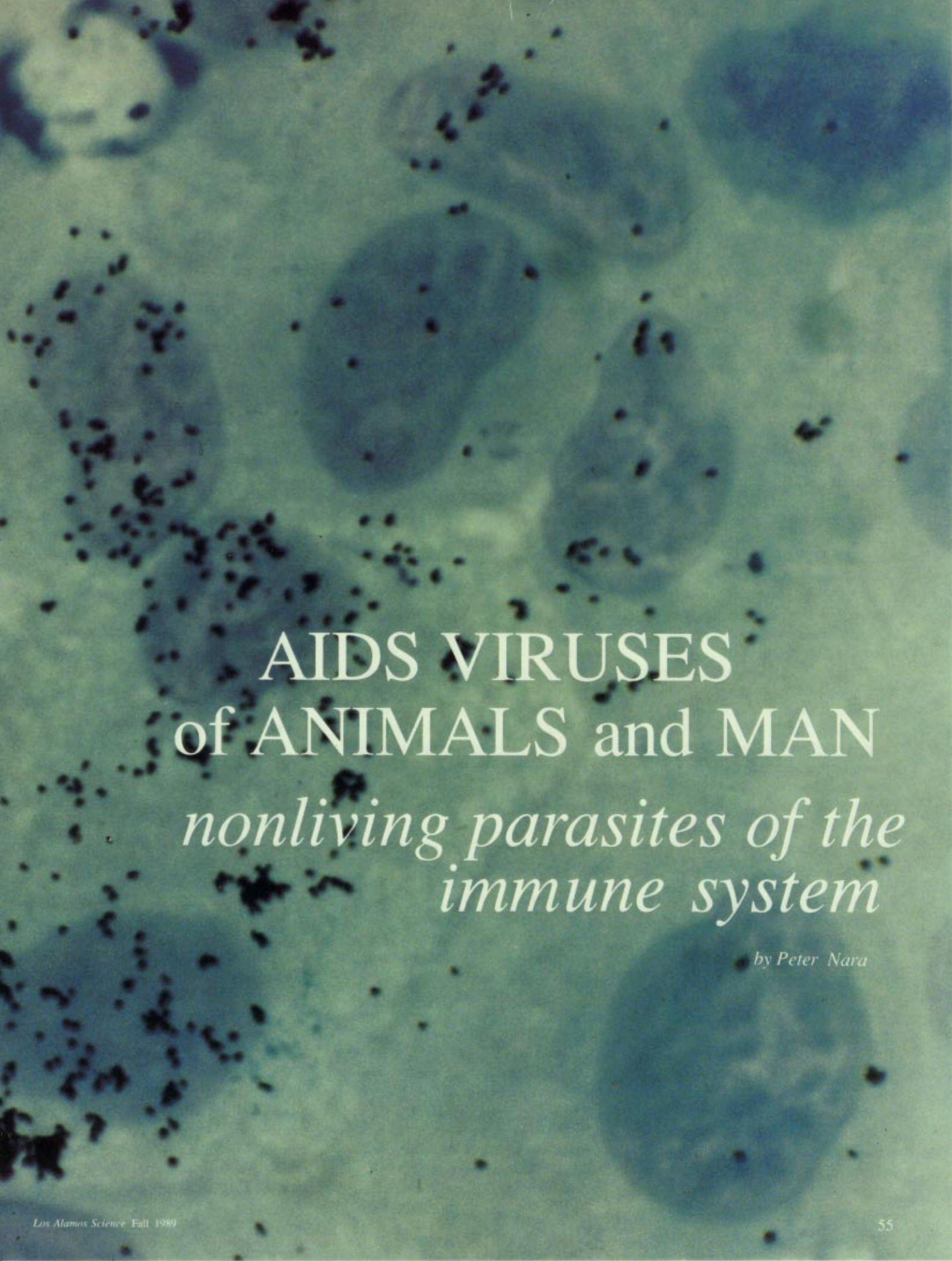


AIDS VIRUSES of ANIMALS and MAN

nonliving parasites of the immune system

by Peter Nara

From one of the earliest known descriptions of a viral disease, namely, the report in 1904 of a disease affecting horses called swamp fever, to the sudden appearance of a wasting disease of sheep, which reached epidemic proportions in Iceland in the 1940s, the *lentiviruses* remained known only among a small group of virologist as a curiously slow infectious agent and were totally unappreciated as a potential source for a global human viral pandemic. Then, in the late 1970s and early 1980s, subtle patterns of a new clinical disease gave way to the parallel epidemics of AIDS in captive primates and humans. Characterized by seemingly unrelated opportunistic infections, the lentiviruses thrust themselves into the scientific, genetic, moral, and cultural fabric of mankind throughout the world.

HIV (human immunodeficiency virus), the cause of AIDS, is a member of the lentiviruses, a subfamily of a larger family called *retroviruses.* That large family is well known for containing viruses that cause cancer in humans and other animals. Although lentiviruses do not cause cancer, they do present a formidable challenge to the host. First, lentiviruses integrate themselves into their host's genetic blueprint. Second, they contain numerous regulatory genes that allow them to control their rate of replication in both dividing and nondividing cells. Third, and most important, they have evolved, interacted, and survived completely within the cells of the host's immune system—the only viruses described to date that spend their entirety in such cells.

In this article we intend to retrieve from anonymity the lentiviruses associated with animals other than humans' and focus attention on their various strategies for survival. We will explain how the AIDS virus and other lentiviruses outsmart the host's immune system and show why traditional approaches to vaccine development will most likely fail against this type of virus. Finally, we will turn to models of host adaptation, in particular, the African green monkey and the chimpanzee, as a probable source of inspiration for understanding and, therefore, developing a successful strategy against AIDS and AIDS-like diseases.

Retroviral Life Cycle and Family History

All viruses are parasitic in nature. They require a host to replicate but unlike parasites, which are living organisms, viruses are functionally nonliving. A virus is best described as an infectious chemical made up of an outer envelope or protein coat that encapsulates the viral genome, the genetic blueprint for constructing more viruses. What they lack are the protein-synthesis and energy-generating capabilities required to manufacture progeny. They infect the host cell by binding to and fusing with the cell membrane and then depositing the viral genes within the cell where they are free to be read by and interact with the host's manufacturing machinery (Fig. 1).

The "retro'' viruses are so-called because at the beginning of their life cycle they reverse the usual flow of genetic information. In all living organisms and in many other viruses, genetic information is stored as deoxyribonucleic acid, or DNA, and later transcribed into ribonucleic acid, or RNA, which serves as a template for protein synthesis. By contrast, retroviruses store their genetic information as RNA and also contain the unique enzyme, reverse transcriptase, which catalyzes the "reverse" transcription of the RNA genome into a DNA copy. The resulting proviral DNA is oftentimes perceived by the host cell as its own and is integrated into its DNA where the provirus can remain dormant or latent for weeks, months, or even years without being expressed. In fact, some retroviruses (for example, those of chickens and mice) have assured their persistent association by integrating into the germ cells of the host. As integrated viruses (so called proviruses), they are transmitted vertically to the next generation without an infectious cycle. There are no known methods of eliminating such retroviruses.

STRUCTURE AND LIFE CYCLE OF HIV

Fig. 1. The structure (a) and Iife cycle (b) of HIV. The cycle starts with the binding of the viral envelope protein *gp120* to a CD4 receptor on the surface of the target cell, the fusing of the viral and cellular lipid bilayers, and the entry of the viral core, containing the RNA genome and the enzyme reverse transcriptase, into the cell's interior. The cycle ends with the production of new viral genomes and viral proteins and the assembly of viral cores and budding of new virus particles. Step 4, reverse transcription of the viral genome into proviral DNA, and step 5, integration of proviral DNA into the host cell's genome, are unique to retroviruses. In some cases, after step 4, the DNA will spontaneously close on itself and this circular DNA will remain in the cytoplasm as episomal DNA. Also shown is the possibility that steps 4-6 will be bypassed and the positive strand of genomic RNA will serve directly as a template for protein synthesis, that is, it will be translated directly into viral proteins by ribosomes within the cell.

