Review

Progress on new vaccine strategies against chronic viral infections

Jay A. Berzofsky,[1] Jeffrey D. Ahlers,[2] John Janik,[3] John Morris,[3] SangKon Oh,[1] Masaki Terabe,[1] and Igor M. Belyakov[1]

[1]Molecular Immunogenetics and Vaccine Research Section, Vaccine Branch, The Center for Cancer Research, National Cancer Institute,
[2]Division of AIDS, National Institute of Allergy and Infectious Diseases, and [3]Clinical Trials Team, Metabolism Branch,
The Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland, USA.

Among the most cost-effective strategies for preventing viral infections, vaccines have proven effective primarily against viruses causing acute, self-limited infections. For these it has been sufficient for the vaccine to mimic the natural virus. However, viruses causing chronic infection do not elicit an immune response sufficient to clear the infection and, as a result, vaccines for these viruses must elicit more effective responses — quantitative and qualitative — than does the natural virus. Here we examine the immunologic and virologic basis for vaccines against three such viruses, HIV, hepatitis C virus, and human papillomavirus, and review progress in clinical trials to date. We also explore novel strategies for increasing the immunogenicity and efficacy of vaccines.

Vaccines have proven among the most cost-effective strategies for preventing infectious diseases — following only the provision of safe drinking water and sanitation. During the 20th century, vaccines for bacterial toxins and many common acute viral infections were developed and made widely available. Vaccines have changed the face of viral disease as much as antibiotics have affected the course of bacterial disease. They have been most successful in cases in which acute natural infection is self-limited and leads to long-lasting protective immunity if the patient survives the initial infection. In these cases, the best vaccine has usually been the one that most closely mimics the natural infection, such as a live, attenuated virus. Indeed, just this year, a new, live, attenuated influenza vaccine was licensed for intranasal aerosol administration (1).

However, development of a vaccine that is effective against viruses that cause chronic infections, such as HIV, hepatitis C virus (HCV), and human papillomavirus (HPV), may require consideration of a paradigm different from that described above. These viruses cause chronic infections with different frequencies; virtually 100% of cases of HIV infection, 55–85% of cases of HCV infection, and over 30% of cases of HPV result in chronic viral infection. In most of these cases, the immune response to the natural infection is not sufficient to eradicate the infection. Therefore, a vaccine that just mimics natural infection is not likely to be adequate to induce protection. Also, there is much concern about the use of live attenuated viruses for vaccination against these diseases. These viruses have evolved to escape or evade the immune system, not to act as an optimal vaccine. The challenge for the 21st century is to apply the latest fundamental knowledge in molecular biology, virology, and immunology to developing vaccines that are more effective at eliciting immunity than the natural infection and, consequently, effective against chronic viral and other infectious diseases in addition to cancer, which do not fit the classic paradigm.

Although advances in molecular biology have raised great hope for the development of new vaccine strategies and much effort has been invested in this endeavor, only one recombinant viral protein vaccine — a hepatitis B surface antigen vaccine — has been licensed to date, and that advance occurred about 17 years ago (2, 3). In the last 5–10 years, however, many new vaccine strategies have been designed based on substantial increases in fundamental knowledge of the immune system, and some of these vaccines have advanced to clinical trials. Most of these strategies are based on improved ways of inducing antibodies, which can prevent infection if present at high enough levels at the time of exposure, or inducing CTLs that can detect and destroy cells infected with virus and thereby control and ultimately clear infection. These CTLs can detect any viral protein made within the infected host cell even if it is not present on the cell surface. They are able to respond to peptide fragments of these proteins produced by proteasomal cleavage and transported to the endoplasmic reticulum. Here they bind newly synthesized class I MHC proteins, such as HLA-A, -B, and -C in humans, which carry the peptides to the cell surface and present them to T cells.

In addition to CD8^{+} T cell responses, CD4^{+} T cell responses have been found to be critical in the maintenance of adequate CD8^{+} T cell function and control of viremia in both HIV and HCV infection (4–7). However, HIV-specific CD4^{+} T cells may be preferentially infected and deleted by HIV (8), limiting the ability of vaccines to induce crucial T cell help after the early stages of infection. In addition, memory CD8^{+} T cells have now been subdivided into effector memory T cells, which home to tissues, and central memory cells, which recirculate in the body (9–11). Chronic antigen stimulation during a persistent infection may inhibit the transition of memory CD8^{+} T cells to central memory cells. However, central memory cells are more effective at protection because they are better able to proliferate when reexposed to antigen (12). Thus, chronic viral infection may perpetuate itself by preventing the development of the most effective form of T cell memory. Therefore the challenge for an effective vaccine is to induce long-lived central memory CD8^{+} T cells as well as CD4^{+} helper T cells. While space limitations preclude comprehensive coverage, this review article will attempt to highlight some of the exciting progress in vaccine development, primarily for three chronic infections on which much research has been focused: HIV, HCV, and HPV.

Table 1
Vaccine strategies for prevention of selected chronic viral infections

| Virus	Desired immune response	Major strategies in development
HIV	Generation of CD4+ Th cells and CD8+ CTLs	Heterologous prime-boost
		Cytokine-adjuvanted DNA delivery
		Mucosal immunization
	Generation of broadly cross-reactive neutralizing antibody	Delivery of stabilized trimeric envelope protein
		Delivery of prefusion intermediate HIV envelope structures
		Delivery of modified and variable loop-deleted envelope proteins
		Delivery of multiclade and multiquasi species envelope immunogens
HCV	Generation of neutralizing antibody against HCV envelope proteins (E1 and E2)	Delivery of recombinant proteins (recombinant E1 vaccine is in clinical trial)
		Delivery of plasmid DNA and recombinant vectors (E1/E2)
	Generation of T cell–mediated immunity against HCV proteins	Delivery of plasmid DNA vaccine (E1/E2 and NS proteins)
		Delivery of synthetic peptide vaccines (Core, NS, and envelope proteins)
		Delivery of recombinant viruses (NS3, E2)
HPV	Prevention of HPV infection: generation of humoral mucosal immunity	Delivery of: L1 or L1/L2 virus–like particles (VLPs).
	Therapy of HPV-associated tumor: generation of cellular immunity directed against oncogene products E6/E7.	Delivery of vaccinia vector expressing E6/E7
		Delivery of chimeric VLPs (with E6/E7 peptides or L1/L2-E7 fusions)

NS, nonstructural.

One may ask how viruses that cause chronic infections differ from those that cause acute, self-limited infections. Each of these viral types has specific mechanisms of evading or attacking the immune system, but certain common features may be discerned. A number of factors probably play a role, including the size of the virus inoculum, the kinetics of viral replication, the viral genotype, host genetics, and the competence of the host immune system. For example, for hepatitis B virus (HBV), chronicity occurs in about 90% of cases of vertical (maternal) transmission when the immune system of the recipient is immature but in only about 10% of cases of horizontal transmission to immunocompetent adults (2). The common scenario for chronic viral infections, we suspect, is that the initial, acute infection does not usually cause very severe disease and does not provoke an immune response that is adequate to eradicate the infection, only one that can reduce the viral load. Consequently, a balance is struck between the immune system and the virus in which a steady-state level of virus is maintained within the host. The immune system keeps the virus partially in check, but the virus also evades or in some cases inhibits the immune system. Finally, disease is due primarily to the chronic insult of the lower level of viral infection over time, rather than to high viremia. When viremia does increase again, as occurs in the case of HIV, it is usually after the virus has taken its toll on the immune system as well as after viral mutations have allowed a transition to a more aggressive phenotype. In contrast, viruses that cause acute infections usually cause more severe disease initially and provoke an immune response that clears the infection. For example, in the case of hepatitis B and C, individuals who develop chronicity often experience an initially milder acute hepatitis syndrome and weaker cellular immune responses than those that clear the infection and do not develop chronic infection (13–15). The goal of vaccines in the case of viruses that cause chronic infections is therefore to tip this balance in favor of the immune response. Thus, the real hope for dealing with chronic viral infections is the development of a new generation vaccine, important to developing countries. Effective vaccination could be accomplished either prophylactically, by establishing an early immune advantage via the generation of pre-existing vaccine-induced immune memory, or therapeutically, by increasing the strength or quality of the immune response beyond that controlling the level of steady-state viral load during chronic infection. For HIV, HCV, and HPV, we will explore the approaches being undertaken to accomplish these goals (see Table 1).

