

# **CALIFORNIA ASSOCIATION FOR MEDICAL LABORATORY TECHNOLOGY**

## **DISTANCE LEARNING COURSE**

### **HIV/AIDS Part II**

### **Life and Times of the Human Immunodeficiency Virus**

(Note: this course is designed to follow CAMLT Basic Level Course 968:

*An Introduction to HIV, HIV Infection, and AIDS*)

by

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**Course Number: DL-970**

**3.0 CE/Contact Hours**

**Level of Difficulty: Intermediate**

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# HIV/AIDS Part II—Life and Times of the Human Immunodeficiency Virus

By Elizabeth Crabb Breen, M.T. (A.S.C.P.), Ph.D.

## MEASURABLE OBJECTIVES:

Upon completion of this course, the reader should be able to:

- Identify the viral and cellular components involved in HIV binding and entry into potential host cells
- List three enzymes unique to the HIV life cycle that are targets for anti-viral drug therapy, and match each enzyme to the step of the life cycle at which it acts
- Identify at least three obstacles to effective HIV vaccine design
- Describe trends in reported AIDS cases in the U.S. before and after the introduction of HAART

Preface/Author’s Note: The community of persons living with HIV struggles constantly with the death of friends, while the HIV/AIDS research community rarely has to deal with the loss of one of its own. However, in 2000, Dr. Janis V. Giorgi, an incredibly dedicated and talented HIV/AIDS researcher at UCLA, lost her battle with cancer. This course is dedicated to her memory as a celebration of her life and times.

## FREQUENTLY USED ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
HIV	Human Immunodeficiency Virus
T <sub>H</sub>	T helper cell (CD4-positive T cell)
MO	monocyte/macrophage
RNA	ribonucleic acid
DNA	deoxyribonucleic acid
ssRNA	single-stranded RNA
dsDNA	double-stranded DNA
gp	glycoprotein
<i>vif</i>	viral infectivity factor (HIV molecule)
<i>nef</i>	negative effector molecule (HIV molecule)
RT	reverse transcriptase (HIV enzyme)
PR	protease (HIV enzyme)
IN	integrase (HIV enzyme)
MHC	major histocompatibility complex
NRTI	nucleoside reverse transcriptase inhibitor (a type of anti-retroviral drug)
NNRTI	non-nucleoside reverse transcriptase inhibitor (a type of anti-retroviral drug)
PI	protease inhibitor (a type of anti-retroviral drug)
HAART	highly-active anti-retroviral therapy; also known as combination therapy or “anti-HIV cocktail”
CDC	The Centers for Disease Control and Prevention

## **INTRODUCTION**

In a previous course on the topic of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS), several basic aspects of HIV, HIV infection, and AIDS were reviewed (1). These included an overview of the history of the HIV/AIDS epidemic, an introduction to the biology/virology of HIV, a discussion of HIV transmission (especially relating to occupational exposure), and descriptions of laboratory testing used to diagnose and evaluate persons along the continuum of HIV infection. In this intermediate-level course, the primary focus will be on HIV itself. A description of the viral structure and life cycle will provide the basis for discussions of anti-viral treatments and the challenges of HIV vaccine development. The course will conclude with a brief review of the state of the HIV/AIDS epidemic both in the U.S. and worldwide. The goal of the course is to provide clinical laboratory scientists, regardless of their area of expertise or specialization, with a more in-depth understanding and appreciation of the workings of HIV as the medical and scientific communities attempt to control its growth and combat its spread.

## **THE STRUCTURE AND LIFE CYCLE OF HIV**

Viruses are typically classified based on physical characteristics, such as the type of genetic material, which can be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), and size and/or shape. Viral classification can also utilize biological characteristics, such as distinguishing features of viral life cycles. When first isolated, however, the names initially given to viruses often reflect the interests and/or observations of the scientists who isolate them. The virus that is now known as HIV-1 was first isolated in late 1983 at the Pasteur Institute in Paris, France, and in early 1984 at the National Institutes of Health (NIH) in the U.S. (2, 3). It was originally given different names by these two laboratories, e.g., Lymphadenopathy-Associated Virus (LAV) and Human T-Lymphotropic Virus type III (HTLV-III), respectively. The consensus name of HIV was agreed upon in 1986, and when a second, closely-related virus was isolated in the same year, the original virus was designated HIV-1, and the newer virus HIV-2. For the remainder of this course, HIV will refer to HIV-1 unless otherwise noted.

The change of name from LAV/HTLV-III to HIV acknowledged an improved understanding of the clinical behavior of the virus. The new name recognized that: 1) the virus primarily infects and only causes disease in humans (as described below), and 2) the hallmark of disease caused by the virus was the development of immunodeficiency (1-4). HIV also provided a neutral alternative to the names associated with the two labs that first isolated the AIDS-associated virus and laid to rest competing claims to its discovery. For those readers interested in the early disputes over scientific (and financial) claims to HIV, the journal *Science* published many of the key scientific and editorial articles on this subject throughout the 1980s. As an historical footnote, it is interesting to note that Dr. Jonas Salk (the inventor of the “killed” polio vaccine, who was well-known for his very public disputes during the 1950s and 60s with Dr. Albert Sabin, the inventor of the “live” polio vaccine) played a very important role in resolving the disputes over HIV during the 1980s.

The first determinations of some of the physical characteristics of HIV, such as size and overall shape, were made by electron microscopy. Electron microscopy was necessary due to the small size of viruses, which cannot be seen using light microscopes. HIV particles were

observed to have an average diameter of 100 nanometers or 0.1 microns. (By comparison, an average small lymphocyte is 10 microns or one hundred times larger). It was also quickly determined that HIV carried RNA as its genetic material, contained within a cone-shaped viral core (Figure 1).

Further studies of the biological properties of HIV revealed that it was a member of a specialized subtype of RNA viruses known as retroviruses, which are capable of reverse transcription (1-6). Many primate species are infected with retroviruses, and HIV appears to be descended from a primate retrovirus that may have first entered the chimpanzee species, and then was introduced into humans (5). Based on genetic studies and mathematical modeling utilizing a range of HIV and other primate retrovirus isolates, it is estimated that HIV entered the human population in sub-Saharan Africa around 1930 (5). Primate retroviruses can often infect more than one species, but cause disease in only certain species. In the case of HIV, the virus can infect both humans and chimpanzees, but only causes immunodeficiency in humans.

Since its isolation more than twenty years ago, HIV, its genes and gene products, and its life cycle have been studied intensively, making it perhaps the best-characterized of all known human viruses (4-6). A detailed understanding of the structure and life cycle of HIV has provided the foundation needed for the development of effective drug treatments and rational vaccine design. A basic outline of the structure and life cycle will be provided here, followed by discussions of both drug and vaccine design.

There are several molecules found in an HIV particle (as shown in the schematic drawing in Figure 1) that play key roles in the viral life cycle (3-6). The first two to be called into play are the surface glycoproteins gp41 and gp120. The abbreviation “gp” is used for glycoproteins; proteins that are glycosylated (have sugars attached to them). When describing viral components, glycoproteins (gp) and proteins (p) are named according to their molecular weight. Gp41 and gp120 have molecular weights of 41 and 120 kilodaltons (kD), respectively. These two proteins are originally made as a single larger glycoprotein with a molecular weight of 160 kD (gp160). During the assembly of new HIV particles, gp160 is cleaved or cut into the two smaller products, which associate with each other. Gp41 and gp120 then become associated with the outer coat of the viral particle, which is a complex mix of lipids and proteins derived from the membrane of the host cell in which the particle was produced. Gp41 remains embedded in the outer coat (also known as the envelope) and serves as a tether for gp120, which extends outward from the surface of the virus. As will be described in more detail below, gp120 serves to bind HIV to a target cell, while gp41 facilitates the entry of the viral RNA into the cell.

The genetic material of HIV consists of two identical molecules of single-stranded RNA (ssRNA). It is these molecules that are detected in the test known as “HIV RNA viral load”, also known as “plasma viral load” or “HIV viral load”. Therefore, measurements of viral load are expressed in terms of the number of viral copies or viral equivalents of HIV per milliliter of plasma. Viral load quantifies the amount of HIV a person is carrying per milliliter of blood relative to the amount of viral RNA known to be present in one particle of HIV (see reference 1 for a review of HIV viral load measurements).

