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HIV Vaccines: A Progress Report

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Interests:

Mucosal vaccines
Clinical HIV vaccine trials
Metabolic effects of antiretroviral therapy
Hepatitis C/HIV co-infection

HIV Vaccines: A Progress Report

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I. The need for an HIV vaccine: global and local perspectives.

In 1984, U.S. Secretary of State Margaret Heckler reported that an HIV vaccine would be developed in the next two years (1). One and one half decades later, that goal is nowhere in sight. Each day, 16,000 new infections take place; and a total of 36.9 million people worldwide are currently living with HIV (2). About 90% of these new infections take place in developing countries where the average annual income falls far short of the cost of paying for even one month of HAART (Highly Active Anti-Retroviral Therapy). Over half of all infections are in young adults, with devastating social, demographic, and economic consequences for the hardest-hit countries in sub-Saharan Africa (3). The World Health Organization announced in May of this year that HIV is the world's most deadly pathogen, having overtaken tuberculosis and diarrheal illnesses to become the fourth leading cause of death in the world (Figure 1). Prevention measures such as education and access to condoms do reduce the risk of HIV transmission. However, these interventions do not reduce the transmission risk to zero and are not globally available (International Aids Vaccine Initiative (IAVI): Scientific Blueprint for AIDS Vaccine Development, 1998).

In the industrialized world including the U.S.A., the picture is somewhat less gloomy. AIDS has been transformed into a chronic, treatable disease for which there are now 14 FDA approved anti-HIV drugs (4). Great strides have been made in preventing maternal-fetal transmission through screening and prenatal HAART. However, close to 1 million people are living with HIV in the US, at a cost in 1996 dollars of $20,000 per person per year (5). These costly drugs have recently halved HIV-related mortality, and yet they are incapable of eliminating replication-competent HIV from lymphoid tissue (6).

At Parkland Hospital, approximately 3500 individuals receive HAART, which costs 8.5 million dollars per year- or 18.6% of the annual pharmacy budget. To put this figure into perspective, consider that the cost for diabetes specific drugs is about 2 million dollars per year, or 4.4% of the annual pharmacy budget (figures from Parkland Drug Information and Policy Analysis). Because the mortality rate from AIDS has been cut in half since 1996, our clinic census is swelling at a rate of 50 to 60 new patients per month without a compensatory attrition rate. A recent national survey of newly infected patients, in which Dallas was a participant, demonstrated that drug resistant HIV is being transmitted (7). To meet the challenge of treating and preventing drug-resistant HIV, costly tests, such as ultra-sensitive HIV viral load assays, phenotyping, and genotyping are now being added to our standard of care.

Leading Causes of Death in the World, 1998

Disease	Estimated Deaths, 1998
1. Ischemic heart disease	7,375,000
2. Cerebrovascular disease	5,106,000
3. Acute lower respiratory infections	3,452,000
4. HIV/AIDS	2,285,000
5. Chronic obstructive pulmonary disease	2,249,000
6. Diarrheal diseases	2,219,000
7. Perinatal conditions	2,155,000
8. Tuberculosis	1,498,000
9. Cancer of the lung/ bronchus/trachea	1,244,000
10. Road traffic accidents	1,171,000

From: World Health Report, 1999, WHO, Geneva, 1999.

Figure 1

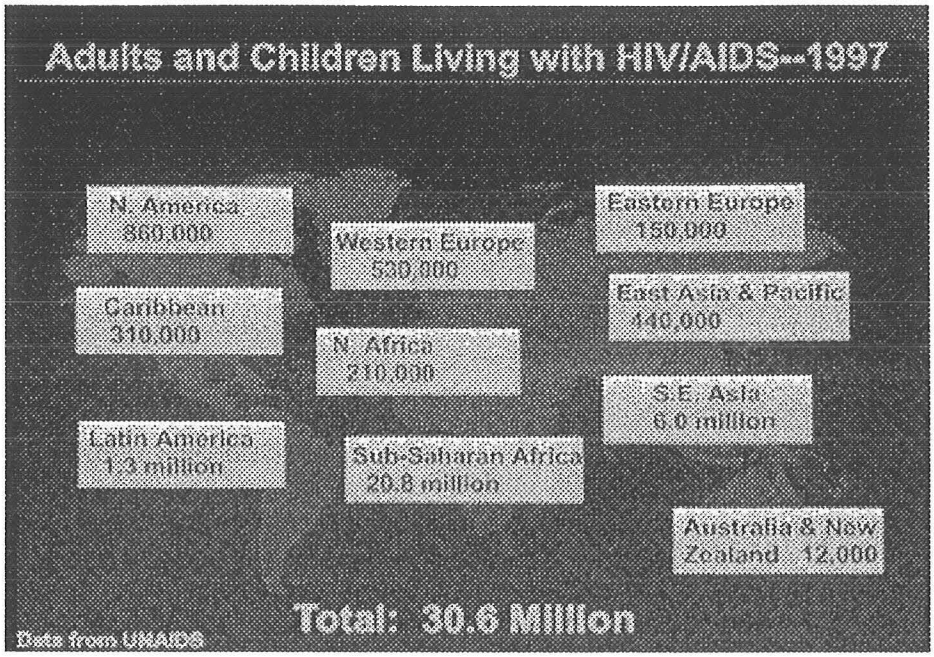


Figure 2

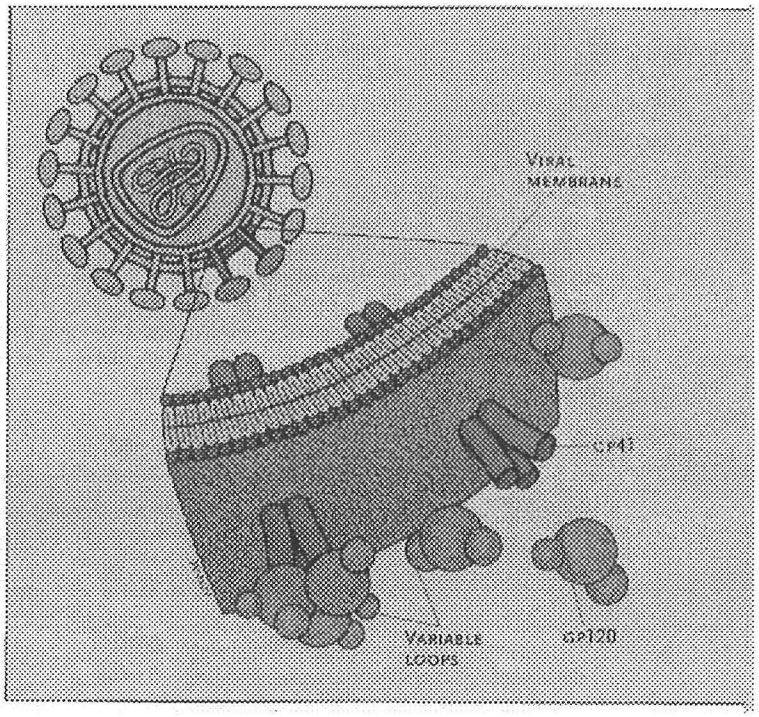
Thus, the human and economic costs of the AIDS epidemic have created an urgent need for a preventive HIV vaccine. Today's progress report will review the scientific obstacles to HIV vaccine development as well as new strategies developed to overcome these obstacles. A summary of clinical trials in humans and primates will be presented, as well as a brief listing of the ethical and logistical issues that present challenges to these vaccine trials. Dr. David Baltimore, chairman of the NIH AIDS Vaccine Research Committee, recently noted that "*It is an embarrassment that the scientific community, with all its power, can't outwit an organism of 10,000 nucleotides*". This review may provide us with the perspective necessary to evaluate that statement.

