Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Review Article

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Md. Mahmudul Hasan
C/O-Shahjahan Chowdhury
NIPORT Hostel (4th Floor)
NIPORT Building, Azimpur
Dhaka 1205, Bangladesh

E-mail: mahmudbt@hotmail.com
Fax: +88-041-731244, 731521

ANSInet
Asian Network for Scientific Information

J. Med. Sci., 3 (1): 1-23
January-February, 2003

Different Methods of HIV Vaccination, Efficacy and their Delivery System: A Review

Md. Mahmudul Hasan and Mahbub-E-Sobhani

Two genetically different but related forms of HIV, called HIV-1 and HIV-2, have been isolated from patient with AIDS and also closely related viruses are found in many species of non-human primates. Significant progress has been made in preclinical and clinical research in the worldwide efforts to develop an effective HIV vaccine. Many approaches like attenuated and whole inactivated vaccine products, along with recombinant DNA, protein and peptide approaches and some novel construct including other lentivirus have been studied in various animal models of HIV infection. Currently, 2 gp160 vaccines, 2 gp120 products, 1 envelope yeast-derived protein and 1 vaccinia gp160 recombinant vaccine has entered the clinical trials in United States and gag particle product has been studied in the United Kingdom. No immunization strategy has been demonstrated to be completely effective in preventing HIV infection ***in vivo***. Major scientific obstacles blocking the development of a successful preventive vaccine are the extraordinary variability of HIV, the lack of exact animal model of HIV-induced AIDS and understanding of the correlates of positive immunity to HIV.

Key words: HIV, vaccine, immunogenicity, viral load, attenuation, humoral immunity

Biotechnology Discipline, Khulna University, Khulna-9208, Bangladesh

Introduction

Human immunodeficiency virus (HIV) is a human retrovirus that belongs to the lentivirus family. Feline immunodeficiency virus, simian immunodeficiency virus (SIV), visna virus of sheep and the equine infectious anemia virus are also included in this group. These nontransforming retroviruses have several common features (Cotran ***et al.***, 1994). Like 1. Long incubation period followed by a slowly progressive fatal outcome, 2. Tropism for hematopoietic and nervous systems, 3. An ability to cause immunosuppression and 4. Cytopathic effects ***in vitro***.

Two genetically different but related forms of HIV, called HIV-1 and HIV-2, have been isolated from patient with AIDS and also closely related viruses (SIVs) are found in many species of non-human primates (Cotran ***et al.***, 1994; Klinger ***et al.***, 1998). HIV-1 is the most frequent type associated with AIDS in the United States, Europe and Central Africa, whereas HIV-2 causes a similar disease principally in West Africa (Cotran ***et al.***, 1994).

HIV infection is a major threat to public health and the prevention or control of AIDS by immunization is a principal goal of vaccine research today. The induction of a strong and long lasting immunity characterized by both humoral and cells mediated-immune (CMI) response is one of the most important considerations in developing an effective HIV vaccine (Kent ***et al.***, 1998; Okuda ***et al.***, 1997).

The need for an effective vaccine is urgent, but progress towards this goal has been slowed in part by the inability of any vaccine candidate to elicit antibodies capable of neutralizing the infectivity of primary HIV isolates from infected individuals (LaCasse ***et al.***, 1999).

To date, most viral vaccines registered for human use are composed of whole killed or live attenuated virus particles with the exception of hepatitis recombinant subunit vaccine. Nevertheless, in case of various types of HIV/AIDS vaccine different modes have been applied to determine which is the most effective.

Different methods of HIV vaccination

Live attenuated vaccine

Live, attenuated vaccines have been the most successful vaccines in monkey models of HIV-1 infection. However, there are several safety concerns about using such anti-HIV vaccine in humans, which includes reversion of the vaccine strain to virulence and recombination with endogenous retroviral sequences to produce new infectious and potentially pathogenic viruses. As testing in humans would inevitably carry a substantial risk, Berkhout ***et al.*** (1999) set out to test the genetic stability of multiply deleted HIV constructs in perpetuated tissue culture infections. The Delta3 candidate vaccine strain of HIV-1 contains deletion in the viral long terminal repeat (LTR) promoter and the vpr and nef genes. This virus replicates with delayed kinetics, but a profound enhancement of virus replication was observed after culturing. Analysis of the revertant viral genome indicated that the three deletions were maintained but a 39-

nucleotide sequence was inserted in LTR promoter region. This insert was formed by duplication of the region including three binding sites for Sp1 transcription factor. The duplication of the Sp1 region was demonstrated to increase the LTR promoter activity and a concomitant increase in the virus replication was measured. In fact, duplication of Sp1 sites increased the fitness of Delta3 virus to the level higher than that of the singly deleted Delta vpr virus. The result indicates that deleted HIV-1 vaccine can evolve into fast-replicating variants by multiplication sequence motifs and their safety is, therefore, not guaranteed (Berkhout ***et al.***, 1999). The use of live attenuated has potential risk of the emergence of pathogenic revertant viruses. Furthermore, this approach has not yet been successful in controlling the HIV infection ***in vivo*** (Kim ***et al.***, 1997b).

The macaque model

In macaques, immunization with live attenuated simian immunodeficiency viruses (SIV) has induced the most potent protective immunity. Recent evidence supports involvement of both cytotoxic T-lymphocyte and neutralizing antibodies in protective immunity against infection by SIV, but more detailed studies are needed to document their relative importance (Johnson ***et al.***, 1998).

Inactivated whole-virus vaccines

The live attenuated viruses are effective against ***in vivo*** infection. The complete inactivation of virus infectivity is usually confirmed by prolonged cell culture (Oxford and Jeffs, 1996). The inactivation can also be done by series of chemical and physical steps in order to ensure complete virus inactivation. An important question after such a rigorous procedure is whether the immunogenicity of the viral proteins has been impaired. Antibodies induced in animals immunized with HIV-1 RF and IIIB bind with high titers to both homologous and heterologous viral proteins in ELISAs indicating that immunogenicity of conserved epitomes has been preserved. Furthermore, peptide-binding studies show that these antibodies bind across the entire length of the gp120 molecule (Race ***et al.***, 1995).

The inactivation procedures involving heat or formalin appear to be adversely effective to the viral envelope proteins. Arthur ***et al.*** (1998) inactivated the HIV-1 with compound 2,2′-dithiodipyridine, which inactivates infectivity of retroviruses by covalently modifying the nucleocapsid zinc finger motifs. HIV-1 inactivated with Alrithiol-2 retained the conformational and functional integrity of the virion and virion-associated cellular protein on the viral membrane. The virion gp120 subunit appeared to be completely unreactive with Aldrithiol-2. Analysis of gp120-CD4 mediated post fusion events showed that the inactivated virus could enable CD4 dependent fusion with efficiencies similar to that untreated virus. These inactivated virus preparations is useful in whole killed-particle vaccine preparation (Arthur ***et al.***, 1998).

Envelope-based subunit vaccine

As the HIV envelope protein mediates the early binding and entry steps in infection, many vaccine strategies have focused on this target (LaCasse *et al.*, 1999). Present knowledge of HIV-1 immunobiology has been derived almost exclusively from analysis of subtype B viruses, yet such viruses represent minority of strains currently spreading worldwide (Fig. 1).