The HIV RNA molecules are contained within a viral core composed of a 24 kD protein (p24). The somewhat cone-shaped viral core, as shown in Figure 1, can actually be seen on an electron micrograph of HIV (2, 3). Each of the HIV RNA molecules has attached to it one

molecule of an enzyme known as *reverse transcriptase* (RT). This enzyme is not produced by any type of mammalian cell, but is absolutely required for the retroviral life cycle (Figure 2). Therefore, all retroviruses (including HIV) must carry the gene for their own RT enzyme into their host cell, and utilize the protein-producing machinery of the host to produce this essential molecule. The viral core of HIV (Figure 1) also contains two additional unique viral enzymes known as *protease* (PR) and *integrase* (IN). As with RT, these enzymes are not produced by any human cell and so must be produced from HIV's genes by the host cell, and be packaged inside each HIV particle, in order to ensure successful completion of the viral life cycle.

In order for HIV to establish an infection, it must first gain access to the tissues and/or bloodstream of a potential human host. As reviewed in the previous course on HIV/AIDS (1), HIV transmission occurs as a result of exposure to blood, semen, or vaginal fluid from an HIV-infected person, or from an HIV-infected mother to infant during pregnancy, birth, or via breast milk. Once it is inside the body, HIV can bind to cells that carry the CD4 molecule on their surface (3-6). The most likely targets are two types of white blood cells, CD4-positive T helper cells ( $T_H$  cells) and monocyte/macrophages (MO).  $T_H$  cells carry high numbers of CD4 molecules on the surface of each cell, while MO have a lower but detectable level of CD4 expression. When tested in the laboratory, some strains of HIV are more efficient at infecting  $T_H$  cells, while other strains are more efficient at infecting MO (the underlying mechanism for this cell preference or tropism is described below). Other possible target cells for HIV infection that express low levels of CD4 are a type of antigen-presenting cell known as a dendritic cell and macrophage-like cells in the brain known as glial cells.

The ability of HIV to bind to a CD4-expressing cell is due to the interaction of gp120 on the surface of an HIV particle with the CD4 molecule on a cell surface (Figure 2). This interaction is similar to the binding of antigen to antibody or an enzyme to its substrate. There is a deep cleft in gp120 into which a specific portion of the CD4 molecule fits, bringing the CD4-expressing cell into contact with the viral particle like a key fitting into a lock (5, 6). From the earliest days of the AIDS epidemic, CD4 was considered likely to be the HIV receptor, since it was CD4-expressing  $T_H$  cells that were lost in persons with AIDS (1-4). The role of CD4 as the cellular receptor was confirmed experimentally by artificially expressing CD4 on the surface of human cells that normally could not be infected with HIV in the laboratory, i.e., skin cells or B lymphocytes (2). When CD4 was expressed on these cells, they became susceptible to infection with HIV. If the CD4-expressing human cells were treated with antibodies that blocked interactions between CD4 and gp120, then infection by HIV was blocked. Surprisingly, in other experiments utilizing non-human cells, it was observed that expression of human CD4 failed to make cells susceptible to HIV infection. For example, mouse cells could not be infected with HIV, even if they artificially expressed human CD4. In other words, CD4 was required for HIV infection, but it could not enable infection on its own. This suggested that there was a second "co-receptor" molecule, found on human but not other mammalian cells, that was also required for successful infection by HIV.

The identification of a family of molecules on human cells that act as co-receptors for HIV infection has answered a number of questions about the process of HIV infection (3-6). These molecules, known as chemokine receptors, are naturally-occurring cell-surface proteins that normally bind to chemokines (proteins that stimulate cells to undergo chemotaxis or directed

cell movement). It is only in the presence of HIV that they become co-receptor molecules that facilitate viral infection. It became apparent that every mammalian species has its own unique family of chemokine receptors, but only the human chemokine receptors can act as co-receptors for HIV infection. Thus, the inability to render non-human cells susceptible to HIV infection by artificially expressing human CD4 was due to a lack of the appropriate human chemokine co-receptors.

The human chemokine receptor family is a large one, consisting of many different proteins that are similar in their size, structure and function, but differ in their amino acid sequences. Several different chemokine receptors have been shown to act as HIV co-receptors, but only certain ones are expressed on T cells, while others are expressed only on MO. It has turned out that strains of HIV that preferentially infect T cells can only bind to chemokine receptors expressed on T cells. The predominant chemokine receptor that facilitates T cell infection by HIV is known as CXCR4; viral strains that infect using this receptor are often referred to as “X4” viruses (5, 6). Conversely, HIV strains that infect MO can bind to chemokine receptors found on MO but not on T cells. The predominant chemokine receptor facilitating MO infection is CCR5; viral strains that infect MO using this receptor are referred to as “R5” viruses. Hence, the interaction between virus strains and co-receptors on host cells appears to dictate the type of cell that a particular virus will infect most efficiently. Most primary infections (immediately after entry of HIV into the body) are established via mucosal membrane or intravenous transmission in MO by R5 viruses, utilizing CD4 plus CCR5. Later in HIV disease, X4 viruses capable of spreading to T<sub>H</sub> cells via CD4 plus CXCR4 are more commonly found (6). With the identification of chemokine receptors as co-receptors for HIV infection, it was then possible to determine the role of the other HIV surface glycoprotein, gp 41, in the successful infection of target cells (as described below).

The **binding** of HIV to the target cell surface via the gp120-CD4 interaction (Figure 2, top left) has two purposes (5, 6). First, it facilitates the initial attachment of the virus to the surface of a potential host cell. Second, the binding of gp120 changes the shape of the CD4 molecule (shown as a cone-shaped molecule in Figure 2) on the target cell. This permits a subsequent interaction between gp120 and chemokine receptors immediately adjacent to the CD4 molecule on the cell surface (represented in Figure 2 as rounded molecules flanking CD4). This gp120/chemokine receptor interaction triggers the viral gp41, which is coiled like a spring, to extend itself like a harpoon towards the target cell. The tip of gp41 becomes imbedded in the target cell membrane, then recoils, drawing the membranes of the cell and the virus particle together. The two membranes undergo **fusion**, enabling the contents of the viral particle, i.e., two strands of viral ssRNA and their associated molecules, to be released into the cytoplasm of the cell. While the virus has now gained entry into the cell, it has not yet established an infection. The ability of the virus to successfully complete the next few steps in its life cycle will determine whether the cell will actually become infected.

HIV belongs to a special class of RNA-containing viruses known as retroviruses. This name refers to the next step in the viral life cycle, during which HIV undergoes a process known as **reverse transcription**. It is important to remember that human genes, which are composed of DNA, are transcribed into RNA (DNA⇒RNA) which in turn is translated into protein. In contrast, the genes of retroviruses are made of RNA which is transcribed into DNA

(RNA $\Rightarrow$ DNA) (1). Since this is the opposite direction of transcription compared to human genes, it is known as reverse transcription. The family of viruses that use this process were given the name retrovirus, derived from the Latin word *retrogress* meaning “backwards” (3).

For many parts of its life cycle, HIV depends on and parasitizes the machinery of its host cell to accomplish various processes. However, this is not possible for reverse transcription, as the host cell does not have the enzymes necessary to make DNA copies from RNA genetic material. Therefore, as mentioned above, HIV must provide its own RT to catalyze this process. Since each of the HIV viral RNA strands has a molecule of RT attached to it, they are poised to undergo reverse transcription once they have been released into the cytoplasm following fusion.

DNA molecules are usually double-stranded sequences (dsDNA) composed of four basic chemical building blocks (adenine [A], cytosine [C], thymine [T], and guanine [G]). The first step in reverse transcription is to make a single-stranded DNA (ssDNA) copy of the HIV ssRNA. In the cytoplasm of a host cell, RT catalyzes the assembly of A, T, C, and G bases (provided by the host cell and using the viral ssRNA as a template) into a complementary single strand of DNA (ssDNA). The original HIV ssRNA and the newly reverse-transcribed ssDNA form a double-stranded RNA/DNA hybrid molecule. The cell then recognizes the presence of this unstable hybrid molecule and takes over the rest of the process. The cellular machinery degrades the relatively fragile HIV RNA strand of the hybrid, and in its place, synthesizes a second complementary strand of DNA. This results in a stable dsDNA molecule that is a copy of the original HIV viral RNA. Just as the original HIV RNA had RT enzyme molecules attached to it, the DNA copy of HIV remains physically associated with some of the viral proteins that entered the cell inside the original viral particle. This dsDNA copy of HIV, known as a provirus, is now in the same form as the dsDNA that makes up the chromosomes of the host cell. The compatibility of the HIV provirus with the cellular chromosomes will permit the final step in the establishment of infection.