II. Features of HIV biology relevant to vaccine design

A. Anatomy of the virion

At the core of this retrovirus are two single strands of viral RNA surrounded by a protein core (p24 antigen). The outer membrane is a lipid sheath pierced by envelope glycoproteins: transmembrane gp41 spikes, which are non-covalently associated with outer membrane trimeric gp120 knobs (Fig. 3). The gp120 knobs are composed of a conserved core from which variable loop structures protrude, and the whole complex is so heavily glycosylated that it has been described as a "sugar dome" which hides important epitopes from the immune system. One role of the envelope glycoprotein is to facilitate virus entry into cells. Within gp120 is a deep cleft that binds to CD4, a viral receptor on the host cell surface. After binding, a conformational change occurs in gp120 exposing another binding site for co-receptors (8). HIV uses the chemokine receptors CCR5 or CXCR4 as secondary docking points. Most primary isolates of HIV are M-tropic (macrophage tropic) and use CCR5 as the co-receptor for entry. Later in the disease, the virus is preferentially T-cell tropic, using the CXCR4 molecule as co-receptor (9). These features are important for vaccine design because most envelope-based vaccines to date have been directed against the T-tropic strain of virus. By virtue of their more accessible Env-epitopes, T-tropic Env-based vaccines elicit better NA (neutralizing antibodies; those which prevent viral particles from entering and infecting a host cell) than M-tropic virus-based vaccines. Unfortunately, NA raised against T-tropic viruses are ineffective against the M-tropic strains which actually cause primary infection. Both the variable regions of gp120 and the shield of complex carbohydrates contribute to the difficulty in making good NA against HIV (9).

Figure 3: Anatomy of a virion. (8)

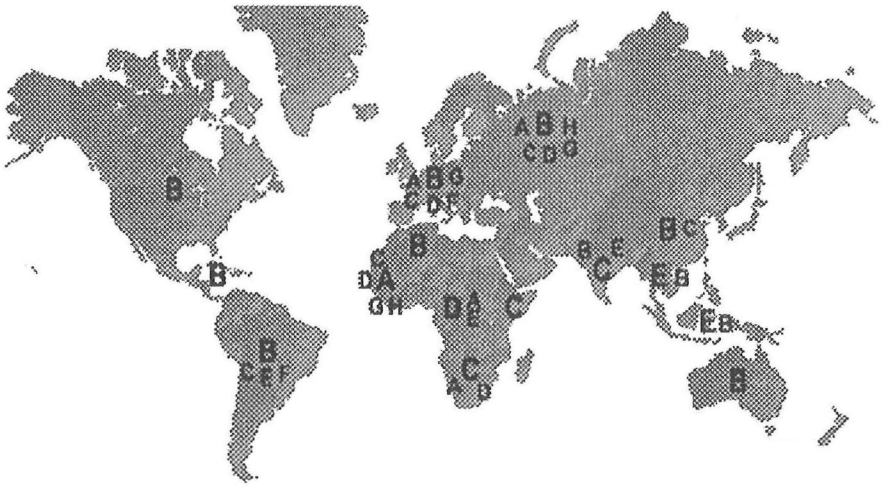


B. Viral Diversity

Based on DNA sequencing, HIV has been divided into ten genetic subtypes known as clades. The clades are designated by letters of the alphabet from A-J; clade O is an additional genetic subtype representing a small outlying group. In the United States, Europe and Australia, clade B is predominant. Antibodies induced in human trials based on clade B viruses have only rarely neutralized viruses from the other clades. Generation of protective cytotoxic T lymphoctye-mediated immunity is also hampered by the potential need to elicit clade specific responses.

The vast genetic diversity of HIV is due to the enormous speed of replication (one billion virions are produced daily in an infected host), (10); as well as the error-prone nature of HIV-1 reverse transcriptase (11). Viral diversity is an important mechanism by which HIV escapes immune surveillance. These issues have prompted ongoing extensive searches for conserved epitopes that elicit cross-clade immunity as well as the suggestion that vaccines should be customized for the geographic region in which they are to be used, or administered in a multivalent cocktail (12). Consider the vastly less complex scenario presented to those who designed globally effective vaccines to prevent polio, which has 3 distinct serotypes, each conferring life-long type-specific immunity (13). Measles and Varicella have only one relevant strain each.

Figure 4: Viral Diversity: Global Distribution of Clades

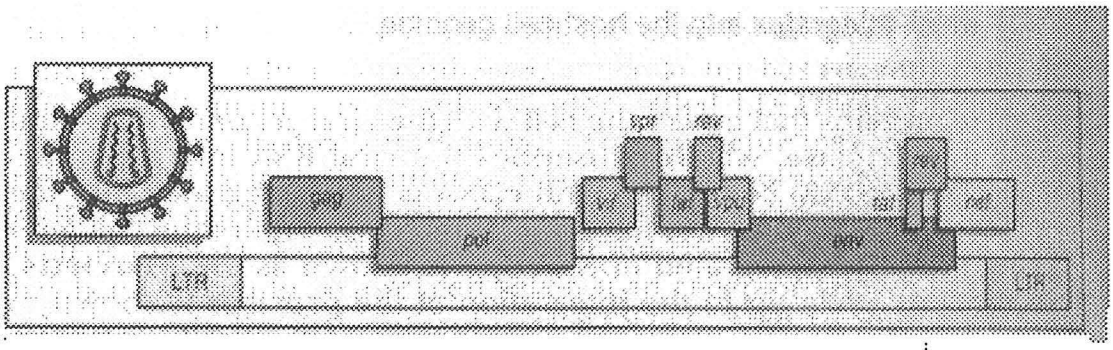


C. Complexity of the viral genome.

HIV consists of 9 genes flanked by long terminal repeat (LTR) sequences required for integration into host DNA, and binding sites for gene regulatory proteins. Three major genes are similar in all other retroviruses: *gag, pol,* and *env*. *Gag* encodes structural proteins of the viral core, *pol* encodes enzymes for replication and integration, and *env* encodes the envelope glycoproteins (14). Six virally encoded regulatory/accessory proteins (Tat, Rev, Vif, Vpr, Vpu, and Nef) impart complexity to HIV replication that is not seen in other members of the lentivirus family. ***Tat*** is a transcriptional activator that

regulates high-level transcription of DNA from the integrated DNA form of the virus. Tat is produced early after infection and is essential for virus infectivity and replication. Extracellular Tat induces CCR5 and CXCR4 co-receptors, thereby enhancing cell to cell spread of virus. Historically, vaccine strategies have focused on Env, Gag, Pol and Nef as targets (6). All of these epitopes can induce CD8+ T cell mediated immunity, and Env has the potential to induce NA (15). Tat has received attention recently as a potential vaccine target because it has also been shown to be immunogenic. Importantly, Tat is conserved in its immunogenic epitopes among most of the different clades. (16). Rev is a regulator of viral expression that allows export of unspliced transcripts from the nucleus. *Nef* is associated with increased viral loads in animal models. Nef protein confers an immune-escape strategy for HIV-1 by down-regulation of MHC class I as well as CD4 expression on the cell surface. (17). Infection with a strain of HIV that is defective in *nef* appears in some cases to result in a much milder form of HIV disease (the long-term non-progressor state). Therefore, use of *nef*-deleted viruses is an important vaccine strategy in primates, which we will review in more detail.

