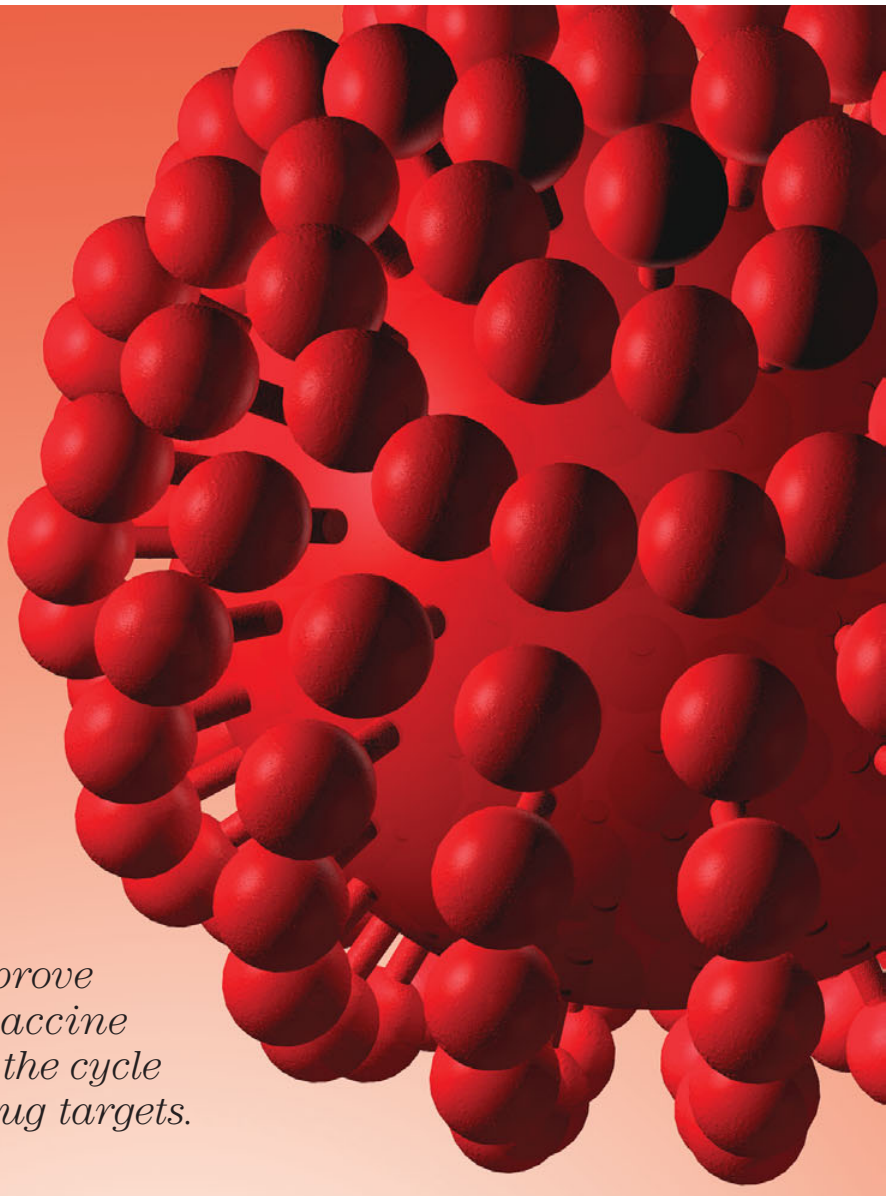


HIV—The BIOGRAPHY of a KILLER

As simple measures of attack prove inadequate and hopes for a vaccine diminish, researchers focus on the cycle of the whole disease for new drug targets.

BY MARK S. LESNEY

Perhaps the greatest hope of eventually conquering the AIDS plague is the power of modern molecular biology to spy into every facet of the HIV life cycle, including each response of the human body to invasion. On the virus side, the tools of biotechnology have been harnessed to sequence the viral genome and the amino acids of the relatively few proteins it produces. Topological mapping and functional analysis of these proteins have also proceeded apace, coupled to determining their interactive role with human cells to complete the viral replication process.