It was recently discovered that a closely-related group of normal human enzymes (known as the APOBEC family), which have the ability to modify host cell RNA and DNA, appear to act as natural anti-viral agents during retroviral infection (5, 7). It has been suggested that these enzymes act by mutating the HIV proviral DNA at the reverse transcription step, thereby preventing the formation of a functional dsDNA provirus. DNA-modifying enzymes have been found to be packaged inside HIV particles along with the virus' own RT, so that they are present and ready to act as soon as they are released into a new host cell along with the HIV RNA and RT. While this discovery was initially greeted with great excitement, this enthusiasm has been tempered by the even more recent observation that HIV appears to have evolved a means of dealing with this natural anti-retroviral activity. HIV produces a viral protein (*vif* or viral infectivity factor) that degrades the APOBEC enzymes within the host cell, thus preventing them from being incorporated into some, if not all, viral particles being produced by that cell. The success or failure of retroviral infection, therefore, may depend at least in part on the balance between viral proteins like *vif* and cellular proteins such as the APOBEC enzymes.

In order to permanently infect a cell, the HIV provirus must become part of the genetic material of the cell. This final step in infection requires that the provirus must first be transported from the cytoplasm to the nucleus of the cell. While the precise mechanism of provirus transport across the membrane that separates the cytoplasm from the nucleus is still

being defined, it appears that it is an active process mediated at least in part by the viral proteins associated with the provirus (6). It is estimated that the HIV provirus and its associated proteins make up a complex approximately the size of a ribosome, which is twice the size of the pores in the nuclear membrane. This ensures that, whatever the mechanism, entry into the nucleus “must involve considerable molecular gymnastics” (6).

Once the proviral DNA has gained access to the nucleus, it is then inserted into the dsDNA of the chromosomes. This process, known as **integration**, is facilitated by the unique HIV enzyme *integrase* (IN), which is one of the viral proteins carried along by the provirus into the nucleus. IN breaks the host chromosome, trims the ragged ends of the dsDNA HIV provirus left by the reverse transcription process, and then joins the loose ends of the provirus to the breaks in the chromosome (5, 6). In most HIV-infected cells, there are multiple proviruses present at different locations throughout the chromosomes. Once integration has occurred, the HIV provirus is now a part of the DNA that makes up the genome of the host cell. A cell containing one or more proviruses is considered to be infected, and therefore, has the potential to be infectious to other cells.

There are two ways in which HIV infection is maintained in and/or spread through the body. First, because of its ability to be reverse transcribed and permanently integrated into the genetic material of its host cell, HIV (like all retroviruses) can be passed on through many generations of cells simply by the normal process of cell division (Figure 2). Each time a cell divides, the DNA of the chromosomes is duplicated so that two complete sets of chromosomes can be divided between two daughter cells. If the parent cell contains an HIV provirus in its DNA, then the chromosomes passed onto its daughter cells also contain the provirus, giving rise to two new HIV-infected cells.

This kind of infection is a true latent infection, i.e., the virus is completely dormant. The proviral DNA quietly resides within the chromosomes, with no transcription of the viral genes contained in the provirus, and no production of viral proteins or new viral particles. HIV remains latent for the entire lifetime of an infected cell, *as long as that cell remains in a resting, non-activated state* (Figure 2, bottom right). Then, if the infected cell were to die without dividing again or becoming activated, the proviral DNA would be degraded and recycled along with other chromosomal DNA following the death of the cell. Since  $T_H$  cells live only a few days, and monocytes and macrophages live for weeks or months, if all HIV-infected cells remained resting, the number of HIV-infected cells would gradually decline, and in theory, could eventually disappear (4). However,  $T_H$  cells and MO are part of the immune system, and are designed to become activated if they encounter the proper stimulus (3). Therefore, during the course of normal day-to-day responses by the immune system unrelated to HIV infection, some fraction of T cells and MO are always becoming activated. Unfortunately, such activation disrupts the quiet, non-productive latent infection, and opens wide the door to the second way in which HIV infection is sustained and spread.

When a T cell or MO receives an external activation signal (Figure 2, bottom left), it begins to express cellular genes on its chromosomes that have previously been inactive. These genes are transcribed to produce messenger RNAs (mRNA), which are in turn translated into proteins that alter the behavior of the cell. Activated cells then divide and/or acquire new or enhanced functions such as the secretion of growth factors known as cytokines, or increases in

phagocytosis or intracellular killing. In a cell that contains one or more HIV proviruses, activation also results in viral **transcription**, the expression and transcription of the integrated DNA copies of the HIV viral genes. Taking advantage of the fact that the machinery of the cell has been mobilized in response to an activating stimulus, the HIV provirus is transcribed into more than a dozen HIV-specific mRNA molecules. The viral mRNAs are transported out of the nucleus into the cytoplasm, where they drive the **synthesis** of new viral proteins. At the same time, some of the transcription produces full-length ssRNA copies of the DNA provirus, providing the necessary genetic material for new HIV viral particles. One of the HIV proteins, known as the “negative effector” protein or *nef*, impacts the function of the infected cell in order to optimize viral replication (6). *Nef* decreases the expression of CD4 (as shown in Figure 2, activated cell at bottom left), as well as another critical immune system molecule, major histocompatibility complex (MHC) class I, which reduces the ability of the infected cell to be detected and destroyed by other cells of the immune system. *Nef* also blocks the normal pathway to cell death (known as apoptosis), thereby prolonging the life of an HIV-infected cell.

Due to the nature and organization of the HIV genome, some of the viral proteins are initially produced as large immature precursor proteins (polyproteins), which are then cut into smaller, final protein products, e.g., gp160 cleaved into gp41 and gp120. In addition to RT and IN, the HIV genome carries a gene for yet another unique enzyme, known as viral *protease* (PR), which is responsible for the proper cutting (or cleavage) of polyproteins. As the proviral genes are transcribed and long viral polyproteins are synthesized, PR cuts itself from one of the polyproteins, so that it is ready to be packaged into an immature viral particle with the remaining polyproteins.

Viral **assembly** takes place on the interior side of the cytoplasmic membrane of the cell, as the structural, genetic, and other HIV components come together. For reasons not yet understood, certain regions of the host cell membrane are favored for the assembly of new viral particles. It is at this assembly step that host anti-viral factors such as APOBEC enzymes may be incorporated into viral particles (unless thwarted by HIV through the action of *vif*). Assembly ultimately results in new (but immature) viruses **budding** from the surface of the activated cell. While only a single virus is shown budding from the cell at the bottom of Figure 2, it has been shown by electron microscopy that a single HIV-infected cell can have multiple new viruses budding from its surface simultaneously. Once a new, but still immature, HIV particle is released from the cell, it becomes fully mature and infectious as a result of **cleavage** of viral polyproteins by the HIV PR.

An infected cell that has been activated and begins to make viable HIV particles is no longer latently infected, but rather, is considered to be productively infected. During the productive phase of HIV infection, new viruses are budding out from the infected cell into the circulation and/or tissues. At this point, the infected cell is capable of spreading the infection not just to daughter cells via cell division, but to any other CD4-positive cell that might bind and take up the new free-floating viruses. The potential for spread of the HIV infection is mind-boggling when one considers that the estimated number of viral particles produced throughout an infected person’s body in a single day may be as high as one billion ( $10^9$ ) (3).

## HIV AS A TARGET FOR DRUG THERAPY

Viral infections have always posed a challenge in terms of antimicrobial therapy directed against the virus itself. This is because all viruses are parasites, taking up residence in host cells and utilizing the cellular machinery to complete their life cycle. Since they parasitize normal cellular functions in order to reproduce, it is nearly impossible to eliminate viruses, and difficult to interfere with their reproduction or replication, without having a detrimental effect on the cells of the body. Historically, most treatments for viral illness and disease have merely addressed the virally-induced symptoms, not the underlying viral infection. However, true anti-viral therapy for persons infected with HIV (directed against the virus itself, not just the symptoms) does exist and has been used with varying degrees of success. It is important to stress that anti-HIV therapy is not intended to kill off all traces of the virus in the body—to do so would probably do more damage to the host cells than the virus itself. ***The goal of anti-HIV therapy is not a cure, i.e., ridding the body of its HIV infection. Rather, the goal is to control the growth of the virus so as to minimize its impact on the body and reduce the severity and/or length of associated disease.*** When a retrovirus such as HIV has steps in its life cycle that are unique to the virus (and are, therefore, not dependent on normal cellular processes), it may be vulnerable to anti-viral therapy targeted to those virus-specific elements. Fortunately, HIV has multiple steps in its life cycle that have been and/or are currently being targeted with anti-viral therapy as a means of reducing the overall rate at which HIV reproduces. If antiviral drug resistance does not develop, then these therapies are often successful in slowing and/or reversing the damage to the immune system, postponing the immune deficiency that ultimately leads to the development of AIDS (3, 4).