Figure 5: The Viral Genome (14)



Gene		Gene product/function
gag	Group-specific antigen	Core protein
pol	Polymerase	Reverse transcriptase, protease, and integrase enzymes
env	Envelope	Transmembrane glycoprotein; gp120 binds CD4; gp41 is required for virus internalization
tat	Transactivator	Positive regulator of transcription
rev	Regulator of viral expression	Allows export of unspliced transcripts from nucleus
vif	Viral infectivity	Affects particle infectivity; helps assemble virion
vpr	Viral protein R	Positive regulator of transcription; Augments virion production
vpu	Viral protein U	Unique to HIV-1; Downregulates CD4
nef	So-called negative-regulation factor	Augments viral replication in vivo and in vitro; Downregulates CD4

D. Viral life cycle. Figure 6 is a schematic representation of events in the HIV life cycle. The requirement for co-receptor usage to facilitate fusion with the host cell membrane is illustrated. The integration of virus into the host genome can last for many years, allowing escape from the immune response. New virions may capture host cell membrane determinants in the budding process, presenting another major hurdle for vaccine design: some vaccines that protected monkeys did so by virtue of the immune response directed against cellular determinants (interposed in the budding virus), rather than by virtue of virus-specific immunity (6).

Figure 6

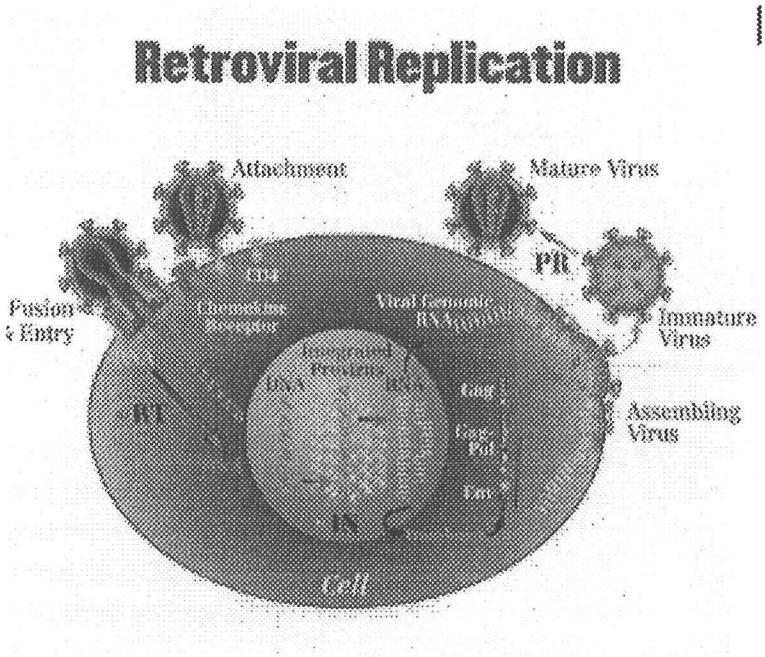
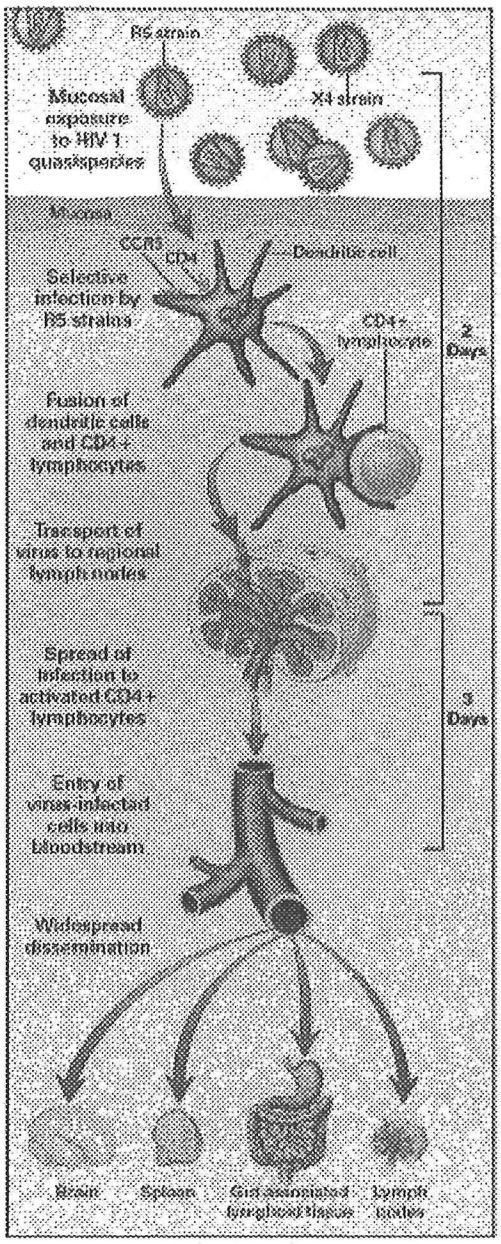


Figure 7 (2)



E. Pathogenesis

Most HIV-1 infections take place at a mucosal surface, yet little is known regarding how to induce effective immune responses in rectal and vaginal mucosa. Further, both antibodies and CTLs have been detected at mucosal surfaces, but it is still unclear what role they play in immune protection

The first cellular targets of the virus are tissue Langerhans dendritic cells of the lamina propria (Fig. 6). Infected dendritic cells fuse with $CD4^+$ lymphocytes, and these fused cells then spread to deeper tissues. Virus can be detected in draining lymph nodes two days after experimental infection, and systemic dissemination occurs shortly thereafter. four to 11 days elapse from mucosal infection to initial viremia, presenting a critical window of opportunity for immune interception. Langerhans dendritic cells, which are the earliest viral target, express CCR5 but may not express the CXCR4 coreceptor, explaining why R5 viruses are usually predominant in acute infection (2).