The retrovirus family, evolutionarily speaking, is quite old. It contains three subfamilies: the oncoviruses, the spumaviruses, and the subfamily of most interest to us, the lentiviruses (Table 1). The oncoviruses, or cancer-causing viruses, are found to be transmitted both by host-to-host contact and as integrated viruses in germ cells. When integrated into the host's DNA, oncoviruses efficiently "transform" the host cells into cells that have a tumor-

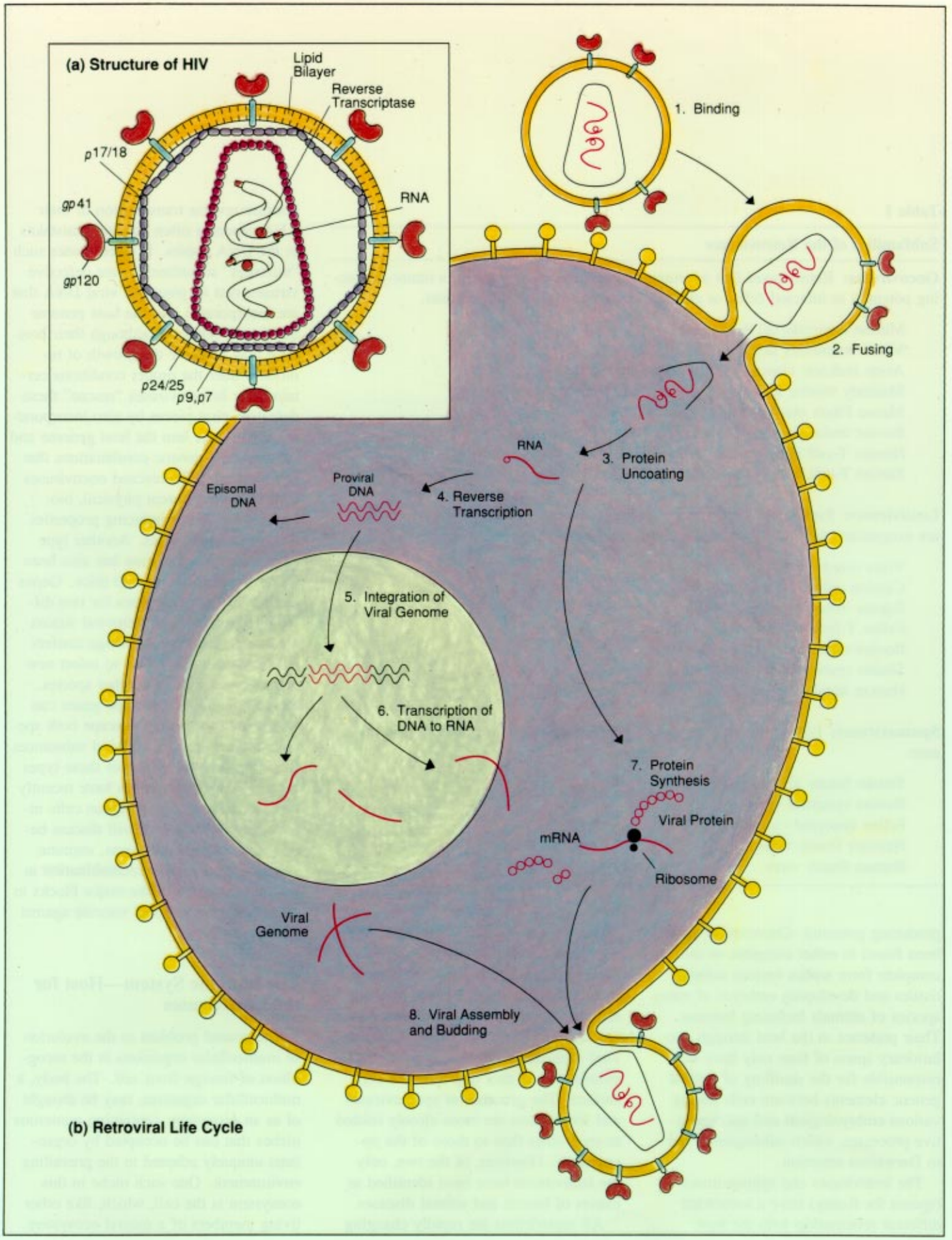

(a) Structure of HIV
Lipid Bilayer
Reverse Transcriptase
p17/18
gp41
gp120
RNA
p24/25
p9,p7
1. Binding
2. Fusing
3. Protein Uncoating
RNA
Proviral DNA
Episomal DNA
4. Reverse Transcription
5. Integration of Viral Genome
6. Transcription of DNA to RNA
7. Protein Synthesis
Viral Protein
mRNA
Ribosome
Viral Genome
8. Viral Assembly and Budding
(b) Retroviral Life Cycle

Table 1

Subfamilies of the Retroviruses

Oncoviruses: Retroviruses that are transforming (that is, they create a tumor-producing potential in infected cells) or closely related nontransforming viruses.

Murine intercisternal A (Type A)
Mouse mammary tumor virus (Type B)
Avian leukosis virus (Type C, avian subgroup)
Moloney murine leukemia virus (Type C, mammalian subgroup)
Mason Pfizer monkey virus (Type D)
Bovine leukemia virus (BLV/HTLV)
Human T-cell lymphotropic virus (BLV/HTLV, Types I and H)
Simian T-cell lymphotropic virus (BLV/HTLV, Type I)

Lentiviruses: Pathogenic slow viruses that cause persistent multiorgan disorders and are exogenous, that is, they do not integrate themselves into the host's germ cells.

Visna maedi virus
Caprine arthritis encephalitis virus
Equine infectious anemia virus
Feline T-lymphotropic virus
Bovine immunodeficiency-like virus
Simian immunodeficiency virus (SIV)
Human immunodeficiency virus (HIV, Types I and H)

Spumaviruses: Foamy viruses that cause persistent infections without clinical disease.

Simian foamy viruses (9 serotypes)
Bovine syncytial virus
Feline syncytial virus
Hamster foamy virus
Human foamy virus

producing potential. Oncoviruses have been found in either complete or incomplete form within various normal tissues and developing embryos of many species of animals including humans. Their presence in the host through evolutionary spans of time may have been responsible for the shuffling of critical genetic elements between cells during various embryological and differentiative processes, which subsequently led to Darwinian selection.

The lentiviruses and spumaviruses (spuma for foamy) have a somewhat different relationship with the host. These viruses do not integrate into the host's germ cell lines and do not cause cancer. In vivo both produce lifelong infection of the host cells but may not kill the infected cells. In vitro they infect and kill host cells through massive viral replication and tearing of the cell membranes as they bud from the cell surface. The genomes of spumaviruses and lentiviruses are more closely related to each other than to those of the oncoviruses. However, of the two, only the lentiviruses have been identified as causes of human and animal diseases.

All retroviruses are rapidly changing because reverse transcription of their RNA genomes often produces mistakes in the DNA copies. In oncoviruses such "mistakes" sometimes create defective viruses, that is, pieces of viral DNA that are incorporated into the host genome but cannot replicate, although their presence may promote the growth of tumors. Under the proper conditions certain other helper viruses "rescue" these defective viral pieces by also incorporating themselves into the host genome and creating new genetic combinations that can replicate. The rescued oncoviruses usually have different physical, biological, and tumor-inducing properties than the original virus. Another type of genetic recombination has also been found in experiments with mice. Genes that specify the envelopes for two different retroviruses or retroviral strains are exchanged. The exchange confers on the viruses the ability to infect new cell types or cells of another species. Recombination of envelope genes can also enable the virus to escape both specific and nonspecific antiviral substances found in the host. Many of these types of genetic recombination have recently been found to occur in human cells infected by HIV. As we will discuss below, spontaneous mutations, immune selection, and genetic recombination in HIV presents one of the major blocks to developing a traditional vaccine against AIDS.

The Immune System—Host for the Lentiviruses

The central problem in the evolution of multicellular organisms is the recognition of foreign from self. The body, a multicellular organism, may be thought of as an ecosystem containing numerous niches that can be occupied by organisms uniquely adapted to the prevailing environment. One such niche in this ecosystem is the cell, which, like other living members of a natural ecosystem,