Early on in the epidemic, when it became clear that CD4 was serving as the receptor for HIV (Figure 2, **binding**), considerable effort was made to utilize a soluble form of the CD4 molecule as an anti-HIV therapy. The rationale was that if sufficient soluble CD4 (sCD4) was present in the circulation, then it would act as a decoy for HIV. In other words, it was hoped that free-floating HIV particles would bind the sCD4, saturating the gp120 molecules on the surface of the virus, thus preventing the virus from attaching to CD4 molecules on the surface of cells. The greatest potential drawback was that the normal role for CD4 on the surface of T<sub>H</sub> cells was to interact with MHC molecules in the course of antigen-specific immune responses, and it was feared that sCD4 would interfere with or prevent such responses. While sCD4 had little or no detrimental effects on normal T<sub>H</sub> cell responses and was very effective in preventing HIV infection in laboratory cultures, it was unable to interfere with the widely variable HIV isolates encountered in actual infection in vivo. However, over the intervening years, the idea of blocking the gp120/CD4 interaction has remained an attractive one, especially since it occurs outside the cell, and agents directed against it might be much less toxic to normal cells. Armed with a better understanding of the interactions involved (as described above), several new treatment candidates are under development and testing, including small molecules that would directly inhibit gp120/CD4 binding, and antibodies or drugs that could stabilize the CD4 molecule so that it could not undergo the change in shape required for further interactions with chemokine co-receptors (5).

The discovery of the chemokine receptor family as the co-receptors for HIV infection in human cells opened up a variety of approaches that are being explored which target these molecules. However, like CD4, the chemokine receptors serve an important function during

normal immune responses, so there were concerns as to whether they can be targeted to prevent HIV infection without interfering with these normal functions. In addition, because of the predominance of R5 viruses early in HIV infection, and more virulent X4 viruses targeting T cells later in HIV disease, there were also concerns that blocking the CCR5 chemokine receptor to prevent new cells from becoming infected with HIV might accelerate the transition to the production of X4 viruses (5). Since the chemokine receptors interact with viral gp120 during infection, it is hoped that decoy molecules (analogous to sCD4) or drugs that bind to either the appropriate region of gp120 or the chemokine receptor(s) would serve to block HIV fusion and infection without compromising normal immune responses. There is a great deal of interest in developing treatments related to the role of chemokine receptors in HIV infection, and thus far chemokine blocking does not appear to compromise immune responses or result in an accelerated predominance of X4 viruses (5).

Since the relatively recent description of the precise mechanism by which gp41 facilitates the **fusion** of viral particles and target cell membranes, i.e., the uncoiling, “harpooning”, and recoiling of gp41, drugs have been designed specifically targeting this process. One such drug, enfuvirtide or Fuzeon<sup>®</sup>, is already approved, and binds to the uncoiled gp41 molecule, preventing it from retracting and bringing the viral particle and target cell into close proximity (5, 8). Other fusion inhibitors that utilize similar strategies are under development.

The approaches that have had the most effort, and, therefore, the most success thus far in developing anti-HIV treatments, have been in identifying and/or designing drugs that specifically target two of the unique enzymes of HIV, i.e., RT, and PR. The first drugs approved for use in the mid- to late 1980s for treating AIDS and/or HIV infection targeted RT, involved in **reverse transcription**. In the mid-1990s, an entirely new class of drugs emerged designed to interfere with PR, which is responsible for **cleavage** of viral proteins. Drugs targeting these two steps of the HIV lifecycle will be described in detail below. Much less is known about the other HIV enzyme, IN, which facilitates **integration**, but it is actively being investigated as a potential target for anti-HIV drugs.

Following its isolation in 1983-84, it was quickly shown that HIV was a retrovirus dependent on RT for **reverse transcription** (Figure 2), a process that is completely absent in normal human cells. Therefore, it is not surprising this was the first HIV-specific enzyme targeted for anti-viral therapy. Any intervention that interrupts the process of reverse transcription would not affect the ability of HIV to *enter* a potential host cell. However, it would prevent the formation of a dsDNA copy of the HIV genome, which in turn, would prevent permanent *infection* of the cell (no dsDNA, no provirus, no integration). Such a treatment could not rid an already-infected cell of its provirus nor prevent infected cells from producing new viruses, but it would protect uninfected cells from becoming infected.

The earliest anti-HIV drugs were nucleoside analogues, counterfeit versions of the A, C, T, or G nucleosides used to make a strand of DNA. The first of these, azidothymidine (AZT, also known as zidovudine or Retrovir<sup>®</sup>) is a chemically-modified form of the T nucleoside (thymidine). If AZT is incorporated by RT into the DNA copy of the viral RNA during reverse transcription, no additional nucleosides can then be added to the DNA strand. If the DNA strand cannot be completed, then reverse transcription fails, and no provirus is created. AZT and other drugs of this type are known as Nucleoside Reverse Transcriptase Inhibitors (NRTIs). The

drawback of nucleoside analogues is that they could potentially be taken up by and disrupt normal cells' duplicating their DNA prior to dividing. It turns out, however, that the viral RT takes up AZT (and other NRTIs that came later) far more efficiently than the corresponding cellular enzyme involved in cell division. Therefore, the drug would be more likely to interfere with reverse transcription than cell division. AZT can still be too toxic for some persons with HIV infection or AIDS to take, especially in high doses as was done when first used in the mid-1980s. Now that there are many other retroviral drugs available, AZT is typically used at much lower doses in combination with other drugs (see below). Due to its relatively low cost, AZT alone is also still being used, especially in developing countries for the prophylactic treatment of HIV-infected pregnant women in order to reduce transmission to their newborn infants. Other NRTIs similar to AZT include ddi (didanosine, Videx<sup>®</sup>), ddC (zalcitabine or HIVID<sup>®</sup>), 3TC (lamivudine or Epivir<sup>®</sup>), and abacavir (Ziagen<sup>®</sup>)(3-5, 8). Treatment with any single NRTI over several months can result in the development of resistant strains of HIV (analogous to antibiotic-resistant strains of bacteria), but combination and/or sequential treatment with different NRTIs, particularly when combined with newer types of drugs (see below), can be an effective treatment approach. Typically, at least two of the NRTIs are included in multidrug treatment regimens. As a result, the latter two drugs are now formulated in combination with AZT as Combivir<sup>®</sup> (AZT plus 3TC) and Trizivir<sup>®</sup> (AZT plus 3TC plus abacavir), which has helped to simplify and/or reduce the number of pills needed to be taken daily. It is interesting to note that while these drugs were developed to treat HIV infection, some are also used clinically to treat other viruses with reverse transcription steps in their life cycles.

In addition to the NRTIs, there is another group of anti-retroviral drugs that also target **reverse transcription** (3-5, 8). These are known as Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) because rather than being nucleoside analogues incorporated into proviral DNA, they act on the RT enzyme itself. They bind directly to a specific site on RT of HIV-1, preventing it from catalyzing any further nucleoside additions to the new proviral DNA strand. Interestingly, they are so specific that they do not have any activity against RT from HIV-2, and a mutation that confers resistance to one member of this class of drugs makes a virus resistant to all members of the class (5). The earliest NNRTI was nevirapine (also known as Viramune<sup>®</sup>), which is extremely potent at very low concentrations. However, when it was used alone, nevirapine resulted in the rapid emergence of resistant strains of HIV in approximately six weeks, as indicated by a rebound in viral load to pre-treatment levels. This was in sharp contrast to the eighteen months or more that usually passed when resistance was observed to develop to treatment with AZT alone. Nevirapine, as well as another newer NNRTI, efavirenz (Sustiva<sup>®</sup>), are now commonly used to treat HIV-infected persons or persons with AIDS in combination with other drugs; one additional member of this class of drugs is delavirdine (Rescriptor<sup>®</sup>). NNRTIs have been considered for use in post-exposure prophylaxis (PEP), e.g., to treat persons exposed to HIV due to occupational or other exposure in order to prevent establishment of infection (1), but no NNRTIs are part of the current PEP recommendations from the CDC.

The most successful anti-HIV drugs developed to date have targeted the viral protease (PR), which, like RT, is produced from one of the genes of HIV and is very specific to the HIV life cycle (3-6). These protease inhibitors (PIs) prevent the **cleavage** of viral polyproteins into the smaller final protein products (Figure 2). It is only *after* cleavage that a viral particle is fully

mature and infectious. Therefore, if PR function is inhibited, there cannot be a productive infection from an activated infected host cell. Unlike the RT inhibitors, PIs do not prevent the establishment of new *infections* by HIV. Instead, PIs act on infected cells to prevent them from *producing* new infectious viruses following cellular activation, thereby limiting the spread of HIV to uninfected cells.