After the initial rise in viremia there is a marked reduction from the peak viremia to a steady-state level of viral replication. This steady state level of viremia, referred to as the viral setpoint, varies widely from individual to individual, and is correlated closely with prognosis. Persons with the highest viral set points have the most rapid rates of progression to AIDS and death (18). Lowering the set point during primary infection, a potential outcome of a non-sterilizing AIDS vaccine, could result in a lower setpoint, and therefore in an improved prognosis. Study of individuals who control viremia well during acute infection is one important way to gain insight into correlates of immunity to HIV.

III. Scientific obstacles to HIV vaccine development.

Why is it so hard to make an AIDS vaccine?

1. The correlates of protection are poorly defined.

Despite 15 years of study, we do not fully understand which kinds of immune responses are necessary for vaccine-induced protection from HIV. Progress has been made by studying infected people who are either long-term non-progressors, or multiply exposed but HIV-seronegative (19). Additional insight has come from animal vaccine trials, and

from the nature of the vigorous immune response to primary infection which drastically reduces viremia after the acute infection (20). The main kinds of immune responses observed are CTL (CD8+ T lymphocyte cytolysis of virally infected cells), CD4+ T lymphocyte proliferative responses, neutralizing antibody responses, and finally ADCC, or antibody-dependent cellular cytotoxicity (15). ADCC is defined as the destruction of antibody coated target cells by natural killer (and other) cells. Most neutralizing antibodies have ADCC activity, but not all ADCC have NA activity. In addition, soluble factors from $CD8^+$ cells are inhibitory for HIV (21).

A. Primary Infection: During acute HIV retroviral syndrome, there is a steep rise in plasma viremia. Vigorous $CD8^+$ CTL responses appear quickly and are temporally associated with declining viral titers (20). One in 17 $CD8^+$ T cells in peripheral blood may have HIV specificity during this acute phase (2). Individuals with CTLs of especially broad specificity in early HIV have lower viral set points and slower disease progression, providing further evidence that CTL contain viremia (22). In individuals who receive early, potent HAART during acute infection, a gradual generation of virus-specific $CD4^+$ cell proliferative responses can be demonstrated as viral load is suppressed to undetectable levels (23). The role of $CD4^+$ T cells in antiviral immunity probably relates to providing help for CTL production, increased production of cytokines, or augmented antibody responses. Neutralizing antibodies are not detectable until weeks to months after reduction in replicating virus (20).

B. Non progressors: These patients remain clinically asymptomatic 7 to 10 years after primary infection and have stable $CD4^+$ T cell counts; they comprise about 10 to 15% of the HIV-infected human population (19). These patients have high levels of CTL that do not decline with time. They also have strong $CD8^+$ non-MHC-restricted HIV suppressor activity, and significant aby (antibody) levels. High NA levels with broad cross-reactivity have been found, suggesting that NA levels may be important for the control of HIV in non-progressors. On the other hand, anti-gp120 antibodies displaying ADCC (but non-neutralizing) activity against cells infected with divergent strains of HIV have recently been isolated from a non-progressor as well, suggesting that we should not be solely focused on abys that are capable of neutralizing (24). Picker et al showed that CD4+ memory T cells are detectable in most subjects with active HIV infection, but frequencies of these cells are considerably higher in non-progressors. Continuous HIV suppression with HAART is associated with a reduction in frequencies of gag-specific CD4+ memory T cells. Thus, functional HIV-1-specific CD4+ T cells are commonly available for support of anti-HIV responses in active disease, but their decline with HAART indicates that immunologic participation in long-term HIV-1 control will probably require vaccination (25).

C. Multiply exposed, HIV seronegative: These individuals also show T cell responses to HIV proteins. Mucosal immunity is postulated: if CTLs are present at the site of challenge, adequate control of infection may be achieved (19). Seronegative female sex workers in the Gambia who are repeatedly exposed but uninfected have HIV-specific CTL activity (detected in peripheral blood), in the absence of HIV infection by PCR, and in the absence of seroconversion (26). Their exposure to live or defective virus particles could have immunized these women; it is unlikely that this represents cross-reactivity from CTL primed by similar epitopes in another pathogen. A comparable group of

women in Nairobi show that a few remain uninfected over long periods of time, appearing to have lasting immunity. A small subgroup of highly exposed, uninfected people are homozygous for a 32-bp deletion in CCR5. These individuals resist infection with R5 strains, but can be infected with X4 viruses (2, 27).

TABLE 1: Obstacles to vaccine development

The correlates of immunity to HIV are incompletely defined
HIV only poorly elicits neutralizing antibody
There are multiple HIV-1 clades
HIV mutates rapidly after infection, resulting in immune escape mutants
Viral integration into the host genome
Animal models have limitations

IV. What animal models have taught us.

1. Chimpanzees

Pan troglodytes troglodytes is the source and ultimate reservoir for SIVcpz (Simian Immunodeficiency Virus, cpz = chimpanzee), which is the immediate genetic ancestor of HIV-1 (28). Humans probably became infected with this virus by blood contact during the butchering of these animals for food. With one exception, (a chimp-passaged isolate of HIV-1) HIV-1 does not cause AIDS in chimpanzees. Patient isolates maintain low levels of replication in these animals, with no detectable viral RNA in the plasma (6). Vaccines that have been reported to protect chimpanzees have actually only protected against these weak isolates. While challenge with the single more virulent HIV strain would be a better test of vaccine protection, chimpanzees are an expensive and endangered species. The ethics of deliberately infecting a chimp with a virulent strain have been questioned. Furthermore, there is no standard way to give mucosal challenges and large numbers of female chimpanzees are not available for research. Thus most challenge studies use either IV or rectal challenges, which may not predict the outcome of exposure to virus through heterosexual sex (6).

2. Rhesus macaques (*Macaca mulatta* and related macaques)

Macaques are susceptible to SIV infection. While non-pathogenic in its natural host (African macaques), SIV causes an AIDS-like disease in Asian (Rhesus) macaques (29). SIV bears significant homology to HIV-1. This fact, coupled with the greater availability of macaques for vaccine research, has made the SIV macaque model an extremely valuable tool. SIV and HIV are divergent in Env sequences, however. Thus, a genetically engineered hybrid virus, the SHIV, has been developed for vaccine studies. SHIVs have an SIV backbone with HIV *env, tat, rev*, and *vpu* (some also have HIV *nef* and *vpr)*, and produce AIDS in macaques (30, 31). Thus, macaques can be immunized with HIV-1 Env or Tat-based vaccines, and then challenged with SHIV. Strategies that have shown protection in the macaque model are listed in Table 2. In general, *nef*-deleted vaccines are considered the best approach to provide "working vaccine" level protection, by preventing infection from high doses of virulent SIV (32). Unfortunately, the live-attenuated *nef*-deleted vaccines may be unsafe (33), as discussed below.