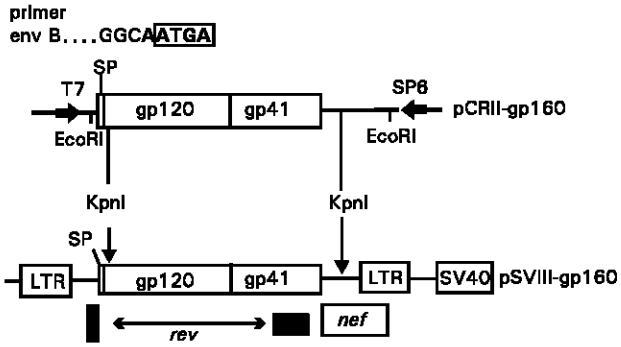

Fig. 1: Schematic representation of different envelop expression plasmids

PCR-derived full-length gp160 sequences were subcloned into pCRII by T/A overhang generating the pCRII-gp160 constructs. The positions of T7 and SP6 promoters, as well as the location of the plasmid polylinker in relation to the inserted env genes, are indicated. The relative positions of gp120 and gp41 coding regions, nef, rev, the HIV-1 LTR and simian virus 40 (SV40) origin of replication are indicated. The composition of the Kpn plasmid has been described as SP, signal peptide (Gao *et al.*, 1996).

Analysis of deduced amino acid sequences of HIV-1 isolates collected from major epicenters of AIDS pandemic revealed several important differences from prototypic subtype B strains, including changes in the number and distribution of cysteine residues, substantial length differences in hyper variable regions and premature truncations in gp 41 domain (Gao *et al.*, 1996).

The HIV envelope protein orchestrates a complex series of protein-protein interactions and structural changes that ultimately result in fusion of the virus and cell membranes and infection of the cell. Upon binding to CD4, the envelope protein undergoes conformational change. Interaction with either coreceptor induces further conformational change in the envelop protein and exposure of the hydrophobic fusion domain of the transmembrane gp 41 subunit, which then mediates the fusion of apposed cell and virus membranes. Based on this dynamic model of HIV binding and entry, many approaches have been taken to develop HIV vaccine immunogens that explicitly incorporate these functional intermediate structures (LaCasse *et al.*, 1999).

There is compelling evidence, which correlates the induction of specific antibody responses to HIV envelope glycoproteins with either protection against infection or significant modification to the predicted clinical course of disease (Jones ***et al.***, 1995).

The p6 (gag) is a protein of HIV-1 that is produced as a carboxyl-terminal sequence within the gag polyprotein. Site-directed mutations at the minor cleavage site and within the hydrophobic tail showed that all the single amino-acid-replacement exhibited either reduced or undetectable cleavage at the site yet almost all were nearly as infectious as wild-type virus, demonstrating that processing at site is not important for viral replication. One exception, a virus with two substitutions of p6, could not infect susceptible cells. The mutant was not rescued by an HIV-1 env with a truncated gp 41 cytoplasmic domain, showing that it is phenotypically different from the previously described mutants that do not incorporate their full-length env proteins (Ott ***et al.***, 1999).

The efficacy of envelop-based vaccines would be greatly enhanced if their structure could be based on three dimensional structure of gp 120/160. If this is understood, it may be possible to design molecules and functionally disable them. In arrangement and function, the HIV envelop glycoprotein has many similarities to that of influenza hemagglutinin but presents a far greater challenge as not only reliance have to be placed on recombinant DNA technology for structural studies (Oxford and Jeffs, 1996).

The gp120 approach

Direct immunization with preparations of HIV envelope glycoproteins (Berman ***et al.***, 1990; Girard ***et al.***, 1991) or the passive transfer of monoclonal antibodies specific for V3 region of HIV gp120 (Emini ***et al.***, 1992), protect chimpanzees from challenge with live virus (Jones ***et al.***, 1995) and for some other reasons the HIV gp120 molecule is still a strong candidate for incorporation into a recombinant sub-unit HIV vaccine.

The molecule is expressed on external surface of the virus particle (Jones ***et al.***, 1995) and contains the human cell CD4 receptor binding region (Lasky ***et al.***, 1987; McDougal ***et al.***, 1986) possesses neutralization epitomes (Jones ***et al.***, 1995; Steimer ***et al.***, 1991) and amino acid sequences which act as targets for CD4+ and CD8+ human cytotoxic T-lymphocytes (CTL). Hypervariability within the HIV gp 120 is responsible for the generations of virus variants which are believed to result in the evasion of the host immune system (Jones ***et al.***, 1995). The glycoprotein is reported to be important in determining both the cytopathic characteristics (Chang-Mayer ***et al.***, 1991; Jones ***et al.***, 1995) and the cell tropism of individual virus isolates. Finally, the involvement of gp 120 in specific post-binding mechanisms is understood to be crucial to the fusion between virus envelope and cell surface membranes which ultimately lead to the establishment of infection (Jones ***et al.***, 1995).

Huang *et al.* (1997) have prepared glycosylated analogues of the principal neutralizing determinant of gp 120 and studied their conformations by NMR and circular dichromism spectroscopies. Binding and monoclonal antibody mapped data show that glycosylation of V3 loop peptides can affect their conformations as well as their interactions with antibodies. The design of more ordered and biologically relevant conformation of immunogenic regions from gp 120 may aid in the design of more effective immunogens for HIV-1 vaccine development.

The gp160 and p24 approach

Both structural p24 and gp 160 induce strong immune reactivities, of which functional p24 and gp160 reactivity acts through cytolytic cells and the anti-gp 160 reactivity by neutralization of envelope proteins and possibly antibody dependent cytolytic cells. Induction of all these reactivities in the absence of infectivity might give an immune protection similar to that of an attenuated live virus (Shiver *et al.*, 1997).

Recombinant subunit proteins

For the production of an efficient synthetic vaccine care should be taken that it should contain different T and B cell epitomes of HIV antigens and the B epitope regions in the vaccine and the HIV should be conformationally similar (Morris, 1999). The genetically engineered, noninfectious HIV-1-like particles containing processed envelope glycoproteins represent potential candidate immunogens for vaccine against HIV-1. However, since the gp 120 glycoprotein is known to be rapidly lost from the surface of infected cells and purified virions as a result of its low-affinity interaction with gp 41, shedding of this extracellular subunit could compromise the immunogenic potential of particle-based HIV-1 vaccine candidates. Monkey kidney Vero cell can be transfected with an inducible human metallothionin-based expression vector that can be genetically modified to introduce safety mutations and destroy the major cleavage site of the HIV-1 envelope glycoprotein. A stably transfected cell line can be isolated and may secrete HIV-1 like particles containing unprocessed gp 160. Thus, these novel HIV-1 like particles represent alternative candidate immunogens for the development of a particle-based AIDS vaccine (Rovinski *et al.*, 1995).

Antigenic determinants of cellular and humoral immunity are blocks for the vaccine design. From experimentally studied HIV-1 T and B cell epitomes, Eroshkin *et al.* (1995) constructed a sequence of four-helix protein TBI (T and B cell epitomes containing immunogen). The gene of protein was synthesized and the protein was produced in C600 *Escherichia coli* cells under a recA promoter from *Proteus mirabilis*. CD spectroscopies of the domain demonstrate that 30% of amino acid residues adopt an alpha-helical conformation. Mice immunized with TBI have shown both humoral and cellular responses to HIV-1. The obtained data showed that the design is successful. The synthesized gene structure makes possible further reconstruction and improvement of the protein vaccine structure.