The first PI drugs were introduced in late 1994, and then became more widely available in 1995. These included zidovudine (ZDV), zalcitabine (ddC), and didanosine (ddI). Serendipitously the new technologies that permitted the determination of plasma HIV viral load became available at about the same time the PIs were introduced. If measurements of viral load had not been possible, it would have taken much longer to evaluate and understand the effects of these new drugs. Used alone, each of the PIs was clearly among the most potent anti-viral drugs to date. They reduced HIV viral loads in many HIV-infected persons to 1/100-1/1000 of the pre-treatment levels, often to below the level of detection (50-500 viral copies or viral equivalents per ml of plasma, depending on the test). However, as was observed for the NNRTI nevirapine, development of resistant strains of HIV was seen within 6-8 weeks of treatment with a single PI. Based on this observation, and drawing on historical experience with resistant bacteria (especially tuberculosis), PIs became widely used for the treatment of persons with HIV infection and/or AIDS as part of multi-drug treatment regimens. This type of combination therapy, known as “highly active anti-retroviral therapy” or HAART, usually consists of simultaneous use of three or more drugs: 2 NRTIs (as described above) plus at least one PI (or in some cases, a NNRTI) (5, 9). Two PIs are often used in HAART, as it has been found that zidovudine, which by itself is poorly tolerated in higher doses, can be used in low doses to dramatically boost or enhance the activity of other PIs. This has greatly improved the dosing of the other PIs, which can now be given fewer times or with fewer pills per day when combined with zidovudine. There is a newer PI, lopinavir, which is given only in combination with zidovudine, and so is formulated with zidovudine in a single pill (Kaletra<sup>®</sup>) (5, 8). Other commonly used PIs include nelfinavir (Viracept<sup>®</sup>), amprenavir (Agenerase<sup>®</sup>), and atazanavir (Reyataz<sup>®</sup>, which requires only one pill daily). Similar to NRTIs, resistance to PIs (when part of a multidrug regimen) can develop over a period of many months, and is usually dealt with by changing from one PI to another within the HAART regimen. Although the viral RT is a target unique to HIV and is not found in human cells, the PIs still have some toxicity associated with them. In particular, after extended periods of use, some of the PIs interfere with lipid metabolism that can result in redistribution of body fat (lipodystrophy), and increased blood levels of LDL cholesterol and triglycerides (hyperlipidemia) (5). One of the newest PIs, atazanavir, not only has the best dosing of PIs (once daily), it does not appear to cause hyperlipidemia.

HAART has also been dubbed “the anti-HIV cocktail” or simply an “HIV cocktail”. Contrary to the image conjured up by the term “cocktail”, a person receiving HAART is not free to take the drugs at his or her leisure, all at one time or in any order he or she may please. When HAART was first introduced in the mid-1990s, a typical regimen entailed swallowing 16 or more pills a day on a very rigid schedule that depended on the exact drugs in the combination being prescribed for a given person. This pill count did not include antibiotics or any other medications needed for other reasons related to HIV infection or other health conditions! However, with more experience with these anti-retroviral drugs, and the improved dosing made possible by

combining NRTIs into single pills and/or boosting PIs with ritonavir, HAART has been simplified considerably since its introduction (5, 9). This has made it easier for a person living with HIV infection and/or AIDS to follow the correct schedule of doses. This has important clinical implications, as non-adherence to HAART (skipping doses and/or failing to take all medications exactly as prescribed) greatly increases the risk of the development of resistant viruses, and has been estimated to account for about half of all treatment failures (4, 5, 9). The development of viral resistance affects not only the individual, but also increases the likelihood that resistant viruses will be transmitted into the population at large, posing an even greater threat to the containment of the HIV/AIDS epidemic. It should be noted that medication adherence is not the only challenge associated with HAART, since the cost of combination anti-retroviral therapy (for just the drugs themselves) can be as much as \$15,000 per person per year. Even if an HIV-infected person is willing and able to adhere to a HAART regimen, its cost may be a significant barrier to obtaining and continuing treatment.

Failure of anti-HIV therapy is currently defined by virologic (unable to achieve and/or maintain undetectable viral load levels in the blood), immunologic (failure to increase CD4 T<sub>H</sub> cells by 25-50 cells/mm<sup>3</sup> during the first year), and clinical (occurrence/recurrence of HIV-related condition) parameters (9). This highlights the importance of both HIV viral load testing and T cell subset monitoring, as described in the previous course on HIV/AIDS (1). Medical opinion as to the appropriate time to initiate therapy has changed over time. The initial approach with HAART in the mid-1990s was to treat aggressively as early as possible in HIV infection, with the idea that it might be possible to eradicate the virus with long-term HAART (4). However, even in persons on HAART who had successfully maintained plasma HIV viral loads below the limits of detection for three years or more, biopsies showed that HIV was still present in some tissue sites. This demonstrated that anti-HIV drug therapy should indeed be viewed as an attempt to control the growth of the virus, not a means of eradicating the HIV infection (as can be accomplished with antimicrobials and bacterial/fungal infections). While guidelines for treatment will continue to change over time, current recommendations for adults defer initiation of treatment in most asymptomatic HIV-infected persons until the absolute CD4 T<sub>H</sub> cell count drops below 350/mm<sup>3</sup> (Table 1) (9). While some clinicians might choose to initiate HAART in asymptomatic HIV-infected individuals with CD4 counts >350 cells/mm<sup>3</sup>; in general, such persons are viewed to be at low risk for opportunistic infections and other AIDS-defining conditions, and so are spared the cost, toxicity, and possible development of resistance associated with longer-term therapy initiated earlier in HIV disease (5, 9). In contrast, any symptomatic HIV-infected person, e.g., with a clinically defined AIDS condition or other severe symptoms, or an asymptomatic person with an AIDS diagnosis based solely on a CD4 count of <200 cells/mm<sup>3</sup>, is recommended to be treated, regardless of HIV viral load (Table 1) (9). For the latest treatment recommendations and other HIV-related guidelines, please see reference 9, which is updated regularly by the U.S. Department of Health and Human Services.

### **THE VACCINE CHALLENGE POSED BY HIV**

The challenges posed by treatment of HIV infection, as described above, highlight the need to *prevent* HIV infection in the first place by educating people about the modes of transmission (as reviewed in reference 1). Similarly, the development of effective HIV vaccines

that would prevent infection and/or the development of immunodeficiency disease, would be preferred over having to rely upon drug treatment to attempt to manage HIV disease once established (10).

Historically, the approach to the development of viral vaccines has been to throw the whole virus at the immune system, and let the body sort out which part of the virus was necessary to stimulate a protective immune response (3). Until the advent of recombinant DNA technology that provided the first genetically engineered viral vaccine (Recombivax HB<sup>®</sup> for hepatitis B), virtually all viral vaccines consisted of preparations of whole viruses that were either live/attenuated or killed/inactivated. The use of killed viruses was known to induce the production of virus-specific antibodies. These antibodies, secreted by B lymphocytes of the immune system, were sufficient to provide protection against subsequent disease caused by some, but not all viruses. Protection against other viral diseases was obtained only when the body was exposed to live attenuated strains of virus, i.e., viruses capable of reproducing in the human body, but that were no longer capable of causing disease. As our understanding of the immunologic responses to natural viral infections and live viral vaccines improved, it became clear that these immunizations induced cell-mediated immunity. This type of immunity is mediated by CD8-positive cytotoxic T lymphocytes (CTLs) that protect from a recurrence of the same viral disease by killing virally-infected cells. (For an overview of antibody and cell-mediated immune responses, see references 3 and 12). Whether antibody or cell-mediated immunity is effective for protection against future disease caused by a given virus is dictated by a number of factors such as route of infection, incubation time between initial infection and development of disease, nature and duration of the virus life cycle, etc. Viral vaccination mimics this process by inducing the appropriate type of viral-specific immune responses in the absence of the actual disease, which will protect the vaccinated individual from future disease caused by the same virus.

There are a number of reasons why development of an HIV vaccine poses a great challenge (3, 10). First and foremost, successful vaccines induce protective immune responses that mimic the response to natural infection that leads to recovery and immunity. In the case of HIV, it is not clear what kind of immunity, if any, is protective against HIV infection or disease. Virtually all HIV-infected persons develop vigorous HIV-specific antibodies and HIV-specific cell-mediated immunity, which appear to control the initial infection. The virus, however, is able to escape complete elimination, and persists as a chronic, mostly latent infection. Over time, circulating antibodies fail to prevent the spread of HIV to new target cells, and the cellular immune response against infected cells becomes ineffective, due at least in part to the loss of T<sub>H</sub> cells (10). As a result, after many years, the vast majority of HIV-infected persons develop the serious immunodeficiency known as AIDS (1, 3, 4). Since there is no clear natural response to HIV that provides protection, it is difficult to know what type of vaccine might be appropriate to try to artificially induce protection against infection, but more importantly, against the loss of immune system function and the development of AIDS.

