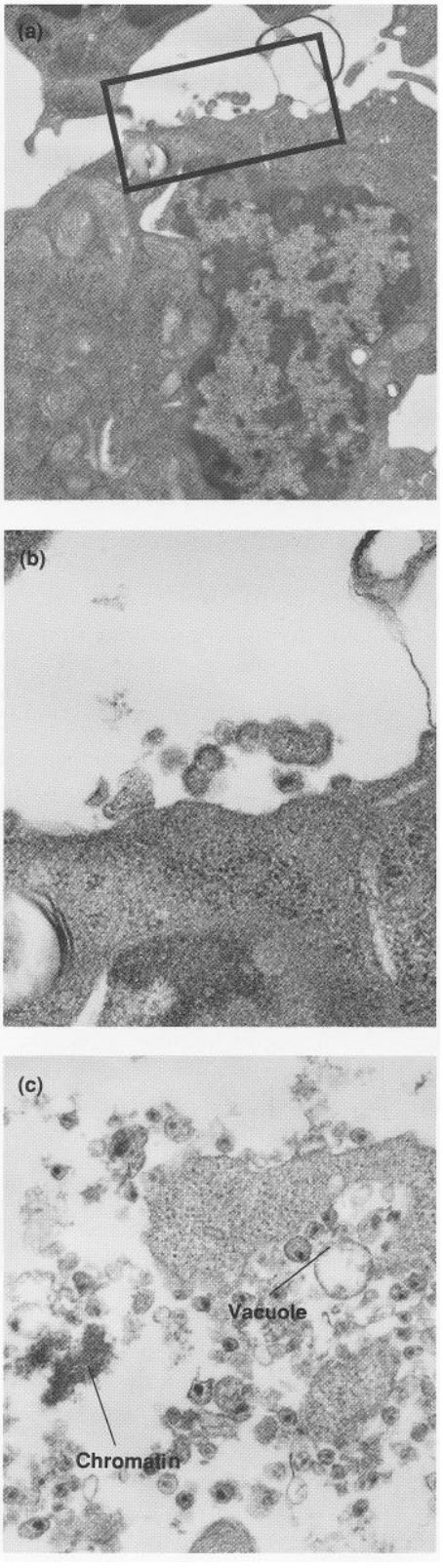
chimpanzee remains functionally intact during the persistent HIV I infection.

Studies following in vitro infection with HIV I show that all normal T4 lymphocytes from the blood of uninfected chimpanzees are capable of controlling the replication of the virus so that only small numbers of complete infectious virus particles are produced at any one time in these cells (Fig. 13). Also, the viral infection does not appear to kill the lymphocytes. More important, the monocytes and macrophages derived from the blood- and bone-marrow stem cells of experimentally inoculated animals do not appear to be readily infected by the virus. In support of this in vivo observation is the fact that current attempts to infect cultured chimpanzee monocytes and macrophages with human monocyte HIV I variants have failed. One other interesting finding is the naturally larger concentration of circulating T8 cells in the blood of chimpanzees. That population of cells in uninfected chimpanzees may be responsible for the antiviral controlling effect against HIV I. In contrast, in vitro studies of lymphocytes from the blood of HIV-infected humans and SIV-infected monkeys show that the presence of T8 lymphocytes seems to slow down but does not eliminate viral replication in the other lymphocytes.

The fundamental differences in the chimpanzee's response to HIV infection are being actively explored for application to the prevention and treatment of the human condition. Studies of the other SIV-adapted African primates mentioned previously should also contain clues to the various factors responsible for the carrier state present in these animals. As described in the section on SIV, the African green monkey and various other African primates appear to be successfully adapted to their virus, whereas the same virus when put into Asian primates leads to AIDS. The HIV I-infected chimpanzees also appear to be successfully adapted to HIV I. That fact suggests that an ancestral HIV I-like virus should be present in wild chimpanzee populations in central Africa. Furthermore, it looks like HIV I-infected humans are the counterpart to the SIV- infected Asian monkeys.