A glycoprotein derived from a mammalian expression system and substituted with complex carbohydrate side chains similar to those synthesized in human cells, is more likely to posses the correct conformations of the peptide backbone when compared with products derived from insect, yeast or bacterial cells. The use of recombinant gp120, which maintains native conformation and glycosylation patterns, has been shown important in generating "broadly reactive" antibody responses capable of neutralizing with diverse HIV strains (Olshevsky ***et al.***, 1990; Steimer ***et al.***, 1991). Further, the generation of neutralizing antibodies to functionally important regions of the molecule, such as the CD4 binding site may need the maintenance of structural integrity through the whole molecule (Jones ***et al.***, 1995).

The SIVnef approach

Current findings suggest that the strain and nature of the attenuating mutations in ***Salmonella typhimurium*** have a considerable effect on the expression of SIVnef and the immunogenicity of this construct in rodents has yet to be determined. Pumpens ***et al.*** (University of Latvia, Latvia) tried an alternative approach to the presentation of protective antigens by using a viral capsid as carrier. By precise positioning of HIV antigen on the surface of such carriers, the immunodominant epitomes will be in the optimal spatial configuration for presentation to the host immune system. A precursor for such studies is a knowledge of the structure of the carrier particle and this has now been determined for RNA phages fr and QB, as well as the hepatitis B core antigen (HBcAg). With this information, full-length V3 loop sequences (MN and HAN2) have been inserted into phage QB and HBcAg. Preliminary results indicate that the V3 loop is fully exposed on HBc particles and immunogenic. These chimaeric particles can also elicit T-cell responses. Genetically modified vaccinia (Ankara strain) (MVA) which are avirulent in human cells and animal cells has been found to be highly efficacious and exceptionally safe small vaccine, being particularly well tolerated for the risk groups such as the elderly, allergic or patients with other chronic conditions. Recombinant MVA-vaccine have been shown to reduce viral loads and significantly reduce the severity of disease caused by SIV challenge in macaques following prior inoculation with MVA expressing the gag-pol and env genes of SIVm (Oxford and Jeffs, 1996).

Nucleic acid based vaccine

Immunity can be achieved to HIV-1 regulatory proteins by vaccination with genes (Wahren ***et al.***, 1995). Such responses may establish resistance to early viral replication events if it were to be induced in individuals subjected to risk of infection (Shiver ***et al.***, 1997). One of the fastest moving fields in the vaccine is related to the development of nucleic acid vaccination. The great advantage of nucleic acid vaccine over subunit vaccines is that the antigen is presented to the immune system in native form and synthesized by host in a similar way to that by which the invading pathogen would make them (Oxford and Jeffs, 1996).

The nucleic acid or DNA immunization has been shown to mimic aspects of live attenuated vaccines. Both humoral and cellular responses can be induced upon injection of a nucleic acid sequence directly into a host target tissue, without the risk of potential viral reversion of the live attenuated vaccines (Ulmer *et al.*, 1993; Wang *et al.*, 1993a; Wang *et al.*, 1993b; Wolff *et al.*, 1990). This vaccination technique is being explored as an effective strategy to immunize man against a variety of pathogen including HIV-1. DNA immunization induces antigen specific cellular and humoral immune responses through the delivery of non-replicating transcription units that drive the synthesis of specific foreign proteins within the inoculated host (Kim *et al.*, 1997b). However, DNA vaccine can induce adequate activation of type-1 helper T cells. Because of their *in vivo* neosynthesis of antigen in host cells, these vaccines bear a striking resemblance to live attenuated vaccines in producing robust immunity in the host (Okuda *et al.*, 1997).

Risk associated with nucleic acid vaccination

The main disadvantage with nucleic acid vaccination arise from safety fears that inoculated DNA will integrate into host genome leading to incorrect transcription of host mRNA or the activation of hitherto silent oncogenies. A further possible worry derives from the persistence of pDNA intra cellularly in absence of replication, which may induce pathogenic instead of protective immune responses. These fears could be circumvent by using RNA but this is expensive to produce and notoriously unstable, although vector-based approaches using the Semiki-Forest virus (SFV) have been attempted (Oxford and Jeffs, 1996).

Probable solution of the problem using animal model

Following the demonstration that high levels of cellular and humoral immunity with prolonged memory could induced in mice to influenza A antigens using both naked RNA and SFV particles and that such a mice survived the challenge with lethal doses of flu virus, macaques were immunized with HIV or SIV envelope genes in SFV vectors. Although only low levels of anti-envelope antibodies and barely detectable CMI responses were induced, all the SFV-immunized animals survived with a clear reduction in viral load. The HIV env-inoculated animals became infected when challenged with SHIV-4, but again showed a clear reduction in viral load compared with control monkeys (Liljestrom, Karolinska Institute, Sweden).

This technology has now been extended to potential HIV vaccines, initially by testing all HIV-1 genes in both rodents and primates to examine immune responses. Weiner and Coworkers (University of Pennsylvania, USA) have mouse lymphoma challenge tumor model where the challenge tumors are engineered to express HIV antigens. A protective Th1-type response was produced when gag/pol/rev, env/rev and whole HIV genome except env plasmid constructs were used. A particularly interesting observation was that following inoculation of mice in the quadriceps, 10-12% of anti-HIV antibodies in vaginal washes was found sIgAs. Similar results were

obtained in macaques. Challenge studies in macaques which had been inoculated with a strain Z6 gp160 plasmids and challenged with a non-pathogenic SHIV showed a 50% reduction in viral load and protection from infection in a subset of DNA-vaccinated animals. The utility of this approach for humans was validated by the demonstration of neutralization antibody production, T-cell proliferation and CTL responses in HIV positive and negative chimpanzee inoculated with plasmid/gp160 and plasmid/gag+pol constructs, coupled with a dramatic reduction in viral load and boosting of immune responses in HIV infected chimpanzees (Oxford and Jeffs, 1996).

Mucosal immunization

Transmission of HIV-1 can occur via mucosal exposure with free virus or with cell-associated viruses (Wang ***et al.***, 1997; Klavinskis ***et al.***, 1997). By this route of exposure, HIV-1 apparently infects susceptible cells such as Langerhans cells, macrophages, T cells and perhaps epithelial cells in the female genital tract prior to initiation of a systemic infection (Alexander, 1990; McGhee and Mestecky, 1992; Milman and Salma, 1994). To obtain control of primary HIV infection it will be necessary to create mucosal immunity. As the HIV transmission occurs primarily via vaginal and rectal routes during intercourse, a mucosal vaccine delivery system is very much useful to control it (Shiver ***et al.***, 1997; Klavinskis ***et al.***, 1997). Mucosal immunity is the first line of immune defense against human pathogen to prevent systemic infection, particularly of sexually transmitted diseases (STD) (Wang ***et al.***, 1997). Immunoglobulin present in mucosal secretions is one of two origins: locally produced immunoglobulin, most usually secretory IgA, which is considered to be a true mucosal response (Mestecky, 1987). Either of these immunological responses could prove to be beneficial for host. In parotid saliva, the immunoglobulin is local in origin. Genital secretions usually contain both transduced and local immunoglobulins (Mestecky ***et al.***, 1994).

Among the viral gene, Rev is a small basic protein that is located in the cell nucleus and is essential for the expression of the viral structural genes gag, pol and env. Two proteins that occur in low levels and only around 20-40% of HIV infected individuals develop humoral or cellular responses to these proteins (Shiver ***et al.***, 1997).

A study by Gorse ***et al.*** (1995) performed with non- HIV-infected volunteers who received the same candidate HIV vaccine as in their study (Immuno AG), revealed the presence of anti-envelop glycoprotein IgG and IgA antibodies in whole saliva following immunization with a recombinant rgp 160 vaccine. A study by Burnett ***et al.*** (1994) evaluated HIV- seropositive-indivual IgA immune responses in serum to neutralize HIV ***in vitro***.