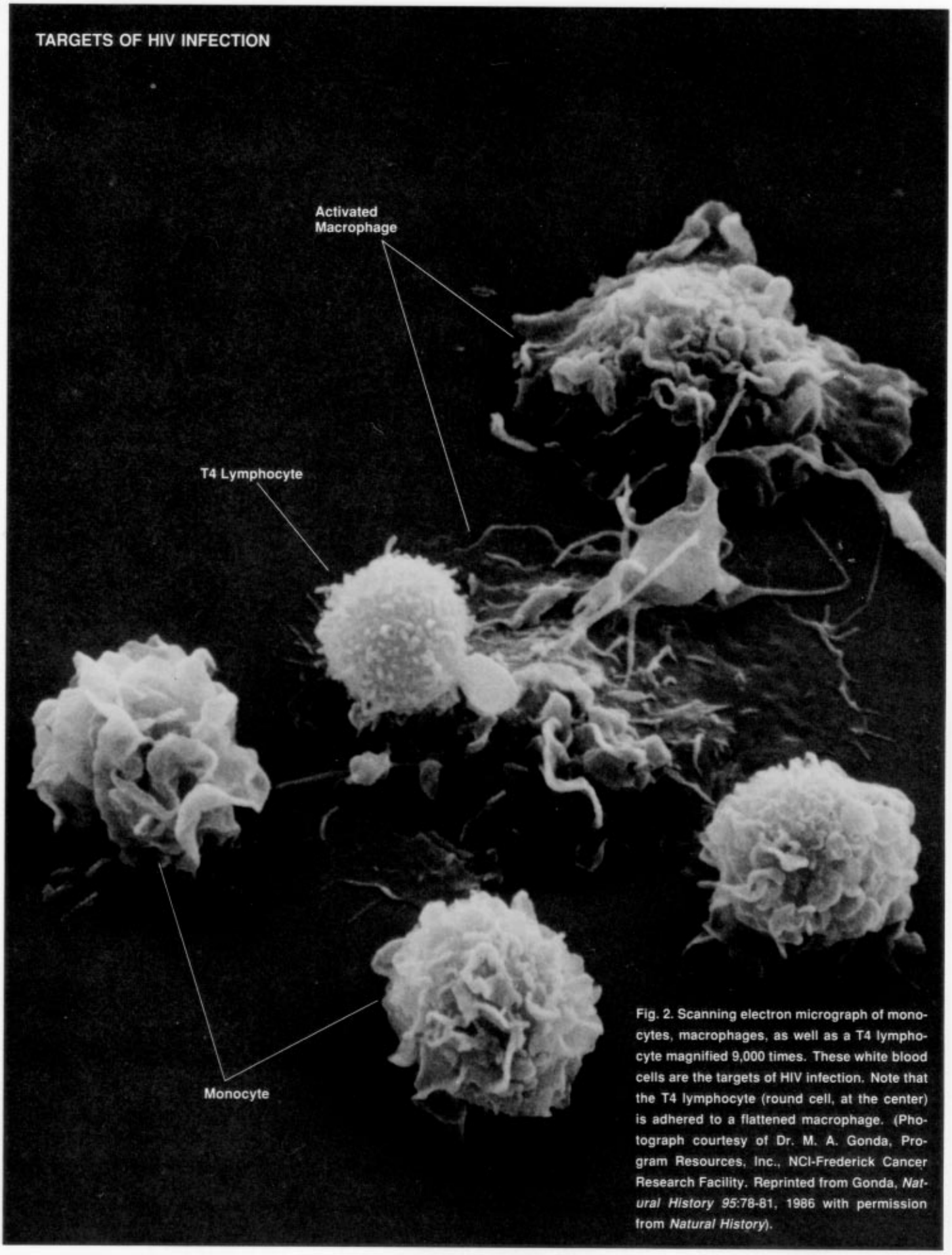
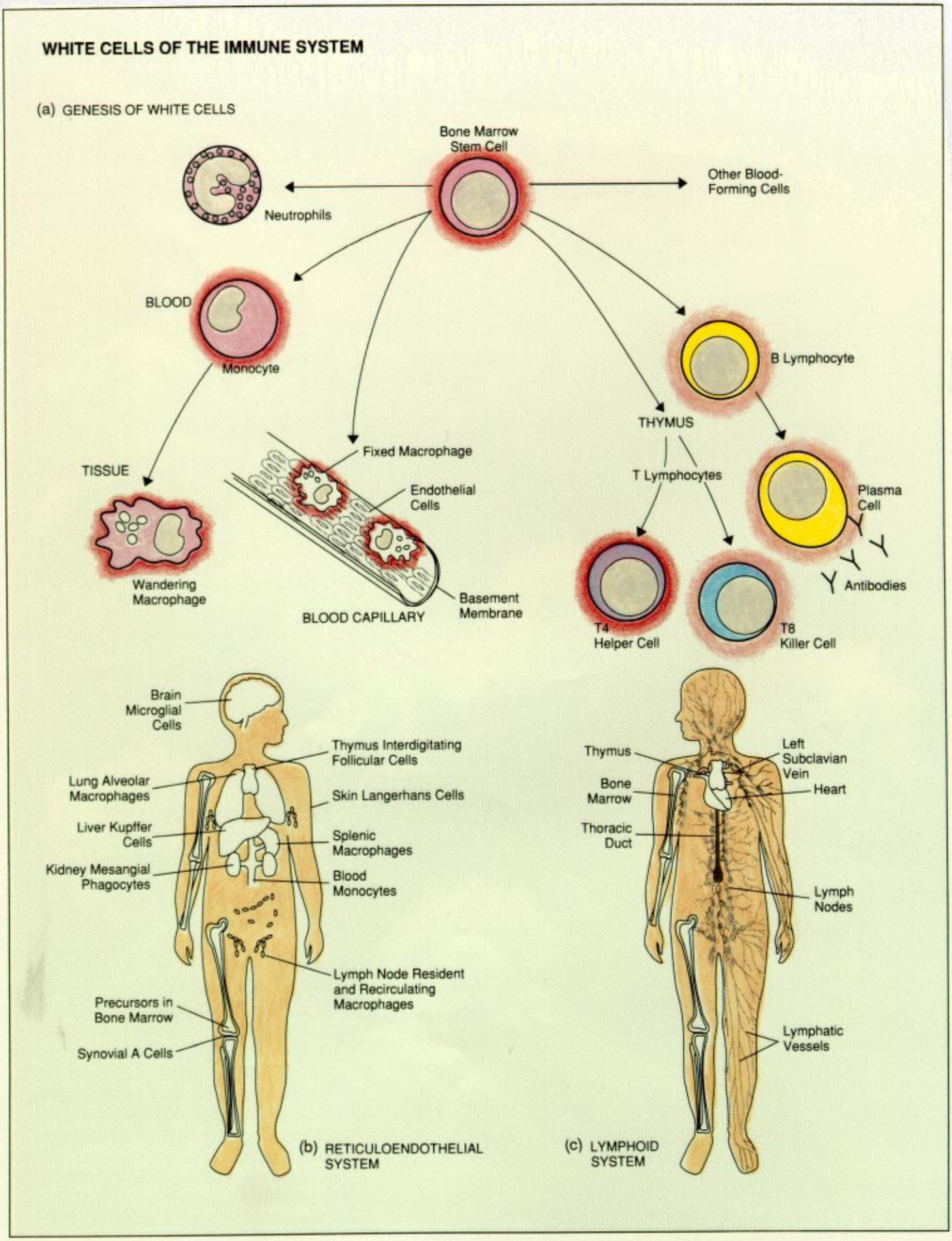


Fig. 2. Scanning electron micrograph of monocytes, macrophages, as well as a T4 lymphocyte magnified 9,000 times. These white blood cells are the targets of HIV infection. Note that the T4 lymphocyte (round cell, at the center) is adhered to a flattened macrophage. (Photograph courtesy of Dr. M. A. Gonda, Program Resources, Inc., NCI-Frederick Cancer Research Facility. Reprinted from Gonda, *Natural History* 95:78-81, 1986 with permission from *Natural History*).


WHITE CELLS OF THE IMMUNE SYSTEM
(a) GENESIS OF WHITE CELLS
Bone Marrow Stem Cell
Neutrophils
Other Blood-Forming Cells
BLOOD
Monocyte
B Lymphocyte
THYMUS
T Lymphocytes
TISSUE
Fixed Macrophage
Endothelial Cells
Plasma Cell
Antibodies
Wandering Macrophage
Basement Membrane
BLOOD CAPILLARY
T4 Helper Cell
T8 Killer Cell
Brain Microglial Cells
Thymus Interdigitating Follicular Cells
Lung Alveolar Macrophages
Skin Langerhans Cells
Liver Kupffer Cells
Splenic Macrophages
Kidney Mesangial Phagocytes
Blood Monocytes
Lymph Node Resident and Recirculating Macrophages
Precursors in Bone Marrow
Synovial A Cells
(b) RETICULOENDOTHELIAL SYSTEM
Thymus
Left Subclavian Vein
Bone Marrow
Heart
Thoracic Duct
Lymph Nodes
Lymphatic Vessels
(c) LYMPHOID SYSTEM

THE IMMUNE SYSTEM

Fig. 3. (a) White cells of the immune system. The cells surrounded by deep red halos are the primary targets of HIV infection; those surrounded by pale red halos are less frequent targets. Among the primary targets are monocytes and macrophages (upper left), part of a functional system of scavenging cells (phagocytes) and antigen-presenting cells called the reticuloendothelial system (b). Whereas monocytes circulate in the blood, macrophages are strategically located in various organs of the body and in the lymph nodes. (Note that the monocytes and macrophages have kidney-shaped nuclei.) Another line of immune defense is provided by the lymphocytes (upper right), an adaptive system that acts against specific foreign antigens (proteins). T lymphocytes mature, differentiate and acquire their antigen-specificity in the thymus. In the presence of specific foreign antigens, the T4 helper lymphocytes direct the activities of other immune cells by sending out chemical messages and the T8 killer lymphocytes send out cytotoxins, which kill the foreign cells. B lymphocytes, in the presence of specific foreign antigens, mature into plasma cells and manufacture Y-shaped antigen-specific protein molecules called antibodies, which bind to the foreign antigens. Most white cells of the immune sytem flow through the bloodstream, and at specific sites in capillary walls, they exit to the lymphatic system (c), a network of tiny vessels permeating the body whose walls are only one-cell thick. These tiny vessels act as conduits for white cells and collect all the extracellular lymphatic fluids in the body. These cells and fluids are then recirculated back to the bloodstream through vessels that merge into even larger lymphatic ducts, like the streams in a watershed, and eventually converge into the large thoracic duct, which empties into a large vein at the base of the neck called the left subclavian vein. The flow of white cells through the lymphatics and bloodstream provides continual immune surveillance of the entire body.

can be invaded by disease-producing organisms. An additional problem for the body's ecosystem is the spontaneous generation of mutant cells, that is, tumor cells that could threaten its own survival. Thus, depending on the size of the multicellular organism, a unique set of cells had to evolve to assure that no members of the organism's ecosystem be parasitized by intracellular pathogens or altered in a way that would damage their critical day-to-day functions. In a large number of animals, including man, that set is composed of two special types of white cells (Fig. 2): the *monocytes* and *macrophages*, and the *T lymphocytes* (the T stands for thymus-derived). Although evolutionarily they are among the oldest cells of the immune system and are well-adapted to perform their functions, these cells have provided the perfect niche for certain nonliving parasites—namely, the lentiviruses! To understand the impact of lentiviral infections, we will first outline the genesis and normal functioning of monocytes, macrophages, and T lymphocytes.