CONTROLLED VIRAL REPLICATION IN CHIMP LYMPHOCYTES

Fig. 14. Transmission electron micrographs of an HIV I-infected lymphocyte from a chimpanzee. The cell was taken from a culture that had been infected for 5 days and was at maximum virus production. (a) Only the boxed area of the cell membrane of this peripheral blood lymphocyte could be found budding HIV I particles (magnified 3500 times). (b) The boxed area in (a) at a magnification of 10,000. (c) To contrast with the chimp lymphocyte, we show a completely degenerated portion of an HIV-infected human lymphocyte magnified 10,000 times. Note the remaining portions of nuclear chromatin and cytoplasmic vacuoles as well as the presence of numerous viral particles.

Prospects for an AIDS Vaccine

Given our current understanding of lentiviral infections, it appears that conventional vaccine strategies are unsuitable for direct application to the prevention of lentiviral diseases. Since the time of Jenner and Pasteur, all successful human and animal antiviral vaccines have been made either from virus attenuated in tissue cultures (called modified-"live" virus particles), or from a part or parts (termed subunit) of the virus that activates the immune system. The vaccines confer immunity by eliciting what is termed an *anamnestic response,* which basically means to "not forget." On subsequent introduction of wild-type virus (other than lentiviruses) into the body, infection occurs, but the immune system, previously sensitized through vaccination, rapidly responds and elim-

inates the viral invader. The success of these vaccines was due primarily to the nature of the viruses involved. Viruses successfully blocked by vaccine-induced protection generally do not integrate themselves into the host's genome and do not exclusively parasitize the immune system of the body. A prototype for a subunit retroviral-based vaccine has been developed for cats against feline leukemia virus infection. However, further work is still required to optimize its usefulness and application to the prevention of lentiviral diseases. Recently, studies with a formalin-fixed Type D retrovirus were found to completely protect primates from a simian Type D retrovirus. As yet no vaccines against any of the retroviruses have been made from approaches using modified-live virus.

The immune activation induced by a successful vaccine is controlled by the immune system's activator and suppressor networks. With time, the induced protective state falls to levels of nonprotection, but the state is permanently programmed into the immune system's memory network. Reinfection of the host cells with the real pathogenic viral agent causes release of chemical signals that rapidly recruit and deploy the appropriate memory cells. These cells produce antibodies, T4 cells, and T8 cells that eliminate the virus before it can spread to a critical number of susceptible target cells and cause significant life-threatening disease.

A key point with regard to AIDS is the fact that the immune response generated by our current viral-based vaccines does not always prevent the initial infection. Since HIV is capable of integrating itself into the genetic material of infected cells, a vaccine would have to produce a constant state of immune protection, which could totally block the initial infection of the host cells at all times. Such complete and constant protection has never before been accomplished and works in direct opposition to the normal immune suppressor network, which dampens, or turns down, specific immune responses. A state of perpetual immune activation may have as yet undefined detrimental consequences to the host. Only through extensive and creative research will we be able to design the type of new generation vaccines required to defeat the AIDS virus and its distant relatives.

Analysis of the comparative spectrum of lentiviral diseases of animals and man are providing important clues to pathogenesis and host response. The nonsusceptible versus susceptible states that occur, respectively, in adapted versus nonadapted species need to be studied further. For lentiviral diseases it appears that protective immunity will not be conferred through the classical immune mechanisms but rather will require a state similar to that of host adaptation. The mechanisms of host adaptation has never been investigated nor understood in detail. Now the investigation of such mechanisms seems urgent. We will have to understand novel viral-controlling mechanisms, the nature of the immune state that prevents the initial infection, and methods for establishing persistent but nondeleterious states of protective immune activation. As yet, no antiviral vaccine can claim such protection. Nevertheless, continued studies of all lentiviruses, as well as other primate retroviruses, will surely reveal important clues to the understanding and control of the disease process in the animal kingdom. ■