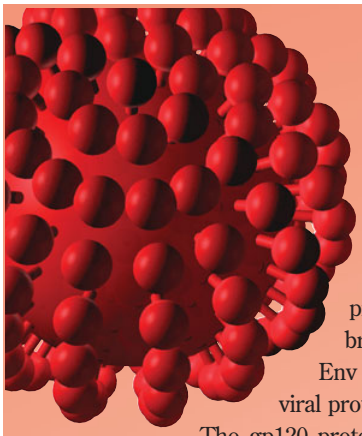
HIV is a member of the retrovirus family. Its genome consists of two copies of a single-stranded RNA (making it diploid) and several replicative and accessory proteins within a lipoprotein shell designed for efficient infection of specific cell types (see Figure 1). Upon infection, the viral RNA replicates itself through the use of a viral reverse transcriptase that creates the equivalent of a cDNA copy of the virus. This copy integrates into human chromosomal DNA, where it operates as a provirus. In the human nucleus, the integrated virus transcribes multiple copies of its own genome and provides messenger RNA to produce the necessary viral proteins to complete the replication process. Ultimately, this results in new, completely packaged virus particles that can bud free of the infected cell to seek out fresh victims.

Because the human immune system is essentially incapable of dealing with HIV, the development of therapeutic drugs and vaccines, coupled to efficient prophylaxis (whether through abstinence, barriers such as condoms or dental dams, or, perhaps one day, a functional vaccine), seem the only means possible to save literally tens of millions of lives in the coming decades. Since the early 1980s, AIDS has killed 25 million people. Currently, 40 million individuals live with HIV, most of them in sub-Saharan Africa (see box, "The widening chasm").

Infection

Everyone knows that HIV is transmitted sexually except for a few cases caused by tainted blood products and laboratory accidents. It enters the vagina or rectum through contaminated semen, or the penis through blood or vaginal or rectal secretions. Virus from an infected pregnant woman can also infect the fetus. As the virus is

ILLUSTRATION: TONY FERNANDEZ



present in human milk, breast feeding of newborns is also a mode of transmission.

HIV infects its target T cells by a process of receptor interaction and membrane fusion. The HIV *env* gene codes for the Env protein (gp160), which is cleaved by the viral protease to form the gp120 and gp41 proteins. The gp120 protein on the viral surface binds to the CD4 receptor on the surface of the host immune cells. Efficient binding is also mediated through interaction with the surface chemokine receptors CXCR4 and CCR5. Upon close binding by the gp120, the gp41 protein mediates fusion of the viral and cellular membranes.

The infection process is complex. Researchers have found, for example, that women can be simultaneously infected by two different strains of HIV, one in the vaginal mucosal cells, the other in blood plasma. Significantly, mucosal infection appears to use a different route than that of T cells, one that does not involve the much studied host receptor, CD4. Evidence shows that dendritic cells can participate in the development of infection involving a host protein called DC-SIGN, which also interacts with gp120. For these reasons, gp120 has been one of the most significant candidates for developing an AIDS vaccine.

Gag and Gag–Pol

The viral genome is a model of economic packaging. Rather than producing only one protein from a single gene, the virus uses complex processing—both in the production and cutting of the mRNAs produced and in the production and cleaving of the final polypeptides—to generate multiple proteins that can multitask in the host cell. The most prominent example of this is in the production of the Gag and Gag–Pol fusion proteins.

The *gag* gene codes for HIV's structural proteins, and *pol* codes for three enzymes crucial for replication. The *gag* gene gives rise to a 55-kDa precursor protein (p55) that associates with cellular membranes when it is time for the virus to escape the host cell. There, it induces the coming together of the genomic RNA and other viral and cellular proteins needed to trigger budding of the nascent virus particle. After budding, p55 is cleaved by the viral protease encoded by *pol* into the capsid protein (p24), matrix protein (p17), and nucleocapsid protein (p9).