A few reports of the anti-HIV antibody response detected in whole or parotid saliva of non-HIV-1-infected volunteers parentally immunized with another candidate AIDS vaccine, a baculovirus- expressed with HIV-1 LAI recombinant rgp160, have been published (Archibald ***et al.***, 1990; Funkhouser ***et al.***, 1993; Vasudevachari ***et al.***, 1992). In one study, envelope-specific

antibodies of IgG class were observed in whole saliva but not parotid saliva of some volunteers immunized with 640 or 1,280 μg doses of rgp160 (Vasudevachari *et al.*, 1992).

Klavinskis *et al.* (1997) investigated a model system, systemic and mucosal immune responses elicited to firefly luciferase generated by DNA immunization incorporating DNA into liposomes with cationic lipids. It enhanced luciferase expression in nasal tissue and was associated with induction of a humoral response in serum and vaginal fluids and also a proliferative and CTL response and iliac lymph nodes draining the genital and rectal mucosal.

Recombinant DNA vaccine

The immunity to HIV by vaccination with regulatory genes may establish resistance to early viral replication events. Plasmids carrying the genes for certain HIV proteins will evoke immune responses in the host. Foreign naked DNA introduced into muscle cell is effectively transcribed to produce immune protective responses against other infectious diseases (Ulmer *et al.*, 1993). Enhancement of DNA vaccine ability to elicit cellular immune response can occur by the co-delivering of the plasmids encoding costimulatory molecule B7 as well as those encoding various cytokine genes with DNA vaccine for HIV-1. Induction of T cell immune response is a complex process that requires engagement of T cell with professional APC and the production of different cytokines, which regulate the clonal and differentiation of antigen reactive cells. The dramatic increase in T cell proliferation and CTL response by the co-delivery of b7 and IL-12 genes is observed (Kim *et al.*, 1997b).

Plasmid DNA and recombinant fowlpox virus (rFPV) vaccines are among the most promising safe HIV-1 vaccine candidates. However, the immunity induced by either vaccine alone may be insufficient to provide durable protection against HIV-1 infection (Kent *et al.*, 1998).

HIV vaccine including vif, vpr, vpu and nef as well as accessory genes can be taken as a part of multi component immunogen. Ayyavoo *et al.* (1998) developed such type of vaccine that molecularly cloned and analyzed the sequence variation and immunogenic potential present in genes those derived from viral isolates. They observed that attenuated accessory genes can effectively induce both humoral and cellular responses in mice and the resulting immune response is directly correlated with DNA concentrations delivered and the number of boosts. A sub type E HIV-1 isolate from the Central African Republic was adapted to grow on chimpanzee peripheral blood mononuclear cells PBMCs by co-cultivation irradiated and infected human PBMCs with chimpanzee PBMCs. The resulting virus was passed in chimpanzee PBMCs to generate a stock of chimpanzee-adapted virus. After demonstrating in one animal each that the passed virus could infect chimpanzees following intravenous (i.v.) or cervical inoculation, the i.v. infectious titer of the stock was determined. Exposure of three chimpanzees to different doses of the virus indicated that the titer was between 2 and 5 TCID50. Thus, the HIV-1 E/90CR402 chimpanzee challenged stock established persistent infections in chimpanzees by both i.v. and

genital routes and should be valuable for future HIV-1 vaccine studies to evaluate cross-protection between HIV-1 subtypes (Barre-Sinoussi *et al.*, 1997).

Delivery of recombinant DNA vaccine

Several gene delivery techniques, such as intradermal or intramuscular injection and systems are used to induce immunity with genes. They include toxic or irritating agents including regeneration of tissues, carriers lipids, gold particles or liposomes, proteosomes and toxins bound to antigens such as cholera toxin and pertussis toxin.

In present cases, the DNA vaccines given without any adjuvant by the nasal route proved to be the most effective. Due to the dichotomy of the systemic and secretory immune systems, mucosal immunization at gastrointestinal or respiratory sites may be far more effective than parental immunization including mucosal responses (Walker *et al.*, 1994).

Use of the intravenous route to establish immunity also in other mucosal sites, such as the vagina, where direct immunization attempts with gene mixtures, gives less prominent local humoral responses (Shiver *et al.*, 1997).

The mouse model

Direct inoculation of DNA for HIV-1 envelope, gag/pol and vif inserted in plasmid expression cassettes induces specific antibody in mouse model. On the other hand, the pre-immunization sera from the identical animal as well as the sera collected from the control animals injected with the plasmid backbone did not show any antibody against the HIV-1 antigens.

Cytotoxic T cells target and destroy virus-infected cells by recognizing processed viral fragments on the infected cell surface associated with the host specific major histocompatibility complex (MHC) class I antigens. Targeting immune responses against viral proteins through the development of specific CTL responses would allow induction of a more efficacious immune response against target virus (Kim *et al.*, 1997b). In mice, the rFPV approach induced greater HIV-1 specific immunity than either vector alone and protected it from challenge with a recombinant vaccinia virus expressing HIV-1 antigens (Kent *et al.*, 1998).

Other animal model

The preclinical evaluation of potential vaccines against AIDS requires challenge models. The experimental infection of macaques with SIV, HIV-2 or chimeric viruses are valuable. The progresses made using simian models to asses the efficacy and identify the correlates or mechanism of protection by whole inactivated virus; live attenuated virus or recombinant subunit vaccine can be studied (Stott *et al.*, 1998).

Klinger *et al.* (1998) constructed an infectious chimeric SIV/HIV-1 (SHIV) with the envelope of Thai subtype E HIV-1 strain for use in a non-human primate model. This SHIV was recovered by co-cultivation from human PBMC after transection with human rabdosarcoma cells. Rhesus

macaque and baboon PBMC were screened ***in vitro*** for susceptibility to infection with SHIV. After successful infection baboon PBMC, four animals were inoculated intravenously with SHIV and monitored for plasma viral RNA. The results showed that the SHIV was able to infect PBMC in 12 out of 14 baboons. This chimeric virus established infection and induced antiviral antibodies in baboons inoculated by the intravenous route with cell-free virus. Thus, infection of baboons with SHIV can serve as an important animal model for studies of HIV-1 vaccine efficacy (Klinger ***et al.***, 1998).

In macaques, a dramatic boosting effect on rFVP DNA vaccine-primed HIV-1-specific helper and CTL responses, but a decline in HIV-1 antibody titers, was observed following immunization. The vaccine regimen protected macaques from an intravenous HIV-1 challenge, with the resistance most likely mediated by T-cell responses. These studies suggest a safe strategy for the enhanced generation of T-cell-mediated protective immunity to HIV-1 (Kent ***et al.***, 1998).

An edible HIV vaccine

A study of expression of HIV proteins in transgenic plants reported by J.de Johng, The Netherlands, whilst certainly not at the point of clinical application, is nevertheless intriguing. The gag gene of HIV-1 was cloned in a plant expression plasmid and stably integrated in the chromosomal DNA of potato plants by ***Agrobacterium***-mediated transfer of DNA. The transformants with the highest expression of antigen were selected based upon Northern-blot, micropropagated and regenerated to whole plants. Extracts of potato leaf and tubers were analyzed for the presence of gag protein in both extracts. Sucrose gradient analysis showed that p55 gag protein extracted from the tubers sedimented at different density (1.18 g cm^{-3}) than p55 gag protein produce by chimeric vaccinia viruses (1.16 g cm^{-3}) (Oxford and Jeff, 1996).