The immune system is a complicated network of white cells and their chemical products (Fig. 3), which interact synergistically to eliminate foreign invaders, abnormal cells, and toxic cell products. White cells generally originate from stem cells found in foetal liver and bone marrow. As they mature, they differentiate into many cell types with separate or overlapping functions (Fig. 3a). Most white cells in the blood are short-lived scavenger cells (neutrophils) that engulf and digest foreign microbes and die. The pus seen in bacterial infections are primarily these dead scavenger cells.

The monocytes and macrophages are also scavenger cells, but they may live months or years. They are particularly good at detecting, engulfing, and digesting tumor or virally infected cells. Some monocytes circulate in the bloodstream and later in their life receive immune signals from the lymphocytes that cause the monocytes to migrate into tissues and transform into tissue-specific macrophages. There they either wander freely through the connective tissue in organs or attach to the basement membranes of the tiny capillaries in those organs. Other monocytes differentiate directly into tissue-specific macrophages. Macrophages are concentrated and strategically located in the liver, lungs, and lymph nodes—organs that receive blood from parts of the body exposed to the outside world, such as the gastrointestinal or respiratory tract. Should the invading pathogen escape this early level of immune defense, other macrophages located in the spleen, kidney, joints, and brain provide a second level of defense. Together, monocytes and macrophages form what is known as the *reticuloendothelial system* (Fig. 3b), one major line of defense in the immune system.

Another major line of defense in the immune system is the *lymphoid system*, a set of glands, organs, and cells (Fig. 3c). The lymph nodes, which are distributed throughout the body, serve as way stations, storage facilities, and manufacturing and shipping sites for specific cells of the immune system, including the T and B *lymphocytes* (literally meaning cells of the lymph). In the process of maturing, the lymphocytes differentiate into hundreds of thousands of lymphocyte subgroups, each very small and each designed to recognize and mount a defense against a specific foreign protein, or *antigen*. But how does each antigen-specific subgroup prepare its attack when its target antigen enters the body?

Macrophages entering the lymph nodes or interacting with lymphocytes in tissues have the job of "presenting" foreign antigen to the appropriate lymphocyte subgoup and thereby activating it. More specifically, when macrophages engulf and digest foreign microbes or

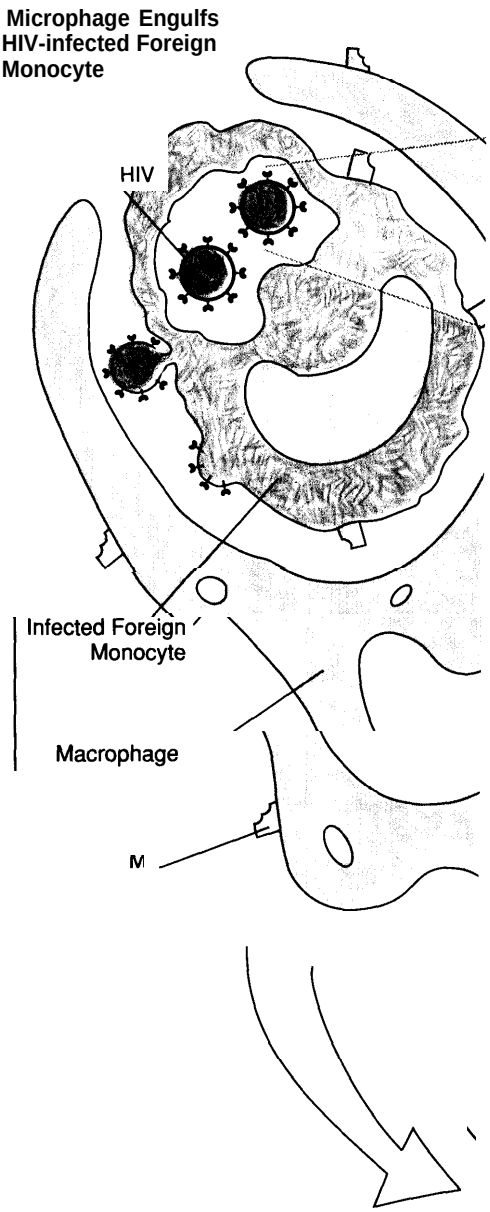
infected cells, they incorporate into their surface membranes the proteins of the foreign invaders (Fig. 4). The foreign antigens are inserted on the microphage surface next to other normal receptors, called *MHC antigens* (for antigens of the major histocompatibility complex). The MHC antigens are part of the associative recognition network of surface receptors that enable the macrophages and lymphocytes to recognize each other as parts of the self and to receive appropriate instructions from each other. When a foreign antigen is present in the microphage surface, only those lymphocytes that recognize or bind to both an MHC receptor and the specific foreign antigen are activated. Figure 4 illustrates this dual recognition by lymphocytes. In this case, we have chosen to show how a special type of lymphocyte called a *T4 helper cell* recognizes both an MHC II receptor and the antigen *gp120,* one of the envelope proteins of HIV. Although this example is particularly relevant for our story, it also illustrates the normal phenomenon of recognition between antigen-presenting macrophages and lymphocytes.

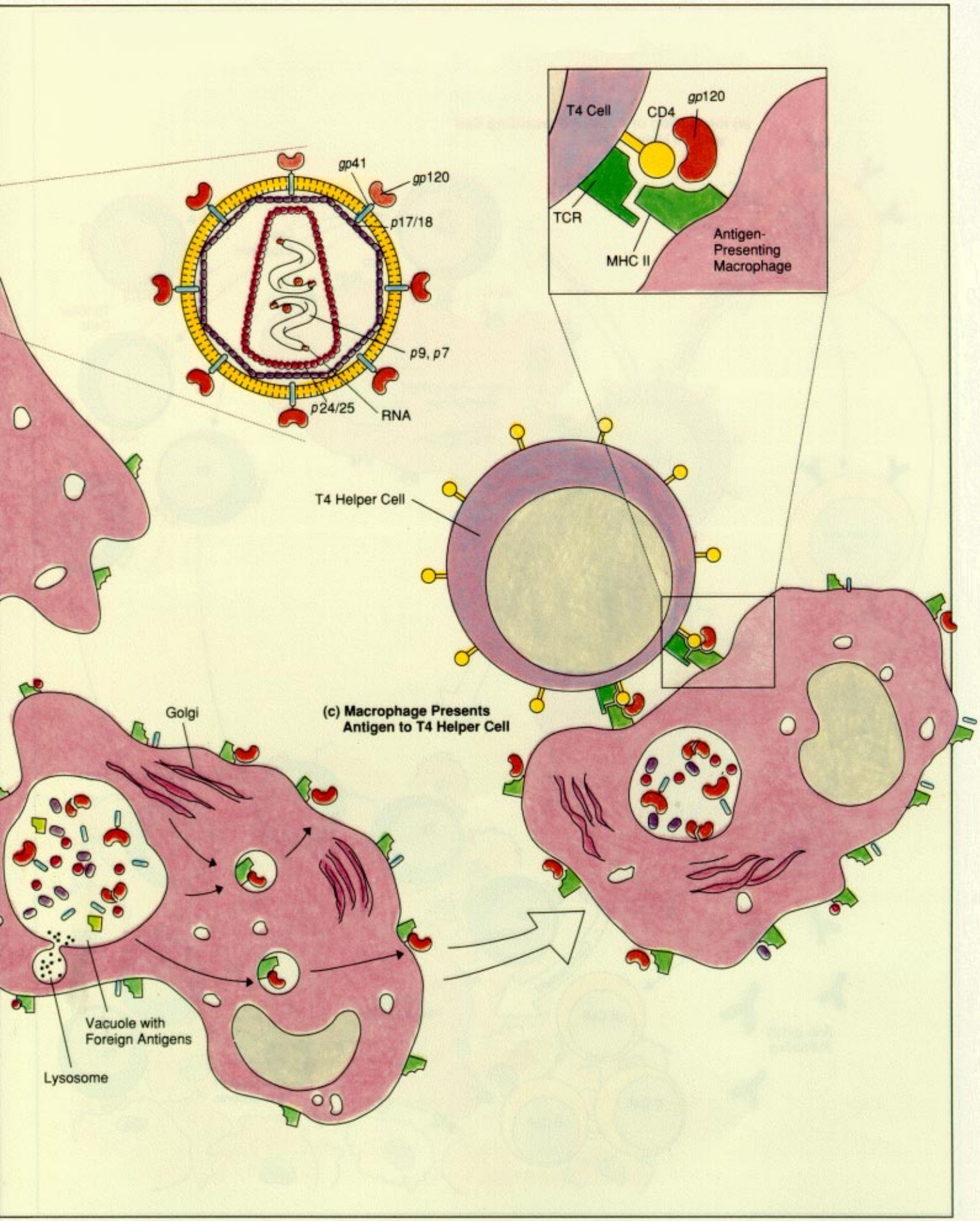
Figure 5 goes on to illustrate the many immune responses induced by the dual recognition between a T4 helper cell and an antigen-presenting microphage. Contact between the T4-cell receptors and the MHC and foreign antigens of the microphage stimulates the T4 lymphocyte to send out chemical instructions to other immune cells. The chemical instructions induce a variety of effects: they activate monocytes and macrophages and thereby enhance their ability to engulf and destroy the invading pathogen; they stimulate cytotoxic lymphocytes, called *T8 killer cells,* to proliferate and kill cells that display the foreign antigens on their surfaces; and they stimulate B lymphocytes (the B stands for bursal or bone marrow-derived) to proliferate and produce antigen-specific antibodies capable of binding to the foreign antigen. The antibodies produced by B lymphocytes help the macrophages and other cells to carry out their function either as scavenger cells or as killer cells.