Table 5

Problems with Vaccine Development against HIV

1. Integration of HIV genetic material into cellular DNA.
2. Regulatory genes responsible for controlled, low-level viral expression.
3. Similar cell populations serve as both targets of viral infection and vehicles of immune protection.
4. Viral activation and spread from antigen-presenting macrophages to T cells during normal immunogenic episodes.
5. Molecular mimicry between viral envelope proteins and MHC II molecules.
6. Rapid rate of envelope mutations.
7. Hypervariability of a single immunodominant neutralization epitope may act as a decoy to antibody producing cells.
8. Viral envelope proteins are poor immunogens due to high carbohydrate content.
9. Rapid shedding of viral envelope glycoproteins.
10. Cell-to-cell fusion, resulting in transmission of viral RNA without complete assembly of virus particles.
11. Presence of antibody induces viral latency.
12. Absence of complement-mediated cytolysis or direct complement activation.
13. No efficacious vaccine developed for any lentiviral infections of other species.
14. The need to maintain a constant level of protective immune activation in the face of an immune system suppressor network.

The Search for Protective Host Responses

How does a host usually develop a state of protection against an invading virus? Three major host responses to invading viruses include activation of complement, production of neutralizing and complement-fixing antibodies, and cell-mediated immune responses. Traditionally, when a new viral disease is recognized in a species, efforts to understand the protective immune states are derived from its surviving members. These individuals serve as immune benchmarks, and subsequent studies often reveal important clues to the eventual production of a vaccine. Here we will review studies of the major antiviral immune responses to HIV and see that none of them are completely effective, although some avenues of developing traditional vaccines for AIDS are still open.

Complement. One possible response to HIV would be the activation of the complement system, known to be a powerful, continuous, ever-present, microbe-eliminating system (see Fig. 11 in the main article). Complement is a group of serum proteins circulating in the bloodstream that bind to, become activated, and destroy invading microbes by creating holes in their surface membranes. Complement proteins are synthesized by activated macrophages, liver cells, and epithelial cells. Complement inactivates some Type C oncoviruses directly due to the presence of as yet undefined receptors on the viral envelopes. Complement can also work in conjunction with antibodies. The antibodies produced in response to a viral infection may bind both to complement and to the virus or virally infected cells, resulting in destruction of the intruder. The destruction of virally infected cells through this mechanism is called antibody-dependent, complement-mediated cytolysis (ACC). The lentiviruses as a group, unfortunately, appear to be resistant to destruction by complement.

Studies performed in my laboratory in 1987 showed no evidence of ACC activity in humans at various stages of AIDS despite the presence of large amounts of antibody directed against both HIV and HIV-infected cells. The absence of ACC is also documented in visna-maedi. No ACC activity has been reported for the other animal-lentivirus systems mentioned here. Both my lab and others have shown that human complement is incapable of inactivating HIV either directly or in the presence of neutralizing antibody. Recently, we have discovered a heat-sensitive serum factor in various laboratory and wild animal species that does inactivate the human AIDS virus in vitro. Further studies are underway to characterize this factor, or factors, and to understand how to recruit its activity in humans and why human complement does not work against HIV.

Neutralizing Antibody. Neutralizing antibodies have been shown to be one of the major lines of defense in viral diseases of human and other animals. Following the infection of the host by the AIDS virus, plasma cells produce antibodies directed against various parts of the virus. The antibodies are of two major types, functional (when they bind to the virus they inactivate or destroy it) and nonfunctional. A nonfunctional antibody recognizes various parts of the virus; however, they do not mediate any antiviral effects in vitro or in vivo. Also the nonfunctional antibodies can coat the virus and thereby block or interfere with otherwise effective antibodies, such as neutralizing or complement-fixing ones. An antibody that is coating a virus can also, as previously described, facilitate entry of the virus into monocytes and macrophages, thus infecting these cells. Some evidence for this type of antibody-facilitated infectiv-