The capsid protein forms the conical core of the virus particles. The matrix protein, upon infection, helps transport the viral DNA (produced through reverse transcription) into the nucleus. The nucleocapsid protein helps with RNA packaging in the completed virion and assists in subsequent reverse transcription upon infection (Figure 1).

The Gag–Pol fusion protein (p160) is produced by a ribosomal frame-shifting event (occurring roughly 5% of the time) that causes a jump over the stop signal for the Gag protein and continues into the *pol* gene region. The viral protease cleaves the Gag and Pol polypeptides apart. Subsequent cleavages produce new protease (p10), an RNAse H (p15), the reverse transcriptase (p50), and the viral integrase (p31) from the initial Pol polypeptide.

It is obvious from the life-cycle components that the viral protease (which is an aspartyl protease) is a unique and critical function in HIV replication. Analysis of this protease has provided one of the most significant therapeutic targets leading to the so-called protease inhibitors, which have helped inaugurate the HAART (highly active antiretroviral therapy) era of AIDS therapy in the developed world.

In October 2001, the National Institute of Allergy and Infectious Diseases began Phase I clinical trials of its first vaccine, which contains DNA for the *gag* and *pol* genes. Gag and Pol are considered good candidates for developing AIDS vaccines because they are relatively constant across different virus strains and account for a large percentage of total virus protein.

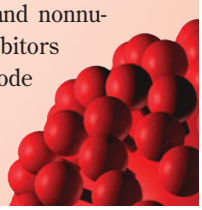
The widening chasm

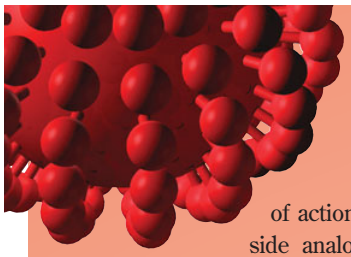
No disease has made more of a mockery of the concept of global community and the vacuous promises of modern medicine than has AIDS. The near-miraculous success of HAART therapy drugs in preventing AIDS-related deaths in the industrialized West has had essentially no comparable impact on the developing world—other than to set the stage for what looks likely to be a series of vicious patent disputes, which will put the earlier battles over AZT to shame. All of which may turn out to be moot if the phenomenal proliferation of HAART-resistant strains continues to develop, such that each new drug developed has an increasingly limited lifespan of effectiveness, even in individual patients.

Combined therapeutic vaccines and drug therapies are being proposed as a means of countering such resistance, but they look likely to require even more intensive (and costly) medication and doctor–patient interactions—making them even less apt to transfer to the developing world and to poorer populations in the developed world. These are the very groups who even now cannot get access to lifesaving antibiotics and fully effective vaccines for more traditional killers—despite the fact that these therapeutics have negligible prices compared with AIDS drugs. International programs, including collaborations between the pharmaceutical industry, governments, and nonprofit agencies, have been initiated to address this issue, including a commitment of more than $1 billion for research and treatment. But few consider these efforts adequate to the magnitude of the epidemic—which is greater in potential scope than that of the black plague of the late Middle Ages.

Reverse transcription

As mentioned, the viral reverse transcriptase (p50, also known as RT) is coded for by the *pol* gene. RT has both RNA- and DNA-dependent modes. RT makes a DNA copy of the viral RNA. The viral RNA is then digested away by the viral RNAse H, allowing synthesis of complementary DNA to the original strand. The newly double-stranded DNA can then integrate into the host genome. RT was the target of the first wave of anti-AIDS drugs and continues to be a key focus of drug discovery research. The RT inhibitor drugs are generally defined as nucleoside RT inhibitors (NRTIs) and nonnucleoside RT inhibitors (NNRTIs). The mode





of action of the NRTIs is fairly simple—these nucleoside analogues are phosphorylated in host cells, and when incorporated into the developing viral nucleic acids, they cause chain termination because of the lack of an appropriate 3′ end for extension.