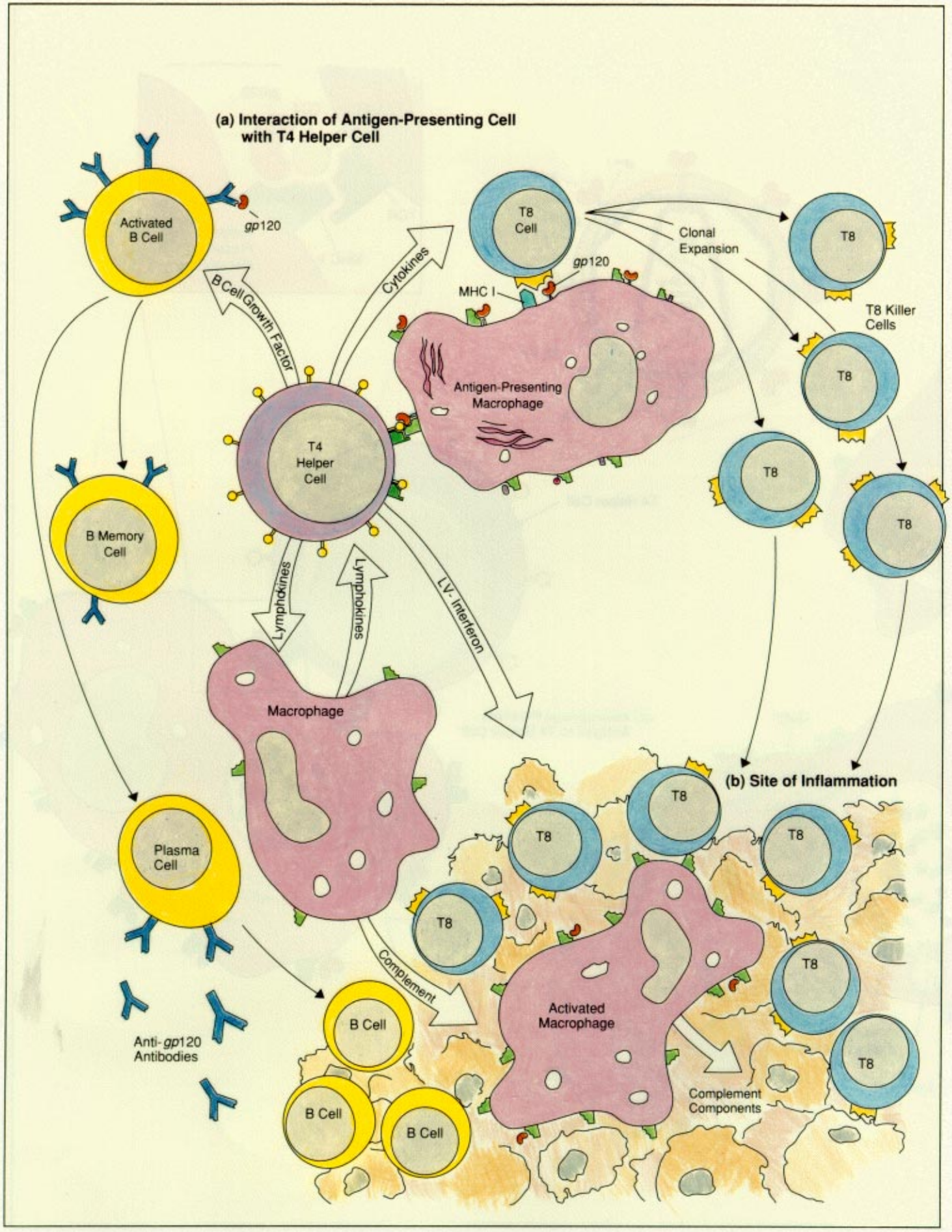
In addition to all this complex activity against a foreign invader, the body must keep the immune response from getting out of hand. Control is accomplished by conveniently activating other T lymphocytes, called *T8 suppressor cells,* which produce chemical messages capable of slowing or stopping the immune reactions. Thus, when the macrophages and lymphocytes interact, they mount

ANTIGEN PRESENTATION TO T4 CELLS

Fig. 4. (a) A microphage engulfs a foreign monocyte infected with HIV. Some virus particles have budded into a vacuole in the infected monocyte. (b) The engulfed cell Is enclosed within a vacuole of the microphage where it is partially digested by Iysosomes and other enzymes. For purposes of illustration, some intact foreign antigens are shown inside the vacuole but, in reality, antigen is broken down into much smaller pieces. Foreign antigens as small as eight amino acids in length, when presented on the microphage surface, may initiate an immune response. The large vacuole breaks up into smaller and smaller vacuoles that bring the foreign products to the cell surface where they are either released or are presented on the surface In conjunction with MHC II antigens produced by the microphage. (c) Finally, the microphage is shown presenting the foreign antigens, in this case *gp120,* to a T4 helper cell. The blowup shows the dual recognition by the T4 cell's receptor CD4 of both MHC II and *gp120.* Note that CD4 appears in conjunction with TCR (T cell receptor) and both are involved in the recognition of MHC II antigens on macrophages and other cells. The dual recognition by the T4 cell of both the self antigen MHC II and the foreign antigen stimulates the T4 cell to orchestrate a defense against the foreign invader.




T4 Cell
CD4
gp120
TCR
MHC II
Antigen-
Presenting
Macrophage
gp41
gp120
p17/18
p9, p7
p24/25
RNA
T4 Helper Cell
(c) Macrophage Presents
Antigen to T4 Helper Cell
Golgi
Vacuole with
Foreign Antigens
Lysosome


(a) Interaction of Antigen-Presenting Cell with T4 Helper Cell
Activated B Cell
gp120
B Cell Growth Factor
Cytokines
T8 Cell
Clonal Expansion
T8
gp120
MHC I
T8 Killer Cells
Antigen-Presenting Macrophage
T4 Helper Cell
T8
T8
B Memory Cell
T8
Lymphokines
Lymphokines
LV- Interferon
Macrophage
(b) Site of Inflammation
Plasma Cell
T8
T8
T8
T8
T8
Complement
Activated Macrophage
B Cell
Anti-gp120 Antibodies
B Cell
B Cell
Complement Components

INTERACTION OF MACROPHAGES AND LYMPHOCYTES

Fig. 5. (a) Immune responses induced by antigen-presenting cells. As in Fig. 4, an antigen-presenting microphage displaying MHC II and foreign antigen interacts with a T4 cell carrying antigen-specific receptors. in this case the foreign antigen is *gp120.* The interaction produces a series of immune responses with the T4 helper cell as the central player, sending out chemical messages to B lymphocytes, T8 killer lymphocytes, and macrophages. At the upper left, a B cell is activated by the binding of *gp120* to an anti-*gp120* antibody on its surface. B cell growth factor from the antigen-driven T4 lymphocyte nonspecifically stimulates the activated B cell to proliferate and mature into memory clones and antibody-secreting plasma cells. A T8 killer cell (top center) is carrying an antigen-specific receptor that recognizes MHC I and *gp120* on the antigen-presenting microphage. The T8 cell also receives chemical messages (cytokines) from the T4 helper cell. Both signals work in conjunction to stimulate proliferation and maturation of the T8 cell either into T8 killer cells that travel through the body and destroy infected cells with cytotoxins or into T8 memory cells. in addition, the T4 cell secretes lymphokines that enhance the ability of macrophages to engulf and destroy infected cells and that stimulate the macrophages to produce some of the so-called complement proteins, which stick to and help destroy foreign invaders nonspecifically. (See Fig. 11 for some functions of complement and antibodies.) Lentiviral infections cause chronic stimulation of immune responses shown here. They, in turn, lead to proliferation of lymphocytes and chronic inflammation of lymph nodes, joints, and other organs containing infected macrophages. (b) The invasion by immune cells at a site of inflammation. One specific response associated with the lentiviral infection of sheep, called visna-maedi, is the secretion by T4 lymphocytes (see figure) of a unique γ-like interferon (a cytokine released by virally infected cells or lymphocytes to protect other cells from viral infection). The γ-like interferon seems to suppress viral replication and at the same time induces a persistently high expression of MHC II and some viral antigens on the surface of infected macrophages. The persistent expression of both MHC II and viral antigen is involved in the overactivation or dysregulation of the immune system and inflammation of lymph nodes and infected organs characteristic of lentiviral infections. Similar mechanisms of inflammation for HIV have not been thoroughly investigated.

a multi-leveled, self-controlled defense against the viral invaders.