There are some viral vaccines that stimulate the immune system so as to prevent infection from ever being established (3). Vaccination of this type often depends on the production of virus-specific antibodies, and so is usually successful against viruses that are stable over long periods of time, i.e., they do not mutate. HIV presents an enormous challenge on this front, as it

replicates very rapidly, and is very prone to mutations due to its retroviral life cycle. (RT is very prone to errors while catalyzing reverse transcription, leading to a high rate of mutation throughout the viral genome.) To put this in perspective, HIV is estimated to mutate 65 times faster than the influenza virus, which is notorious for requiring changes in vaccine strains on an annual basis (3). High rates of mutation in non-essential portions of the surface glycoproteins gp41 and gp120 lead to significant differences in these molecules from different strains of HIV. As a result, antibodies that might block infection by one isolate of HIV are incapable of recognizing and preventing infection by a different isolate (3, 4). Not surprisingly, early human vaccine trials utilizing purified recombinant gp120 or gp160 from one or a few HIV isolates have shown no protection from HIV infection.

Mutation is not the only means by which HIV appears to evade antibody responses intended to prevent infection. With more detailed studies of the interactions involved in HIV binding and fusion, it has been discovered that the essential portions of gp120 and gp41 that interact with CD4 and/or chemokine receptors are not accessible to antibodies due to structural constraints and/or heavy glycosylation (5, 6, 10). This may at least partially explain why the virus-specific antibodies present in HIV-infected persons ultimately fail to limit the spread of HIV infection within the body. Until portions of HIV molecules are identified that do not mutate so rapidly (or have less strain-to-strain variation), or are physically accessible to antibodies, the potential for development of a widely-effective vaccine designed to prevent infection remains limited.

There are examples among other successful viral vaccines where vaccination does not prevent infection, but rather, enables the immune system to recognize and control the virus produced from infected cells, so that the virus cannot cause disease (3). Because of its retroviral lifecycle (as well as the challenges described above), this may be the most appropriate type of vaccination approach for HIV. Once HIV establishes an infection following entry into the body, it persists as a latent integrated provirus in resting cells (Figure 2, bottom right). In this form HIV is undetectable by the immune system, making it impossible to mount an immune response that could recognize latently infected cells and eliminate them. However, it may be possible to develop an HIV vaccine that could help to control and eradicate infected activated cells that were actively producing virus (Figure 2, bottom left). This would require a vaccine that would stimulate the cellular arm of the immune system, creating a reservoir of HIV-specific memory  $T_H$  cells and CTLs. During acute infection, these pre-existing HIV-specific cells would work together quickly to eliminate productively-infected cells, and therefore, might be able to resolve the bulk of the initial infection in a shorter period of time, resulting in a lower persistent viral load (10). Later in HIV infection, such a vaccine could potentially limit the spread of the virus within an individual as it is produced by activated cells, maintain a low viral load, and presumably slow or prevent the development of immunodeficiency and the progression to AIDS. Since lower viral loads are thought to reduce the likelihood of HIV transmission from one individual to another, this type of vaccine would also have the wider public health benefit of reducing the spread of virus among the human population.

Vaccines capable of inducing cell-mediated immune responses usually consist of live but attenuated forms of the pathogenic virus (3). Because of the usual progression of HIV-infected persons to AIDS and subsequent death, there are enormous concerns about the safety of any

kind of live attenuated HIV vaccine, since there are very rare instances of other attenuated viral vaccines reverting to full virulence (e.g. the live polio vaccine). Although there are naturally-occurring mutants of HIV that appear to be greatly reduced in their virulence, there is no strain of HIV that is considered to be safe for use as a vaccine (3, 4). This need for a safe but live vaccine has led to the development of a number of different recombinant vector vaccines, where one or more genes of HIV are genetically engineered into a live (non-HIV) virus that is not pathogenic (3, 4, 10). This includes viruses such as canarypox and different strains of vaccinia virus that have previously been used as smallpox vaccines. There are even some approaches using recombinant versions of mycobacterial BCG (the tuberculosis vaccine) as an HIV vaccine vector. Because the HIV proteins are expressed by a live virus (or mycobacterium) that carries them into the body (the “vector”), this type of vaccine has been shown to induce cell-mediated immunity against HIV proteins. At the same time, because only isolated HIV genes and not a complete HIV genome are present in the vector, the recombinant vector vaccines generally pose no health threat to those receiving the vaccines. It is encouraging to note that the ability of HIV to mutate and escape from cell-mediated immunity seems to be limited by structural constraints, making it less likely that it can evade this type of vaccine (10). However, because these are recombinant vaccines based on the genes from one or a few HIV strains, it is not clear if this approach will be effective against a genetically diverse population of wild virus.

Another approach being tested is the use of purified DNA of selected HIV genes (known as “naked DNA vaccines”) that is injected under the skin and taken up by underlying cells. The cells then transcribe the vaccine DNA, and synthesize and express HIV proteins on their surfaces, stimulating cell-mediated immune responses similar to live viral vaccines. There are animal studies and human trials underway throughout the world, utilizing naked DNA vaccines as a “priming” vaccine, followed by a recombinant vector vaccine containing the same viral genes as a “boost”. These studies have provided contradictory results, but one preliminary human trial completed in 2004 reported a surprisingly poor result, with only 20% of immunized subjects demonstrating appropriate evidence of HIV-specific cellular immune responses (11). It is hoped that other ongoing human trials utilizing different combinations of prime/boost vaccines may provide better results, but this remains to be seen.

Many of the challenges discussed above focus on issues relating to the life cycle or structure of HIV itself. The means by which HIV is transmitted from one person to another also poses some unique challenges (3). Most viral vaccines protect against viruses to which persons are rarely exposed, or are exposed to on a seasonal basis (such as influenza). Most of these exposures occur via the respiratory system or the gastrointestinal tract. Worldwide, HIV is most frequently transmitted via sexual activity, gaining access to the body via the genitourinary tract. When injecting drug use is the mode of transmission, the virus is introduced directly into the bloodstream. While some individuals may be rarely exposed to HIV, those persons at highest risk of HIV infection (and with the greatest need for an effective vaccine) have repeated and frequent exposures to the virus through sexual activity and/or shared needle use. Since both the route and frequency of HIV exposure are unlike any viruses for which vaccination has been successful in the past, an HIV vaccine must provide a level of protection that is unprecedented in vaccine development to date (3).

Finally, the development of HIV vaccines has been hampered by the lack of a small and/or non-primate animal model for preliminary evaluation and testing of candidate vaccines. During vaccine development, preliminary experiments for determining safety and efficacy are usually conducted in laboratory animals such as mice, rats, or rabbits, or occasionally larger animals such as pigs, dogs, or sheep. Such preliminary animal testing determines if candidate vaccines induce the desired type of immune response, and most importantly, are able to protect a vaccinated animal against the development of disease following exposure to the pathogenic virus. HIV cannot establish an infection in any mammals other than primates, and among the non-human primates, only chimpanzees can be infected. However, even chimpanzees are not a suitable species for evaluating HIV vaccines, as they do not develop disease as a result of HIV infection. The only HIV-related vaccine research that has been possible in animals is in certain types of rhesus monkeys. These monkeys can be infected with simian immunodeficiency virus (SIV), the monkey equivalent of HIV, and they develop an AIDS-like immunodeficiency as a result of the infection. There are a limited number of monkeys available worldwide for research studies, and the cost of housing and caring for these animals can be prohibitive, which further limits the size and scope of SIV/HIV vaccine studies. While some valuable information has been obtained from such studies, it has been difficult to determine if rhesus monkey studies are directly applicable to human vaccine development.

Further development and testing of HIV vaccines designed to impact infection and/or disease progression may ultimately result in an effective means of controlling the HIV/AIDS epidemic. However, the success of such endeavors may hinge on improving not only the current understanding of virologic, immunologic, and genetic factors related to HIV infection, but also on improving the extent of scientific and governmental collaboration around the globe. It is appropriate, therefore, to examine the extent to which this pandemic has impacted not only the United States, but the entire world community.