Preventive and therapeutic vaccine

For the reduction of viral load both preventive and therapeutic vaccine programmes provide substantial benefit. The effect of a therapeutic HIV vaccine on the epidemic outcomes depends markedly on whether the therapeutic vaccines reduce the infectivity of the vaccine recipient. The relative merits of preventive and therapeutic vaccines depend on the stage of epidemic.

Therapeutic vaccination has been proposed as a strategy to augment immune mechanisms to control viral replication and slow clinical progress of HIV infection to disease. Following recombinant gp 160 (rgp160) immunization in three clinical trials, plasma HIV-1 RNA and cellular proviral DNA were assessed by quantitative polymerase chain reaction (PCR) in 76 HIV-1 seropositive subjects with CD4+ T cell counts >or = 300 mm^{-3}. Immunization increased HIV-1 specific cellular immune responses (e.g., CTL activities, lymphocyte proliferative responses) (Kundu ***et al.***, 1997).

Owens ***et al.*** (1998) used an epidemic model to evaluate the population effects potential

preventive and therapeutic vaccines in early and late-staged epidemics for a population of homosexual men in San Francisco, California. In the model, a preventive vaccine prevented 3877 cases of HIV infection during a 20-years period, reduced the projected prevalence of HIV infection from 12 to 7% in a late-staged epidemic.

Use of adjuvant with vaccine

It is important to explore the immune responses to conformationally native regions in an attempt to exploit general cross-reactive immune responses, which lead to loss infectivity or reduction in viral survival. Study with using an extremely well characterized and native rgp120 suggests that alhydrogel and certain oil-based adjuvant systems may denature the rgp 120, thus potentially compromising the generation of broad-based efficacy (Jones ***et al.***, 1995).

The adjuvant QS-21 provides a number of advantages compared with other adjuvants such as Freund's adjuvant (VA) and alum. QS-21 formulations accelerated the production of antibodies to MN rgp120 and elicited complete seroconversion after a single immunization. QS-21 shifted the antigen dose-response curve for antibody production by as much as three orders of magnitude, enabling a more economical use of antigen. Antibody titers of MN rgp120 and to principal neutralizing determinant in the V3 domain were higher in animals receiving QS-21 formulations than in animals immunized with other adjuvants and correlated with higher virus neutralization titers in an ***in vitro*** assay (Powell ***et al.***, 1995).

Several observations indicate that both the antigen and the adjuvant require optimization together. Observations suggest that the gp120-alum interactions weak, where in buffer concentrations such as phosphate, sulfate and bicarbonate may cause the desorption of gp120 from alum. Comparison of gp120 with other protein shows that the weak binding of gp120 to alum is not an anomaly. Serum and plasma also cause desorption of gp120 from alum with a half-life of only a few min., where in this half-life may be faster than ***in vivo*** recruitment of antigen presenting cells to the site of immunization. Immunization of guinea pigs, rabbits and baboons with gp120 formulated in alum or saline demonstrated that alum provides adjuvant activity for gp120, particularly after early immunizations, but the adjuvant effect is attenuated after several boosts (Weissburg ***et al.***, 1995).

Monoclonal antibody as adjuvant

Many cytokines such as IL-2, IL-4, IL-7, IL-1beta, IL-12, IFN-gamma, TNF-alpha and granunolocyte macrophage CSF (GM-CSF) are effective as adjuvant (Ahlers ***et al.***, 1997; Kim ***et al.***, 1997a) to promote CTL response, T-cell proliferation, cytokine and antibody production. Novel synergies such as GM-CSF synergies with IL-12 for CTL induction in BALB/c mice concominant with suppression of Th2 cytokines IL-4 and IL-10. TNF alpha also synergied by a different mechanism induced IFN-gamma production in BALB/c mice and thus shifted the response to a Th1 phenotype (Ahlers ***et al.***, 1997).

Trial of HIV vaccine

Current HIV vaccines containing the HIV gp120 envelope have been tested in phase I and II trials but they have had a major limitation of neutralizing only T-cell tropic laboratory-adapted HIV strains grown in T-cell lines, but not neutralizing HIV primary isolates. Phase III trials of monovalent HIVgp 120 envelope vaccine are being planned in Thailand, but in US concern has been raised that recombinant monovalent gp 120 may not be an appropriate immunogen for an efficacious HIV vaccine. Because the immune response is probably responsible for controlling the viral load in some long-term survivors of HIV infection, studies are now being carried out to induce similar immunity against a broad spectrum of strains of HIV primary isolates with targeted HIV experimental immunogens (Haynes ***et al.***, 1996).

As of January 1998, more than 85 vaccines for 24 clinical indications are currently licensed in the United States. From the time of discovery of the etiologic agent to the development of a licensed vaccine, many years have usually been required. Although many vaccines have been licensed based on one efficacy trial, multiple vaccine concepts and multiple efficacy trials (both in the United States and internationally) have at times been necessary. Over a relatively short period, there has been remarkable progress in HIV vaccine development, with over 34 different HIV candidate vaccines having been tested in phase 1 trials and 3 having been tested in phase 2 trials. The fast phase 3 efficacy trial has been initiated in US and tentative plans have been announced for three other phase 3 efficacy trials with the most advanced HIV candidate vaccines to begin in the next three years. Like many previous vaccine development efforts, these initial HIV vaccine efficacy trials could be the first of many large-scale efficacy trials in the future, testing various vaccine design concepts among different high-risk populations in both developed and developing countries. The choice of when and how to proceed to phase 3 trials remains a complex decision, but it is likely that only through such trials will further knowledge be gained to advance this important effort and reach our goal of a safe and effective HIV vaccine (Heyward ***et al.***, 1998).

The trial of envelope based vaccine

The NIAID-sponsored AIDS vaccine evaluation group was established in 1988 to perform phase I/II clinical trials with candidate preventive HIV-1 vaccines. This report includes safety data from 1398 HIV-negative, healthy volunteers who were enrolled into 25 phase I and 1phase H multicentered, randomized, double-blind studies evaluating seven recombinant HIV-1 envelope vaccine, two V3 loop synthetic peptide vaccines and two live poxvirus-vectored recombinant envelope vaccines. Of all studies three were placebo controlled. The placebo was either the adjuvant alone or, in studies of recombinant poxvirus vaccines, it was the vector with no gene insert or a non-HIV gene insert. All candidate vaccines were generally well tolerated. The only adverse effects were occasional acute local and systemic reactions that were associated with

adjuvants. There were no serious adverse laboratory toxicities and no evidence of significant immunosuppressive events after receipt of the candidate vaccines. However, few volunteers experienced symptoms that might relate to an underline immunopathologic mechanism (rash, hemolytic anemia, arthralgia), but their presentations were mild and their incidence was low. In conclusion, the envelope-based recombinant or synthetic candidate HIV-1 vaccines appear to be safe and this work has prepared the way the testing of increasingly complex candidate HIV-1 vaccines (Keefer ***et al.***, 1997).

Since the isolation and molecular characterization of HIV from AIDS patients, a variety of vaccine constructs and strategies have been explored to combat this disease. Currently, no immunization strategy has been demonstrated to be completely effective in preventing HIV infection ***in vivo*** (Kim ***et al.***, 1997b). Major scientific obstacles blocking development of a successful preventive vaccine are the extraordinary variability of HIV, the lack of exact animal model of HIV-induced AIDS and the lack of understanding of the correlates of positive immunity to HIV (Haynes ***et al.***, 1996). Although the immunological basis for potential HIV vaccine efficacy presently is unknown and controversial, there is ample reason to believe that preexisting neutralizing antibody may offer protection against infection or disease (LaCasse ***et al.***, 1999).

