that programmed cell death induction is related to AIDS pathogenesis (*4*). Chimpanzees that have been infected with HIV-1 are productively infected but do not develop disease and do not show any immune deficiency,and simian immunodeficiency virus (SIV)–infected macaque rhesus monkeys show a rapid CD4 cell decline leading to AIDS-like disease. Programmed cell death did not occur in T cells from HIV-1–infected chimpanzees and, by contrast, cell death by apoptosis was observed upon activation of T cells from SIV-infected macaques. The absence of apoptosis in HIV-infected chimpanzees suggests that T cell programmed cell death is not an obligatory consequence of HIV infection, but reflects a more complex array of interactions between HIV and the immune system.

Is there in vivo evidence that binding of CD4 receptors on lymphocytes by gp120 occurs in AIDS patients? The site of the CD4 receptor (D1 domain), which binds to gp120, is often masked in AIDS patients, presumably by the gp120 itself (*13*). Viral particles can shed free gp120, which could bind rapidly to CD4 receptors on lymphocytes or could be complexed by specific antibodies. Upon treatment of patients with AZT, a transient unmasking of CD4 occurs.

Recognizing the importance of apoptosis in AIDS pathogenesis may have dramatic consequences for the conception of new strategies of research and treatment for combating the disease. In order to prevent apoptosis, it is essential to dissect the subtle interaction of molecules involved in the process. Particularly important will be the identification of novel surface markers for an early diagnosis of apoptosis. The biochemistry also needs to be clarified. The role of oxidative stress and mitochondrial alterations should be analyzed. Antioxidants such as *N*-acetylcystein, vitamins C and E, and superoxide dismutase should be included in clinical trials in combination with antiviral therapy.

The masking of CD4 receptor by gp120 may be used as a surrogate marker for the clinical evaluation of antivirals, as suggested by the rapid, although transient, unmasking of this receptor by AZT. Soluble CD4 could prevent the binding of gp120 to the CD4 receptor. As a viral inhibitor, soluble CD4 has been rather disappointing, but it could have a beneficial effect on the course of the disease by preventing the abnormal signaling of the CD4 lymphocytes due to the binding of the viral glycoprotein. We suggest therefore that the pharmaceutical industry reenter this neglected field and make sufficient amounts of soluble CD4 for clinical trials.

Finally, it would be very important to identify the superantigens, if any, that activate $CD4^+$ and $CD8^+$ T cells. Because of the existence of superantigens in animal retroviruses (*14*), it has been proposed that HIV encodes a superantigen. However, there is no convincing evidence at the present time in favor of this suggestion. Another explanation is that there is more than one superantigen coming from a microorganism such as mycoplasma (*15*). Association of some mycoplasma species with HIV and AIDS has been described (*16*, *17*).

Antiviral therapy of AIDS patients or of HIV-infected individuals has shown little success. The time has come to fight this complex disease by combinations of several treatments including antivirals, antibiotics, and anti-apoptotic drugs. The validation of the efficacy of these drug combinations by appropriate clinical trials will be difficult, but in our opinion this is the only way to respond correctly to the challenge of AIDS.

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A Strategy for Prophylactic Vaccination Against HIV

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A prophylactic vaccine against human immunodeficiency virus (HIV) infection represents the best hope for controlling the continuing and devastating worldwide AIDS epidemic. In this commentary, we outline evidence suggesting that the goal of immunization to prevent or control HIV infection should be activation of the cell-mediated, rather than the antibody-mediated, arm of the immune system. Accordingly, we propose a vaccination strategy intended to ensure that a stable cell-mediated response occurs after exposure to HIV.