TABLE 2: Protective strategies in macaques

Strategy	NA	CTL	Cross-protection	Reference	Comment
*Nef-attenuated					Best protection vs truly virulent challenge
(SIVΔ2 or Δ3)*	no	yes	vs. SHIV, yes	(31)	
SIVmac239 *	yes	vs 1° isolate		(34)	
HIV-1_{SF2} rgp120 ISCOMS	yes	Not done, but + Th1 Cytokines	Vs $SHIV_{SF13}$ yes	(35)	+ $CD4^+$ lymphoproli-feration
DNA/pox Prime/boost strategy	No	Yes (low)	Vs. $SHIV_{89.6}$	(36)	Intradermal DNA better than gene gun
Pox/subunit Prim/boost	Poor. Pox alone: no.	yes	Vs low pathogencity	(37)	Safe strategy
Tat HIV_{IIIB} protein	No	Yes	Vs.$SHIV_{89.6}$	(16)	Novel approach
HIV-2	No	Yes	Vs SIVsm	(38)	Mucosal challenge

3. The Hu-PBL SCID Mouse Model

Severe combined immunodeficient (SCID) mice have been populated with human peripheral blood mononuclear cells and infected with HIV-1. Koup at al demonstrated that a potent neutralizing human monoclonal aby (IgG1b12) at high dose is able to completely protect even when given hours after viral challenge (39). The results support the notion that aby induction could contribute to an effective vaccine.

4. The CD4/CCR5 Transgenic Mouse Model

Transgenic mice whose T cells express human CD4 and CCR5 have been genetically engineered. T cells from these mice permit virus entry, but do not support replication, indicating that there are blocks to HIV replication in mouse cells. After in vivo inoculation, HIV infected cells can be detected by PCR in the spleen and lymph nodes, but HIV could not be cultured from these cells. These should prove useful for the assessment of potential vaccines (40).

V. HIV compared with other vaccine-preventable virus infections.

Measles and varicella induce good natural immunity. Once you've had the disease, you don't get it again. We have excellent vaccines for these diseases. Historically, prospects for developing a successful vaccine are not good when natural infection fails to elicit long-term protective immunity.

Measles and polio are not persistent for the life of the host. Thus, in the case of measles a live attenuated vaccine is both immunogenic and safe; there is no concern that the attenuated virus will become integrated into the host genome, and later undergo recombination events causing a return to the pathogenic state.

Measles and polio vaccines produce non-sterilizing immunity. Thus, despite protective immunization, infection occurs, but produces no clinical disease. Non-sterilizing immunity may not be adequate protection from persistent pathogens. In the case of HIV, good natural immunity is not induced, the infection is persistent, so non-sterilizing immunity may not be good enough. The persistence of HIV infection (even at a very low level, as might result when viral setpoints are lowered by nonsterilizing immunization) results in gradual attrition of the $CD4^+$ cell population and eventual immunodeficiency.

VI. Current strategies for HIV vaccine development

1. Live-attenuated virus

Historically, this is a very successful approach to immunizing against viral pathogens, as evidenced by the smallpox, MMR (measles, mumps, rubella), polio, and varicella vaccines. Adult monkeys vaccinated with *nef*-deleted SIVmac have been protected in multiple challenge studies (31), but neonatal macaques mucosally immunized with a triply attenuated *nef*-mutant (deletions in *nef, vpr*, and *nre*) subsequently developed AIDS from the vaccine (33).

Mechanism for protection: Strong CTL activity, evidence for broad specificity, some instances of NA (NA is probably not a major mechanism for protection with this strategy) have all been observed (31). In addition, a "viral interference" model has been proposed, in which the attenuated virus occupies all the available "niches" thereby preventing the pathogenic virus from infecting (41).

Weaknesses: No safe attenuation strategy has yet been demonstrated in primates. An experiment of nature has yielded data on *nef*-deleted mutants in humans. The Sydney Blood Bank Cohort consists of a blood donor who unwittingly infected eight transfusion recipients with a naturally attenuated HIV-1, containing mutations in *nef* and an overlapping regulatory region (LTR). Two of the recipients died of unrelated causes, and one died of an AIDS-like illness after immunosuppressive treatment. Over 14 years later, the 6 remaining individuals (including the donor) are asymptomatic. Three of the six have undetectable viral loads, but two recipients and the donor have detectable viremia and decreasing CD4 counts, suggesting progression. (42). Thus, despite significant potential for efficacy, safety concerns currently preclude vaccination of humans with the prototypical *nef*-attenuated viruses (32). One of the major safety concerns stems from the possibility of recombination-mediated repair with parts of the host cell genome, e.g.,

endogenous retroviral sequences (43). Additional attenuation to reduce virulence could reduce immunogenicity. Nevertheless, continued experimentation in animal models is warranted to define correlates of immunity (41).

Future: Gorelick *et al* recently described a nucleocapsid protein zinc-finger mutant of SIV that is replication defective in macaques because it produces RNA-deficient viral particles. Immunogenicity/challenge studies have yet to be performed (44).

2. Killed (inactivated) virus

Description: The inactivated HIV immunogen known as REMUNE (whole, chemically inactivated, irradiated, gp120-stripped HIV-1) has been studied in phase I and II human clinical trials (45). This vaccine is presently entering phase III trials as a *therapeutic, rather than a preventive* strategy.

Mechanisms for protection: In general, killed vaccines work by inducing neutralizing antibodies (41). Although Remune is not yet being studied as a preventive vaccine, it may have some potential as a vaccine *component* because it elicits lymphocyte proliferation to purified native p24 (46).

Weaknesses 1. It is difficult to produce useful quantities of *primary* isolate inactivated virions. 2. The chemical processing results in stripping of gp120, removing the major neutralizing determinants against which antibodies should be elicited.

Future: Remune-elicited immune responses are of similar magnitude to those observed in some long-term non-progressors, justifying larger studies on the effect of Remune plus HAART. Remune is entering Phase III clinical trials as an adjunctive immunotherapeutic agent for infected individuals who have been able to achieve suppression of viremia using HAART. The Parkland AIDS clinic is one site for this trial, which will begin enrollment this fall.

3. Subunit and peptide vaccines

Description: The term subunit vaccine refers to proteins, which are to be distinguished from peptide vaccines in that they elicit different immune responses. Peptides are classified according to which class of MHC molecule they bind. See Table 3.

Table 3. Immunity elicited by subunit versus peptide vaccines.

	Class I peptide (8-10 aa)	Class II peptide (12-16 aa)	Subunit (protein) (many aa)
CTL	yes	Possible	Possible
T cell help (CD4)	No	Yes	Yes
Ab to discontinuous epitopes	No	No	Yes
Ab to continuous epitopes	Possible	Possible	Yes
Needs to be tailored to HLA types in population	Yes	Yes	No

Most studied subunit vaccines for HIV have been based on Env, and some recently, on Tat (in macaques). The proteins or peptides are delivered parenterally with adjuvants such as IFA or RIBI. There are also strategies for mucosal delivery of peptide or subunit immunogens by wrapping them inside particles such as PLGLs (poly-lactide-co-glycolide derived constructs) or cochleates, which surround the peptide inside of a rolled lipid bilayer. By virtue of their size, particles such as these can be taken up by antigen sampling cells in the gut and thus deliver an immune stimulus to the underlying gut-associated lymphoid tissue.

