for permeant movement in the channel that is higher in one direction than in the other" (*14*, p. 860). Loewenstein suggested that the asymmetry could be provided by electrostatic interactions between the permeant and the channel, interactions through hydrogen bonding, or rapid conformational changes in the channel. An additional possibility, we suggest, is that the asymmetry is attributable to differences in pore diameter at either side of a heterologous gap junction (Fig. 3). Molecules of an appropriate size may be able to enter the junction through the larger pore and then pass through to the other side. However, similar molecules would be unlikely to pass in the opposite direction because they would be blocked by the smaller pore. This variation of Loewenstein's model implies that ions and molecules smaller than a certain size will be able to pass freely in both directions. It also predicts that gap junctions composed of the protein connexin 43 (astrocytes) have a larger pore diameter, and hence a higher unitary conductance, than those composed of connexin 32 (oligodendrocytes). Presumably, gap junctions in Müller cells have an even smaller pore diameter.

Our findings have two implications. First, they support the idea that gap junctions composed of a particular connexin protein have a characteristic channel diameter and permeability. It follows from this that the exchange of certain trophic and signal molecules may be limited to gap junctions composed of particular connexins. Second, and more important, as the bidirectional exchange of molecules through gap junctions is thought to provide a basis for intercellular communication (*1*), the unidirectional transfer of molecules provides the potential for a hierarchy of command.

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TECHNICAL COMMENTS

Protection from HIV Infection or AIDS?

One of the major goals of AIDS research is the development of an efficacious vaccine providing broad, long-lasting protection against human immunodeficiency virus–type 1 (HIV-1) infection. In the past, for induction of protective immunity, viral vaccine development aimed to elicit humoral immunity (that is, to produce strong neutralizing antibodies) that would protect one from infection. In contrast, cellular immunity was in principle thought to be associated with recovery from viral disease and convalescence. In a provocative Perspective, Jonas Salk and his colleagues hypothesize that a protective vaccine against HIV-1 infection should induce cellular rather than humoral immunity (*1*). Not only do they propose that cell-mediated immunity (CMI) can protect one from HIV-1 infection, but they also suggest that antibody responses are associated with increased *susceptibility* to such infection. This hypothesis is based on two distinct sets of data on the immunology of HIV-1 infection, in part published and in part presented during the IXth International Conference on AIDS held in Berlin in June 1993.

First, Clerici *et al.* (*2*) found that a large percentage of individuals exposed to HIV-1 who tested negative for the virus, but a small percentage of unexposed or low-risk subjects, showed evidence of HIV-1–specific CMI. Peripheral blood mononuclear cells from the former group released measurable amounts of interleukin-2 (IL-2) (which prompts lymphocyte proliferation) when stimulated with HIV-1 envelope peptides. From six persons who were studied longitudinally, two eventually became seropositive for HIV-1 antibodies. In subsequent studies, larger groups were included, and cells from up to 50% of the individuals responded with IL-2 production in the HIV-1 peptide assay (*3*). Furthermore, macaque monkeys inoculated with low doses of simian immunodeficiency virus (SIV) frequently had CMI responses that were detectable up to 64 weeks after inoculation, yet they did not produce SIV-specific antibodies or show evidence of infection (*4*). In contrast, all but one of the animals that received higher doses of virus became infected, tested seropositive, and showed no CMI response against the SIV peptides 64 weeks after infection. Salk *et al.* conclude from

these studies that T helper cell–type 1 (T_H1) responses (CMI) protect against HIV-1 infection in humans (or SIV in macaques) and that development of a T helper cell–type 2 (T_H2) (antibody) response correlates with susceptibility to infection with HIV-1 in humans or SIV in macaques. They apparently assume that the CMI responses were induced by infectious virus, probably present in infected cells that were successfully eradicated from the recipient by these responses.

An alternative interpretation that explains these observations was not mentioned in the Perspective by Salk *et al.* The CMI responses could have been evoked by doses of virus too low to establish infection, by noninfectious viral particles, or by transmission of viral proteins. Antibodies are elicited only after productive infection, but macaques were not able to produce IL-2 after an infection as a result of virus-induced immunodeficiency.

Measurement of IL-2 production against a particular set of envelope peptides was the only evidence of CMI obtained in these studies. Production of IL-2 by class II restricted helper T cells can be induced by cells that present antigen (macrophages or dendritic cells) without a need for de novo protein synthesis. Detection of class I restricted $CD8^+$ T cell responses would be a reliable indicator of transient virus infection.

