Appendix A
A Technical Review of the Evidence for Adverse Reactions to HIV Vaccines

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This appendix reviews the various theoretical risks that have been proposed by various investigators to be potentially associated with HIV vaccines for prophylactic and/or therapeutic use. The theoretical basis for these risks, as well as their proposed mechanisms and experimental support are also examined. As is explained below, some of the risks reviewed here are unlikely or are entirely theoretical (i.e., are currently without experimental support). Suggestions for research initiatives to uncover clues to potential adverse reactions from HIV vaccines are provided.

A key point to remember throughout this analysis is the high rate of genetic mutation of HIV (2, 5, 7, 25, 32, 58); these mutations may allow the virus to become resistant to antiviral drugs and to escape immune surveillance. On average, the virus makes one genetic "mistake" every time it replicates. This is because the unique enzyme that allows the virus to turn RNA genetic information into DNA genetic informationa process called reverse transcriptionis a *low fidelity* enzyme that makes many errors. Such errors are called mutations, and may be lethal (i.e., incompatible with viral replication) or may be tolerated.

Unfortunately, HIV appears to tolerate an extraordinary number of mutations throughout the length of its genome. Under certain conditions these mutations even confer a selective advantage to the virus. This is the basis for the high rate of evolution of new viral mutants (or *quasispecies*). For example, if the mutation interferes with the ability to bind active metabolites of the antiviral drug AZT (zidovudine), the resulting mutant virus may be resistant to AZT. If the infected patient (the host) is treated with AZT, the mutant virus will have a selective advantage and over a period

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of months to years become the predominant type of virus in the patient (the *dominant quasispecies*) (12, 56).

Similarly, if a mutation occurs at a site previously recognized by the patient's neutralizing antibodies, the virus carrying this mutation (the *escape mutant*) may evade immune detection and emerge as the dominant quasispeciesat least until a new set of antibodies are formed that can recognize and block the mutant virus (20, 46). Thus, HIV is continually evolving under the selective pressure from the host's immune response and from antiviral drugs. This evolution occurs not only at the level of the overall population of infected people, but also within a single infected individual over the course of disease.

Enhancing Antibodies

The possibility that HIV vaccination could induce antibodies that facilitate viral entry into immune phagocytic cells has been studied in the laboratory using a variety of cell types[143]. Results have been inconsistent among studies, and the evidence for this phenomenon has recently been comprehensively reviewed by a study group sponsored by the National Institutes of Health (NIH) (39). Some have suggested that anti-HIV antibodies that are protective or inactive at one concentration may be enhancing at a lower concentration (50). To date, there has been little laboratory evidence of antibody dependent enhancement in the sera of HIV vaccine recipients, but this may be due to the limited number of laboratories that are examining this potential problem. More importantly, the activity of HIV in humans may not be adequately approximated by laboratory studies. Investigators have presented evidence that macaques that were vaccinated with SIV protein subunit vaccine (17) or transfused with anti-SIV antibodies (26) showed enhanced rates of infection and disease progression when subsequently exposed at mucosal membranes to SIV.

Original Antigenic Sin

HIV infection induces an abundance of antibodies, including neutralizing antibodies; however several groups have shown that the generation of neutralizing antibodies tends to lag behind the generation of viral escape mutants by several months or even years. One explanation for this observation involves the phenomenon of *original antigenic sin* (OAS), the fixing of an immune response in a nonadaptive pattern.

OAS was first observed in immune responses to sequential influenza A virus infections. Investigators observed that, in some instances, exposure of an individual to one strain of influenza A virus triggered the production of antibodies that were predominantly directed at another strain of influenza A virus that had infected the individual in the past. The antibodies that were produced had weak affinity for the newly encountered strain of influenza A virus. OAS is a particularly important problem with organisms that mutate frequently. OAS has also been observed in some bacterial infections as well, but its mechanism has never been fully elucidated.

Some investigators have argued that, during the course of HIV infection, an OAS pattern occurs with respect to antibodies recognizing the V3 loop and other variable regions of HIV envelope proteins (31, 44).