Human immunodeficiency virus

The basis for current HIV vaccine strategies. There is little direct evidence for immune correlates of protection against HIV in humans since no individual has mounted an immune response capable of spontaneously clearing the infection, even though there are some long-term nonprogressors who have remained infected without developing AIDS. Nevertheless, there is much evidence in animal studies and indirect evidence in humans that CD4+ and CD8+ T cells, broadly neutralizing antibodies, and innate immunity all play an important role in the control of infection with HIV and its close cousin, simian immunodeficiency virus (SIV), in macaques.

Antibodies neutralizing AIDS viruses clearly play an important role in protection. Passive transfer of IgG1 monoclonal antibodies was shown to be sufficient to protect macaques against i.v. challenge or against mucosal transmission (16, 17). However, a high level of monoclonal antibody is required to achieve complete protection while partial protection could be achieved with a lower-antibody titer. Therefore early studies primarily focused on the HIV envelope protein gp160 as the primary target of neutralizing antibodies. However, while it was possible to achieve neutralizing antibodies against a specific virus strain grown in the laboratory, the difficulty of obtaining antibodies that neutralized a broad array of strains, particularly primary isolates, provided incentive both to devise novel approaches for the induction of the relevant antibodies and to target T cell immunity as an alternative strategy.
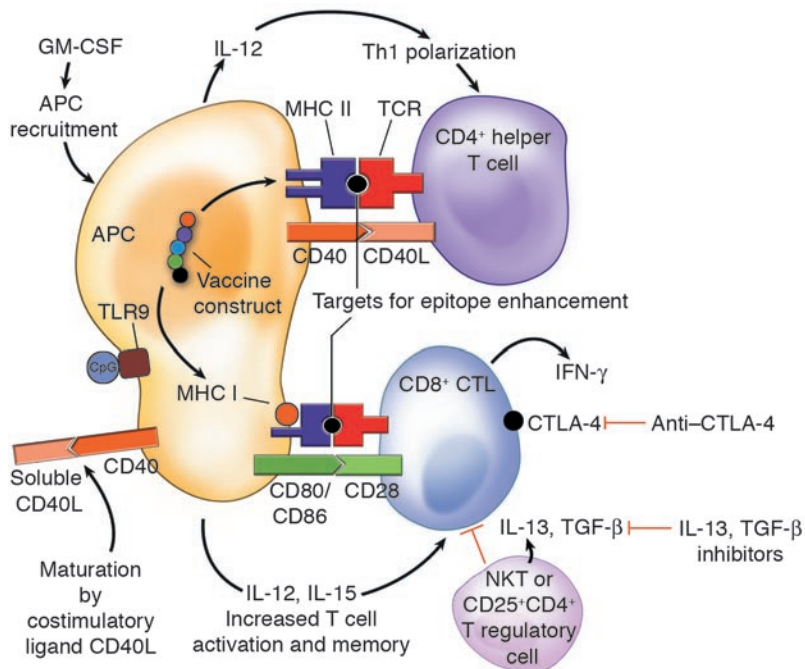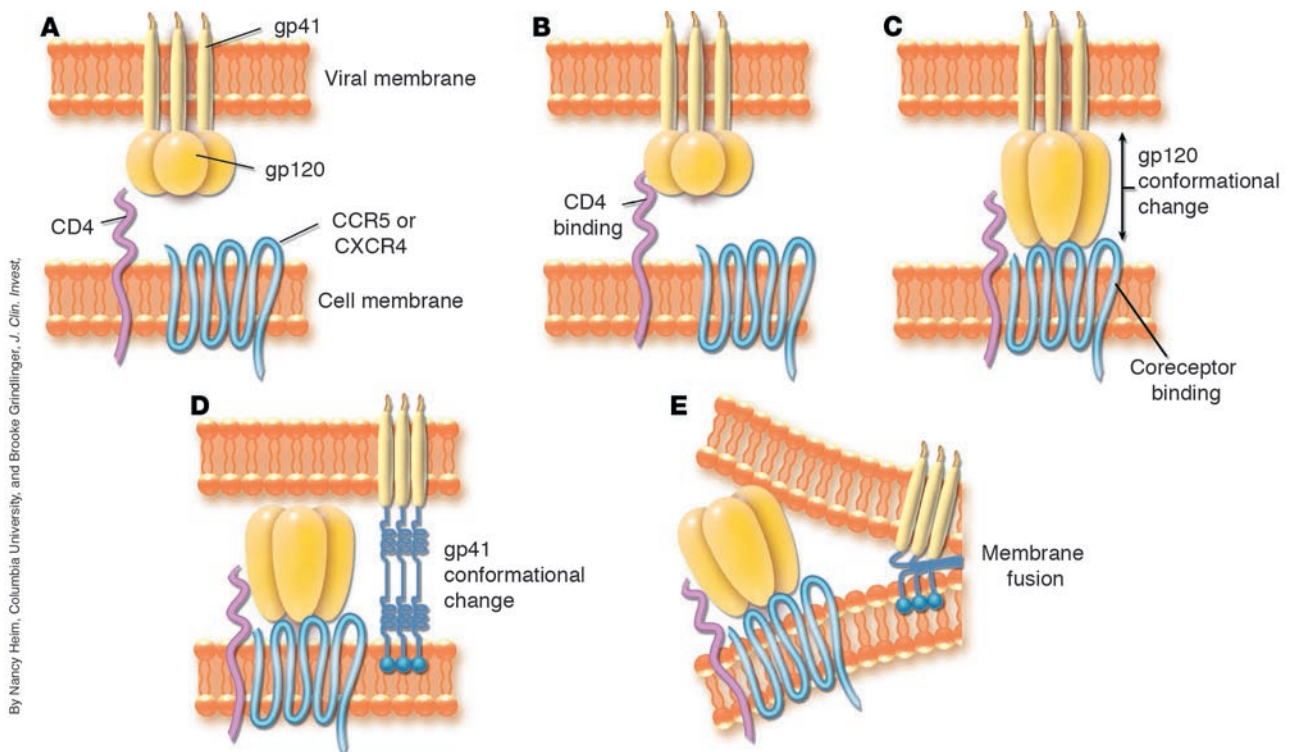
Figure 1
New strategies for second generation vaccines based on cellular immunity. CD4+ helper T cells mature and activate APCs through recognition of epitopes presented by class II MHC molecules (MHC II) and interaction of CD40 and CD40 ligand (CD40L). The CD40-CD40L interaction causes the APC to upregulate expression of costimulatory molecules such as CD80 and CD86 and to secrete cytokines IL-12 and IL-15. The costimulatory molecules interact with CD28 on the CD8+ CTL to provide a second CTL activation signal in addition to T cell receptor (TCR) recognition of an antigenic peptide presented by a class I MHC molecule (signal 1). IL-12 also contributes to activating the CTL and polarizing the T helper cell to produce Th1 cytokines, such as IFN-γ. IL-15 contributes to induction and maintenance of CTL memory and longevity. Regulatory T cells, including NK T cells and CD25+CD4+ T cells, can dampen or inhibit the CTL response in order to prevent autoimmunity, but also reduce the immune response to the vaccine. Various strategies may be employed to improve the natural T cell response. Epitope enhancement of class I or class II MHC–binding peptides can increase their affinity for the respective MHC molecules and their immunogenicity. Incorporation of (a) cytokines such as IL-15 to recruit more memory CTLs; (b) IL-12 to steer the T helper cell population towards a Th1 response; (c) GM-CSF to recruit dendritic cells; or (d) costimulatory ligands such as CD40L to activate and induce maturation of the dendritic cells recruited by the GM-CSF can synergistically amplify the immune response. In addition, CpG-containing oligonucleotides can act through toll-like receptor 9 (TLR 9) to activate dendritic cells. Increased levels of costimulatory molecules can selectively induce higher-avidity CTLs that are more effective at clearing virus infections. Agents that block factors secreted or induced by regulatory T cells, such as IL-13 and TGF-β, can synergize with other strategies to allow the CTL response to reach its full potential. Similarly, blockade of the inhibitory receptor, CTLA-4, on the T cell, can increase the T cell response. See multiple references in the text for the other strategies.