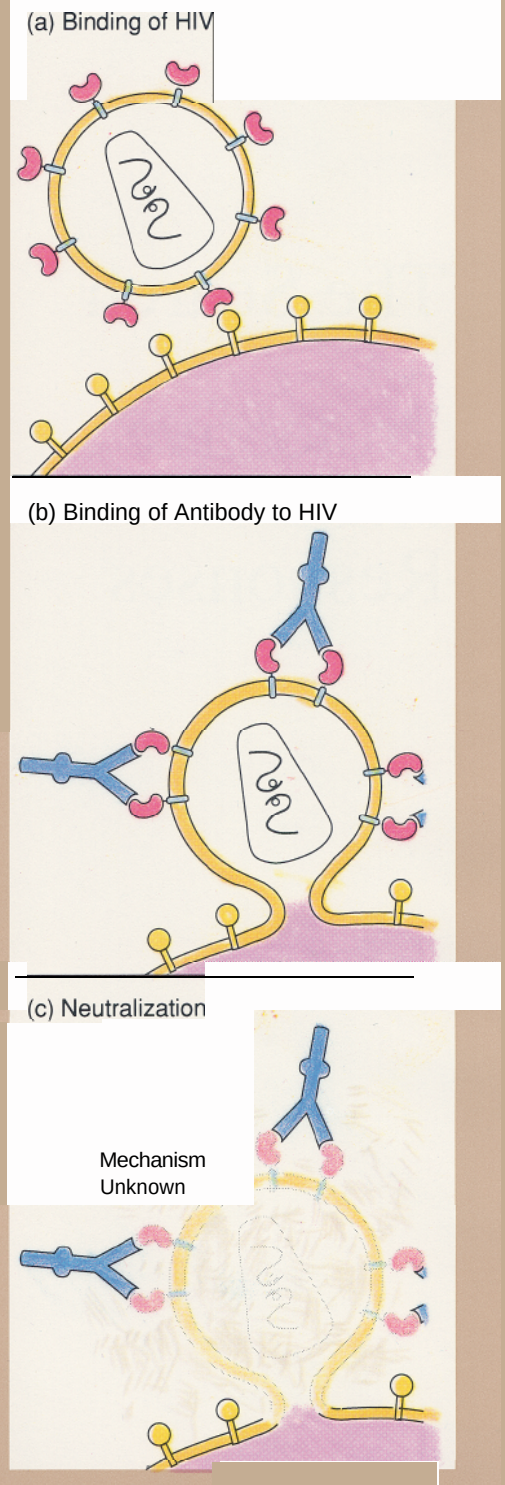
ity has been reported in the visna-maedi system.

Functional neutralizing antibodies are produced by plasma cells derived from B lymphocytes that have been specifically activated against a particular antigen. We have already indicated that the ability of the neutralizing antibody to inactivate lentiviruses is highly variable. Horses infected with the EIA virus make antibody that is capable of neutralizing the initial infecting viral strain. However, antigenic drift eventually produces viruses that can avoid those neutralizing antibodies.

Our information on visna-maedi is more detailed. In vitro, antibody against visna-maedi is capable of neutralizing the virus so that it cannot infect a cell. However, neutralization of the virus occurs only if the virus and antibody are allowed to interact for 15 minutes or longer before being introduced to the target cells. It appears that after this preincubation the antibody prevents the virus from attaching to the sheep's cells. However, when the virus and antibody are added to the target cells simultaneously, no neutralization of the virus occurs. These studies suggest that the antibody produced during the infection is not biologically functional in vivo. In the host the virus probably encounters and infects target cells before neutralizing antibody has sufficient time to neutralize it. The virus's escape from antibodies appears to be related to the high sugar content of the viral envelope proteins, which conceal neutralization epitopes (protein shapes that serve as antibody binding sites).