In infected individuals, there may emerge a predominance of neutralizing antibodies directed against HIV species present at some earlier time of infection, but not to the contemporaneous HIV species.[144] While this may simply reflect a delay in the development of measurable titers of anti-

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[143] This antibody dependent enhancement may occur in the presence (50) or absence (15) of complement.

[144] The mechanism by which OAS occurs has been investigated. The current hypothesis is that previously stimulated B lymphocyte clones bearing surface receptors with high affinity for previously circulating strains of virus may be sufficiently cross-reactive (due to membrane surface-arrayed multivalent binding) to be triggered by new viral strains; but the B lymphocytes secrete antibody that, in soluble monomeric form, have only low affinity for the new strains.

body directed against the more recent circulating strain of HIV, this delay may have potentially significant immunologic consequences, and its mechanism remains unclear. There is also some recent laboratory evidence that an OAS pattern can be observed in B and T lymphocytes from uninfected volunteers following vaccination with recombinant HIV protein subunit vaccines (54).

Related to the observation of a lagging antibody response to HIV escape mutants is that of a limited and relatively fixed diversity of antigenic specificity seen among antibodies created in response to HIV antigens (14, 31, 43, 44, 45, 61). Whether this is a cause or effect of the delayed antibody response to emergence of new strains of virus is unclear. In either case, this low diversity antibody response to HIV is probably detrimental to the host's ability to suppress infection.

It should be emphasized, however, that the evidence supporting the view that there is a limited diversity in antibody response to HIV rests largely on the finding that certain antibody *variable region* genes, the genes that code for an antibody's antigenic specificity, are used disproportionately for the immune response to the dominant antigenic regions of the virus. This still allows for greater diversity to be generated during the course of the immune response by a process called *somatic mutation*, as demonstrated by Andris and colleagues (1). Thus, studies showing that only a restricted number of variable region genes are used for the production of anti-HIV antibodies probably underestimate the true diversity of the antibody response.

Vaccine-induced OAS may occur when a vaccinated individual is exposed to a noncross reactive strain of HIV that induces the production of antibodies specific for the vaccine strain that are unable to neutralize the newly encountered strain. When exposed to HIV, however, vaccinated individuals exhibiting OAS may be no worse off than unvaccinated individuals because unvaccinated individuals also have a lag in generation of antibody to HIV because their immune response has not been "primed" by vaccination. It is not known whether the lag in antibody production in unvaccinated individuals is greater than the lag in the production of antibody directed to contemporaneous HIV strains in vaccinated individuals exhibiting OAS.

Expansion of V_3H family or other families of B lymphocytes

Investigators have found that certain genes for a particular family of antigen receptors on B lymphocytes (the V_3H family) are expressed much more frequently among HIV-infected individuals than uninfected individuals. Because more than half of all HIV-infected individuals express these antibodies, which bind to viral proteins, we know that the virus induces their expression (43, 61). Muller and colleagues have shown that such antibodies were even further elevated in 40 of the 44 HIV-infected patients with B cell lymphomas (24). Because these antibodies are not necessarily protective and seem to be associated with lymphomas, their presence may not be desirable.

Recently Schwartz and colleagues examined the sera of vaccinees receiving various HIV envelope-based vaccines for the presence of these antibodies. They found that many vaccinees made them at some point after immunization, generally at times of peak total antibody response (unpublished data). Thus, envelope based-vaccines are, at least transiently, inducing antibodies that mimic this aspect of the host response to HIV infection.

Recently, a group of investigators presented evidence that HIV-envelope (gp120) protein can function as a superantigen for B lymphocytes carrying another family of antigen receptors (4). By binding to a common portion of the surface immunoglobulin receptors of B lymphocytes, gp120 - envelope protein initially induces stimulation and then exhaustive depletion of those B lymphocytes carrying surface receptors from that family of genes. Other investigators (34) believe such B lymphocytes can support infectious replication of HIV and also may contribute to B cell lymphomas in HIV-infected patients. Hence, a concern that

most HIV envelope vaccines in development could expand this pool of B lymphocytes and induce B cell lymphomas exists.