During the past few years it has become clear that apparently harmless, and possibly protective, encounters with HIV can occur. Some individuals who have been exposed to the virus and are therefore at high risk for HIV infection remain apparently uninfected; they do not have antibodies to HIV in their blood, and neither HIV nor its nucleic acids can be detected in blood samples. Nevertheless, in one study of 97 such individuals seronegative for HIV, 49% exhibit cell-mediated immunity to HIV (their T cells respond to HIV peptides in vitro), whereas only 2% of 163 individuals not known to be exposed to HIV exhibit responses to these peptides (*1*, *2*). Such HIV-specific, cell-mediated responses have been seen in gay men with known sexual exposure, intravenous drug users, health care workers exposed by accidental needle stick, and newborn infants of HIV-positive mothers (*1*, *2*). HIV-specific lymphoproliferation or cytotoxic T lymphocyte (CTL) activity, hallmarks of cell-mediated responses, have also been observed by other investigators in some exposed, but apparently uninfected, subjects (*3*).

These findings have been extended by monitoring HIV-exposed individuals over time (*1*, *2*). HIV status was followed in six gay men who exhibited cell-mediated immunity in the absence of antibody to HIV or detect-

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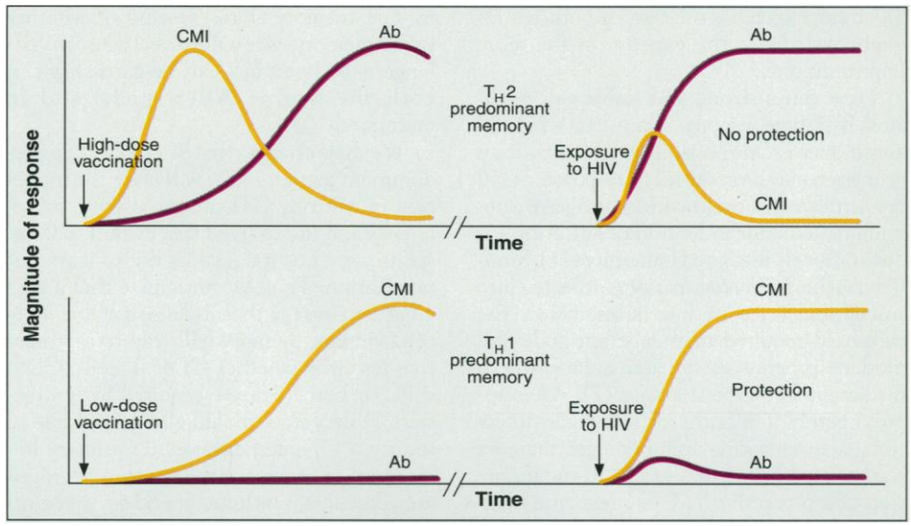
able virus in their blood. Two of the six individuals eventually seroconverted (antibodies to HIV became apparent in their bloodstream); in both cases seroconversion was associated with the appearance of detectable virus in blood and a decline in cell-mediated immunity (*1*). Although only correlative, these observations suggest that cell-mediated immunity might control HIV infection, regardless of the route of viral entry, and that protection may be lost if cell-mediated immunity declines (*1*).

There is precedence for this suggestion. In many infections, cell-free pathogens or toxins (for example, influenza, polio, and rabies viruses; pneumococcus bacteria; diphtheria; and tetanus toxins) can be effectively neutralized in the circulation or extracellular fluid by the humoral arm of the immune system through antibodies that bind to the pathogens or toxins and thereby lead to their inactivation or destruction (*4*). However, for many intracellular pathogens, it is cell-mediated immunity [consisting of $CD4^+$ T cells that mediate delayed-type hypersensitivity (DTH), $CD8^+$ CTLs, macrophages, and other cell types] that protects the organism against invaders. These include the bacterial infections of tuberculosis and leprosy, the spirochetal infection of syphilis, and the protozoan infections of cutaneous and visceral leishmaniasis (*5*, *6*). In fact, a limited infection ensues when such pathogens induce stable cell-mediated immunity (*5–7*). On the other hand, chronic, progressive, or fatal disease will occur if an antibody is produced and the cell-mediated response declines (*5–7*).