Despite the naturally elicited immune response seen following HIV exposure, where the infection with HIV elicits a vigorous immune response, the host directed immune response is not effective in clearing HIV or in controlling its replication to prevent progressive organ damage (Lambert ***et al.***, 1997).

The failure of natural HIV type 1 infection to elicit a protective immune response, the complexities of viral replication and persistence, and the propensity of the virus for genetic change have suggested that the development of an effective AIDS vaccine will be difficult (Bolognesi ***et al.***, 1994; Graham and Wright, 1995; Haynes 1993; Moore and Anderson, 1994)

Naturally, occurring recombinant HIV strains have been found in infected patients in regions of the world where multiple genotypic variants cocirculate. One recombinant HIV strain has spread rapidly to millions of persons in Southeast Asia. Attaining multi-drug-resistant, recombination also poses theoretical problems for the development of a safe HIV vaccine (Burke, 1997). The development of an effective AIDS vaccine requires immunization strategies that can achieve the necessary maturation of immune responses to HIV-1 antigens in the minimum amount of time.

The development of the protective immunity SIV and equine infectious anemia virus models can demonstrate the ability of the immunization strategy to elicit broad and enduring immune protection from virus exposure. The development of protective immunity by these attenuated virus vaccines; however, has been shown to be time dependent and are associated with a complex and lengthy maturation of immune responses. The accomplishment of optimum vaccine protection is associated with fully mature immune response, which is characterized by relative steady-state antibody responses that are maintained indefinitely (Montelaro ***et al.***, 1998).

At least two major obstacles exist in binding well to the envelope spikes of HIV-1 and SIV, which can induce the immune system to produce antibody against these. The first is that very little of the envelope spike surface of primary viruses appears accessible for antibody binding (low antigenicity), probably because of oligomerization of the constituent proteins and a high degree of glycosylation of one of the proteins. Secondly the mature oligomer constituting the spikes appears to stimulate only weak antibody responses (low immunogenicity). Viral variation is another possible obstacle that appears to present fewer problems than anticipated. Vaccine design should focus on presentation of an intact mature oligomer, increasing the immunogenicity of the oligomer and if they bind well with the spike can offer protection or benefit if present at appropriate concentration before viral exposure (Burton, 1997).

Given this degree of diversity, it is widely believed that a vaccine based on a single strain or subtype of HIV-1 will not be successful against the larger spectrum of globally circulating HIV-1 variants (Lambert ***et al.***, 1997). The env genes of primary HIV-1 isolates collected worldwide can vary considerably in their genetic, phylogenetic and biological properties (Gao ***et al.***, 1996). Most vaccine formulations in clinical trials contain immunogens derived exclusively from subtype B viruses, the predominant genotype in the United States and Europe (Graham and Wright, 1995). By contrast, little emphasis has been placed on the development of candidate vaccines on non-subtype B viruses, although this causes the vast majority of HIV-1 infection in developing countries, where they continue to spread extremely rapid (Gao ***et al.***, 1996). Two major subtypes of HIV-1 have been identified from infected persons in Thailand (McCutchan ***et al.***, 1992; Ou ***et al.***, 1993). The subtype B HIV-1 was mainly transmitted among injection drug users (IDUS) in Bangkok (McCutchan ***et al.***, 1992; Ou ***et al.***, 1993; Kalish ***et al.***, 1995; Wasi ***et al.***, 1995). Recent data suggest that the frequency of subtype E HIV-1 is also increasing significantly in newly infected IDUS in Bangkok (Kalish ***et al.***, 1995; Wasi ***et al.***, 1995; Subba-Rao ***et al.***, 1998). The high prevalence and incidence of HIV-1 subtype E infection in Thailand indicates that prophylactic HIV vaccine should be based on this subtype (Wnag ***et al.***, 1998).

HIV-1 subtype E isolates from patients with AIDS in northern Thailand were shown to have more extensive inter-isolate variation in C2-V3 region of gp120 (Yu ***et al.***, 1995) as well as other variable regions in gp120 (Wnag ***et al.***, 1998). Recent studies suggest that diversity within subtype E HIV-1 among Bangkok IDUS has significantly increased (Wasi ***et al.***, 1995). The India, which is facing the largest burden of HIV worldwide the subtype C of HIV-1, is the most commonly transmitted type (Lole ***et al.***, 1999).

To be an effective HIV vaccine it should contain common syncytium inducing (SI) and nonsyncytium inducing (NSI) antigenic structures that can stimulate the immune response to produce a broad spectrum of neutralization response.

Certain vaccination regimes in chimpanzees and human volunteers with clade B SI type HIV-1 derived candidate vaccine induce neutralizing antibodies against intraclade B SI type primary HIV-

1 isolates, but not against intraclade B NSI type of viruses. To be effective against the antigenic spectrum of primary HIV-1 isolates candidate vaccine should contain immunogen of primary isolates representative of the whole antigenic spectrum of HIV-1 and to improve their immunogenicity identification of this immunogen is necessary. In developing countries the vaccine should be developed against the more prevalent clades C, A and E (van-der-Groen ***et al.***, 1998).

Exactly which epitopes are expected to be important for an HIV-1 vaccine is yet unknown. However, it would seem prudent to ensure that any candidate vaccine component is produced as near to structurally "native" as possible (Jones ***et al.***, 1995).

The power of DNA delivery ***in vivo*** is for both the production of a new generation of more effective vaccines as well as an analytical tool for the molecular dissection of the mechanisms of immune function (Kim ***et al.***, 1997b).

The intravaginal nucleic acid vaccines may provide a novel strategy for generation of mucosal immunity. The combination of systemic and mucosal immunization of DNA might be the ideal vaccine against HIV infection. DNA immunization against HIV-1 represents a model system, wherein the feasibility of the particular impact on application to other mucosally transmitted diseases (Wang ***et al.***, 1997).

Since an effective combination of HIV-1 immunoglobulin and HIV-1 vaccine given to the HIV-1 exposed newborns to prevent HIV-1 transmission similar to the viral hepatitis B model is not firmly established at present, post exposure antiretroviral prophylaxis and non-breast-feeding are advocated for infants born from the HIV-1 infected mothers (Phuapradit, 1998).

Because of a unique combination of challenges facing HIV vaccine developers, a number of traditional and novel vaccine designs are being evaluated essentially in parallel. Monomeric proteins, poxvirus vectors, peptides and particle based candidate vaccine entered or will soon in human trials. Other designs are at early stage of development. All candidates evaluated to date in phase I/II trials have proven safe and immunogenic. One or more of the most promising designs will soon progress to 'test concept' clinical trials to determine the efficacy (Johnston, 1995).

References

Ahlers, J.D., N. Dunlop, D.W. Alling, P.L. Nara and J.A. Berzofsky, 1997. Cytokine in adjuvant steering of the immune response phenotype to HIV-1 vaccine constructs: granulocyte-macrophage colony-stimulating factor and TNF-alpha synergize with IL-12 to enhance induction of cytotoxic T-lymphocytes. J. Immunol., 15; 158: 3947-58.

Alexander, N., 1990. Sexual transmission of human immunodeficiency virus:Virus entry into the male and female genital tract. World Health Organization, Global Programme on Acquired Immune Deficiency Syndrome. Fertilization and Sterilization, 54: 1-18.

Archibald, D.W., C. A. Hebert, D. Sun and C.O. Tacket, 1990. Salivary antibodies to human immunodeficiency virus type1 in a phase I AIDS vaccine trial. J. Acquired Immune Deficiency Syndrome, 36: 35-41.

