# 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-

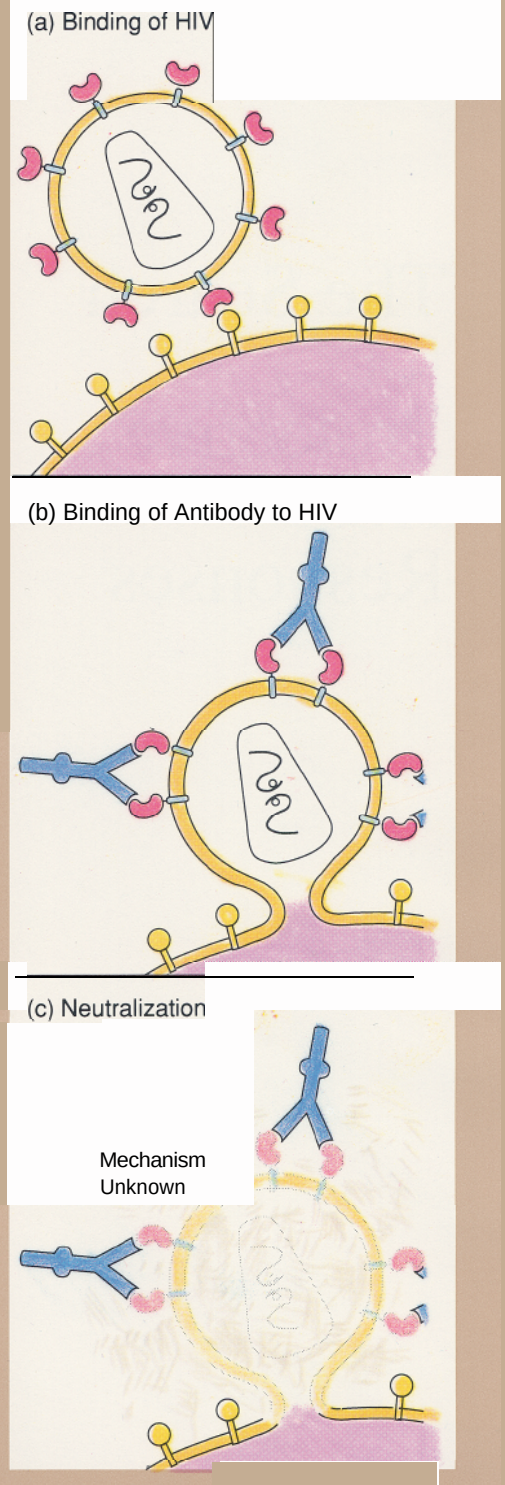
ity has been reported in the visna-maedi system.

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 $4.0 \times 10^{-9}$ 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

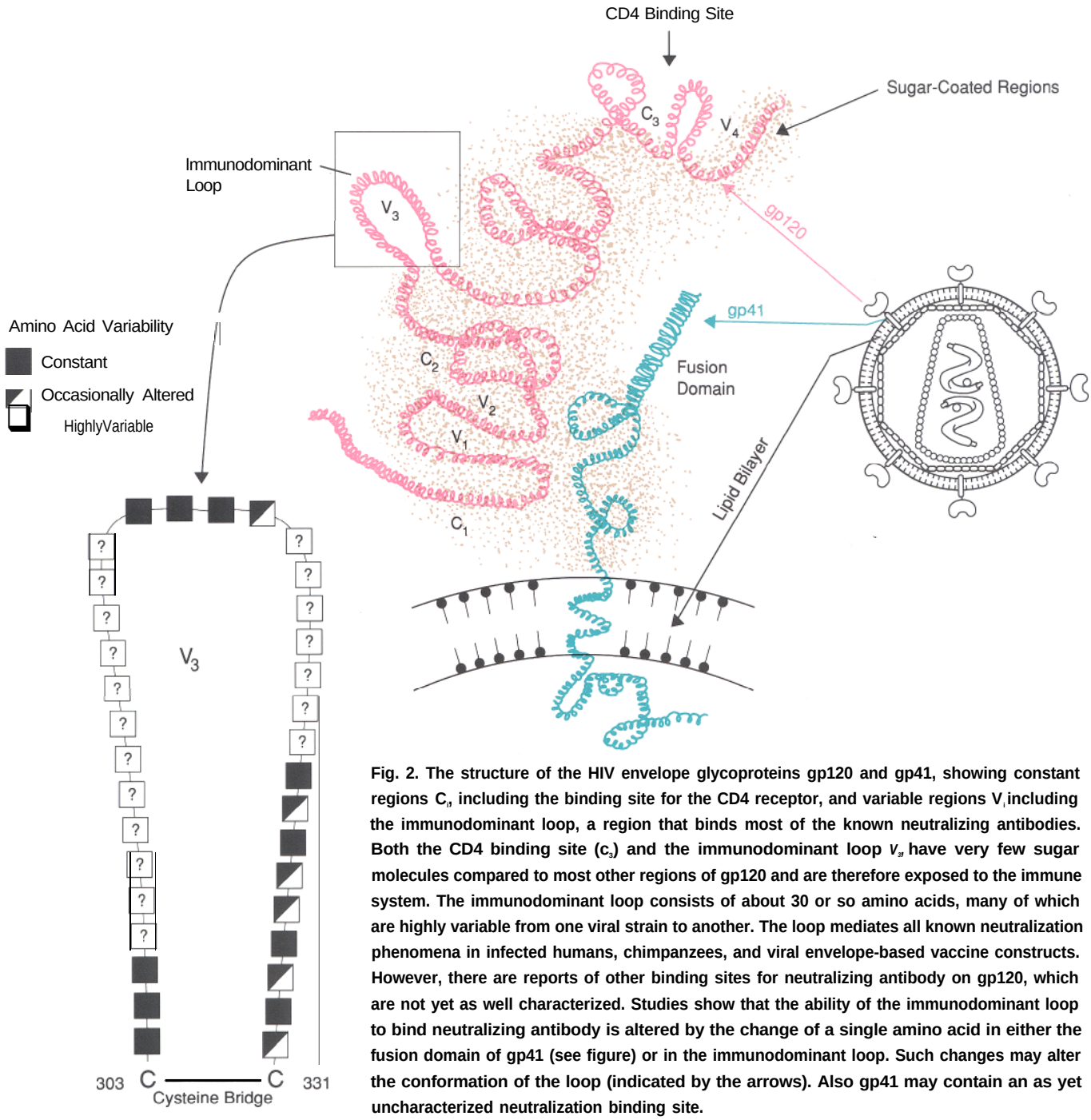



**Fig. 2. The structure of the HIV envelope glycoproteins gp120 and gp41, showing constant regions $C_i$, including the binding site for the CD4 receptor, and variable regions $V_i$ including the immunodominant loop, a region that binds most of the known neutralizing antibodies. Both the CD4 binding 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.

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