Salk *et al.* designate antibody responses in general as being T_H2 responses; this is not correct. Although only T_H2 cells are involved in immunoglobulin E (IgE) antibody production, T_H1 and T_H2 cell responses both involve antibody responses of several other isotypes (5). To protect from heterosexually transmitted HIV-1 infection, efficacious vaccines preferably should elicit mucosal immunity that is characterized by a T_H2-type immunoglobulin A (IgA) antibody response. Indeed, T_H2 cell responses in animal models inhibit T_H1 and cytotoxic T cell responses, but this is a result of the regulatory cytokines (6), not of the forming of antibody. Thus, antibodies per se do not curtail cell-mediated responses and cannot be considered a failure of the cell-mediated immune response. Long-term, asymptomatic HIV-1–infected individuals, for example, have good antibody responses and cell-mediated immunity to HIV-1 and yet have not progressed to AIDS in more than 8 years (7). This shows that efficacious vaccines might induce T_H1- and T_H2-type responses at the same time. Irrespective of the interpretation of how the T cell response was induced, it will be of interest to see whether the macaques that appeared resistant to a low-dose inoculum of SIV and had signs of T cell priming are protected when challenged with a high dose of SIV.

In a second set of experiments, Clerici *et al.* demonstrated that 50% of HIV-1–infected asymptomatic patients initially had a good T cell response; IL-2 was released after activation with influenza virus or with HIV-1 peptides (8). These patients showed a gradual shift from a predominance of T_H1- to T_H2-type responses in the course of HIV-1 infection. Loss of IL-2 responses to soluble antigen or HIV-1 peptides is often accompanied by increased IL-4 production in vitro (9). Others have reported similar findings, including our own group, which analyzed T cell clones and primary lymphocyte cultures (*10*). The fact that HIV-1–infected patients initially respond with IL-2 release seems inconsistent with the idea that T_H1 responses are protective against HIV-1 infection. These early T_H1 responses thus do not protect.

The effect of HIV-1 infection on helper T cell reactivity is reminiscent of the polarization of T-helper responses by parasites such as *Schistosoma mansoni*, an effect that is correlated with protection against parasitic disease (6). On infection with parasites, T_H1 cell responses are often protective, whereas a shift to T_H2 and shutoff of T_H1 responses are associated with lack of protective immunity to disease, although protective T_H2 responses have also been described (6). In mice infected with *S. mansoni*, suppression of T_H1 reactivity by dominant T_H2 reactivity has been shown to result in the failure of virus-specific cytotoxic $CD8^+$ T cell responses (*11*). Shifts in T_H2 response occur in the already infected host and are associated with protection against the disease and reduced replication of the parasite. Salk *et al.*, however, erroneously imply that T_H2 shifts are a determinant of susceptibility to infection.

Down-regulation of T_H1 responses may be one of the mechanisms by which HIV-1 infection gradually perturbs the cellular immunity that is required to maintain the asymptomatic state and to protect against disease development and progression to AIDS (*12*). Preservation of T_H1 responses, IL-2 release, and strong cytotoxic T cell (CTL) responses that are class-I restricted and HIV–1 specific has been observed in long-term asymptomatic men (7). In conclusion, patterns of immune reactivity to HIV-1 in the naïve host do not support the hypothesis proposed by Salk *et al.* (1), that a prophylactic vaccine should induce cellular immunity to protect against HIV-1 *infection*, rather than an antibody response. However, we agree with Salk *et al.* that evidence is rapidly accumulating which suggests that T_H1 responses and accompanying class I restricted CTL responses might protect one against the development of AIDS.

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Response: In our Perspective we provided a reasoned justification for the hypothesis that the induction of stable CMI memory might control HIV infection, regardless of the route of viral entry. On the basis of this hypothesis, we proposed a strategy for prophylactic vaccination against HIV using low doses of noninfectious antigens together with appropriate adjuvants and immunomodulators, and outlined steps to test this strategy.

Miedema *et al.* note that T_H1-like, HIV-specific CMI responses (IL-2 release) observed in peripheral blood lymphocytes of HIV-exposed, but apparently uninfected individuals might have resulted from exposure to subinfectious doses of HIV or to noninfectious HIV antigens [see also (*1*)], and hence may not signify that CMI responses alone can clear an initial active infection. Nevertheless, at least some of the homosexual males and intravenous drug users studied are likely to have been exposed repeatedly to infectious virus; this is consistent with the concept that such CMI responses, however they were initially induced, may have protected against subsequent infection. The presence of HIV-specific MHC class I–restricted CTL activity in apparently uninfected infants born to HIV-infected mothers (*2*)

suggests that such infants have experienced a transient infection that was subsequently cleared, conceivably with the participation of the CTL.