in vivo led to increases in viral load that were later reversed when the T cells reappeared (22–24). For this reason, most strategies studied in nonhuman primates today are based on eliciting an effective HIV- or SIV–specific CTL response. Although CTLs can be elicited by peptides and other constructs, the most straightforward approaches have involved agents that induce endogenous expression of the viral antigens in a professional antigen-presenting cell, such as a dendritic cell, because the most efficient and natural way to load class I MHC molecules with peptides for presentation to CD8+ T cells is endogenous expression of the protein within the cell. This can be accomplished with DNA vaccines or with viral vectors, which can introduce the antigen gene into antigen-presenting cells. A recent study indicates that a DNA vaccine augmented by IL-2 can induce a strong HIV-specific CTL response and can control pathogenic viral challenge and prevent AIDS in rhesus macaques (25).

Compared to repeated DNA vaccination or viral-vector–based vaccination alone, one of the most effective strategies for eliciting HIV-specific immunity is a heterologous prime and boost regimen, which involves administration of a DNA vaccine followed by a viral-vector vaccine, which induces a stronger CTL response than can be achieved by priming and boosting with the same agent (26), or a recombinant protein boost, which induces a significant level of neutralizing antibodies (27). HIV DNA vaccines were shown to be effectively boosted by a recombinant vaccinia virus, such as modified vaccinia Ankara (MVA) (28) or replication-deficient recombinant adenovirus (29), which induced high frequencies of CD8+ CTLs and neutralizing antibodies and protected against viral challenge. MVA is a highly attenuated vaccinia virus that has lost the ability to replicate in primate cells and can be considered a safe vaccine. In a study comparing SIV gag recombinant MVA and recombinant adenoviral vectors in prime-boost regimens in macaques, DNA prime–recombinant adenovirus boosting was most effective at eliciting long-lasting CD8+ IFN-γ responses and protection against viral challenge (30). However, mutation of AIDS viruses, leading to escape from immune control mediated by CTLs (31), and the breadth of the protection against more distant strains of challenge virus remain concerns. Indeed, several of these studies used the highly pathogenic simian-human immunodeficiency virus strain 89.6 (SHIV 89.6P) as the challenge virus as it has an atypically rapid disease course, but this virus may not be representative. Protection against heterologous viral challenge will be critical to demonstrating the breadth of vaccine efficacy.

These difficulties led to the development of new vaccine strategies against AIDS viruses as described below (reviewed in ref. 32), including but not limited to the following: (a) creation by

A number of lines of evidence implicate CD8+ T lymphocytes (especially CTLs) in controlling HIV or SIV infection (reviewed in refs. 18–21). The acute viremia in both HIV and SIV was found to decline concomitant with the rise of the CTL response and prior to the appearance of neutralizing antibodies. Many HIV-infected long-term nonprogressors have expressed a high level of HIV-specific CTLs, and African sex workers that had been exposed to HIV but remained uninfected possessed high CTL responses. However, the most direct evidence comes from studies of HIV-infected chimpanzees and SIV-infected macaques, in which depletion of CD8+ T cells

Figure 2
Interactions of HIV envelope glycoproteins, CD4, and chemokine receptors CCR5 or CXCR4 trigger fusion and entry of HIV. These interactions determine critical regions of the HIV envelope glycoprotein against which neutralizing antibodies could be raised. After the envelope protein interacts with CD4 on the target cell (**A** and **B**), it undergoes a conformational change allowing its interaction with a chemokine receptor (**C**). This second interaction induces a further conformational change in the gp41 portion of the envelope glycoprotein that mediates the fusion event (**D** and **E**). Blockade of any of these three steps can prevent viral entry.

sequence modification of enhanced epitopes that bind with higher affinity to MHC molecules; (b) targeted induction of mucosal immunity; (c) use of synergistic combinations of cytokines, chemokines, or costimulatory molecules to enhance the immune response; (d) relief of negative regulatory or suppressive mechanisms that inhibit the immune response; (e) use of dendritic cells as vaccine vehicles; (f) induction of alloimmunity for protection against HIV; and (g) formulation of the vaccine to incorporate agents inducing innate immunity (Figure 1).

Mucosal vaccination of mice with an HIV peptide induced systemic and mucosal CTL responses, while parental vaccine administration induced predominantly systemic CTL responses (33). Systemic CTLs were not sufficient to protect against mucosal virus transmission, so it is important that the CTLs be locally present in the mucosa (34). Mucosal delivery of the vaccine is usually the most effective route to induce mucosal immunity although, interestingly, transcutaneous immunization can also induce mucosal CTLs and may provide an alternative vaccination option (35). Moreover, mucosal vaccination against AIDS viruses, which induced CD8+ CTLs in the gut mucosa of immunized Rhesus macaques, more effectively cleared the major reservoir for SIV replication in the gut and thus reduced plasma viral load below the level of detection. The same vaccine administered subcutaneously was less effective, leaving residual viremia (36). Also, control of SIV infection was associated with mucosal CTLs (37). These findings make a strong argument for mucosal delivery of an AIDS

vaccine even if some partial protection against mucosal challenge can be observed with a systemic vaccine (38).

Cytokine and chemokine gene codelivery along with DNA-encoding immunogens can modulate the direction and magnitude of immune responses (39) and can improve vaccine efficacy compared to delivery of DNA alone (25). For example, RANTES coinjection induced high levels of CD8+ CTLs, as did a synergistic combination of GM-CSF and CD40 ligand when negative regulatory mechanisms were blocked (40).

Overall it is widely believed that induction of both antibodies and T cells will be needed for an effective AIDS vaccine. Several major strategies are being studied for development of a preventive vaccine against AIDS viruses (reviewed in ref. 41). Most of these approaches focus on the generation of either neutralizing antibodies or CTL responses, but ultimately some combination of these approaches may be needed.