The mechanisms that regulate which type of immune response is induced by various pathogens have become clearer with the identification of distinct $CD4^+$ T helper cell subsets. These cell types are categorized by their different functions and by the constellation of cytokines they produce (*8*). T helper cells–type 1 (T_H1) secrete interferon γ (IFN-γ) and interleukin-2 (IL-2) and contribute to cell-mediated responses such as DTH and macrophage activation (*8*). T helper cells–type 2 (T_H2) produce IL-4, IL-5, and IL-10 and help B cells to generate antibody responses (*8*). These T helper cell subsets, originally defined in the mouse (*8*), have also been identified in humans (*9*).

There is a tendency for either the cell-mediated or the antibody response to predominate in any particular immune response (*8*, *10*, *11*). This tendency is thought to result from cross-regulation, whereby T_H1 cells (or other coordinately induced cells) inhibit the induction of T_H2 responses, probably by production of IFN-γ, and T_H2 cells (or other coordinately induced cells) inhibit the generation of T_H1 responses through production of cytokines such as IL-4 and IL-10 (*12*, *13*).

The induction of protective cell-mediated immune responses and of nonprotective antibody responses in some infectious and parasitic diseases can be understood in terms of T_H1 and T_H2 cell subsets. For example, C3H mice, which are resistant to infection with *Leishmania*, develop a T_H1-type immune response after infection, whereas susceptible BALB/c mice develop a T_H2-type response (*14*). Administration of antibody to IFN-γ ablates the protective response in C3H mice, and administration of antibody to IL-4 renders BALB/c mice resistant to infection (*14*). In addition, protective immunity against *Leishmania* infection in BALB/c mice (induced by immunization) can be transferred by injecting cells with a T_H1 phenotype to another animal, whereas transfer of cells with a T_H2 phenotype causes exacerbation of disease, perhaps through cytokine-mediated inhibition of T_H1-associated protective mechanisms (*15*). Similar associations between T_H1-type responses and protection against infection and T_H2-type responses and susceptibility have been observed in a number of other infectious and parasitic diseases (*6*, *13*, *16*, *17*).



A proposed vaccination strategy. Postulated effects of vaccination with high doses and low doses of HIV antigens on the initial immune response, the establishment of immunologic memory, and protection against subsequent exposure to HIV. CMI, cell-mediated immunity; Ab, antibody.

These relationships may also apply to HIV infection and AIDS. Peripheral blood mononuclear cells from seronegative, but HIV-exposed, individuals respond to HIV envelope antigens with a T_H1-like response, that is, they produce IL-2 (*1*, *2*). Moreover, as asymptomatic, HIV-seropositive individuals progress towards AIDS, their peripheral blood lymphocytes shift from a T_H1-predominant to a T_H2-predominant pattern of cytokine production (*18*, *19*). These observations are consistent with the hypothesis that T_H1-type cell-mediated responses are associated with resistance to HIV infection, progression to AIDS, or both, and that T_H2-type antibody responses are associated with susceptibility (*17*, *19*).

Other observations also support the hypothesis that cell-mediated responses are important in controlling HIV infection. Virus-specific CTL responses have been observed early in the course of HIV infection in humans and simian immunodeficiency virus (SIV) infection in monkeys, followed by antibody production (*20*). A sharp decline in the amount of virus in the bloodstream occurred just after the cellular response, but preceded the appearance of neutralizing antibody in the human subjects and seroconversion in the monkeys, suggesting that cell-mediated immunity, rather than antibodies, may be primarily responsible for the initial control of acute HIV and SIV infection. Data from mice also suggest that a cell-mediated response may accompany resistance to infection. In a murine model of AIDS, spleen cells from C57BL/6 mice, which develop immunodeficiency disease after infection with the LP-BM5 mixture of murine leukemia virus, show a T_H2-like pattern of cytokine production after infection, whereas spleen cells from A/J mice, which resist disease, display a T_H1-like pattern (*21*).