Because the viral RT does not have proofreading machinery, a high accumulating mutation rate occurs during replicative cycles—up to several point mutations in each copy of the viral genome. This leads to rapid evolution and proliferation of different viral mutants—even in an infected individual—and is one of the chief sources of the pernicious drug resistance that seems to develop so rapidly in AIDS patients.

Worldwide, more than a dozen subtypes of the major (M) strain of HIV-1 are currently recognized, all of which evolved from the original transmission from chimpanzees to humans. Major subtypes are named alphabetically (A, B, C, . . .) and tend to be regionally located. For example, subtype B is most prevalent in North America, subtype C in South Africa and Asia.

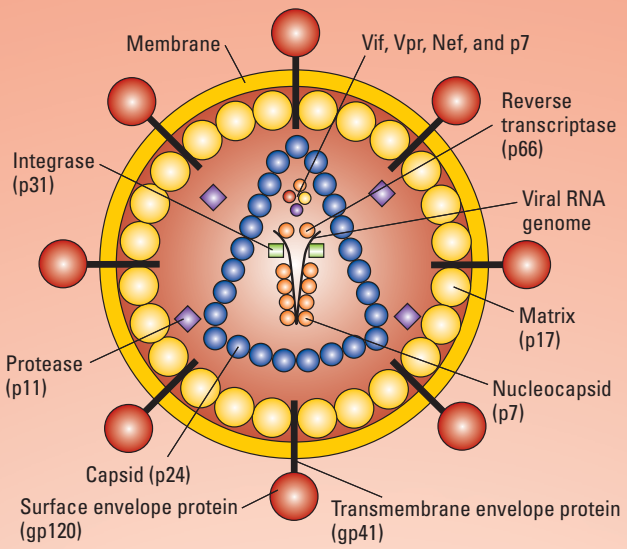
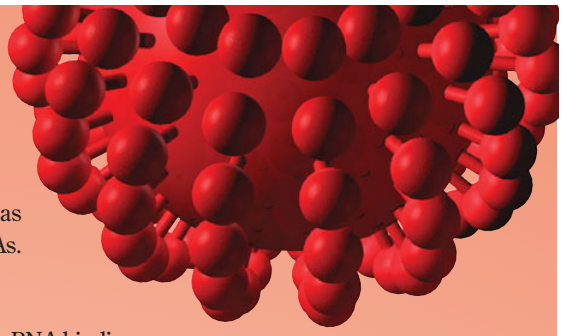


Figure 1. Portrait of a killer.

The existence of such geographical subtypes provides an added complication in humanity's current sexual "global village" because diploid RNA viruses can undergo a pseudomating process whereby related strains exchange material in a doubly infected individual through homologous recombination. Such recombinant strains (called circulating recombinant forms or CRFs) are often named after the parental "alphabetical" strains from which they are derived (e.g., CRF01-AE or CRF05-DF).

Although the process of multiple infections has always been assumed to take place (and was



one of the chief reasons for researchers arguing against the potentially deadly—and not uncommon—practice of HIV+ individuals having unprotected sex with one another), it was only recently that clinical evidence of this process leading to treatment implications in the patient was obtained (see ACRIA Forum Summary March 2002).

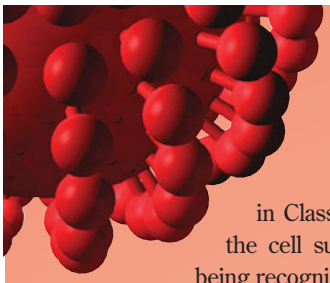
Integration

As a typical retrovirus, HIV integrates itself into the host chromosome by the use of a virally coded enzyme called integrase, which has multiple functions. It acts first as an exonuclease to trim the viral complementary DNA produced by RT to proper size, then as an endonuclease that cuts host DNA and facilitates insertion of the DNA copy of the virus. Finally, it acts as a ligase that fuses the host and viral DNAs into a seamless whole. Integration seems to occur in easily accessible regions that tend to be transcriptional "hotspots", thereby facilitating virus expression. Viral genes are efficiently transcribed only from this proviral DNA, making it a key target for antiviral discovery efforts. Recently, some drugs have been developed in an attempt to inhibit this integration step, and they are currently in clinical trials. The inserted DNA transcribes the mRNA required for producing the proteins needed for processing and incorporation into the final virus, as well as the viral genomic RNAs.