Another major hurdle for HIV vaccine development is the extraordinary diversity of the virus and its ability to rapidly mutate within each infected individual. The genetic subtypes of HIV, clades A–E, are responsible for the main epidemics in different parts of the world: clade B is prevalent in North America and Europe; clades A, C, and D in Africa; and clades E and B in Thailand. In addition to the diversity of viral subtypes that must be targeted by a vaccine, the high level of mutability, due to the error-prone nature of reverse transcriptase, facilitates escape mutation. Some of the major neutralizing epitopes of HIV are so

variable that it is hard to find broadly cross-reactive neutralizing antibodies, but the existence of a handful of monoclonal antibodies that are broadly neutralizing implies that it is possible, in principle, to do so. These antibodies bind to at least three different sites on the HIV envelope protein: the CD4 binding domain, the chemokine receptor–binding domain, and the stalk of gp41 that must change conformation in order to mediate fusion with the cell membrane (Figure 2) (42). In addition, the conserved conformation of the V3 loop, despite high sequence variability, has allowed production of broadly cross-reactive antibodies to this principal neutralizing region (43, 44). Thus, with respect to antibodies, a goal is to develop vaccines that direct the immune response to conserved sites such as these that cannot vary without a resulting loss of function, but this has not proven straightforward through the use of existing forms of recombinant envelope proteins. In the case of T cells, which can target internal viral proteins and are not limited to neutralizing epitopes, one approach has been to focus on sequences that are conserved for functional or structural reasons and cannot tolerate modification by escape mutations. This approach is supported by the finding that more cross-clade reactivity has been observed among T cells than antibodies (45, 46).

HIV-1 vaccine clinical trials. This year heralds the deployment worldwide of multiple vaccine trials in an effort to stem the ongoing HIV epidemic. Safety and immunogenicity data for multiple vaccines and immunization platforms currently moving into phase I/II studies will establish the criteria for phase III efficacy studies. A listing of ongoing preventive trials of HIV vaccines is available at the International AIDS Vaccine Initiative website (http://www.iavi.org/trialsdb/basicsearchform.asp), and the ongoing and planned protocols of the HIV Vaccine Trials Network in association with the National Institute of Allergy and Infectious Diseases can be found at http://chi.ucsf.edu/vaccines/.

Therapeutic vaccine trials. Early clinical studies tested the ability of HIV-1 clade B envelope protein (gp160 or gp120) vaccines or peptide vaccines based on the V3 loop of gp120 that had been identified as the principal neutralizing determinant (PND), in order to elicit neutralizing antibodies. Other vaccine trials in HIV-1 infected individuals initiated during the pre–highly active antiretroviral therapy (pre-HAART) era attempted to elicit both cellular and humoral immune responses using synthetic peptides containing promiscuous CD4+ T helper cell epitopes linked to the PND located at the crown of the V3 loop (47, 48). Various strategies (e.g., the use of adjuvants and multimeric and multivalent immunogens) were employed to increase vaccine immunogenicity and cross-reactivity. Although these approaches proved capable of eliciting high-titered type-specific neutralizing antibodies to tissue culture lab–adapted virus strains in addition to some lymphoproliferative responses in immunized patients, these antibodies failed to neutralize primary viral isolates (49–51).

An early approach targeting specific cellular immunity used gp120-depleted, whole, killed virus (52). The idea was to elicit cellular immunity to internal viral proteins while avoiding induction of so-called enhancing antibodies, specific for the envelope protein, that facilitate virus uptake by cells, as well as avoiding induction of other potential deleterious effects of the envelope protein. A large multicenter, double-blind, placebo-controlled, randomized trial was conducted on a whole inactivated Zairian HIV-1 isolate in incomplete Freund's adjuvant given intramuscularly at 12-week intervals. A total of 1262 subjects of 2527 HIV-1 infected patients received the HIV-1 immunogen. There were no statistically significant differences between groups in plasma HIV RNA loads although patients in the vaccine group had an increase in average CD4+ T cell counts. Recent studies have shown that this vaccine is able to enhance specific CD4+ T cell responses in patients with chronic HIV infection (53, 54). However, the clinical benefit of enhancing such responses in the therapy or prevention of HIV infection is yet undetermined.

Current therapeutic vaccines entering phase I/II trials are aimed at boosting immune responses in HIV-1–positive patients where plasma viral load is controlled by antiretroviral therapy. These include immunization with a recombinant canarypox vector (VCP1452) expressing clade B gag, protease, reverse transcriptase, gp120 and nef, and immunization with gag, pol, and nef lipopeptides, in addition to peptide-pulsed autologous dendritic cell immunization plus IL-2.

Preventive vaccine trials. Recombinant vaccinia virus vectors have proven effective at eliciting CD8+ T cell responses in small animal models; however, concern about the effect of disseminated vaccinia on immunocompromised patients and the effect of prior smallpox vaccination on immunogenicity of the vaccine led to the development of a number of live, attenuated pox-vectored HIV vaccines that do not replicate in human cells and can be administered repeatedly. In a study by Evans et al. (55), recombinant canarypox expressing only gp160 of HIV-1MN (also known as vCP125) elicited anti-HIV env CD8+ CTLs in 24% of low-risk subjects. Anti–HIV-1 env CTLs were detected in 12 subjects and anti–HIV-1 gag CTLs were detected in 7 of the 20 vaccine subjects receiving canarypox virus (known as ALVAC) expressing HIV-1 *env*, *gag*, and protease (vCP205) vaccine alone or with clade B HIV-1 strain SF-2 recombinant gp120 protein (called rgp120 SF) (56, 57). Coadministration with SF-2 rgp120 vaccine enhanced lymphocyte proliferation in response to HIV-1 envelope glycoprotein and broadened envelope-stimulated cytokine secretion. Belshe et al. (58) reported a cumulative positive response frequency of 33% for anti–HIV-1 env or gag CTLs among 170 subjects in a phase II trial of vCP205. The vaccines were safe, and all patients developed binding antibody to monomeric gp120; approximately 60% developed antibody to gag p24.

Overall, lymphoproliferative responses to gp120 varied among ALVAC vCP205 studies, with from 50–100% of vaccinated subjects demonstrating CD4+ T cell proliferation. Fifteen to twenty percent of vaccinees developed CD8+ CTL responses, mostly against the envelope protein, with cross-clade reactivity seen in some subjects (45, 59). The maximum positive CTL response (35/84; 42%) was observed after four immunizations (57). It is noteworthy that the first successful phase I vaccine study initiated in Africa involved vaccinating uninfected volunteers in Uganda with clade B ALVAC vCP205 (60). Future vaccine strategies involving variations of the canarypox vector currently being developed as combination vaccines include replacing the clade B *env* sequences in vCP205 with clade A or clade E *env* sequences or sequences from a primary clade B isolate and the addition of reverse transcriptase (RT) and nef epitope sequences.

Heterologous prime-boost strategies. DNA vaccines used alone have not proven as immunogenic in humans and nonhuman primates as in mice; however, strategies involving DNA priming and boosting with a viral vector are capable of eliciting potent CD8+ and modest CD4+ T cell and antibody responses in macaques (28, 29). In an effort to improve immunogenicity results obtained with ALVAC vectors, current studies employing multiple viral