Fortunately, the neutralizing antibody present in HIV-infected humans, HIV-infected chimpanzees, and animals that have been vaccinated with the viral envelope protein *gp 120* are more effective. Recent detailed kinetic studies in my laboratory revealed that the serum from these hosts rapidly neutralizes the virus. Subsequent infectivity studies with HIV I demonstrated that the virus can be neutralized at various times, even after it has attached via the CD4 receptor to the host cell (Fig. 1). It appears that the virus binds to susceptible lymphocytes at the diffusion-limited rate of 4.0×10^{-9} M (see "The Kinetics of Viral Infectivity"). After binding, however, the virus only slowly enters the cell by the fusion process. Thus, neutralizing antibody is capable of neutralizing the virus during the 30 to 60 minutes between binding and entry into the lymphocyte. This is a singularly encouraging finding for vaccine development. However, only the sera from HIV-infected humans or HIV-infected chimpanzees were capable of neutralizing more than one HIV I strain. Moreover, these strains may only be a subpopulation of the virus present in any one infected individual. Studies of the role of neutralizing antibody in preventing infection of monocytes and macrophages will have to await the development of new assay methods.

Our studies also show that neutralizing antibody derived from sera of goats infected with the purified envelope of one HIV strain is also capable of neutralizing the virus either before or after it has bound to a target cell. The major limiting feature however was the narrow specificity of the neutralizing antibody produced. We, in collaboration with Jaap Goudsmit, Scott Putney, and others, have discovered that neutralizing antibody reacts only with the immunodominant neutralizing epitope of *gp* 120 shown in Fig. 2. Further, this portion of gp120, which is about 30 amino acids in length, appears to be changing its amino acid content rapidly in infected humans and more slowly in chimpanzees. In particular, even the first viruses isolated from chimps infected with a specific and well-characterized human AIDS virus were resistant to a typing sera made



NEUTRALIZATION OF HIV

Fig. 1. In vitro studies suggest that neutralizing antibody against HIV can neutralize the virus even after it has bound to a target-cell membrane. The figure shows neutralizing antibodies attaching to the viral envelope after the virus has bound to and begun to fuse with the cell membrane. The antibodies somehow prevent infection, but the details of the neutralization mechanism are unknown.

