strains of HIV-1 representing independent cross-species transmission events (groups N and O) are found in Africans living in proximity to chimpanzees harboring highly related SIVcpz (Figure 2). Moreover, HIV-2 in West Africans clearly traces its origins to multiple cross-species transmissions of SIVsmm from sooty mangabeys. Likewise, accidental transmissions of SIVsmm from sooty mangabeys to Asian macaques (SIVmac) in captivity resulted in infection and disease in the new host (Figure 2). One common theme has become evident: SIV in its natural host is typically nonpathogenic (for example, SIVsmm in sooty mangabeys, SIVagm in African green monkeys, and SIVcpz in chimpanzees), whereas transmission to a new host results in the virus becoming pathogenic (HIV-1 and HIV-2 in humans, SIVmac in macaques). Interestingly, the nonpathogenicity in the natural host is associated with a high efficiency of virus replication that is decoupled, for some unknown reason, from the generalized lymphocyte activation that characterizes pathogenic infections in humans or macaques. Herein lies a clue to the secret of AIDS pathogenesis, perhaps.

Conclusion

The AIDS pandemic has presented unprecedented scientific, medical, and moral challenges to humanity. The 25 years since the discovery of HIV-1 have been characterized by remarkable scientific discoveries, as well as dramatic successes and failures in translating science into effective interventions. Overall, significant advances have been made toward effectively tackling this modern-day scourge. However, it is sobering to note that while the AIDS death toll is already staggering, the worst of this epidemic may lie ahead.

REFERENCES

Barré-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., et al. (1983). Science *220*, 868–871.

Daniel, M.D., Kirchhoff, F., Czajak, S.C., Sehgal, P.K., and Desrosiers, R.C. (1992). Science *258*, 1938–1941.

Feng, Y., Broder, C.C., Kennedy, P.E., and Berger, E.A. (1996). Science *272*, 872–877.

Fornerod, M., Ohno, M., Yoshida, M., and Mattaj, I.W. (1997). Cell *90*, 1051–1060.

Garrus, J.E., von Schwedler, U.K., Pornillos, O.W., Morham, S.G., Zavitz, K.H., Wang, H.E., Wettstein, D.A., Stray, K.M., Côté, M., Rich, R.L., et al. (2001). Cell *107*, 55–65.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., and Markowitz, M. (1995). Nature *373*, 123–126.

Keele, B.F., van Heuverswyn, F., Li, Y., Bailes, E., Chen, Y., Takehisa, J., Santiago, M.L., Bibollet-Ruche, F., Liegeois, F., Loul, S., et al. (2006). Science *313*, 523–526.

Korber, B., Muldoon, M., Theiler, J., Gao, F., Hahn, B.H., Wolinsky, S., and Battacharya, T. (2000). Science *288*, 1789–1796.

Kwong, P.D., Wyatt, R., Robinson, J., Sweet, R.W., Sodroski, J., and Hendrickson, W.A. (1998). Nature *393*, 648–659.

Maddon, P.J., Dalgleish, A.G., McDougal, J.S., Clapham, P.R., Weiss, R.A., and Axel, R. (1986). Cell *47*, 333–348.

Mellors, J.W., Rinaldo, C.R., Gupta, P., White, R.M., Todd, J.A., and Kingsley, L.A. (1996). Science *272*, 1167–1170.

Mohri, H., Perelson, A.S., Tung, K., Ribeiro, R.M., Ramratnam, B., Markowitz, M., Kost, R., Hurley, A., Weinberger, L., Cesar, D., et al. (2001). J. Exp. Med. *194*, 1277–1287.

Muesing, M.A., Smith, D.H., Cabradilla, C.D., Benton, C.V., Lasky, L.A., and Capon, D.J. (1985). Nature *313*, 450–458.

Neil, S.J., Zang, T., and Bieniasz, P.D. (2008). Nature *451*, 425–430.

Perelson, A.S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., Markowitz, M., and Ho, D.D. (1997). Nature *387*, 188–191.

Popovic, M., Sarngadharan, M.G., Read, E., and Gallo, R.C. (1984). Science *224*, 497–500.

Sheehy, A.M., Gaddis, N.C., Choi, J.D., and Malim, M.H. (2002). Nature *418*, 646–650.

Stremlau, M., Owens, C.M., Perron, M.J., Kiessling, M., Autissier, P., and Sodroski, J. (2004). Nature *427*, 848–853.

Wei, X., Ghosh, S.K., Taylor, M.E., Johnson, V.A., Emini, E.A., Deutsch, P., Lifson, J.D., Bonhoeffer, S., Nowak, M.A., Hahn, B.H., et al. (1995). Nature *373*, 117–122.

Wei, P., Garber, M.E., Fang, S.M., Fischer, W.H., and Jones, K.A. (1998). Cell *92*, 451–462.