Studies of intracellular microbial infections other than HIV have suggested that T_H1-type CMI responses tend to be protective against such pathogens, whereas T_H2-type responses associated with antibody production tend not to be protective and may in some circumstances increase susceptibility to disease. Miedema *et al.* mention *S. mansoni*, which is a helminthic parasite and not an intracellular pathogen. Nevertheless, the study by J. K. Actor *et al.* (*3*) which they cite demonstrates an association between reduced T_H1-type cytokine responses in mice infected with *S. mansoni*, reduced HIV- and vaccinia virus–specific $CD8^+$ CTL activity, and delayed clearance of recombinant vaccinia virus expressing HIV gp160, a finding that is consistent with the picture we have described. T_H1-type responses appear to be responsible for vaccine-induced protection against infection even in this system, although T_H2-type responses have been associated with protection in chronically infected humans (*4*).

Cell-mediated responses precede the development of antibody under certain conditions of antigen administration (*5*). The transition from T_H1-associated CMI responses to T_H2-dependent antibody responses does not represent a "failure of the cell-mediated immune response," but the normal kinetics and dynamics of the immune response under those circumstances.

Will an immune response to HIV which follows such a pattern be protective against either the establishment or progression of the infection? In intracellular parasitic infections, such as *Leishmania* infection in mice (*6*), protection is associated with T_H1-type responses that are *not* followed by T_H2-type responses, and hence are not subject to down-regulation by T_H2-associated cytokines. Miedema *et al.* seem to have interpreted the transition from T_H1- to T_H2-type patterns of cytokine secretion in HIV-infected individuals to mean that initial T_H1-type responses do not protect. In our view, this transition represents the course of events that is likely to occur when the immune system has not been preconditioned, by appropriate vaccination, to mount a T_H1-predominant response that will remain stable and protective.

Miedema *et al.* correctly point out that T_H1-type responses can lead to the production of certain antibody isotypes (for example, IgG2a in the mouse) (*7*, *8*). However, strong T_H1-like responses can also be associated with the absence of detectable antibody production (*9*), and therefore HIV infections might not lead to antibody production if they occur in the context of a stable T_H1-predominant response.

Miedema *et al.* view the coexistence of anti-HIV antibody and CMI responses in long-term asymptomatic individuals as an indication that prophylactic vaccination regimens that induce a mixed T_H1/T_H2 response may be effective. Although strong T_H1- and T_H2-type responses tend to be mutually exclusive, mixed responses can occur (*7*). In our view, the data from long-term asymptomatic individuals indicate that CMI activity can at times persist, along with antibody, at levels sufficient to exert some degree of control over the HIV infection. However, for the purposes of prophylactic vaccination, the induction of CMI responses that are not subject to the down-regulatory influences of T_H2-associated cytokines would seem preferable.

Rhesus monkeys immunized with an inactivated SIV vaccine containing a strong adjuvant (similar to complete Freund's adjuvant) developed strong SIV-specific CMI responses (lymphocyte proliferation and IL-2 release) and high concentrations of antibody, including neutralizing antibody, yet they were not protected against subsequent challenge with simian-grown SIV (*10*). Because such protection can be induced by vaccination with an attenuated strain of SIV (*11*), the lack of protection in these monkeys may have resulted from a suboptimal T_H1-type response in the context of a strong, anamnestic T_H2-type response.

In our Perspective we described experiments with monkeys (*12*) in which intrarectal administration of low doses of SIV produced strong CMI responses unaccompanied by antibody production or infection, whereas administration of high doses led to both antibody production and infection. Three of the animals that had been exposed to low doses were subsequently challenged with high doses of SIV, in parallel with three controls. Whereas all of the control animals became infected and developed immunodeficiency disease, none of the three experimental animals have become infected after more than a year (*13*). These findings lend further support to the hypothesis we have proposed.

Which, if any, of the approaches that have been suggested for prophylactic immunization against HIV infection and AIDS will ultimately prove effective remains to be determined by experiment. As the strategy we have proposed is supported by findings from a wide range of studies, it would appear useful that it be included among the approaches pursued.

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