Arthur, L.O., J.W. Bess, Jr., E.M. Chartova, J.L. Rossi, M.T. Esser, R. E. Benveniste, L.E. Henderson and J.D. Lifson, 1998. Chemical inactivation of retroviral infectivity by targeting nucleocapsid protein zinc fingers; a candidate SIV vaccine. AIDS Research and Human Retroviruses, 14 (Suppl 3): S311-9.

Ayyavoo, V., T. Nagashunmugam, M.T. Phung, C. Buckner, S. Kudckodkar, P.Le, P.J. Reddy, L. Santiago, M. Patel, L. Tea and D.B. Weiner, 1998. Construction of attenuated HIV-1 accessory gene immunization cassettes. Vaccine, 16: 1872-9.

Barre-Sinoussi, F., M.C. Georges-Courbot, P.N. Fultz, H. Nguyen-Thi-Tuyet, E. Muchmore, S. Saragosti, G. Dubreuil, F. Georges, E. Van-der-Ryst and Girard, 1997. Characterization and titration of an HIV type 1 subtype E chimpanzee challenge stock. AIDS Research and Human Retroviruses-1, 13: 583-91.

Berkhout, B., K. Verhef, J.L. Van-Wamel and N.K. Back, 1999. Genetic instability of live, attenuated human immunodeficiency virus type 1 vaccine strains. J. Virol., 73: 1138-45.

Berman, P.W., T.Z. Gregory and L. Riddle, 1990. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp 120 but not gp 160. Nature, 345: 622-625.

Bolognesi, D.P., L. Corey, S.H. Vermund and D.F. Hoth, 1994. HIV vaccine development: a progress report. Ann. Int. Med., 8: 603-611.

Burke, D.S., 1997. Recombination in HIV: A important viral evolutionary strategy. Emergence of Infectious Dis., 3: 253-9.

Burnett, P.R., T.C. VanCott, V.R. Polonis, R.R. Redfield and D.L. Brix, 1994. Serum IgA-mediated neutralization of HIV type 1. Immunol., 7: 1-7.

Burton, D.R., 1997. A vaccine for HIV type 1: the antibody perspective. Proceeding of National Academy of Sciences of USA, 16; 94: 10018-23.

Chang-Mayer, C., T. Shioda and J. Levy, 1991. Host range replicative and cytopathic properties of human immunodeficiency virus type 1 are determined by very few amino acid change in Th1 and gp120. J. Virol., 65: 6931-41.

Cotran, R.S., V. Kumar and S.L. Robbins, 1994. Pathologic Basis of Disease. 5th ed. Eastern Press Pvt. Ltd., Bangalore-560 029.

Emini, E.A., W.A. Schleif and J.H. Nunberg, 1992. Prevention of HIV-1 infection in chimpanzee by gp120 V3 domain-specific monoclonal antibody. Nature, 335: 728-730.

Eroshkin, A.M., E.A. Karginova, I.P. Gileva, A.S. Lomakin, L.R. Lebedev, Y.P. Kamyinina, A.V. Pereboev and G.M. Ignat'ev, 1995. Design of four-helix bundle protein as a candidate for HIV vaccine. Protein Engineering, 8: 167-73.

Funkhouser, A., M. L. Clements, S. Slome, B. Clayman and R. Viscidi, 1993. Antibodies to recombinant gp160 in mucosal secretions and sera of persons infected HIV-1 at seronegative vaccine recipients. AIDS Research and Human Retroviruses, 9: 627-632.

Gao, F., S.G. Morrisom, D.L. Robertson, C.L. Thornton, S. Craig, G. Karlsson, J. Sodroski, M. Morgado, B. Galvao-Castro and H. Von-Briesen, 1996. Molecular cloning and analysis of functional envelope genes from human immunodeficiency virus type1 sequence subtypes A through G. The WHO and NIAID networks for HIV isolation and characterization. J. Virol., 70: 1651-67.

Girard, M., M.P. Kieny and A. Pinter, 1991. Immunization of chimpanzees confers protection against challenge with human immunodeficiency virus. Proceeding of National Academy of Sciences. USA, 88: 542-546.

Gorse, G.J., J.H. Rogers, J.E. Perry, F.K. Newman, S.E. Frey, G.B. Patel, R.B. Belshe and the NIAID AIDS Vaccine Clinical Network, 1995. HIV-1 recombinant gp160 vaccine-induced antibodies in serum and saliva. Vaccine, 13: 209-14.

Graham, B.S. and P.F. Wright, 1995. Candidate AIDS vaccine. New England J. Med., 333: 1331-1339.

Haynes, B.F., 1993. Scientific and social issues of human immunodeficiency virus vaccine development. Sci., 260: 1279-1286.

Haynes, B.F., S.B. Putman and J.B. Weinberg, 1996. Update on the issues of HIV vaccine development. Ann. Med., 28: 39-41.

Heyward, W.L., K.M. MacQueen and K.L. Goldenthal, 1998. HIV vaccine development and evaluation: realistic expectations. AIDS Research and Human Retroviruses, 14 (Suppl) 3: S205-10.

Huang, X., J.J.Jr. Barchi, F.D. Lung, P.P. Roller, P.L. Nara, J. Muschik and R.R. Garrity, 1997. Glycosylation affects both the three-dimensional structure and antibody binding properties of the HIV-1 IIIB gp120 peptide RP135. Biochem., 9; 36: 10846-56.

Johnson, R.P. and R.C. Desrosiers, 1998. Protective immunity induced by live attenuated simian immunodeficiency virus. Current Opinion in Immunology, 10: 436-43.

Johnston, M.I., 1995. Progress in AIDS vaccine development. International Archive of Allergy and Immunology, 108: 313-7.

Jones, D.H., B.W. Mcbride, M.A. Roff and G.H. Farrar, 1995. Efficient purification and rigorous characterization a recombinant gp120 for HIV vaccine studies. Vaccine, 13: 991-9.

Kalish, M.L., A. Baldwin and S. Raktham, 1995. The evolving molecular epidemiology of HIV-1 envelope subtypes in injection drug users in Thailand: implication for HIV vaccine trials. AIDS, 9: 851-7.

Keefer, M.C., M. Wolff, G.J. Gorse, L. Corey, M.L. Clements-Mann, N. Verani-Ketter, S. Erb, C.M. Smith, R.B. Belshe, L.J. Wagner, M.J. McElrath, D.H. Schwartz and P. Fast, 1997. Safety profile of phase I and phase II preventive type 1 HIV envelope vaccination experience of the NIAID AIDS vaccine evaluation group. AIDS Research and Human Retroviruses, 20; 13: 1163-77.

Kent, S.J., A. Zhao, S.J. Best, J.D. Chandler, D.B. Boyle and I.A. Ramshaw, 1998. Enhanced T cell immunogenicity and protective efficacy of a human immunodeficiency virus type 1 vaccine regimen of consecutive priming with DNA and boosting with recombinant fowlpox virus. J. Virol., 72: 10180-8.

Kim, J.H., J.E. Loveland, K.V. Sitz, S. Ratto-Kim, R.J. Mclinden, K. Tencer, K. Davis, D.S. Burke, R.N. Boswell, R.R. Redfield and D.L. Brix, 1997a. Expanrestricted cellular immune responses to HIV-1 envelope by vaccination: IL-7, IL-12 differentially augment cellular proliferative responses to HIV-1. Clinical Experimental Immunology, 108: 243-50.