gene products in complex combinations of DNA and viral vectors such as MVA, attenuated Vaccinia Copenhagen strain with deletions in virulence genes (NYVAC), fowlpox, adenovirus, and Venezuelan equine encephalitis virus–like replicon particles are underway. Major considerations in the development of recombinant viral vectors are ways to circumvent preexisting immunity and the production of high-titered stable vectors. Current phase I placebo-controlled trials are aimed at defining optimum vaccination regimens for eliciting cellular immune responses by varying the dose (dose escalation), number of doses, intervals between doses, and routes of administration. Results of phase I adenovirus trials in humans show safe, strong, long-lasting CD8+ T cell responses measured by IFN-γ ELISPOT. The University of New South Wales, Australia, has recently initiated a phase I trial of a prime-boost protocol using DNA and a fowlpox vector each expressing clade B gag-pol (without integrase), tat, nef, and gp160 env coding sequences. It is encouraging that recent clinical trials evaluating heterologous prime-boost regimens with HIV-1 and a recent malaria sporozoite protection trial with pre-erythrocytic *Plasmodium falciparum* immunogens have demonstrated the ability of these regimens to elicit strong IFN-γ–secreting CD8+ T cell responses equivalent to or better than those achieved during natural infection (61). Current vaccines moving into clinical trial that incorporate multiple immunogenic viral gene products are designed to address the issues of HLA polymorphism and escape mutation, and to identify correlates of immune protection. Second-generation DNA and viral-vectored vaccines include multiclade gag, pol, and env and may include nef and the accessory gene products tat and vpu. In addition, several novel agents are currently in phase I trials. One examines modified HIV envelope immunogens (e.g., ΔCFI [cleavage site deletion (C), fusion peptide deletion (F), deletion of interspace between gp41 (N), and C heptad repeats (I)]) and clade A, B, and C DNA envelope immunogens developed by the Vaccine Research Center. A second examines a V2-deleted trimeric gp140 protein developed at Chiron Corp. Two other studies (at St. Jude's Children's Hospital, Memphis, Tennessee, USA; and University of Massachusetts Medical School, Worcester, Massachusetts, USA) test multiclade, multienvelope DNA, recombinant vaccinia virus with protein boost strategies in an effort to elicit broadly neutralizing antibody in addition to CD4+ and CD8+ T cell responses. In the Vaccine Research Center phase I DNA vaccine trial with a gag-pol-nef and multiclade env vaccine, CD4+ T cell responses were more frequent than CD8+ T cell responses and were primarily directed toward env but not the gag-pol-nef fusion protein. Coadministration of plasmid DNA expressing IL-2–Ig to enhance cellular immune responses is currently being tested with the Vaccine Research Center DNA vaccine (clade B gag-pol-nef and multiclade env) in a phase I trial. Cytokines IL-12 and IL-15, which have been shown to enhance induction of cellular immune responses and memory to vaccine antigens in small animal models, are scheduled for testing in human phase I HIV-1 vaccine trials in the near future.

Although cross-clade CD8+ CTL responses have been reported with clade B immunogens (45, 46), the importance of clade diversity will only be definitively addressed in phase III trials that compare vaccine candidates in parallel trials in different geographic regions. A recent study of HIV-1 subtype C–specific immune responses during natural infection in individuals in Botswana emphasizes the need to match vaccine epitopes to immunodominant epitopes detected in the target population based upon HLA frequencies (62). Recently,

the first phase I HIV vaccine trial to be conducted simultaneously in Africa and the United States (sites in Gabarone, Botswana; Boston, Massachusetts, USA; and St. Louis, Missouri, USA) was initiated to test a DNA vaccine developed by Epimmune, composed of a promiscuous helper T cell epitope, pan-DR epitope (PADRE), and 21 specific epitopes optimized to elicit CD8+ CTL responses in individuals expressing one of three HLA alleles: HLA-A2, HLA-A3, and HLA-B7. Results obtained from this polyepitope study regarding immunogenicity will provide information for future vaccine design since the immunogen is not specifically selected for epitopes or matched for HLA types prevalent in this African population. Results of a previous single phase I trial in Africa employing a DNA vaccine expressing clade A gag and a stretch of 25 CTL epitopes known to be expressed in the vaccinated population revealed modest CD8+ T cell IFN-γ responses to gag, but no responses to the individual epitopes included in the vaccine were seen. After a single MVA boost (5×10^7 pfu) 6 months to 1 year later, CD8+ IFN-γ ELISPOT responses were detected in 19 of 26 individuals. In addition, an increase in the breadth of responses was seen after boosting. A phase II trial of this vaccine is in progress (63).

Lessons for the future. Only one HIV vaccine construct has yet progressed through large-scale phase III studies testing efficacy. A phase III trial completed in early 2003 (known as VAX003) in the United States, Canada, and the Netherlands and another in Thailand completed in 2004 (known as VAX004), both testing bivalent formulations of gp120 protein subunit vaccine (AIDS-VAX B/B and B/E; VaxGen Inc.) aimed at targeting neutralizing antibodies, failed to demonstrate efficacy. Although a difference in the infection rate of African-American placebo recipients (9/116; 7.8%) versus African-American vaccine recipients (2.6%; 6/233) was found, based upon the small number of infections, further analysis is necessary to determine the significance of these differences. This suggestive result in a retrospective stratification emphasizes the need to adequately power future phase III trials to address differences in immune responses based upon gender and ethnicity. Phylogenetic analysis representing the overall diversity of viral isolates from the complete VAX004 data set showed no differences in any treatment group based upon race, gender, or geography (64). Results from these trials were consistent with those of previous studies, in which monomeric gp120 was not proven effective at eliciting broadly cross-reactive neutralizing antibodies.

A critical component for future vaccine prime-boost regimens is the inclusion of an envelope immunogen capable of eliciting broadly cross-reactive neutralizing antibodies against primary HIV-1 isolates. Although HIV-1–infected individuals are capable of developing neutralizing antibodies to primary viral quasispecies, serial escape occurs; consequently, the neutralizing antibody response lags one step behind the evolution of the viral envelope (65–67). The majority of cross-reactive neutralizing antibodies directed against HIV-1 glycoproteins have been mapped to conserved regions within the CD4 binding site and CD4 inducible epitope, V2, V3, the carboxy-terminus of C5, the leucine zipper-like region of gp41, and the ELDKWAS motif in the transmembrane region of gp41. Monoclonal antibodies directed to these epitopes neutralize primary isolates from multiple clades to varying degrees. Monoclonal antibodies, 2F5 and 4E10, directed to membrane proximal domains in gp41 are the most potent, in that they cross-neutralize 67% and 100%, respectively, of all clade isolates tested (68). The inability of primary isolates to elicit cross-reactive, neutralizing antibody may be explained by the low immunogenicity

of these epitopes, resulting from conformational dynamics within the viral envelope that maintain a structure that makes these sites inaccessible or only transiently exposed. The rational design of new envelope immunogens should focus on engineering structures that expose, and direct antibody responses to, conserved epitopes on native trimers that are recognized by broadly cross-reactive neutralizing antibodies, and that at the same time prevent induction of dominant non-neutralizing antibodies.

An effective HIV-1 vaccine will require both potent and durable cell-mediated immune responses as well as effective neutralizing antibody responses. In the coming years, phase III efficacy trials of vaccines of proven immunogenicity will determine the need to employ immunization strategies focusing on eliciting mucosal immune responses, as noted above, since delivery of current vaccines primarily targets induction of systemic responses. Another important issue is whether sterilizing immunity, in which a vaccine is completely successful in preventing infection and which is likely to require high titers of broadly neutralizing antibodies, is essential or whether a vaccine based only on inducing cellular immunity, which controls viral loads so as to both prevent disease in the individual and reduce the risk of transmission to others (69, 70), will be sufficient to contain this pandemic.

Hepatitis C virus

Most cases of acute viral hepatitis are caused by hepatitis A virus (HAV), HBV, HCV, and hepatitis D virus (HDV). Of those viruses, HBV and HCV are the most important causes of chronic infection and liver-related morbidity and mortality (71). For both HAV and HBV, effective vaccines are currently available and include inactivated whole virus for HAV and recombinant hepatitis B surface antigen for HBV (reviewed in ref. 72). In addition, a new combined HAV and HBV vaccine has recently been approved for use in individuals 18 years and older. Lack of a licensed vaccine for HDV is of less concern because HDV requires HBV for pathogenicity. Unlike HBV, HCV is an RNA virus that does not integrate into the host genome. HCV infection results in persistent infection in 55–85% of patients (reviewed in ref. 72). Chronic HCV and HBV infections are leading causes of liver cirrhosis and hepatocellular carcinoma worldwide. Although chronic HBV infection remains widespread despite the existing prophylactic vaccine and immunotherapeutic approaches are needed, this review will be confined to HCV.