HIV ENVELOPE GLYCOPROTEINS

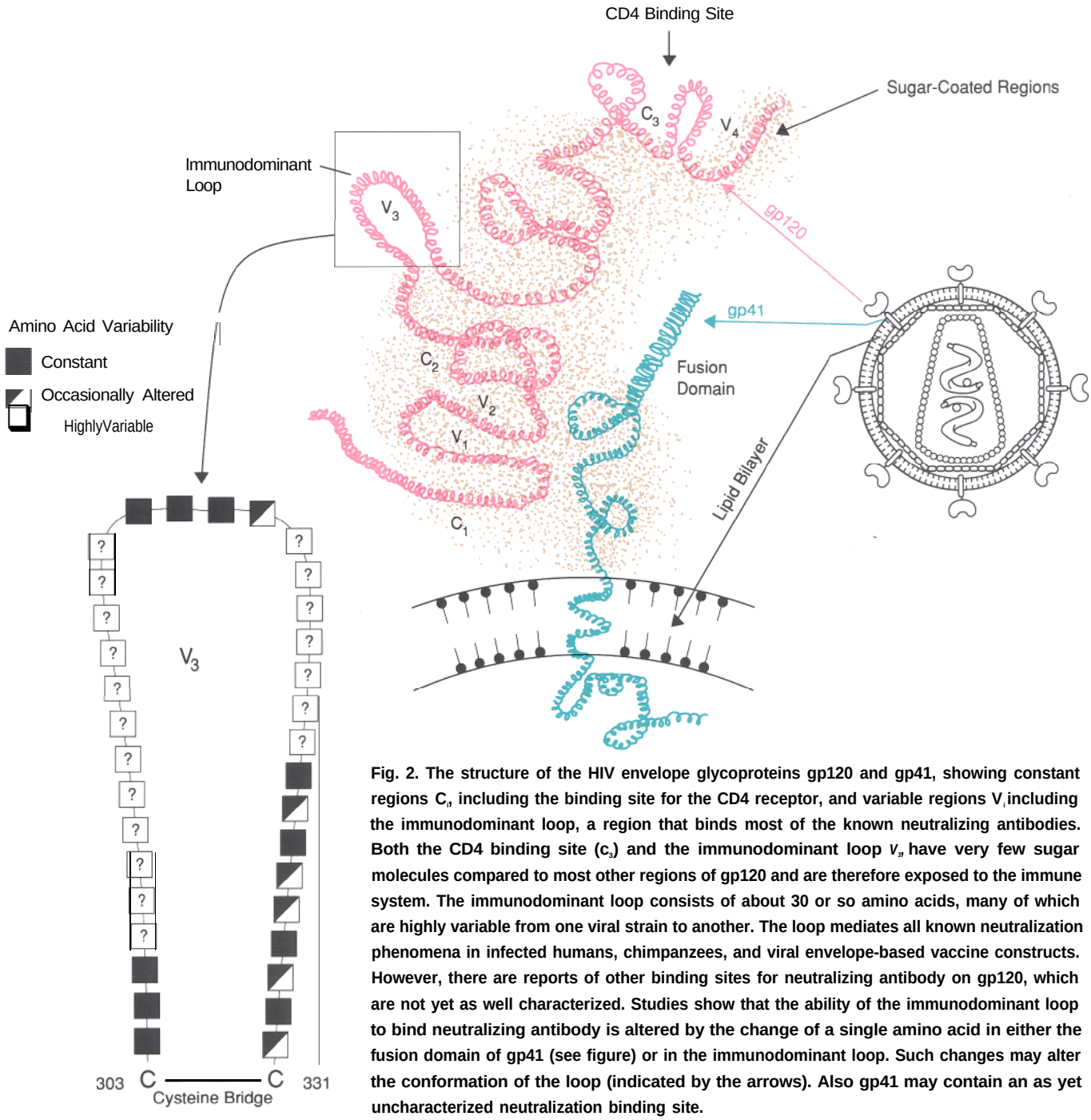


Fig. 2. The structure of the HIV envelope glycoproteins gp120 and gp41, showing constant regions C_i, including the binding site for the CD4 receptor, and variable regions V_i including the immunodominant loop, a region that binds most of the known neutralizing antibodies. Both the CD4 binding site (C_3) and the immunodominant loop V_3 have very few sugar molecules compared to most other regions of gp120 and are therefore exposed to the immune system. The immunodominant loop consists of about 30 or so amino acids, many of which are highly variable from one viral strain to another. The loop mediates all known neutralization phenomena in infected humans, chimpanzees, and viral envelope-based vaccine constructs. However, there are reports of other binding sites for neutralizing antibody on gp120, which are not yet as well characterized. Studies show that the ability of the immunodominant loop to bind neutralizing antibody is altered by the change of a single amino acid in either the fusion domain of gp41 (see figure) or in the immunodominant loop. Such changes may alter the conformation of the loop (indicated by the arrows). Also gp41 may contain an as yet uncharacterized neutralization binding site.

from goats immunized with *gp* 120 of the original innoculated virus as well as sera from other chimps infected with the original virus. We are currently studying the amino acid sequence of the relevant pieces of the viral envelope protein in an effort to identify the location and the types of changes that occur during viral replication. Additional collaborative studies in our lab now indicate that other sites in the viral envelope also must contribute to the interaction between the neutralizing antibody and the immunodominant loop (see Fig. 2). When completed, the molecular study of the viral-envelopes from chimp isolates will provide a map of the mutation sites and allow for a better understanding of its complexity. Perhaps we will be able to identify a limited number of locations and variations that cover the spectrum of gp120 variations made during viral replication. We might then be able to manufacture an anti-gpl20 vaccine that would be effective against all these variations.