Monocytes and macrophages are primary targets of all lentiviral infections especially those of nonprimate species. As we will see below, once infected by a lentivirus, the macrophages chronically stimulate the immune reactions shown in Fig. 5, which, in turn, lead to the abnormal accumulation of immune cells and chronic inflammation characteristic of lentiviral diseases.

In human and nonhuman primates the clinically relevant target of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) is both the monocytes and the cell that we singled out as a key player in the immune system, the T4 helper lymphocyte. A progressive decline in the number of T4 cells is correlated with the progression of AIDS from infection to death. We will address the mystery of that decline later. Here we focus on the mechanism by which the virus enters the cell because it involves the surface receptors we have already met in Fig. 4.

It has been found that HIV and SIV infect T4 cells by binding to a particular protein receptor present in great abundance on the surface of those cells, namely *CD4.* As shown in Fig. 4, the normal function of CD4 is to bind to MHC II on antigen-presenting macrophages and thereby stabilize the interaction between T4 cells and macrophages. CD4 is thus part of the MHC recognition network that assures the body of a controlled destruction of its own cells when they are either infected or tumor-prone. Unfortunately, when HIV meets a T4 helper cell, its envelope glycoprotein *gp* 120 binds to CD4. The stem (transmembrane protein *gp41)* attached to *gp* 120 then inserts itself into the cell membrane, the viral and cell membranes fuse, and the virus dumps its genetic contents into the interior of the cell (Fig. 1). In other words, the virus takes advantage of the recognition network in the immune system to gain entry into cells that are attempting to fight off foreign invaders.

Further research now makes it appear that CD4 receptors are also present on various circulating monocytes, fixed and wandering macrophages, bone-marrow stem cells, B cells, and some T8 killer cells (Fig. 3a). Thus, although it was first thought that HIV is a disease of only the T4 cells, it now appears that their central role in AIDS is mediated through the infection of monocytes and macrophages as well as their bone marrow stem cells.

In addition, studies on the basic science of the immune system have recently provided evidence for an interconnective system between it and the central nervous system. Lentiviral infection of sheep, goat, and nonhuman and human primates have demonstrated strains of the virus that are more neurotropic, that is, more capable of producing neurological disease. Furthermore, studies in the emerging field of neuroimmunology suggest that the two systems have a highly interactive nature. Cell receptors and interactive chemical messages (cytokines) common to both systems are continually being discovered. Numerous reports continue to suggest that HIV, like the other animal lentiviruses, affects the nervous system. This may occur through direct infection of resident brain macrophages (microglial cells), which subsequently alter neural function, or possibly through di-

rect infection of neural cells via CD4 or other receptors common to both the immune and nervous systems.

Very little is currently known about the extent and effect of lentiviral infection in the various microphage subtypes in the body's reticuloendothelial system. Infection of some or all of them may be related to different clinical manifestations of lentiviral disease seen in various species. In any case, AIDS has many features in common with other animal lentiviral diseases. For that reason, a closer, more comparative look at the spectrum of lentiviral diseases is instructive. But before we do that, let's take a closer look at the process of how the lentiviral infection takes place in the whole animal.

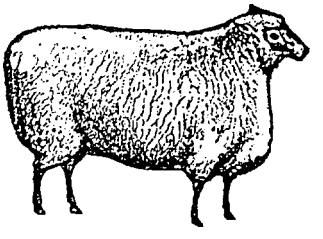
The process of infection. Lentiviral infections are usually introduced into the body by a virally infected foreign cell (Fig. 4). Soon after entry into the host, the infected foreign cell will most likely encounter a strategically located, antigen-trapping microphage of the reticuloendothelial system (Fig. 3b). On the other hand, the infection can be introduced as a cell-free virus, which will most likely interact with the lymphatic system (Fig. 3c). Thus an infected foreign cell will generally be removed by a tissue-fixed microphage, whereas a cell-free virus may infect a lymphocyte. The monocytes or macrophages, although doing their job admirably, are probably infected during their attempts to eliminate the foreign cell or virus.

Once infected, the host's immune cell can begin making or spreading virus. In some tissue-fixed macrophages of various lentiviral infected species, viral replication rates seem to be controlled by a combination of cellular and viral regulatory processes, and the infected cells are usually not destroyed by the infection. However, macrophages infected with certain HIV strains will fuse with neighboring cells, either directly transferring the viral genome or releasing extracellular virus. In some animal lentiviral infections, only infrequently will the macrophages degenerate. In contrast, the infection of other animal species will lead to rapid viral replication and lysis of the macrophages or infected T4 helper lymphocytes. Lysis, or break up, of the cell allows massive release of new virus.

Normal immune interactions shown in Fig. 5 promote the spread of the virus, primarily through cell-to-cell interactions, until a critical majority, if not all, of the susceptible wandering and tissue-fixed macrophages of the reticuloendothelial system are infected. The viral spread may be accomplished through cell fusion, which is facilitated by the binding of *gp120* to CD4 receptors on neighboring cells. Normal interaction between macrophages and lymphocytes may also allow for cell-to-cell transmission of virus. As newborn viruses bud from the cell surface, they coat themselves with the cell's membrane into which they insert their highly sugar-coated, and thereby disguised, envelope receptors. Consequently, the free viruses are poorly recognized as foreign by the immune system, causing the immune responses against them to be ineffectual. Eventually, the immune system is paralyzed as critical immune cells become victimized or eliminated by the virus.

The Animal Models—Examples of Host-Virus Interactions

For any microbe to be a successful pathogen, it must optimize both its rate of producing disease and mortality in the host and its rate of self-replication and transmission. If the two rates are not in balance, then its extinction will be self-assured through natural selection. The slow viruses produce disease in the host only after years of infection. They occupy unique cellular niches, and they are not readily transmitted from host to host without a direct exchange of infected cells or body fluids that contain free virus. Thus lentiviruses have evolved unusual strategies for transmission to assure their procreative investments. Over the past sixty years a few dedicated veterinary and medical researchers interested in persistent viral diseases of various animal species have built a database from which we can derive a multidimensional account of lentiviruses and their various strategies for survival. The variations on the host-virus survival theme seen in animal models lead us to a deeper understanding of the patterns of replication, disease, and adaptation that eventually create successful host-virus relationships in nature. (Not all animal models will be discussed in this article because two have only recently been discovered and others have not been thoroughly researched.)



Visna-maedi

A lentivirus of sheep, visna-maedi, derives its two-word name from the two distinct sets of clinical symptoms it causes: wasting and shortness of breath. These symptoms are associated with dysfunctions of the central nervous and pulmonary systems. We are starting this discussion of animal models with visna-maedi because it caused an epidemic in a manner strikingly similar to that which produced the current worldwide AIDS epidemic. Much like human AIDS, visna-maedi reached epidemic proportions in Iceland in the 1940s, about a decade after the virus was inadvertently introduced into that coun-

BUDDING OF VISNA-MAEDI AND HIV

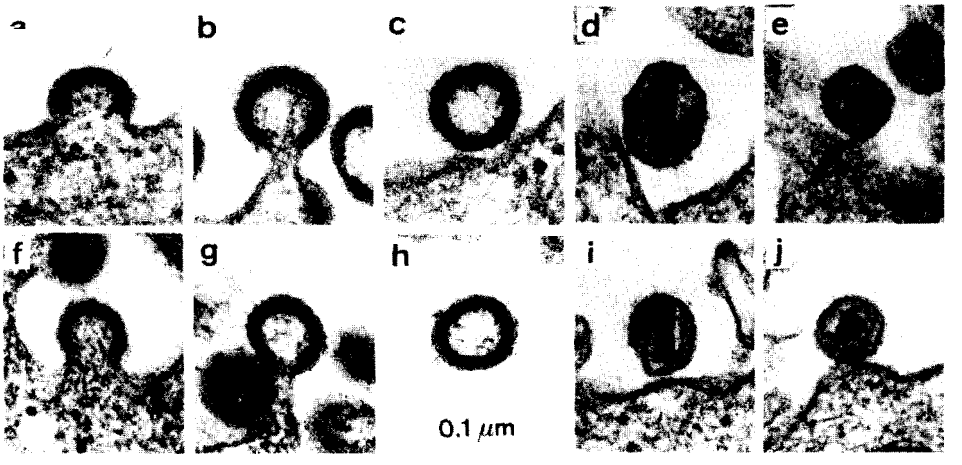


Fig. 6. Transmission electron micrograph of thin sections of cells infected with HIV (top) and vlsna-maedi (bottom). (a, b, f, and g) Virus particles bud at the plasma membrane. (c and h) Immature, extracellular virus particles have not yet assembled viral cores. (d and i) Mature, extracellular virus particles have bar- or cone-shaped cores. (e and j) From other views, the cores of mature, extracellular virus particles have condensed, circular, eccentric shapes. (Photograph courtesy of Dr. M.A. Gonda, Program Resources, Inc., NCI-Frederick Cancer Research Facility. Reprinted from Gonda et al., *Science,* 227:173-177.) Copyright 1985 by the AAAS.

try. In 1933 the Icelandic government imported twenty karakul sheep from a farm near Halle, Germany, with the intention of creating new types of wool through crossbreeding with native Icelandic herds. Small outbreaks of the visna-maedi disease occurred in the late 1930s, and by 1952 over 150,000 sheep had died. In 1957 an Icelandic physician, Bjorn Sigurdsson, reported that a filterable agent (a feature that distinguishes viruses from other pathogens) was responsible for the disease. He introduced the term "slow virus infection" to distinguish this viral disease from other more acute ones.