## **THE HIV/AIDS PANDEMIC**

Since the recognition of AIDS in 1981, AIDS cases in the U.S. have been reported (anonymously) to the Centers for Disease Control and Prevention (CDC). More recently, the CDC has also been collecting statistics on confidential name-based HIV reporting. HIV/AIDS statistics are summarized by calendar year, and published annually by the CDC (13, 14). Starting with 335 AIDS cases reported in 1981, the numbers swelled to about 12,000 new cases per year in 1985, and to more than 48,000 new cases in 1990. The number of new cases per year continued to climb, reaching a peak in 1992 and 1993 at nearly 80,000 new cases in each of those years. As described in the previous course on HIV/AIDS (1), the estimated numbers of both new AIDS cases and AIDS deaths per year dropped dramatically in the mid-1990s due to the introduction of PIs and HAART, then leveled off and remained relatively stable from 1999-2001. In the most recent set of statistics from 1999-2003 (Table 2), while AIDS deaths continued to decline (3%), the number of AIDS cases increased by 4%, in spite of continued use and improvements in HAART (14). Overall, from 1981 through 2003, the CDC estimates that there have been 929,985 cases of AIDS, and more than 500,000 deaths from AIDS (13). More than nine thousand of these cases have been in children under 13 years of age; the remainder has been in adults and adolescents. (It should come as no surprise to anyone involved in health care in

California that over 100,000 AIDS cases, or approximately 14% of the total, have been reported from the state.) The CDC also estimates that, at the end of 2003, there were more than one million HIV-infected people living in the U.S., including those currently living with an AIDS diagnosis (14). It also confirmed its previous estimates that, within the U.S., approximately 40,000 persons are newly infected with HIV each year. This does not bode well for the future of the U.S. healthcare system, which is already struggling to deal with current levels of persons with HIV infection and AIDS, not to mention the demands created by other public health problems.

While the newer antiretroviral drugs, especially the PIs, have clearly provided a reduction (or perhaps just a delay) in AIDS cases and deaths in the U.S. (and other wealthy industrialized countries), such drugs are unavailable to the >95% of HIV-infected persons worldwide who live in low- and moderate-income countries (15). A stark illustration of this fact is that the death rate for persons 15-49 years old living with HIV is up to twenty times higher in poorer countries compared to industrialized countries. On a global scale, the HIV/AIDS pandemic is continuing unabated. According to the December 2004 estimates of the Joint United Nations/World Health Organization Programme on HIV/AIDS, 4.9 million people worldwide were newly infected with HIV during 2004—this translates to 14,000 new HIV infections per day, or more than 1,500 in just the three hours this course is intended to take (15). This brings to a total of approximately 39 million the number of people estimated to be living with HIV/AIDS worldwide. At the same time, it is estimated that there were approximately 3 million AIDS-related deaths during 2004. In Botswana, one of the hardest-hit countries where more than 35% of the population is HIV-infected, it has been estimated that if the rate of new HIV infections does not change, the chance of a boy who is fifteen years old today dying from AIDS is a staggering 85%. In the seven African countries with the highest rates of HIV infection (>20%), the average life expectancy of a person born between 1995-2000 has dropped 13 years due to AIDS, to 49 years, and the overall population is projected to be more than one-third smaller by 2025. The impact of the HIV/AIDS pandemic is not restricted to Africa, as there are estimated to be up to nearly 12 million HIV-infected persons currently living in Asia, with a projected 10 million HIV infections in China alone by the year 2010.

The devastation being wreaked on nations by HIV and AIDS is now being recognized as a threat to global and national security. When weakened by AIDS, individuals, societies, and/or governments are unable to cope with other threats such as droughts, other natural disasters or wars (15). In turn, wars and armed conflict generate many of the conditions, such as poverty, collapse of social structures, and physical or sexual violence, in which the HIV/AIDS epidemic can most readily expand. In July 2000, this led to an historic, first-ever United Nations Security Council discussion and resolution on a health issue, recognizing the spread of HIV/AIDS as a threat to global peace and security. Around the same time, the U.S. government declared AIDS a threat to national security, the first infectious disease ever to receive such a designation. Hopefully, in spite of complacency that has developed regarding HIV/AIDS among some in the U.S., such statements by national and international leaders regarding the magnitude of the HIV/AIDS pandemic will keep this issue before the public for many years to come.

There remain many other aspects of HIV infection and AIDS that have not been covered in this or the previous course on the topic. Hopefully, these two courses will help provide clinical laboratory scientists at least some of the information they need as health care providers

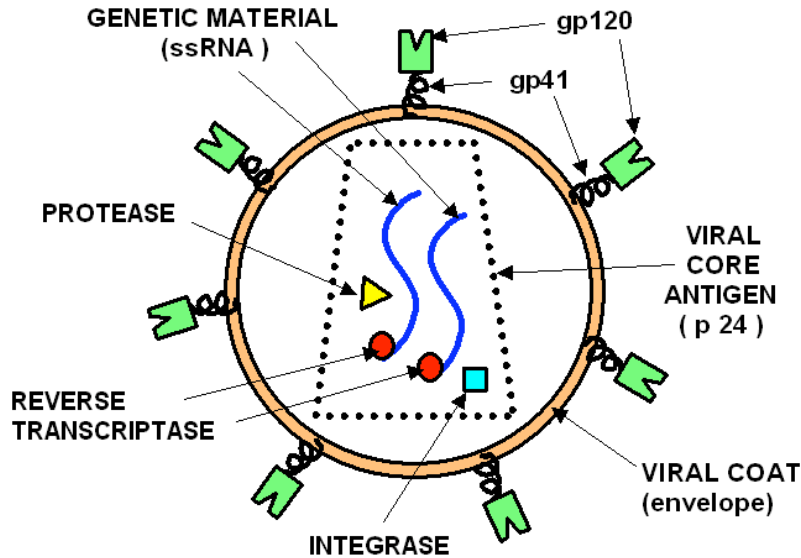
who must deal firsthand with the impact of the HIV/AIDS epidemic. In addition, it is hoped that the references provided in the courses can provide readers with more details, as well as links to other sources.

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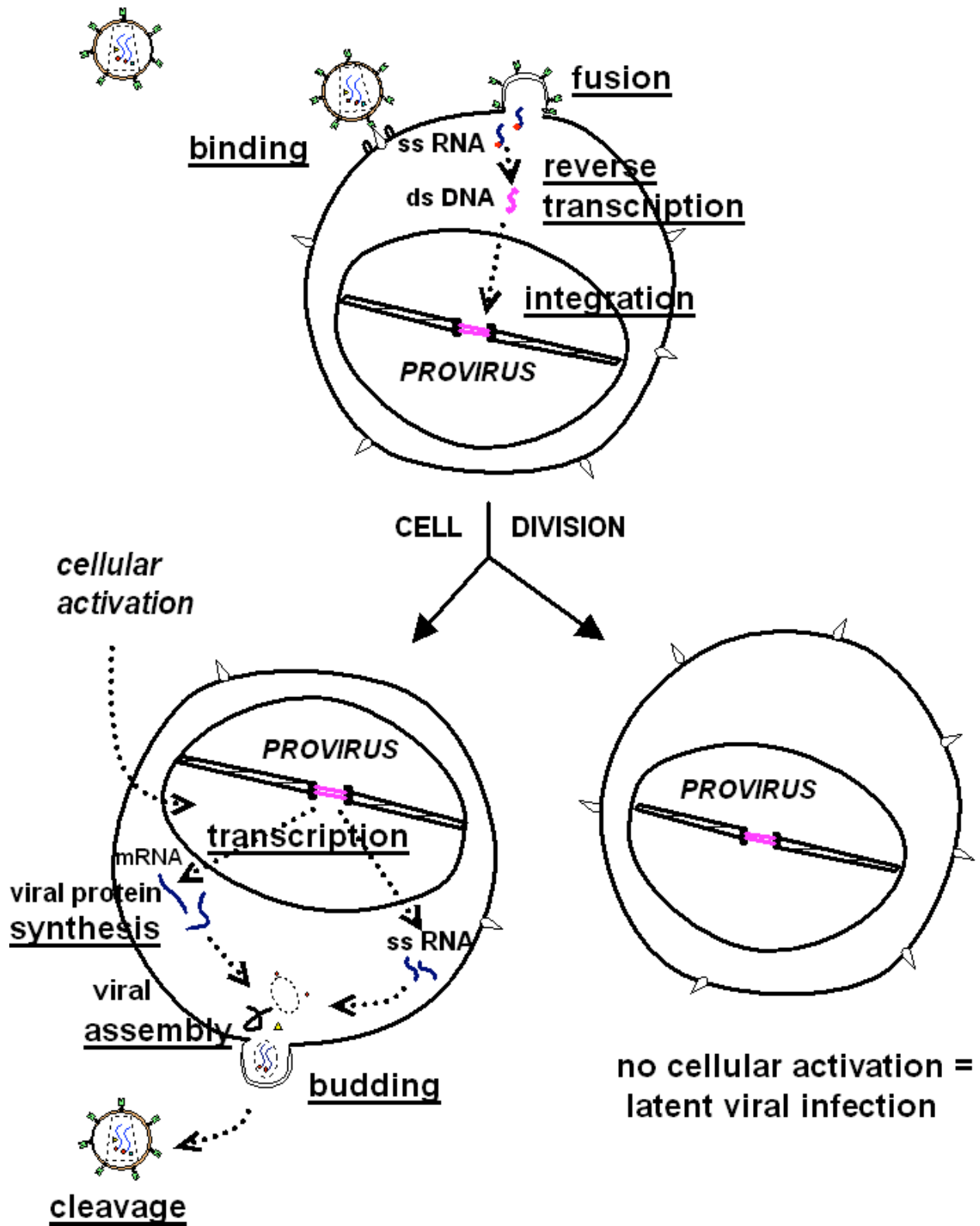
**Figure 1: A Schematic Representation of the Structure of HIV**  
Adapted from references 3 and 6.