Expansion of "Double Jeopardized" CD4+ (T Helper) Cells, Leading to Increased HIV Replication

There may be subsets of CD4+ T lymphocytes that are particularly susceptible to HIV infection early in disease. All CD4+ T lymphocytes can be infected by HIV by virtue of their surface membrane CD4 molecules, which serves as the site of attachment of the virus. However, it has long been appreciated that immune-activated CD4+ lymphocytes are better hosts for HIV entry, integration, and replication than are resting CD4+ cells. Further, cells cannot become infected unless they are brought into proximity either with infected cells or virus. At the earliest stages of HIV infection, the number of infected cells limits the cell-to-cell spread of the virus, and therefore there is a low likelihood that a random CD4+ lymphocyte will come into contact with an infected cell. By contrast, CD4+ lymphocytes with specificity for HIV are constantly "searching" for HIV infected cells to bind to, and thus are at increased risk of coming into close proximity to virus and becoming infected. If HIV undergoes a burst of replication in such cells, this would contribute to early dissemination of virus and poorer long-term prognosis.

Circumstantial evidence supporting the early destruction of HIV-specific CD4+ lymphocytes comes from the results of in vitro *lymphoproliferation assays*, which measure the magnitude of the proliferative response of lymphocytes to a series of *recall antigens* to which the lymphocytes have previously been exposed. These experiments have shown that HIV envelope protein was unable to induce the proliferation of CD4+ lymphocytes obtained from asymptomatic HIV-infected individuals, even though the responses of these CD4+ lymphocytes to other recall antigens were intact.

Experimental evidence also exist for the special ability of antigen-presenting immune cells pulsed with HIV to activate and destroy CD4+ lymphocytes with which they come in contact (8, 38). At the same time, these activated cells can become infected with HIV and support a burst of HIV replication prior to destruction of the infected cells. This might be expected to happen with vaccine-induced CD4+ lymphocytes, which would seek out and proliferate in response to HIV at the earliest stages of infection. A mathematical model this scenario has recently been published (55).

Priming for T Helper 2 (TH2) and T Helper 1 (TH1) Patterns of Cytokine Response

Cytokines are cell-to-cell communication and growth molecules, which can be thought of as short-range hormones. The distinct and to some degree antagonistic cytokine profiles of TH1 and TH2 responses have received increasing attention from HIV researchers. TH1 responses are characterized by the production of the cytokines interleukin-2 (IL-2), IL-12, and Interferon gamma. These cytokines are important in the induction of cytotoxic T lymphocytes. TH2 responses produce IL-4, IL-5, and IL-10—cytokines crucial for the induction and amplification of various antibody responses. Furthermore, the cytokine IL-12, produced by the TH1 response, suppresses TH2 cytokine production, while IL-10, produced by the TH2 response, suppresses TH1 cytokine production. This negative feedback inhibition between TH2 and TH1 responses can accentuate the differences between them.

Although TH1 and TH2 responses were first described in mice, similar though less clearly distinct cytokine profiles have been demonstrated in human cells in vitro, with mitogens (cytokines that induce cell division) and recall antigens inducing predominantly TH1 responses in PBMCs of normal donors and a TH2 profile in PBMCs of HIV-infected individuals (10, 11, 41).

To the extent that TH2 responses in HIV-infected individuals are not protective and are antagonistic to desirable TH1 responses, some researchers have argued that priming for TH2 responses is an inappropriate, counterproductive goal for vaccination, and a likely consequence of recombinant protein subunit vaccines (51).

Schwartz and colleagues at Johns Hopkins University are currently testing the cytokine profiles of vaccinees' PBMCs restimulated in vitro with HIV or HIV antigen. Unpublished preliminary results suggest that the TH1 response remains dominant, thus assuaging some of the concerns that vaccines may prime for TH2 responses.

Induction of Autoimmunity

Any pathogen that binds to or mimics the structure of self-antigens is capable of inducing antibodies directed against the self (autoantibodies). HIV both binds to CD4 receptors of T lymphocytes via it's gp120 -envelope protein and bears sequence homology with several human antigens. There have been several autoantibodies among HIV-infected and envelope vaccinated individuals found, albeit of questionable significance (13, 18, 19, 33, 47, 52). Most intriguing has been the transient, episodic appearance of anti-CD4 antibodies in HIV-infected individuals and in uninfected recipients of rgp160- or rgp120-envelope vaccines (28, 29, 30). Originally a concern because of the potentially immune suppressive effects of such antibodies on CD4+ lymphocytes, the transient appearance of anti-CD4 antibodies has not had detectable effects on healthy vaccinees as judged by their CD4+ lymphocyte counts and the results of in vitro lymphoproliferation assays against recall antigens. Furthermore, Neurath and colleagues have recently demonstrated that hyperimmune rabbit anti-gp120/gp160 antisera had negligible binding activity against a variety of CD4, HLA-I and HLA-II cell surface antigens (30). These authors concluded that detrimental effects from envelope vaccines are improbable.