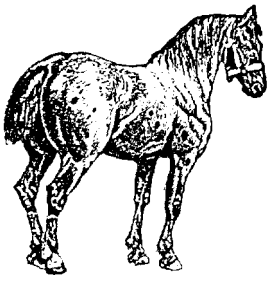
The disease, unlike human AIDS, is usually transmitted among adult sheep through respiratory secretions that contain infected macrophages. (HIV-infected macrophages have been obtained from the lungs of human AIDS patients, although they do not seem to be a major mechanism of transmission.) The vast majority of lambs born to infected ewes are uninfected at birth but become infected after initial suckling of colostrum from the infected mammary glands of the ewe. The visna-maedi virus replicates at the site of entry, generally the lymphoid tissues of the nasal, oral, and upper-respiratory tract, and subsequently spreads via the lymphatic system, the bloodstream, or the cerebrospinal fluid (the fluid in the nervous system). The virus-infected monocytes and macrophages localize in various target organs and cause inflammation and specific pathologies in the lungs, brain, joints, mammary glands, and blood vessels. When first introduced into a nonadapted host (a host that manifests the disease induced by the pathogen as opposed to a host that merely acts as a earner), visna-maedi leads to high rates of morbidity and mortality. After three or four years, most infected nonadapted sheep reach highly diseased states or die, Soon after infection lambs show apparent ill thrift, that is, poor weight gain and abnormalities in muscle development, skin, hair, and central nervous system functioning. In addition some lambs and adult sheep are subject to opportunistic viral and bacterial infections that add to the systemic clinical signs.

The opportunistic infections associated with visna-maedi, however, do not appear to be as life threatening as in human AIDS. Infections with visna-maedi virus can also lead to subclinical, persistent virus-carrier states, as exemplified in the apparently healthy imported karakul sheep. The persistent viral-carrier state is an example of a successful evolutionary adaptation for lentiviruses with their hosts. There is evidence that certain breeds of sheep are more likely to develop primarily carrier states, whereas others seem to have a genetic predisposition for expression of the visna-maedi disease.

The pathogenesis of visna-maedi and AIDS share a number of similarities. Figure 6 shows the similar budding of both viruses from infected cells. In both diseases proviral DNA is carried covertly in monocytes and macrophages; very few cells circulating in the bloodstream are infected (for HIV, possibly one in ten thousand); and the immune reactions of Fig. 5 are chronically induced by infected, antigen-presenting macrophages. The chronic reactions lead to proliferation of lymphocytes and inflammation in the lungs, the central nervous system, and joints (Fig. 7).

There are also significant differences. The profoundly deficient immune state characteristic of AIDS is not observed

in visna-maedi, presumably because visna-maedi virus is confined to monocytes and macrophages and does not noticeably infect or deplete T lymphocytes, Also, the blood from visna-maedi infected animals is not particularly infectious. In contrast, the human AIDS virus does replicate in T lymphocytes circulating in the bloodstream to the extent that cell-free virus and viral antigens are easily detectable in the blood. Consequently the blood and other body fluids of people with AIDS are infectious. In particular, the presence of HIV particles in the blood is detected in HIV-infected individuals both soon after infection and during the later stages of AIDS. This characteristic, as well as the structure of the human placenta, make the human AIDS virus more likely to infect the fetus. Another closely related species, goats, also have a similar lentiviral disease called caprine arthritis encephalitis. The disease affects primarily the joints, central nervous system, and occasionally the lung. Both the virus and its pathogenesis are very similar to visna-maedi.

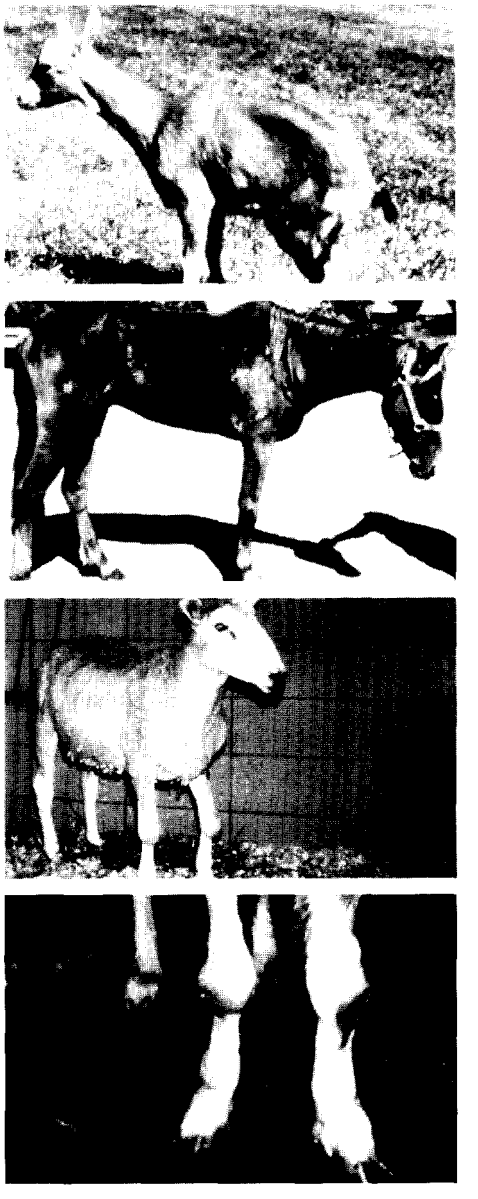


The EIA virus

The earliest known AIDS-like virus is the one that causes equine infectious anemia (EIA), known colloquially as swamp fever. This lentiviral disease occurs in horses and other members of the Equidae family and has been diagnosed worldwide.

The EIA virus is transmitted primarily through blood carried by the biting mouth parts of horse flies and deer flies. This is a particularly efficient transmission process for the virus due to the gregarious social behavior exhibited by the animal hosts. The flies act like flying hypodermic needles, serving solely as mechanical transmission vectors (that is, they physically carry the virus but the virus never replicates in these vectors). The knife-like, slashing mouth parts of the flies cause bites that are painful and elicit host responses, such as tail flicks and shudders, which interrupt the flies's feeding behavior and cause them to move from one horse to another. In this way the virus is disseminated within a herd. This efficient transmission mechanism has evolved for the selection of EIA viral strains that replicate rapidly to high levels of cell-free virus in the blood and are therefore readily transported and transmitted by flies from host to host. Unlike visna-maedi, which results in little cell-free virus in infected sheep, as many as ten million virus particles per milliliter of blood can be found in recently infected horses. The EIA virus, also in contrast to visna-maedi, has been demonstrated to cross the placental barrier and cause fetal infection, although both sheep and horses have placentas containing six functional barriers between the mother and the fetus. The outcome of fetal infection, which is variable and dependent both on the gestational period and stage of infection, leads to either spontaneous abortion or an infected newborn.

The response of adult horses to infection varies, depending on an as yet poorly described host-resistance factor (or factors), viral virulence, and environmental factors, such as stress associated with weather, shipment, and breeding, Infected horses go through various stages of disease. The acute stage, most often associated with the initial exposure to the virus, includes fever and hemorrhages throughout the body within seven to thirty days after exposure. Acute EIA is probably caused



CLINICAL MANIFESTATIONS OF LENTIVIRAL DISEASE

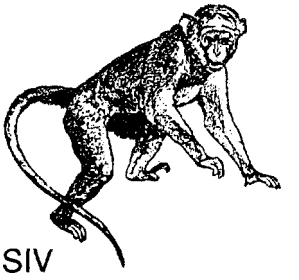
Fig. 7. Animal photographs showing typical manifestations of Ientiviral diseases. Wasting is seen in a goat with caprine arthritus encephalitis (a very close cousin of visna-maedi), and a horse with EIA (top two). Swollen knee joints are seen in a sheep with visna-maedi and another goat with caprine arthritis encephalitis (bottom two). (Photographs by Opendra Narayan of John Hopkins University.)

by the initial high rate of viral replication in monocytes and macrophages, which leads to the destruction of these cells. Large amounts of free virus are present in the blood, but antibodies are not produced rapidly enough to inhibit the spread of the infection, presumably because the rapid destruction of macrophages leaves insufficient time for these antigen-presenting cells to signal the lymphocytes. Subsequently, antibodies that neutralize the virus are made. At the same time, however, other virus variants, the so-called escape mutants that are not neutralized by the antibody, are also produced through viral replication. Details of this process will be presented later.