Cell-mediated Immunity. We have just discussed the ineffectiveness of both complement and neutralizing an-

tibody in preventing infection by cell-free HIV particles. Finally, we turn to cell-mediated responses. As mentioned earlier, T8 killer cells are designed to destroy infected cells and are activated by T4 helper cells. The activation occurs when the T4 cells recognize an MHC-lentiviral antigen pair on the surface of infected macrophages and lymphocytes. The T8 cells then circulate around the body and kill any cells of the body displaying both the MHC and viral proteins. K, or killer cells (a subset of lymphocytes), and certain T cells can also destroy virally infected cells that do *not* present MHC antigens on their surface. One such mechanism, called antibody-dependent cell-mediated cytotoxicity, is the capacity of various antiviral antibodies to bind to the infected cells and thus direct the viral-killing K cells to them (see Fig. 10 in the main article).

Most HIV-infected humans display all these antiviral immune mechanisms and still progress to disease and death. One clue to their ineffectiveness may be the discovery that parts of the envelope of the feline leukemia virus, a member of the oncoviral subfamily, seem to suppress these antiviral immune strategies, thus adding to the persistence of the virus in the cat's body. Some reports suggest that the envelope of HIV may have a similar effect on the human immune system. Thus we have one possible explanation for the ineffectiveness of cell-mediated immune mechanisms against HIV. However, there have been no reports of other similar immunosuppressive effects for the other lentiviral infections of animals.

This short review of protective immune responses suggests that protection against HIV, if it can be developed, will probably have to involve various undefined elements of host-virus adaptive responses in addition to the known antiviral immune responses. ■

Further Reading

Scientific American October 1988. This entire issue is devoted to articles on AIDS, and each article has a valuable reading list of its own.

The Control of Human Retrovirus Gene Expression, edited by B. Robert Franza, Jr., Bryan R. Cullen, and Flossie Wong-Stall. 1988. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Timothy B. Crawford, William P. Cheevers, Paula Klevjer-Anderson, and Travis C. McCuire. 1978. Equine infectious anemia: virion characteristics, virus-cell interaction, and host responses. In ***Persistent Viruses: ICN-UCLA Symposia on Molecular and Cellular Biology, Volume*** XI, 1978, edited by Jack G. Stevens, George J. Todaro, and C. Fred Fox, pp. 727-749. New York: Academic Press.

Alfred S. Evans. 1989. Does HIV cause AIDS? An historical perspective. ***Journal of Acquired Immune Deficiency Syndromes 2:*** 107-113.

Anthony S. Fauci. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. ***Science 239:617-622.***

Matthew A. Gonda. 1986. The natural history of AIDS. ***Natural History 95:4.***

Ashley T. Haase. 1986. Pathogenesis of lentivirus infections. ***Nature*** 322:130-136.

William A. Haseltine, Ernest F. Terwilliger, Craig A. Rosen, and Joseph G. Sodroski. 1988. Structure and function of human pathogenic retroviruses. In ***Retrovirus Biology: An Emerging Role in Human Diseases,*** edited by Robert C. Gallo and Flossie Wong-Stall. New York: Marcel Dekker, Inc.

Peter L. Nara, W. Gerard Robey, Larry 0. Arthur, Matthew A. Gonda, David M. Asher, R. Yanagihara, Clarence J. Gibbs, Jr., D. Carleton Gajdusek, and Peter J. Fischinger. 1987. Simultaneous isolation of simian foamy virus and HTLV-III/LAV from chimpanzee lymphocytes following HTLV-II or LAV inoculation. ***Archives of Virology*** 92:183-186.

Peter L. Nara, W. Gerard Robey, Matthew A. Gonda, Stephen G. Carter, and Peter J. Fischinger. 1987. Absence of cytotoxic antibody to human immunodeficiency virus-infected cells in humans and its induction in animals after infection or immunization with purified envelope glycoprotein gpl20. ***Proceedings of the National Academy of Sciences*** 84:3797-3801.

Peter L. Nara, William G. Robey, Larry 0. Arthur, David M. Asher, Axe1 V. Wolff, Clarence J. Gibbs, Jr., D. Carleton Gajdusek, and Peter J. Fischinger. 1987. Persistent infection of chimpanzees with human immunodeficiency virus: serological responses and properties of reisolated viruses. ***Journal of Virology*** 61:3173-3180.