Interestingly, Letvin and colleagues have shown that the purposeful induction of anti-CD4 antibodies in chimpanzees (63) or administration of anti-CD4 monoclonal antibodies in macaques (48) can protect their cells in vitro from infection with HIV or SIV upon subsequent challenge. Furthermore, immunization of SIV-infected macaques with soluble recombinant RCD4 receptors resulted in both an anti-CD4 and an antiviral response (63). The significance of the low and intermittent anti-CD4 antibody titers seen in the sera of HIV-infected patients is unknown. The possibility of autoimmunity is frequently invoked in discussions of HIV immunosuppression and the destruction of uninfected CD4+ lymphocytes, but there is presently no evidence that anti-CD4 antibodies play a role.

Induction of Endogenous Retroviruses or Oncogenes by HIV Genes or Proteins

In mice, various mammary tumor viruses encode superantigens that can activate dysfunctional lymphocyte proliferation (see discussions of clonal expansion above). Gallo and colleagues were able to induce Kaposi sarcoma-like lesions in male mice transgenically engineered to express only the HIV tat gene (16). Recently, Sekaly and colleagues have shown that transfection of only the HIV gag gene into mice carrying latent mouse mammary tumor virus (MMTV) can cause the induction of active expression of the MMTV viruses, with detrimental MMTV-induced immune consequences. Humans may also carry latent endogenous retroviruses or retrovirus related cellular oncogenes with pathogenic potential. It is possible that introduction of even partial HIV genomes in live vectors carrying, for example, the gag and tat genes, could activate harmful endogenous retroviral genes.

There is a high frequency of tumors in HIV-infected individuals, and in most cases these cells do not harbor HIV. Therefore, secondary effects of HIV infection must be invoked, and these effects may not be dependent on the presence of the complete viral genome. Recently, McGrath and colleagues have identified the HIV genome at constant chromosomal location in the genome of non-B cell lymphoma cells obtained from several unrelated patients with this cancer (57). This further supports the notion that HIV genes may have oncogenic potential.

Induction of Short-Term Immunosuppression

Luban and colleagues have shown that HIV gag proteins bind to cyclophilins (37). These cyclophilins are also targeted by the potent immuno-

suppressive drugs cyclosporin A and FK506. Thus, production of significant amounts of gag gene product for any extended length of time, which may occur as a result of vaccination with a live vector coding for HIV gag gene, might induce immune suppression by the same mechanism as cyclosporin A. Presumably, the vaccine-induced immune response would then eliminate this source of gag.

Cross-linking of CD4 by HIV envelope gp120 or gp160 proteins sends an incomplete signal leading to immune exhaustion (anergy) or subsequent programmed cell suicide (apoptosis) (3, 35, 41, 62). This is thought by some to be a major mechanism of immunosuppression in HIV disease. It is unlikely that the amounts of gp120 used or produced by HIV vaccines would be sufficient to induce any serious immunosuppression, but subtle short-term effects might be induced, especially if anti-gp120 antibodies have also been induced by vaccination (36). Similarly, while apparently not a long-term problem, it is possible that the vaccine-induced production of anti-CD4 antibodies, as described above, could also cause transient immunosuppression.

Short-term immunosuppression following vaccination may occur due to temporary dysregulation of cytokine responses. This is observed after measles vaccination (21) and mimics the more severe immunosuppression accompanying measles infection (22).

The detrimental consequences of transient acute post-vaccination immunosuppression may be much greater in developing countries and other settings where there are high pathogenic burdens (due to other viruses, bacteria, and parasites) found in many third world countries. Subtle immunosuppression of selected T lymphocyte clones—even some HIV specific clones—may not be detected on current routine tests of immune function. Limited data on the course of HIV infection is acquired from several volunteers who became infected during or following immunization with experimental vaccines. It is too soon to know if disease progression will be accelerated in these individuals.