Immune responses to HCV. Although serum antibodies to HCV are detected following virus infection, humoral immunity alone may not play a critical role in viral clearance during acute infection, perhaps because HCV is capable of rapid outgrowth of antibody escape mutants (73). In addition, no data are available to show that HCV-infected patients have long-lasting protective antibody responses. In contrast, cellular immunity does seem to play a role in the virological outcome during acute infection and persists for decades after viral clearance (5, 6, 13–15, 74, 75). A wide variety of vigorous CD4+ T cell responses persists for many years, and memory CD8+ T cells may also be maintained (74). However, these responses are significantly weaker among patients who later progress to chronic infection, suggesting that the intensity of cellular immunity in the early stage of infection is a critical factor in limiting the spread of HCV. The unfortunate consequence of a vigorous cellular immune response to acute hepatitis infection is liver damage, especially that resulting from CD8+ T cells killing infected hepatocytes. Indeed, the possibility has been raised that different subsets of CD8+ T cells

contribute to immunopathology and to viral clearance (6). Thus, the goal of a vaccine against HCV is to induce an initial immune response of sufficient strength and type to clear the infection without causing severe acute hepatitis. Host genetics, including HLA type, have also been shown to contribute to HCV clearance and chronicity (reviewed in ref. 76).

Vaccines for HCV. Development of a vaccine for HCV has been delayed by many impediments, including lack of a suitable small animal model, a high degree of genomic diversity, and inability to grow large amounts of virus in vitro. Recent studies suggest hope for the development of prophylactic and therapeutic vaccines against HCV infection.

An early prophylactic vaccine approach against HCV, targeting recombinant HCV envelope glycoprotein (E1/E2) in chimpanzees (77), was designed to induce neutralizing antibodies. Later, studies using recombinant E1/E2 protein and peptide vaccines showed that antibodies induced could neutralize low levels of homologous HCV challenge in nonhuman primates (78). Although the vaccine failed to protect against high-dose virus challenge, the reduction in risk of chronic infection is a great success because most morbidity and mortality of HCV is a consequence of chronic infection. A recombinant E1 protein is currently being evaluated in clinical trials as a therapeutic vaccine against HCV (reviewed in ref. 79). To achieve humoral immunity with both prophylactic and therapeutic vaccines, several strategies, including use of DNA plasmids, recombinant viruses or bacteria, and virus-like particles (VLPs), are under study. In particular, VLPs are attractive because the particulate multivalent structure is more immunogenic than soluble proteins.

The complementary approach of targeting T cell–mediated immunity has been given an impetus by studies of reinfection after recovery from HCV infection. Although HCV can cause more than one episode of acute hepatitis in the same individual under certain circumstances, such as in thalassemia patients (80), other studies in previously infected humans and chimpanzees indicate that the risk of a second infection becoming chronic is greatly reduced compared to that of a primary infection, and this protection correlates with both CD4+ and CD8+ T cell responses (75, 81–84). Indeed, the critical role of CD8+ T cell recruitment in this protective response was demonstrated by depletion of these cells in chimpanzees, which resulted in high viral loads until the CD8+ T cell population recovered, despite the persistence of a CD4+ T cell response (84). The role of T cells in these protective responses was supported by the results of several studies demonstrating the absence of detectable HCV envelope glycoprotein–specific antibodies in the protected animals (75, 83, 84). Thus, effort has been invested in defining HCV CTL epitopes and designing vaccine constructs (85–90). Such approaches to HCV vaccine development include the use of DNA plasmids (90, 91), recombinant viral vectors expressing HCV antigens (91–93), and HCV virus–like particles (94, 95). To improve on the ability of the wild-type viral sequence to induce T cell immunity, the amino acid sequence of epitopes has been modified to increase affinity for the HLA molecule to make the epitopes more potent vaccines (known as epitope enhancement) (88). This epitope-enhancement approach can be applied to any type of vaccine construct.

However, recent evidence suggests that CD8+ T cell responses alone are not sufficient for protection against HCV infection and that CD4+ T cells may also be critical. Depletion of the CD4+ T cell population from two immune chimpanzees before HCV reinfection led to viral persistence despite continued HCV-specific
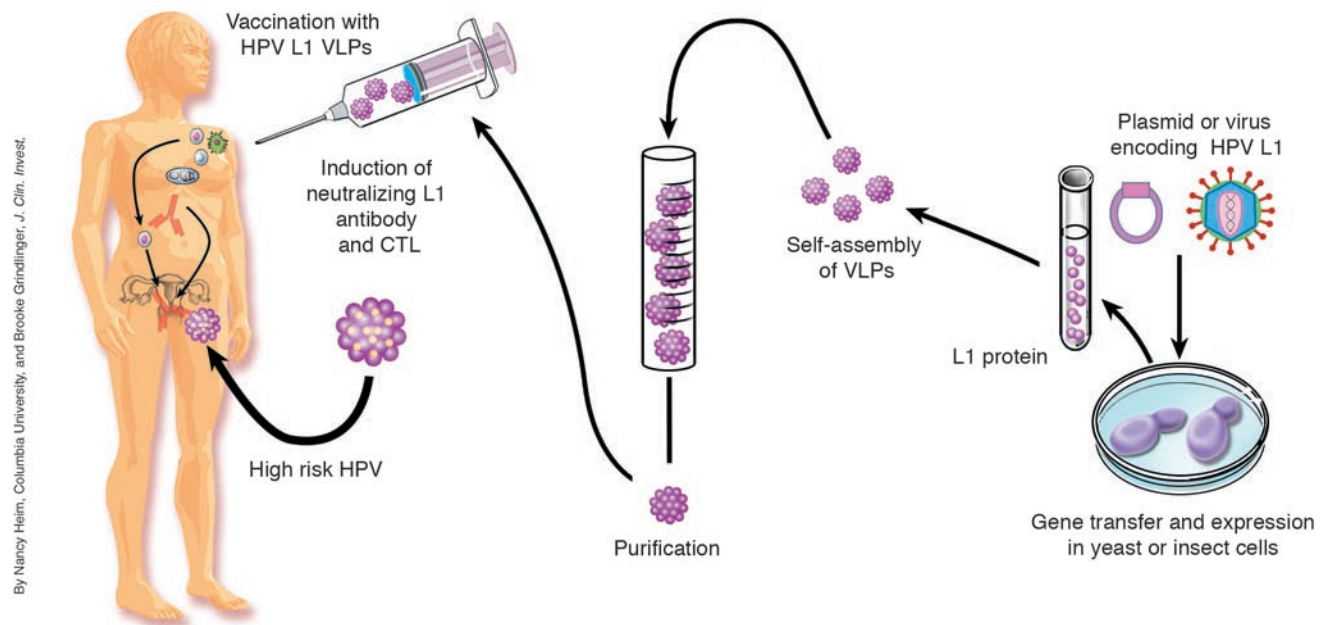
Figure 3
Vaccination against HPV infection using genotype-specific HPV L1 VLPs. Recombinant HPV-16 or HPV-18 L1 capsid protein made in yeast or baculovirus-infected insect cells self-assembles to form VLPs that are very potent at inducing neutralizing antibodies but are not infectious because they lack any viral nucleic acid. Such VLP vaccines show promise for prevention of HPV infection and HPV-associated cervical cancer. Depicted within the vaccinated subject are dendritic cells that present antigen to helper T cells (blue) and B cells (pink), which induces the B cells to become plasma cells (shown as ellipses). Plasma cells then generate antibodies (red) capable of neutralizing the virus.