Historical Failures: In 1993, two gp120 vaccines were candidates for an NIH-sponsored efficacy study. The vaccines had been shown to be safe and to elicit NA against laboratory isolates of HIV. However, the inability of these vaccines to neutralize primary isolates halted the trial effort (47).

Mechanisms for protection: Soluble proteins generally do not elicit strong CD8+ CTL responses unless a specialized adjuvant strategy is used. They can however, elicit antibody responses to discontinuous epitopes. This strategy has yet to elicit neutralizing antibodies that are effective against primary isolates of HIV. A recent Tat vaccine trial in macaques demonstrated non-sterilizing protection which was apparently mediated by CD8+ CTL activity directed against Tat (16). Peptides induce epitope specific CTLs (6).

Weaknesses: Stimulating potent cell-mediated immunity with subunits or peptides is difficult, specialized immunization protocols with potent adjuvants or ISCOMS (immune stimulating complexes) have done so (16, 35). In general, the subunit vaccine strategy is employed to elicit antibodies. However, neutralizing antibody to gp120 is very difficult to elicit for primary isolates, because of the occult nature of the neutralizing determinants on gp120 (48). Peptides present antigen to a limited HLA repertoire, limiting the pool of potential vaccine responders(14).

Current clinical trials: VaxGen's AIDSVAX (bivalent gp120 vaccine, a subunit in alum strategy) was initiated into Phase III clinical trials in late 1998. The company seeks to enroll 5000 individuals in the US, and 2500 individuals in Thailand at high risk for HIV infection via sexual transmission. NIH officials are currently negotiating with VaxGen to provide funding for immunological and virological studies related to the trial. It has been noted that many senior AIDS researchers in the US have warned that the vaccine will not work (49). The makers of the vaccine defend their trial citing 1) that passive transfer of abys to gp120 are both necessary and sufficient to protect primates from infection; 2) the study will allow a "sieve analysis" strategy to select antigens to add in to the next generation of AIDS vaccine; and 3) that a vaccine "may need only lower the probability of infection by an order of magnitude.....(to) halt the transmission of HIV-1" (46).

Future strategies to induce NA:

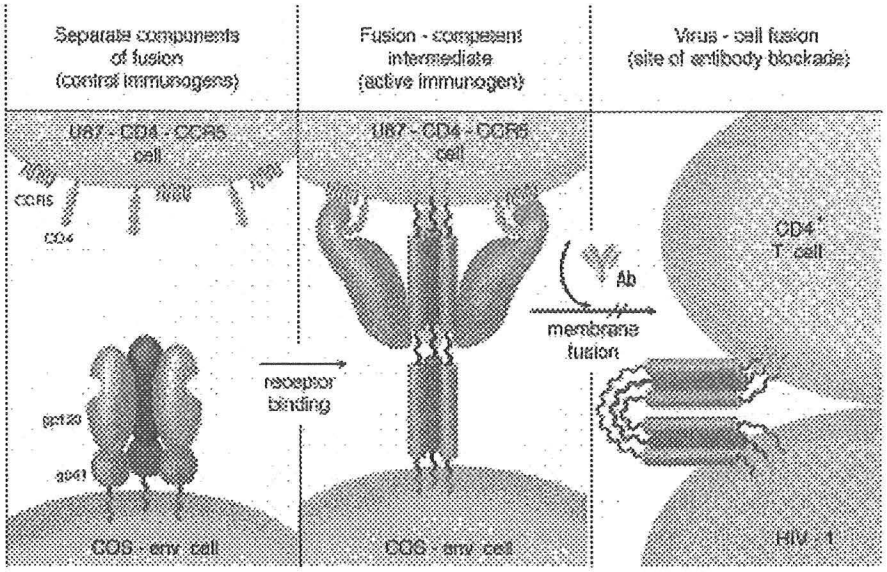


Figure 8: Fusion-competent vaccines (48)
Fusion-competent vaccines are a unique approach to the problem of neutralizing primary isolates (48). Simian fibroblasts were genetically engineered to express functional envelope glycoprotein and allowed to fuse with human neuroblastoma cells expressing CD4 and CCR5. During fusion, envelope glycoprotein conformations change, exposing hidden epitopes and forming neo-epitopes. The fusing cells are fixed with formalin and used to immunize transgenic mice. CD4/CCR5 transgenic mice generated antibodies to these fusion intermediate structures, which were able to neutralize (in vitro), a diverse array of HIV-1 primary isolates. A number of control experiments were done to reduce the possibility that neutralization was due to an antibody response directed against the cells (47). While impractical in their current form (whole cell vaccines), purified fusion-competent complexes could be developed as an inactivated subunit vaccine.

4. Live vectors

Description: Live recombinant vaccines consist of non-pathogenic retroviral, adenoviral, SHIV, canary pox, vaccinia viruses, yeast, or bacteria which have been manipulated to carry HIV genes. When the live recombinant vaccine is administered to a host, an immune response occurs to both the vector and its inserted gene.
Mechanisms for protection: These vaccines elicit cellular immunity with CTL production.
Weaknesses: These vaccines elicit suboptimal humoral immunity to HIV envelope
Selected examples of live vectors:
A. Recombinant viral vectors

i. Vaccinia recombinants This was one of the first vaccines to be evaluated in humans (12). The approach uses smallpox vaccine that has been genetically engineered to express genes from HIV. Recombinant vaccinia vaccines that contain the HIV gp160 envelope protein (vac/env) can elicit class I restricted cytotoxic T lymphocyte (CTL)

responses to HIV envelope. These responses are more frequently seen in persons who are vaccinia naïve. Mucosal immune responses are seen with this approach. However, aby and cellular proliferative responses with vac env immunization alone are poor. Thus, one should optimally combine this strategy with a recombinant subunit protein immunogen (50).

Weakness: Manufacturers cite liability concerns: an attenuated vaccinia virus could not be safely administered to high-risk populations without careful advance screening(12)..

ii. Canary pox This is a member of the vaccinia virus family which expresses proteins in human cells but does not go through an entire replication cycle, making it safer than vaccinia recombinants (12). The strategy elicits good cellular responses, but little humoral immunity unless a prime boost strategy is used. In the prime and boost approach, the live vector vaccine stimulates cell-mediated immune responses, followed by a boost of purified gp120 subunit to induce antibodies.

At least 7 phase I, and one phase II human trials have used ALVAC vCP205 (or a closely related vaccine, AIDSTRIALS database) which contains HIV *env, gag,* and protease for presentation to the immune system. Because of co-expression of these components it is possible that enveloping of the Gag gene product and release from the cell membrane mimic HIV closely enough to potently stimulate immunity. In theory, this should present envelope in the proper configuration to the immune system. Weinhold et al have shown that clade B based canarypox vaccines in humans can elicit broad CTL reactivities capable of recognizing viruses belonging to genetically diverse HIV-1 clades (51). Other studies have shown that the ALVAC combined with a gp120 boost (in humans) can elicit NA directed against a clade B primary isolate of HIV (52).