Recombination in HIV Infected Vaccinees: Retroviruses are capable of genetically recombining with themselves, other viruses, and with host-cell genes (27, 59). This raises the possibility that even multiply deleted, replication incompetent, live vector or naked DNA vaccines might conceivably recombine in the vaccinated host with preexisting or newly acquired HIV or other viruses. There is also the possibility of integration of the HIV genome at a site that has oncogenic (cancer inducing) potential, as is noted above. This is likely to be a rare event, and not readily predicted by preclinical studies.

Activation of HIV from Latently Infected Cells

It has been a goal of HIV vaccine developers to generate protective cytotoxic T lymphocyte responses to HIV. Many other viral infections are thought to be controlled by the constant surveillance and appropriate activation of cytotoxic T lymphocytes recognizing viral antigens in the context of histocompatibility antigens on the surface of infected cells. Recently, however, some studies have raised the possibility that, at least under some conditions, activated cytotoxic T lymphocytes may release cytokines such as TNF-alpha and GM-CSF that can stimulate HIV production in infected cells (6, 23). This concern has caused at least one biotechnology company to discontinue a program of ex vivo expanded autologous anti-HIV cytotoxic T lymphocyte reinfusion, following what they perceived to be a downhill course during treatment of their first patient. However, similar Phase I clinical trials under the direction of Dr. Judy Lieberman at Boston University/New England Medical Center appear to be moving forward with encouraging results.

Possible Adverse Immunological Consequences At The Population Level

There is a possibility that widespread immunization with vaccines could select for more virulent strains of HIV at the population level. Some of the same mutations that permit HIV to avoid neutralization by the immune system may also select for

greater virulence. There is some evidence for this occurring naturally during the course of HIV infection in individuals, in that HIV recovered from patients in later stages of infection is generally more rapidly growing, has a more pathogenic effect, and attacks a wider variety of cells, than HIV isolated from patients in early stages of infection (9, 53, 60). Empirical evidence exists, as well as theoretical reason, to consider these late stage viruses as mutants that escaped host immune defenses. If the effect of vaccination programs were to select for the these late stage viruses early in disease, they might become the dominant circulating strains in the population, leading to more acute disease progression among infected individuals. It is also theoretically possible that large scale vaccination could select for the most infectious strains of HIV[145].

Current studies of early seroconverting cohorts suggest that macrophage tropic, non-syncytium inducing (NSI) HIV strains are the most readily transmitted (40, 65, 64). These also tend to be the strains associated with better health and longer term survival. By contrast, syncytium-inducing (SI) strain emergence is correlated with a downhill course in the host (49). Because of the apparent role of NSI strains in HIV transmission, there has been discussion of focusing vaccine efforts against such strains. If this selective pressure favors transmissible SI strains, it might result in increased prevalence of those more pathogenic strains.

No firm evidence has developed that HIV has evolved toward greater pathogenicity at the population level since the onset of the global pandemic. One reason for this may be the relatively early stage of worldwide host-virus equilibrium in a plague that is still spreading exponentially through many populations. Also because of the high rate of mutation intrinsic to HIV, only the most strongly and consistently selected mutations will remain constant, with reversions occurring as soon as specific selective pressures are removed. Some evidence for population-based selective pressure has come from the reported recovery of AZT-resistant strains in recently infected individuals who had never received AZT, but lived in areas where the use of AZT in infected individuals was high.

Vaccination for particular HIV strains or epitopes would create the conditions for constant and widespread selective pressures that may affect the genotype and phenotype of HIV in the population. If enough members of a population were vaccinated, selection pressures would favor the predominance of escape mutants in the population, resistant to vaccine-induced immune responses. Because of the long-lived nature of successful immunizations, and the fact that a large percentage of uninfected high-risk individuals may have been vaccinated within a given community, the long-term selective effects of vaccination on the circulating strains of HIV may be more difficult to reverse than those of an antiviral drug such as AZT, which can be stopped completely, allowing for rapid reversion to drug sensitivity in the circulating strains of virus.

APPENDIX A REFERENCES

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