CD8^{+} T cell memory, and this inadequate control of viral load in the absence of CD4^{+} T cell help led to the emergence of viral escape mutants (7). Likewise, in humans, clearance of acute HCV infection was associated with a strong CD4^{+} T cell response, and absence or loss of this response was associated with viral persistence or recurrence (5, 6). Thus, the role of CD4^{+} T cell help may parallel that described as occurring during HIV infection. Therefore, recent strategies for inducing both humoral and CD4^{+} and CD8^{+} cellular immunity could potentially provide more complete protective immunity against HCV infection. Some of these vaccines have induced CTLs in HLA transgenic or other mice sufficient to protect against a recombinant vaccinia virus expressing HCV antigens, used as a surrogate challenge virus in mice (91, 93). To induce Th1-type immunity and improve T cell–mediated immunity, inclusion of cytokines and other biological adjuvants may be necessary for both prophylactic and therapeutic HCV vaccines in the future.

Human papillomavirus
Persistent infection with oncogenic strains of HPV is the major cause of cervical cancer. HPV genotypes 16, 18, 31, 33, 45, and 56 account for more than 95% of cases (96, 97). HPV has also been implicated in cancers of the anus, penis, vulva, and oropharynx. As humoral immunity to HPV is genotype specific, effective vaccines must be polyvalent. Approximately 100 HPV genotypes have been identified, about 40 of which infect the genital tract, and of these, at least fifteen are believed to be oncogenic. More than 70% of women who become infected even with high-risk HPV genotypes will clear the infection within 2 years, and only a minority of the women with persistent infection will develop dysplasia and progress to cancer. Vaccine development has been slowed by the large number of genotypes needing to be addressed and the lack of an efficient HPV culture system (98). Two HPV vaccine strategies are currently under study: (a) prophylactic vaccination to prevent primary infection, usually aimed at generating neutralizing antibodies to the L1 major capsid protein; and (b) therapeutic vaccines inducing CTLs, usually against the viral E6 and/or E7 oncoproteins expressed in HPV-associated dysplastic and cancerous lesions and responsible for malignant transformation.

Prophylactic vaccines targeting capsid proteins with antibodies. Prophylactic HPV vaccines have focused on the use of type-specific VLPs generated from recombinant L1 major capsid protein or L1/L2 capsids to induce neutralizing antibodies. Overexpressed L1 capsid protein with or without L2 will self-assemble to generate noninfectious, nononcogenic VLPs (99). In animal models, L1-VLP induced high titers of neutralizing antibodies and protected animals against HPV infection (98). Passive immunization of animals using serum from vaccinated animals confirmed the ability of neutralizing antibodies to prevent infection. L1 or L1/L2 VLP HPV vaccines are currently in clinical trials. Preclinical work includes the development of L1/L2-E2-E7 fusion protein VLPs and the incorporation of plasmid expression vectors into the VLPs (100).

Clinical trials of VLPs have focused on prevention of primary HPV infection (Figure 3). A series of trials showed that an HPV-16 L1-VLP vaccine was well tolerated and generated high levels of antibodies against HPV-16 as well as CD4^{+} and CD8^{+} T cell responses to L1 (97, 101). In a randomized, double-blind, placebo-controlled trial of an HPV-16 L1-VLP vaccine in 1533 women aged 16–23 designed to determine whether such a vaccine could prevent persistent HPV-16 infection, Koutsky et al. found that over 99% of the women receiving the vaccine developed antibodies against HPV (102). In the control arm, the rate

Vaccines for chronic viral infections: future needs and goals

Prophylactic vaccines:

Development of effective multivalent vaccines against the common genotypes of virus.
Reduction in cost of vaccines to broaden access.
Simplified storage, handling, and delivery of vaccines to allow easier implementation of mass vaccination programs in developing countries.
Definition and expansion of target populations for vaccination programs.

Therapeutic vaccines:

Earlier diagnosis and treatment of infected individuals.
Broadened viral protein/epitope content of vaccines to avoid escape mutations.
Use of enhanced epitopes, cytokines, chemokines, costimulatory molecules, and other immunostimulatory agents and agents that block suppressive pathways to boost the immune response beyond that elicited by the chronic infection itself.
Combination of vaccines with other treatments, including antiviral drugs.

of persistent HPV-16 infection was 3.8 per 100,000 woman-years versus none in the vaccinated group ($P < 0.001$). All 41 cases of persistent infection and the 9 cases of HPV-16–related dysplasia occurred in the placebo group, demonstrating a vaccine efficacy of 100%. Bivalent and polyvalent VLP vaccines that include HPV-16 and -18, or HPV-6, -11, -16, and -18 are under development by at least three groups (98), and phase III trials of these promising VLP vaccines are underway. The initial success of these VLP-based prophylactic vaccines for HPV infection offers hope that soon it will finally be possible to save millions of lives by widespread prevention of HPV-associated cervical cancer.

Therapeutic vaccines targeting HPV oncoproteins with CTLs. The major targets for preventing the progression of persistent HPV infection to cancer and for treating cancer are the E6 and E7 oncoproteins. In high-grade dysplasia and carcinoma, genotype-specific E6 and E7 are expressed in virtually all cells. Their expression is both necessary and sufficient for the maintenance of the transformed phenotype (103), ensuring retention of oncoprotein expression by the tumor. Vaccine development for late-stage HPV-associated disease has focused on generating cellular immunity against these antigens.

In patients with recurrent or advanced cervical cancer, a recombinant vaccinia virus encoding nonfunctioning HPV-16 and -18 E6/E7 fusion proteins (known as TA-HPV) produced no clinical responses; however, 2 out of 8 patients remained alive and tumor free 15 and 21 months after vaccination (104). One of three patients tested developed HPV-18 E6/E7-specific CTLs that were not detected prior to vaccination. Unfortunately, complicating the interpretation of this trial was the fact that these patients also received other treatments.

In a phase I/II study, 15 HLA-A*0201–positive cervical cancer patients were vaccinated with HLA-restricted E7 peptides and PADRE in Montanide ISA-51. Proliferative responses were elicited in 4 patients, but no clinical responses or CTLs were observed (105). In another phase I trial, 18 HLA-A*0201–positive women with HPV-16–associated high-grade genital dysplasia were vaccinated with HPV-16 E7 peptides in incomplete Freund's adjuvant (106). Three patients cleared their dysplasia, and 6 others achieved a partial response.

A phase II study of TA-GW, a recombinant HPV-6 L2E7 protein in alum, produced 5 complete responses in 27 men with genital warts (107). Vaccination using a polymer-encapsulated plasmid (ZYC101) expressing HLA-A*0201–restricted HPV-16 E7 epitopes fused with HLA DRA0101 in 12 men with high-grade anal dysplasia produced 3 partial responses, and 10 patients demonstrated antigen-specific IFN-γ–producing T cells for up to 6 months after vaccination (108).

A phase I trial of ZYC101 in 15 women with persistent HPV-16–associated cervical dysplasia reported 5 complete responses, and 11 patients developed HPV-specific T cell responses after vaccination (109). Injection of autologous dendritic cells pulsed with full-length HPV-16 E7 protein in 3 HLA-A*0201–positive patients with HPV-16–associated cervical cancers elicited CD8+ CTLs against autologous tumor cells (110), and one patient had a complete response that lasted 23 months (111). A heat-shock protein-65–HPV-16 E7 fusion protein (HspE7) vaccine that may offer broader immunity against a number of HPV genotypes is currently in clinical trials (112).