It is generally believed that, in order to provide optimal protection, vaccination against HIV should induce both a strong neutralizing antibody response and a strong cell-mediated response. Belief in the potential effectiveness of an antibody response is not without foundation. Antibodies against HIV can neutralize HIV in vitro (*22*), and antibody passively administered to primates can prevent infection with cell-free HIV in chimpanzees and with cell-free SIV in macaques (*23*). However, infection with HIV under natural circumstances is thought to be transferred primarily by infected cells (*24*), which are more likely to be susceptible to a cell-mediated defense. Since the induction of a strong antibody (T_H2-type) response is likely to curtail a cell-mediated (T_H1-type) response due to the effects of cross-regulatory cytokines,

an attempt to have the best of both worlds might well be at the expense of the more important one.

How can a strong and stable cell-mediated, T_H1-type response against HIV be reliably induced? One possibility is that low doses of antigen may promote a T_H1 response. Small quantities of nonreplicating antigens, subimmunogenic for induction of antibody, induce only cell-mediated immunity (*11*). Similarly, viable *Leishmania* parasites injected into susceptible mice in low doses, below the threshold required to induce antibody and produce progressive disease, induce a cell-mediated, T_H1-type response (*7*). After approximately 2 months of low-grade infection, a long-lasting immunologic memory state is established that results in the induction of a protective T_H1-like response upon subsequent injection of a high dose of *Leishmania* parasites, a dose that would normally induce a T_H2-like antibody response and result in progressive and fatal disease (*7*).

Could the strategy of low-dose immunization (see figure) be made effective for protection against HIV infection? Preliminary evidence supports this possibility. Administration of high doses of SIV intrarectally to macaques results in infection and antibody production with minimal cell-mediated immunity, whereas administration of lower doses elicits strong and long-term cell-mediated immunity with neither antibody production nor detectable infection (*25*). In addition, mice immunized with high doses of inactivated HIV generate a transient DTH response followed by an antibody response, whereas mice injected with lower doses generate a DTH response exclusively (*26*).

Immunologic adjuvants and immunomodulators (incluiding cytokines) might be used in conjunction with immunization to help promote a stable cell-mediated response. Cytokines which might be used in this way include IFN-γ and IL-12, which has recently been reported to redirect the antigen-dependent response from a T_H2 to a T_H1 mode (*27*). Live vectors such as Bacillus Calmette-Guérin (*28*) might also be useful, especially if administered in low doses (*29*), and perhaps incorporating T_H1-enhancing cytokine genes (*30*).

Additional studies in mice will determine whether it is possible to lock the immune response to noninfectious HIV antigens into a cell-mediated mode. Studies in mice can also be carried out to determine the contributions of antigen dose, route, and timing, as well as adjuvants, T_H1 immunomodulators, and vectors to the establishment of a locked-in T_H1 memory state. Testing of whether such a memory state will protect against challenge with live virus can be carried out in both the murine AIDS model and in macaques.

We hypothesize that a stable T_H1-predominant memory state will favor the induction of a strong CTL response upon subsequent exposure to live HIV, even if such responses are not induced by the primary immunization. Thus, we anticipate that a vaccination strategy that induces a stable T_H1-predominant memory will lead to a protective response whether $CD4^+$ T cells, $CD8^+$ cells, or both (*31*) are required for protection. However, it should also be possible to induce a CTL response at the primary immunization, if necessary, by using appropriate adjuvants, synthetic peptides, or vectors (*28*, *32*). Since cell-mediated responses against HIV-1 are directed, at least in part, against epitopes of viral proteins that are relatively invariant among different isolates (*33*), the approach suggested here could be protective against all HIV-1 variants.

The concepts we have presented suggest that vaccination strategies favoring the induction of a stable cell-mediated response should protect against subsequent HIV infection, and lend support to the view that efficacious vaccination against HIV will indeed be feasible.

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