The most recent results on human canarypox vaccine trials were reported this summer. An NIAID sponsored study that began in May 1997 involved 435 people, 80% of whom had high risk of HIV infection because of sexual behavior or IV drug use. More than 90% of those who received the prime/boost vaccine developed aby that could neutralize *laboratory* (but not primary) isolates. Moreover, CTL were elicited in only one third of the vaccinees.

B. Recombinant bacterial vectors

Since most cases of HIV are sexually transmitted, mucosal immunity is likely to be critical in preventing infection. Attenuated, recombinant *Salmonella* is an attractive vector because it elicits potent mucosal and systemic immune responses to cloned foreign antigens in humans and in animals. Such vaccines also offer practical and financial advantages over parenterally administered vaccines by obviating the need for a cold chain or for sterile needles. Mucosal immunization focuses the host response to infection at the most common point of entry and has been shown to be important in protecting humans from polio, influenza, rotavirus, and typhoid (53).

A phase I clinical trial involving 46 human subjects using a recombinant *Salmonella typhi* vaccine to prevent HIV was recently conducted in Maryland with disappointing result (AIDSTRIALS database).

5. Nucleic Acid Vaccines

Description: DNA vaccines are plasmids carrying genes encoding antigens from a pathogen. They can be administered by direct IM injection, intradermal injection, or epidermally via a gene gun. Gene guns deliver gold beads onto which DNA has been precipitated. After injection the plasmids express their foreign gene intracellularly under the direction of a strong viral promoter (54).
Mechanisms for protection: MHC class I restricted CTL responses are generated and diverse immunogens can be combined into a single preparation. The plasmids can generate intact proteins rather than peptides, which should allow people of diverse HLA background to be able to respond to the same vaccine. Two chimpanzees vaccinated with *env, gag/pol,* and *rev* encoded plasmids showed no viremia 48 weeks after infection with an attenuated HIV (55). Challenges with virulent isolates have not yet been successful (56).
Weaknesses: The induction of humoral responses has been inconsistent. Mucosal immunity has not been clearly demonstrated. A safety concern remains in that integration of the retroviral DNA into the host genome as well as in vivo recombination are theoretically possible (37).

Future: Here at our own institution, Sykes and Johnston have reported a GLV (genetic live vaccine) strategy with promising early results in primate challenge studies (K. Sykes, personal communication). This strategy involves the generation of expression libraries from viral DNA, producing the immunologic advantages of live vaccines without risk of reversion to the pathogenic state. The fundamental advance of this strategy is that it allows immunization with the entire genome of HIV, thereby eliminating the need to know which pathogen genes should be included for protection. Modifications of the plasmid vectors (fusion of the encoded antigens with either ubiquitin or a secretory protein) can enhance CTL or aby levels (56). See Figure 9.

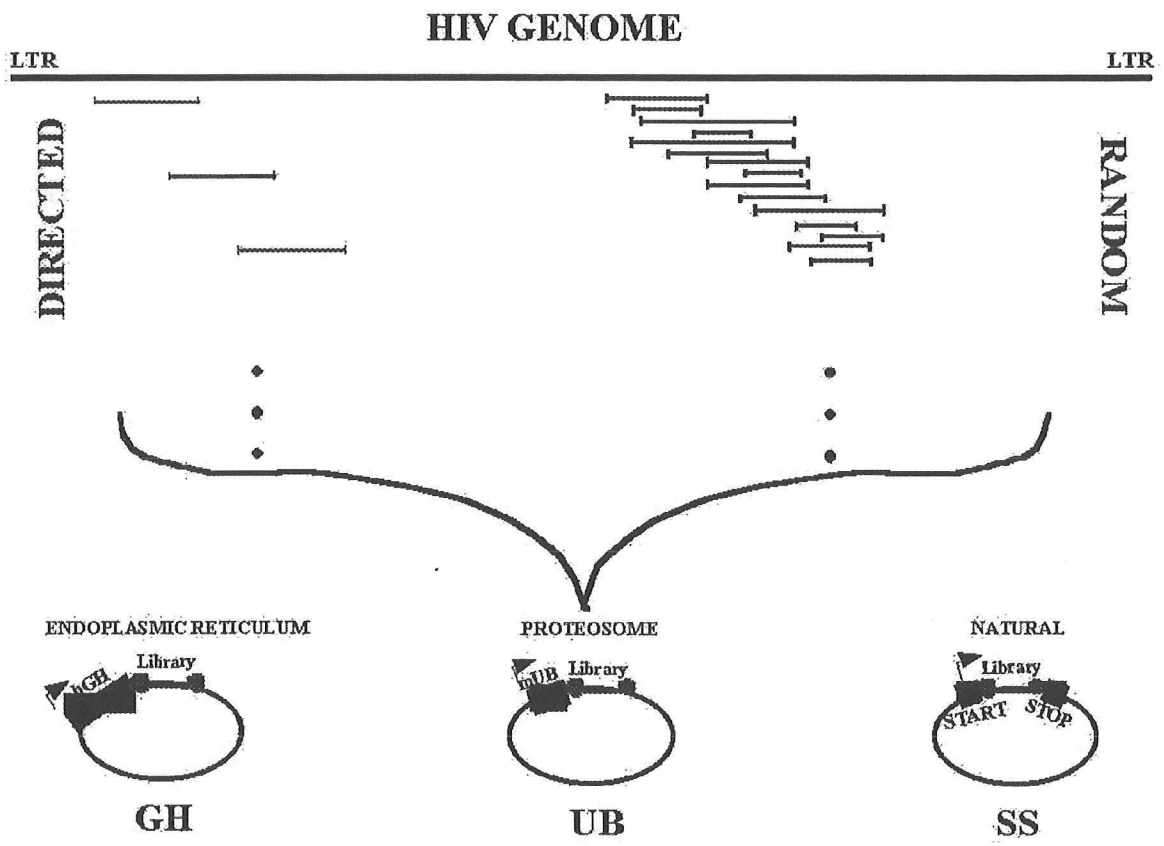


Figure 9: Genetic Live Vaccines (56)
GLVs are plasmid libraries that express the entire genome of a pathogen. The HIV provirus is fragmented into either directed or random subgenes and inserted into a vector designed to target subprotein antigens to the endoplasmic reticulum (GH) or proteosome (UB) or a vector that permits antigens to be naturally targeted (ss). GH= human growth hormone; mUB= murine ubiquitin.

Limitations: Aby against conformationally complex determinants are unlikely to be produced because GLV fragments encode an average subprotein of 200 aa. GLVs may be prohibitively expensive to mass produce. Sorting the library into fewer components would reduce costs (56).

6. Dendritic cell (DC)-based vaccines

Description: DCs were pulsed with HIV antigens and then infused into HIV-infected volunteers (57). Induction of HIV-specific CTL and lymphoproliferation was occasionally noted, but no change in viral load was detected. Current clinical trials are assessing *therapeutic, rather than prophylactic* vaccinations.
Strengths: DCs are potent inducers of immunity (58). Theoretically they could overcome current obstacles to elicitation of strong, durable protective anti-HIV immunity.
Weaknesses: Currently there is no practical way to target DCs in vivo. Cells must be grown ex vivo for expansion followed by adoptive transfer.