The less acute stages of EIA (Fig. 7) are the more commonly diagnosed forms of the disease. Clinical symptoms include loss of weight, loss of appetite, anemia, exercise intolerance, weakness, and fluid accumulation (edema). These symptoms recur in biweekly cycles for one to four months. The frequency and severity of the clinical episodes usually decline in time, so that approximately 90 per cent of all that do occur have occurred within one year of infection. The earliest deaths **due** to EIA usually do not occur until four weeks after infection, at which time antibodies to the EIA virus are present in the blood of the animals. Chronically ill horses may suffer acute episodes at unpredictable times. Studies have shown that these recrudescent episodes are sometimes induced by the administration of immune-suppressing drugs or other environmental factors that lead to changes in the immune system. More recently, experimental inoculation with the EIA virus of young Arabian horses suffering from a combined immunodeficiency syndrome characterized by the absence of T and B lymphocytes caused a rapid, virus-induced anemia and death. Both studies demonstrate that horses must have some aspects of a well-functioning immune system to keep the virus under control. Further studies into these controlling mechanisms should provide much insight into successful host-viral interactions.

Many clinical signs of EIA are attributable to changes induced by immune reactions to viral antigens. For example, the anemia (loss of red blood cells) generally associated with morbidity and mortality is caused by the presence of a hemagglutinin-like protein on the surface of free virus that has an affinity for red blood cells. Once a virus binds to these cells, antibodies directed against the virus also bind to the red blood cells. This binding stimulates a cascade of chemical reactions in the blood serum that produce *complement.* These special proteins accumulate around the activated site on the cell and tear or lyse the cell membrane, thereby destroying the cell. Even in the absence of antibodies, viruses bound to red blood cells will attract and bind complement, resulting in the same destruction. As in visna-maedi disease and AIDS, the immune system is chronically activated and immune cells proliferate or accumulate in all organs of horses dying of the disease. The animals also have hemorrhages and enlarged spleens, livers, and lymph nodes. Most infected horses, however, control their viral infection and exist for many years as healthy carrier animals, serving as a long-term source of the EIA virus, as do the adapted sheep for visna-maedi.

Whether EIA virus infects lymphocytes, as does HIV, is not completely resolved. Cell-free virus in the blood, characteristic of the first stages of EIA, also can occur in humans immediately after infection with HIV and is frequently seen in the later stages of AIDS. Since human placentas have only three functional barriers between the fetus and the mother, compared to the six in horses, HIV has a greater chance of infecting human newborns than does the EIA virus have of infecting a foal.



SIV

The Simian immunodeficiency virus, another prototypic lentivirus, infects numerous nonhuman primate species in central and western Africa. This group of viruses, originally isolated from captive rhesus monkeys in 1985, represents the closest known relatives of the human AIDS viruses. (In particular, SIV is more closely related to HIV II than to HIV I.) SIV and the HIVs have very similar genomes and similar biological and antigenic properties (for example, when the purified viral proteins of each virus are injected into laboratory animals, they induce the production of antibodies that recognize each other's proteins).

The natural history of SIV in healthy, free-roaming African primates is best exemplified by the strains found in the African green monkey, the sooty mangabey, the mandrill, the deBrazza monkey, the Sykes monkey, the talpoins, the quereza colobus, and baboons. All of these strains of SIV live in their respective naturally adapted African primates without producing overt clinical disease. Their presence in the naturally adapted species is detected by the isolation of viral particles from cultured blood lymphocytes and by the presence of antibodies circulating in the bloodstream. Detailed studies of the natural transmission in the adapted species are lacking. However, serological tests of the animals in their native habitats show that not all members of a species have antibodies. Further studies are underway to test susceptibility to

the virus of African green monkeys that do not test positive for the antibody. It would be presumed that through various exchanges of tissue, blood, and bodily fluids during species-specific behaviors, such as territorial and reproductive encounters, as well as integration of the virus into the host genome, the virus is assured continued survival within the species. The evidence of numerous genetically distinct SIVs infecting numerous nonhuman primate species strongly suggests that the primate lentiviruses have existed for long evolutionary times.

In the late 1970s and early 1980s, a number of primate centers began experimental manipulation of African primates to study the biology of lymphoma and leukemia as well as leprosy. Unbeknown to those researchers, the studies provided the arena for the unnatural transmission of various host-adapted African SIVs to nonadapted Asian primates, such as rhesus and pigtail monkeys. Some months to years following the studies, a wasting, debilitating disease, characterized by opportunistic infections as well as tumors, occurred among the primate colonies involved. That somewhat unfortunate event had the serendipitous outcome of creating a very useful primate model for studying AIDS.

If the scenario just described for nonhuman primates is extended to humans, it would suggest that the AIDS epidemic had its origins in Africa, where either ancestral co-evolution or natural virus transmission from adapted nonhuman primates to humans created populations that were at various stages of natural adaptation with an ancestral AIDS-like virus. Whatever the evolutionary route, the result was a worldwide population of susceptible and nonsusceptible humans capable of transmitting the AIDS virus to other humans elsewhere. The cosmopolitan nature and urbanization of Africa and various third-world nations in the 1970s and 1980s then facilitated the transmission of the virus from adapted human HIV carriers to nonadapted members of the species, just as the well-intentioned importation of sheep to Iceland allowed the transmission of visna-maedi.

One or all of the following viral-anthropological schemes may have occurred in Africa and led to the current worldwide epidemic. An ironic possibility is that the AIDS virus was additionally introduced to less adapted Africans through the intense interaction between native Africans and their indigenous primates initiated by the scientific demand in the United States and elsewhere for wild, caught primates to be used in human hepatitis studies in the late 1950s and the 1960s. Anyone experienced and working with primates can tell you of the numerous combative instances that occur during capture. The deep cutaneous wounds from bites and scratches that often occur would serve nicely for introduction of cell-free virus or virally infected cells into the human population. (It is interesting to note that all isolated SIVs grow readily in vitro and kill cultured human T4 helper cells. Therefore, identical biosafety precautions are taken when handling SIV as when handling HIV.)

Another possible transmission route from nonhuman primates to humans involves more customary cultural practices—such as ancestral tribal rituals or hunting primates as a source of sustenance.

An equally plausible explanation for the presence of HIV in central Africa would be the parallel evolution of an HIV-like, host-adapted SIV and modern man, Central Africa is considered by anthropologists to be the evolutionary birthplace of man. Perhaps some ancestors of modern man such as the chimpanzees or gorillas were co-evolving with a host-adapted, HIV-like virus, while others were evolving with a host-adapted, HIV-like SIV, and still other ancestral human primates were not evolutionarily linked to either SIV or HIV. Thus, the stage would be set for very susceptible, moderately susceptible, and less susceptible populations of humans as one sees in the primates and the other animal species previously described. Evidence for such distinct populations will await more extensive and detailed studies.

Ongoing studies in primate centers suggest that, like HIV, SIV can be transmitted through blood containing infected cells or free virus. Whether transmission occurs primarily through semen or other body fluids has not yet been completely characterized. When cell-free SIV is introduced into the vagina of a normal rhesus monkey, the monkey becomes infected as efficiently as when these animals receive virus in the blood. Following entry into the host, the human or simian AIDS viruses infect T4 lymphocytes, monocytes, and macrophages of the monkey, again through the CD4 receptors on the surfaces of these cells. In contrast, recall that the target cells of visna-maedi and the EIA virus are primarily macrophages.

The clinical signs of nonadapted SIV-infected primates parallel those of HIV-infected humans more closely than the other animal models previously described. A prominent swelling of the lymph nodes throughout the body, diarrhea, fever, lack of appetite, depression, inactivity, loss of weight, and necrologic complications characterize the early and later stages of the disease. As the disease progresses, the clinical manifestations caused by the initial viral infection become difficult to identify because they become mixed with those caused by systemic, opportunistic infections that are often fatal.

Also like HIV infection in humans, SIV infection of nonadapted primates leads to progressive loss of T4 helper cells, resulting in severe immune sup-

Table 2

Clinical Manifestations of Lentivirus Infections in Natural Hosts

Host	Lentivirus	Disease Description
Sheep	Visna maedi, progressive pneumonia virus	Generalized wasting Chronic encephalomyelitis Progressive lethal pneumonia Spasticity Paralysis Lymphadenophathy (swollen lymph nodes) Opportunistic infections
Goat	Caprine arthritis encephalitis virus	Generalized wasting Chronic leukoencephalomyelitis Progressive arthritis Osteoporosis Paralysis
Horses	Equine infectious anemia virus	Fever Intermittent anemia General proliferation of lymphoid cells in retriculoendothelial system Glomerulonephritis
COW	Bovine immunodeficiency-like virus	Persistent lymphocytosis Lymphadenophathy Wasting Central nervous system lesion
Cat	Feline T-lymphotrophic virus	lmmunodeficiency-like syndrome Generalized lymphadenopathy Leukopenia Fever Anemia Emaciation
Monkey	Simian immunodeficiency virus (SIV)	lmmunodeficiency Neutropathologic changes Wasting Opportunistic infections
Human	Human immunodeficiency virus (HIV)	lmmunodeficiency Opportunistic infections Lymphadenopathy Encephalopathy Kaposi's sarcoma

pression. It is interesting to note that a particular SIV strain taken from adapted primate species rapidly induced (within seven to fourteen days) viral-associated death in unrelated nonadapted species, whereas other SlVs induced death and disease in nonadapted species only after months and sometimes years. Perhaps some evolutionary relationship exists between a species and its overall susceptibility to lentiviral disease.

At this point we can summarize this