There is real optimism for the development of an effective HPV vaccine in the near future. HPV L1–VLP vaccines appear safe and effective for the prevention of primary infection. Although more progress has been made in the development of prophylactic HPV vaccines, those aimed at the treatment of premalignant lesions and cancer are also promising, albeit still investigational. In the not too distant future, preventative vaccination strategies similar to those used for hepatitis B hold great promise for reducing the burden of HPV-associated cancer.

Future directions

Viruses have evolved to evade the immune system, not to induce an antiviral immune response. New strategies are being developed to improve vaccines so that they will generate immunity beyond that induced by the virus itself and these are needed, particularly in the fight against chronic viral infection. A number of novel strategies are being studied, including epitope enhancement to modify the amino acid sequence of individual epitopes in order to increase their affinity for MHC molecules; incorporation of cytokines, chemokines, and costimulatory molecules to increase and steer the immune response toward the desired type of immunity; blockade of pathways inhibiting immune responses; and development of approaches to increase CTL avidity (32, 113).

Epitope enhancement. As viral sequences may have been selected by immune pressure to differ from sequences that optimally bind to MHC molecules in order to allow viruses to evade immune elimination, modifying the sequence of weaker epitopes may make them more effective vaccines (32, 113). In our early studies of HIV epitopes, we found that the binding of a helper epitope to its class II MHC molecule could be improved by replacing peptide amino acid residues that created adverse interactions (114). Such modification also increased the peptide's efficacy in a vaccine to maximize the CTL response to an attached CTL epitope (115). Further, we found

that the helper response was skewed toward a Th1 cytokine pattern with predominant IFN-γ production. The mechanism could be explained by a reciprocal interaction between helper T cells and dendritic cells in which the higher affinity peptide induced more CD40L expression on the surface of the helper T cells. These in turn induced more IL-12 production by the dendritic cells as well as more costimulatory molecule expression, which skewed the helper T cell phenotype to the Th1 type, made the dendritic cells more effective at activating CTL precursors, and improved protective efficacy (116). Several studies have described epitope enhancement of HIV peptides with low affinity for the most common human class I HLA molecule HLA-A*0201 (117–119), but when modified peptides are used, care must be taken to induce T cells that still respond well to the natural viral sequence (119). This approach has great potential for improving not only peptide vaccines, but any form of vaccine in which such T cell epitopes occur, including recombinant protein, DNA, and viral vector vaccines as well as attenuated viruses.

Use of cytokines, chemokines, and costimulatory molecules. Besides carrying immunogenic epitopes, viruses can also trigger the innate immune system, which alerts the body to danger and helps initiate adaptive immune responses. The signals that transmit these messages from the innate immune system are largely cytokines, chemokines, and costimulatory molecules. Incorporation of these into synthetic vaccines can make the vaccines as effective or more effective than live viruses at eliciting an immune response (reviewed in refs. 32, 120). In addition to individual cytokines, of which the most broadly applicable appears to be GM-CSF, synergies between cytokines such as GM-CSF and IL-12 or the two combined with TNF-α generate more potent immune responses (121–124). IL-15 expressed by a vaccine vector can selectively induce longer-lived memory CTLs (125). Cytokines also synergize with costimulatory molecules to improve the CTL response and antiviral protection (40). A triad of costimulatory molecules can greatly augment CTL responses (126). Chemokines can also be used as adjuvants to enhance immune responses (127, 128). These molecules plus other activators of the innate immune system, such as DNA oligonucleotides of high CpG content, which mimic bacterial DNA (129, 130), can potentiate vaccine efficacy by either triggering or mimicking the innate immune system. They can also steer the immune response toward more protective responses, such as Th1 cytokine production, rather than inhibitory responses. This approach is expected to be a critical component of second-generation vaccine strategies.

Blockade of negative regulatory pathways. Recent work suggests that negative regulation of the immune system is an important braking mechanism that must be overcome to maximize vaccine-induced immune responses (32, 131). CD4+CD25+ T regulatory cells have been found to inhibit other T cell responses (132, 133), and their elimination can improve immune responsiveness to a vaccine (134). Another regulatory T cell subset, CD4+ NK T cells, expresses NK cell markers in addition to conventional T cell receptors and responds to glycolipids presented by CD1 (135). We have found that CD4+ NK T cells can inhibit CTL-mediated tumor immunosurveillance (136–138). These cells act, at least in part, through production of IL-13 (136, 137) and induction of TGF-β (138). Elimination of NK T cells or blockade of IL-13 can increase vaccine-induced CTL responses and protection against an HIV-surrogate virus in a murine model (40). Finally, CTLA-4 has been found to be an inhibitory receptor that binds costimulatory molecules CD80 and CD86 but inhibits T cell responses rather than activating them (139, 140). Blockade of this molecule with antibodies can improve vaccine responses (134,

139), and such anti–CTLA-4 antibodies are now in clinical trials in conjunction with cancer vaccines but may be equally applicable to viruses causing chronic infections. During chronic viral infections, mechanisms that dampen the immune response may contribute to failure to eradicate the virus. Thus we propose that blockade of these regulatory mechanisms may be an important component of a second-generation vaccine strategy for chronic viral diseases.

Induction of high avidity CTLs. High-avidity CTLs are much more effective at eliminating viral infections than low-avidity CTLs (141–143). We recently reviewed the role of high-avidity CTLs in both virus infections and cancer (144). In vitro, high-avidity CTLs can be selectively grown by stimulation with very low concentrations of antigen. However, vaccine strategies to selectively induce high-avidity CTLs in vivo were lacking because very low concentrations of antigen induced no response. However, recently we found that augmentation of costimulation (signal 2) could compensate for a low level of antigen (signal 1) and allow induction of high-avidity CTLs (145). We also recently found that expression of IL-15 by a vaccine vector can select for higher-avidity CTLs that persist longer than low-avidity CTLs, promoting avidity maturation over time (S. Oh, L.P. Perera, D.S. Burke, T.A. Waldmann, and J.A. Berzofsky, unpublished data). We propose that use of such strategies may also be critical for designing the most effective vaccines capable of preventing or eradicating chronic viral infections.

For acute infectious diseases, vaccines have been the most cost-effective agents, saving many millions of lives. However, for chronic viral infections, parasitic and mycobacterial infections, and cancer, the traditional approaches may not be sufficient. Besides implementation of the strategies just described, there are other important goals that need to be attained (see *Vaccines for chronic viral infections: future needs and goals*). Some viruses, such as HIV, invade through mucosal surfaces and grow in mucosal sites, and for those, delivering vaccines by routes that induce mucosal immunity may be critical (32, 34, 36). Mucosal immunization can also provide another benefit in overcoming preexisting systemic immunity to the vaccine vector, such as vaccinia, because of the asymmetry between the mucosal and systemic compartments (146). Use of a DNA-prime and recombinant viral vector boost strategy may also circumvent some preexisting immunity to the viral vector (147). Successful therapeutic vaccination may also require combination with antiviral drug therapy (148). Recent understanding of the immune system has facilitated second-generation vaccine approaches that hold promise for preventing or controlling many of these diseases (149). Some of these new vaccine strategies are being translated into clinical trials, and a combination of these may be necessary to achieve protection against chronic viral infections.

Acknowledgments

Address correspondence to: Jay A. Berzofsky, Vaccine Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 10, Room 6B-12, 10 Center Drive (MSC#1578), Bethesda, Maryland 20892-1578, USA. Phone: (301) 496-6874; Fax: (301) 480-0681; E-mail: berzofsk@helix.nih.gov.

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