Current status in clinical trials: The University of Washington (Seattle) is evaluating therapeutic vaccination. A therapeutic DC-based vaccine has been proposed for study here at UTSW in collaboration with the Baylor Institute for Immunology Research.
Future: Means for in vivo DC-targeting to avoid ex vivo cell manipulations are being studied.

VII. Data from human clinical trials.

Between 1988 and 1998, 22 candidate vaccine formulations were evaluated in >2000 subjects in Phase I/II clinical trials. All of the vaccines have proven safe, and all induce some measurable immune responses. The degree of protection afforded by the vaccines is not known, and awaits phase III efficacy trials such as that recently launched by Vaxgen (46, 59).

The AIDS Vaccine Evaluation Group (AVEG) of the NIH has conducted an analysis of intercurrent HIV-1 infections in phase I/II trials of AIDS vaccine candidates. Twenty vaccine volunteers became infected, of which 13 had received complete immunization, 6 received partial immunization, and 4 received placebo. The vaccine strategies that failed to protect included rgp120 subunits in various adjuvant formulations, as well as Vaccinia-rgp160 with a subunit boost, and finally an rgp160 with alum and deoxycholate. The infections occurred 1-29 months after immunization, and the incidence of infection was 0.38/100 person years in the vaccinated group, compared with 0.30/100 person years in the placebo group. The vaccinated subjects became infected with typical clade B virus, and 46% experienced a symptomatic primary infection, which has been associated with rapid progression to AIDS. Extensive laboratory analysis of these subjects showed no effect of the vaccine on the genotypic or phenotypic characteristics of transmitted virus or the clinical course of infection (59).

Having considered what is known about correlates of immunity from human and animal studies, and having seen the many strategies that fail to protect against HIV, it is possible to construct a list of ideal vaccine characteristics, as noted here:

Characteristics of an ideal vaccine (3, 11, 15, 60, 61)

1. Prevent infection with HIV by inducing immune responses that could block cell-free and cell-associated virus from infecting host cells. HIV can be transmitted by both cell associated and cell-free virus. Strategies to prevent infection will likely require effector T cell responses to eliminate virally infected cells and antibody responses to neutralize cell free virus (50).
2. Rapidly clear host cells that become infected.
3. Provide mucosal protection as this is the point of most common entry
4. Sterilizing immunity may not be necessary if a vaccine changes the dynamics of infection so that disease progression does not occur. Thus, the ideal vaccine may not be required to produce sterilizing immunity (11).

VIII. Ethical Issues and logistics

Ethical concerns include the fact that most vaccine designs have been based on clade B viruses. The industry was concerned that the developing world would not be able to pay for non-clade B virus vaccines (62). Organizations such as the World Bank and the International Aids Vaccine Initiative are working to create incentives for biotechnology companies to work on vaccines that are globally relevant.

Another ethical concern is that in the industrialized world, a vaccinated volunteer who becomes infected will have immediate access to HAART, which will preclude the detection of a long-term non-progressor state (63). Such information could only be gained in developing countries where HAART is unavailable, creating potential mistrust and resentment. Industrialized countries may be seen as using the developing world as guinea pigs to develop a lucrative vaccine.

Vaccine volunteers may have difficulty explaining their seropositivity to life insurance companies, employers resulting in reluctance to participate in trials. Participation in a vaccine trial may stigmatize volunteers because they are automatically labeled as "high risk" (11).

Building a facility that will produce an AIDS vaccine in sufficient quantities to meet demand is costly; ($15 million to design the facility; $150 million to construct it). Typically 5 years are required for the process of design and construction, and these must be underway before efficacy trials begin so that sufficient product is available after approval. In general, industry claims that development of a new pharmaceutical product takes an average 10 years at a total development cost of $150- $250 million (Scientific Blueprint for AIDS Vaccine Development, IAVI, 1998).

IX. Funding for AIDS vaccine development

Internationally: The Rockefeller foundation in partnership with the Merieux Foundation (France) created the International AIDS Vaccine Initiative (IAVI). This independent organization is designed to speed the process of finding an effective HIV vaccine. IAVI goals are to advocate for HIV vaccine research, forge collaborations among the public, private and nonprofit sectors that will stimulate corporate investment, and to support underfunded areas of applied vaccine research.

In May of 1999, Bill and Melinda Gates donated $25 million to IAVI which is the largest charitable gift in the history of the AIDS pandemic. This grant will allow IAVI to more than double its effort.

Nationally: In the US, the NIH Budget for AIDS research this year was about $1.73 billion, of which $180 million was targeted to vaccine development (64).

Collaborations among sectors:

The NIH has supported the following phase I trials through its AVEG (AIDS Vaccine Evaluation Group):

- Env vaccines produced by Immuno Ag, Micro GeneSys, Genentech and Chiron
- Peptide vaccines produced by UBI and Wyeth Lederle

- Vaccinia vectors by Bristol Myers Squibb and Therion
- Canarypox vectored vaccines produced by Pasteur-Merieux-Connaught
- Bacterial vector produced at the University of Maryland (recombinant *Salmonella*)

X. Prospects

In 1997, President Clinton gave us another decade to develop an AIDS vaccine. However, given the many biological challenges and financial restraints, it is unclear whether this is an attainable goal. In the words of David Baltimore,
"*It is unlikely that we will develop a vaccine suitable for wide-scale use in humans in the next five years. Even if the prime-boost combination approach appears to stimulate cellular immunity and generate good broad-spectrum antibodies, large clinical trial will still be needed to demonstrate its value. Those trials alone will take several years.*"
-July 1998 (65).

This may be the first time in the history of vaccinology that the immune effector mechanisms for the vaccine are being studied so thoroughly to design the vaccine itself. This can be thought of as a bottom up approach, distinctly different from historically successful vaccines which were largely developed using a "top-down" approach. This can be thought of as a bottom up approach, distinctly different from historically successful vaccines, which were developed using a top-down approach. Edward Jenner's cowpox immunization experiment was performed in humans without any insight into the correlates of immunity (the "top-down" approach) for small pox (66). A debate continues among scientists who feel that the cost of vaccine trials and the high probability of failure have precluded such an approach for an AIDS vaccine, vs. those who feel that history vindicates a top-down approach (for non-live vaccine strategies).

I will close with an observation from Dr. Neal Nathanson, Director of the Office of AIDS Research at the NIH. He cited the following quote, attributed to David Bodian, at a recent AIDS vaccine lecture:

""*In 1945, Professor Burnet of Melbourne wrote "While I was in America recently I had good opportunity to meet with most of the men actively engaged on research in poliomyelitis... The part played by acquired immunity to poliomyelitis is still completely uncertain, and the practical problem of preventing infantile paralysis has not been solved. It is even doubtful whether it ever will be solved"*
"...most of us doing research on poliomyelitis in ***1945*** *were mainly motivated by curiosity rather than by the hope of a practical solution in our lifetime.*"" (Attributed to David Bodian, 1976).
Ten years later, there was an effective polio vaccine.

XI. LITERATURE CITED

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