P. L. Nara, W. C. Hatch, N. M. Dunlop, W. G. Robey, and P. J. Fischinger. 1987. Simple, rapid, quantitative micro-syncytium forming assay for the detection of neutralizing antibody against infectious HTLV-III/LAV. ***AIDS Research and Human Retrovirus 3:283-302.***

P. L. Nara and P. J. Fischinger. 1988. Quantitative infectivity microassay for HIV-l and ***-2. Nature*** 332:469-470.

Peter L. Nara. 1989. HIV-1 neutralization: evidence for rapid, binding/postbinding neutralization from infected humans, chimpanzees, and gpl20-vaccinated animals. In ***Vaccines 89: Modern Approaches to New Vaccines Including Prevention of AIDS,*** edited by Richard A. Lemer, Harold Ginsberg, Robert M. Channock, and Fred Brown, pp. 137-144. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

P. L. Nara, W. Hatch, J. Kessler, J. Kelliher, S. Carter, J. Ward, D. Looney, G. Ehrlich, H. Gendleman, and R. C. Gallo. 1989. The biology of HIV-IIIB infection in the chimpanzee: in vivo and in vitro correlations. ***Journal of Medical Primatology,*** in press.

0. Narayan, M. C. Zink, D. Huso, D. Sheffer, S. Crane, S. Kennedy-Stoskopf, P. E. Jolly, and J. E. Clements. 1988. Lentiviruses of animals are biological models of the human immunodeficiency viruses. ***Microbial Pathogenesis 5:*** 149-157.

G. Petursson, P. A. Palsson, and G. Georgsson. 1989. Maedi-visna in sheep: host-virus interactions and utilization as a model, ***Intervirology 30*** (supplement 1):36-44.

Howard M. Temin. 1989. Is HIV unique or merely different? ***Journal of Acquired Immune Deficiency Syndromes 2:*** l-9.

Peter Nara received a B.S. in zoology from Colorado State University in 1977 and, by 1986, had received an M.S., a D.V.M. (Doctor of Veterinary Medicine), and a Ph.D. from Ohio State University. For his doctoral work he studied mechanisms of viral inactivation, including identification of the responsible proteins, species distribution, and developmental biology, associated with a particular oncoviral inactivating factor. He is currently chief of the Virus Biology Section, Laboratory of Tumor Cell Biology, at the National Cancer Institute-Frederick Cancer Research Facility in Frederick, Maryland and is a member of numerous scientific committees that deal with AIDS, HIV, and related viruses. Recently, he completed a four-year comparative pathology residency at the Armed Forces Institute of Pathology. His major areas of research interest include comparative pathobiology, cornparative tumor biology, and comparative virology-especially virology that will shed light on the troublesome retroviruses.

Acknowledgments

The author wishes to acknowledge the veterinary and medical researchers who have, over the past 40 years, studied these persistent viral infections of animals and have contributed to the knowledge that has rapidly advanced the research of human AIDS: Bjorn Sigurdsson, Gudmundur Georgsson, Halldor Thormar, Pall Palsson, Opendra Narayan, Ashley Haase, Neal Nathanson, Gudmundur Petursson, Travis McGuire, Lane Perryman, Y. Kono, H. Sentsui, Leroy Coggins, and Timothy Crawford. I would also like to extend my deepest personal regard to Necia Cooper, Dixie McDonald, and Gloria Sharp, editor, office manager, and graphics designer, as well as the rest of the Los Alamos Science staff, for their excellent technical and creative support so critical in the production of a good review article. I would also like to thank my courageous wife, Brenda, and my children, DeAnna and Kelly Ann, for their encouragement and support in these endeavors. Finally, I would like to thank my staff at the Virus Biology Section for their dynamic and enthusiastic support.

The editor and author would like to thank the authors, designer, and publisher of Immunology (by Ivan Roitt, Jonathan Brostoff, and David Male, Grove Medical Publishing, Ltd., London, 1985) for the inspiration their beautifully illustrated volume provided in the creation of this article.