Regulation

The Rev protein is an RNA-binding protein that mediates the transition of the provirus from the production of early virus transcripts to those involved in the late stage required to produce functional virions. Remembering that the virus enters with the proteins necessary for initiating replication, the early genes turned on in the virus are *nef*, *rev*, and *tat*, which are Rev-independent. The mRNA from the later expressed genes, *env*, *gag*, *pol*, *vif*, *vpr*, and *vpu*, all require Rev to be expressed in the cytoplasm. Rev is also critical to the escape of the complete HIV-1 genomic RNA transcript from the nucleus. Normally, unprocessed mRNAs containing introns are not permitted egress to the cytoplasm. Rev acts as a chaperone and interacts with normal host proteins to permit transport.

The Nef protein, one of four so-called accessory proteins, acts in a dual fashion to regulate the behavior of the host cell in response to HIV infection. Nef increases the rate of CD4 endocytosis and degradation. Excess surface CD4 has been found to inhibit the later stages of virus budding from cells. Nef also can create a molecular disguise for HIV-infected cells by triggering a decrease



in Class I major histocompatability protein on the cell surface, thereby preventing them from being recognized and killed by cytotoxic T cells.

Three other HIV-1 accessory proteins are Vif (which appears to counter a cellular antiviral factor), Vpr (which facilitates HIV's ability to infect nondividing cells—rare for retroviruses), and Vpu (which helps increase the level of HIV released from the cell surface). Each of these proteins is an important virulence factor involved in producing and maintaining a successful infection in vivo.

Fighting evolution

The increasingly apparent molecular complexity of HIV—only barely scratched in this review—is a daunting challenge to researchers and anti-AIDS activists alike, even in the face of the near-miraculous HAART treatments. As shown, HIV has a high level of mutagenicity—which often leads to drug resistance. And it possesses the almost sinister ability to integrate into the host and kill off the very cells normally mobilized to fight it. These factors make hopes of easy long-term therapies seem slim—especially the promise of a uniformly effective vaccine.

But the counter to this seemingly hopeless fight against evolution is the fact that the very research that reveals such flaws in our attacks on the virus also gives an ever greater understanding of its most intimate life-cycle secrets and every nuance and possible drug target point in the development of the disease. Perhaps not only the virus is evolving rapidly, but also the technology to fight it. Only time will tell how many lives the ultimate answers will arrive in time to save.

Further reading

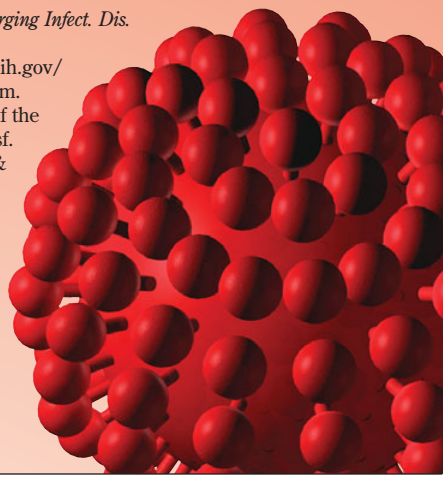
ACRIA (AIDS Community Research Initiative of America) Forum Summary March 2002; www.criany.org/treatment/treatment_edu_forum mar1302.html.

Burke, D. S. Recombination in HIV. *Emerging Infect. Dis.* **1997,** *3* (3), 253–259.

Recent vaccine research; www.niaid.nih.gov/newsroom/releases/vaccinemade.htm.

Structure, Expression, and Regulation of the HIV Genome; http://hivinsite.ucsf.edu/InSite.jsp?doc=kb-02-01-02&page=home-00-00.

The Hopkins HIV Report (bimonthly); www.hopkins-aids.edu/publications/report/report_toc.html.



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