## HUMAN IMMUNODEFICIENCY VIRUS



**Figure 2: The Life Cycle of HIV (figure on next page)**

An illustration of the critical steps (underlined) necessary for HIV to infect, reproduce, and spread utilizing a CD4-expressing host cell. In the bottom half of the figure, the cell on the right is in a resting state, and represents a true latent HIV infection. The cell on the left has been activated, and represents a productive infection.



**Table 1: April 2005 Recommendations: When to Treat HIV-infected Adults\***

Clinical Category	CD4 T <sub>H</sub> Count	HIV Viral Load	Treatment Recommendation
Symptomatic (clinical AIDS or severe symptoms)	Any value	Any value	Treat
Asymptomatic, AIDS defined by CD4 count	< 200 cells/mm <sup>3</sup>	Any value	Treat
Asymptomatic	200-350 cells/mm <sup>3</sup>	Any value	Offer treatment †
Asymptomatic	>350 cells/mm <sup>3</sup>	>100,000 copies/ml	Defer and observe in most cases †
Asymptomatic	>350 cells/mm <sup>3</sup>	<100,000 copies/ml	Defer therapy and observe

\*From reference 9; see additional guidelines (9) for treatment recommendations for HIV-infected pregnant women, and for children

† Patient readiness, probability of adherence, and prognosis based on CD4 count and HIV load need to be considered

**TABLE 2: Estimated AIDS cases, AIDS deaths, and persons living with AIDS in the U.S. 1999-2003\***

	1999	2000	2001	2002	2003
New AIDS cases per year	41,356	41,267	40,833	41,289	43,171
AIDS deaths per year	18,491	17,741	18,524	17,557	18,017
Persons living with AIDS†	311,205	334,731	357,040	380,771	405,926

\*From reference 14

† total number of persons estimated to be living with AIDS at the end of the indicated year

## Review Questions

Course #066-970

Choose the **one** best answer

1. The genetic material of HIV is
  - a) mRNA
  - b) dsRNA
  - c) dsDNA
  - d) ssRNA
2. Which of the following molecules is not an HIV enzyme?
  - a) RT
  - b) PR
  - c) CCR5
  - d) IN
3. Which component of HIV first mediates the binding of the viral particle to the CD4 molecule of a potential host cell?
  - a) gp120
  - b) gp41
  - c) gp160
  - d) p24
4. The role of gp41 in HIV infection is:
  - a) to mediate the fusion of the viral and cellular membranes
  - b) to initiate the binding between the viral particle and potential host cell
  - c) to facilitate nuclear transport of the HIV provirus
  - d) to downregulate target cell expression of CD4
5. What viral enzyme is physically associated with the genetic material of HIV when it enters the cytoplasm of a host cell?
  - a) PR
  - b) RT
  - c) *Nef*
  - d) DNA polymerase
6. Which of the following cell types is not a potential host cell for HIV?
  - a) T<sub>H</sub> cell
  - b) neutrophil
  - c) monocyte
  - d) glial cell

7. What family of molecules on a potential host cell dictates whether a particular virus will infect T cells or MO most efficiently?
- MHC Class I molecules
  - APOBEC
  - chemokine receptors
  - surface immunoglobulins
8. Of the following four steps of the HIV viral life cycle, which one occurs **first**?
- integration
  - viral gene transcription
  - reverse transcription
  - viral protein synthesis
9. Which of the following best describes reverse transcription?
- RNA  $\Rightarrow$  protein
  - DNA  $\Rightarrow$  RNA
  - DNA  $\Rightarrow$  protein
  - RNA  $\Rightarrow$  DNA
10. A provirus is:
- the ssRNA from a retrovirus
  - composed of two strands of viral RNA
  - incapable of being reproduced by a cell
  - a dsDNA copy of a retrovirus
11. Which step of the viral life cycle must be completed before a cell is considered permanently infected?
- binding
  - fusion
  - integration
  - reverse transcription
12. During a true latent infection, HIV will
- actively transcribe its viral genes and make viral proteins
  - have no transcription of the viral genes contained in the provirus
  - infect only CXCR4-expressing cells
  - spread from the blood to the brain

13. HIV switches from a latent to a productive infection as the result of:
- activation of the infected host cell
  - division of the infected host cell
  - integration of proviruses into cellular chromosomes
  - reverse transcription of the viral genome
14. Which of the following drugs acts outside of the host cell?
- nevirapine
  - atazanavir
  - lopinavir plus retrovir
  - enfuvirtide
15. The elimination of viral infections is a greater challenge than eliminating most bacterial infections because:
- viruses parasitize normal cellular functions in order to reproduce
  - viruses are submicroscopic compared to bacteria
  - virus reproduction is completely independent of cellular functions
  - viruses and bacteria never infect the same kinds of tissues and/or cells
16. The primary goal of effective antiretroviral therapy is:
- to completely rid the body of its HIV infection in order to achieve a cure
  - to control the growth of HIV in hopes of reducing its impact on the immune system
  - to control target cell proliferation and propagation of provirus
  - to prevent immune cell activation of any kind
17. NRTIs affect the ability of HIV to:
- enter a potential host cell
  - produce provirus
  - bind to gp120
  - produce mature infectious viruses
18. PIs act on which of the following steps of the HIV lifecycle?
- budding
  - fusion
  - integration
  - cleavage
19. HAART stands for
- HIV-associated anti-retroviral therapy
  - HIV and anti-retrovirus therapy
  - highly active anti-retroviral therapy
  - high-dose analogue and reverse transcriptase therapy

20. Combination therapy is necessary for treating HIV because
- HIV rapidly becomes resistant to certain anti-viral drugs used alone
  - antibiotics alone are ineffective
  - none of the anti-viral drugs have any effect when used alone
  - pharmaceutical companies will not sell the drugs individually
21. An important laboratory test used to track success or failure of anti-HIV drug therapy is:
- HIV antibody ELISA
  - HIV plasma viral load
  - total WBC count
  - Western blot
22. Historically, successful viral vaccines have been
- inactivated purified preparations of individual viral components
  - either live/attenuated or whole killed virus preparations
  - live pathogenic viruses that were likely to cause disease
  - synthetic peptides that cross react with virus
23. It is not known what kind of immunity, if any, might prevent HIV disease because
- the vast majority of HIV-infected persons develop AIDS
  - there is no way to diagnose an asymptomatic HIV infection
  - HIV-infected persons do not develop HIV-specific antibodies or cell-mediated immunity
  - the medical community cannot agree on when to treat HIV infection
24. The rate of mutation in HIV genes is
- not a serious challenge to HIV vaccine development
  - less than that of influenza virus
  - very high, especially in gp41 and gp120
  - low due to its retroviral life cycle
25. Which of the following is not considered an obstacle to HIV vaccine development?
- HIV-specific cell-mediated immunity which appears to control initial infection
  - limits imposed by scarcity and expense of rhesus monkey models
  - extensive glycosylation of gp120 adjacent to the CD4-binding cleft
  - HIV persistence in a proviral form after acute infection

26. An HIV recombinant vector vaccine would most likely contain:
- a combination of SIV and HIV genes to make a cross-species recombinant virus
  - random genetic sequences engineered into a non-pathogenic or attenuated strain of HIV
  - different genes from different strains of HIV to make a complete recombinant live HIV
  - several genes from HIV genetically engineered into a non-pathogenic but live non-HIV virus
27. "Naked DNA" refers to
- a defective HIV particle stripped of its envelope or coat
  - the dsDNA provirus prior to integration
  - the RNA/DNA hybrid that occurs during reverse transcription
  - an HIV vaccine consisting of purified DNA
28. The cumulative number of AIDS cases in the United States that have been reported to the CDC through 2003 is:
- 405,926
  - approximately 500,000
  - more than 900,000
  - 40,000 per year
29. The trend in the number of new AIDS cases in the U.S. per year between 1999 and 2003:
- is now increasing after dramatic declines due to the introduction of HAART
  - has continued downward since the introduction of HAART
  - has shown a 3% decrease during that time
  - indicates that the HIV/AIDS epidemic is near its end
30. According to the UN Programme on HIV/AIDS, the estimated number of new HIV infections worldwide per day during 2004 was:
- 1,500
  - 14,000
  - approximately 3 million
  - 49