Kim, J.J., V. Ayyavoo, M.L. Bagarazzi, M. Chattergoon, J.D. Boyer, B. Wang and D.B. Weiner, 1997b. Development of a multicomponent candidate vaccine for HIV-1. Vaccine, 15: 879-883.

Klavinskis, L.S., L. Gao, C. Barnfield, T. Lehner and S. Paker, 1997. Mucosal imminization with DNA-liposome complexes. Vaccine, 15: 818-20.

Klinger, J.M., S. Himathongkham, H. Legg, P.A. Luciw and S.W. Barnett, 1998. Infection of baboons with a simian immunodeficiency virus/ HIV-1 chimeric virus constructed with an HIV-1 Thai sub-type envelope. AIDS, 28; 12: 849-57.

Kundu, S.K., D. Katzenstein, F.T. Valentine, C. Spino, B. Efron and T.C. Merigan, 1997. Effect of therapeutic immunization with recombinant gp160 HIV vaccine on HIV-1 proviral DNA and plasma RNA: relationship to cellular immune responses. J. Acqu. Immune Deficiency Syndrome and Human Retroviruses, 1; 15: 169-74.

LaCasse, R.A., K.E. Follis, M. Trahey, J.D. Scarborough, D.R. Littman and J.H. Nunberg, 1999. Fusion- competent vaccines: broad neutralization of primary isolates of HIV. Scie., 283: 357-62.

Lambert, J.S., R. Viscidi, M.C. Walker, B. Clayman, M. Winget, M. Wolff and D.H. Schwartz, 1997. Antibody to human immunodeficiency virus type 1 (HIV-1) gp160 in mucosal specimens of asymptomatic HIV-1 infected volunteers parenterilly immunized with an experimental recombinant HIV-1 IIIB gp160 vaccine. The national institute of Allergy and Infectious Diseases-sponsored AIDS vaccine evaluation group. Clinical Diagnostic and Laboratory Immunology, 4: 302-8.

Lasky, L., G. Nakamura and D. Smith, 1987. Delineation of a region of the human immunodeficiency virus type1 gp120 glycoprotein critical for interaction with the CD4 receptor. Cell, 50: 975-985.

Lole, K.S., R.C. Bollinger, R.S. paranjape, D. Gadkari, S.S. Kulkarni, N.G. Novak, R. Ingersoll, H.W. Sheppard and S.C. Ray, 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, the evidence of intersubtype recombination. J. Virol., 73: 152-60.

McCutchan, F.E., P. Higrich and T. Bernan, 1992. Genetic variants of HIV-1 in Thailand. AIDS Research and Human Retroviruses, 8: 887-95.

McDougal, J., M. Kennedy, J. Sligh, S. Cort, A. Mowle and J. Nicolson, 1986. Binding of the HTLV-111/LAV to T4+ T cells by a complex of the 110k viral protein and the T4 molecule. Sci., 231: 382-385.

McGhee, J. and J. Mestechy, 1992. The mucosal immune system HIV infection and prospects for mucosae immunity to AIDS. AIDS Research and Review, 4: 289-312.

Mestecky, J., W.H. Kutteh and S. Jackson, 1994. Mucosal immunity in the female genital tract: relevance to vaccination efforts against the human immune-deficiency virus. AIDS Research and Human Retroviruses, 10: S11-18.

Mestecky, J., 1987. The common mucosal immune system and current strategies for induction of immune responses in external secretions. J. Clini. Immunol., 7: 265-276.

Milman, G. and O. Salma, 1994. Mechanisms of HIV/SIV mucosal transmission. AIDS Research and Human Retroviruses, 10: 1305-12.

Montelaro, R.C., K.S. Cole and S.A. Hammond, 1998. Maturation of immune responses to lentivirus infection: implication of AIDS vaccine development. AIDS Research and Human Retroviruses, 14 Suppl 3: S255-9.

Moore, J. and R. Anderson, 1994. The WHO and why of HIV vaccine trials. Nature (London), 372: 313-314.

Morris, K., 1999. US launches trial of AIDS vaccine in Uganda (news). Nature, 18; 397 (6720): 554.

Okuda, K., K.O. Xin, T. Tsuji, H. Bukawa, S. Tamaka, W.C. Koff, K. Tali, K. Okuda, K. Honma, S. Kawamota, K. Hamajima and J. Fukushima, 1997. DNA vaccination followed by macromolecular multicomponent peptide vaccination against HIV-1 induces strong antigen-specific immunity. Vaccine, 15: 1049-56.

Olshevsky, O., E. Helseth, C. Furman, J. Li, W. Hasetine and J. Sodroski, 1990. Identification of individual HIV-1 gp120 amino acids important for CD4 receptor binding. J. Virol., 64: 5701-5707.

Ott, D.E., E.M. Chertova, L.K. Beusch, L.V. Coren, T.D. Gagliardi and Johnson, 1999. Mutational analysis of the hydrophopbic tail of the human immunodeficiency virus type1 p6 (Gag) protein produces a mutant that fails to package its envelope protein. J. Virol., 73: 19-28.

Ou, C.Y., Y. Takbe and B.G. Weniger, 1993. Independent introduction of two major HIV-1 genotypes into distinct high risk population in Thailand. Lancet, 342: 1171-9.

Owens, D.K., D.M. Edwards and R.D. Shacter, 1998. Population effects of preventive and therapeutic vaccine in early- and late-stage epidemics. AIDS, 18; 12: 1057-66.

Oxford, J.S. and S.A. Jeffs, 1996. New scientific developments towards an AIDS vaccine: report on a workshop organized by EU programme EVA entitled novel approaches to AIDS vaccine development held at the institute Pasteur, Paris. Vaccine, 14: 1712-7.

Phuapradit, W., 1998. Timing and mechanism of perinatal human immunodeficiency virus-1 infection. Australia and New Zealand Journal of Obstetrics and Genecol., 38: 293-7.

Powell, M.F., D.J. Eastman, A. Lim, C. Lucas, M. Peterson, J. Vennari, R.P. Weissburg, T. Wrin, C.R. Kensil and M.J. Newman, 1995. Effect of adjuvants on immunogenicity of MN recombinant glycoprotein 120 in guinea pigs. AIDS Research and Human Retroviruses, 11: 203-9.

Race, E., P. Frezza, D.M. Stephens, D. Davis, N. Polyanskaya, M. Cranage and J.S. Oxford, 1995. An experimental chemically inactivated HIV-1 vaccine induce antibodies that neutralize homologous and heterologous viruses. Vaccine, 13: 54-60.

Rovinski, B., L. Rodrigues, S.X. Cao, F.L. Yao, U. McGuinness, C. Sia, G. Cates, S. Zolla-Pazner, S. Karwowska and T. J. Mattehews, 1995. Induction of HIV type1 neutralizing and env-CD4 blocking antibodies by immunization with genetically engineered HIV type 1-like particles containing unprocessed gp160 glycoproteins. AIDS Research and Human Retroviruses, 11: 1187-95.

Shiver, J.W., M.E. Davies, Y. Yastomi, H.C. Perri, D.C. Freed, N.L. Letvin and M.A. Liu, 1997. Anti-HIV env immunities elicited by nucleic acid vaccines. Vaccine, 15: 884-7.

Steimer, K.S., P.J. Klasse and J.A. McKeating, 1991. HIV neutralization directed to epitopes other linear V3 determinants. AIDS. 3 (Suppl.1): S135-S143.

Stott, J. S.L. Hu and N. Almond, 1998. Candidate vaccine protect macaques against primate immunodeficiency viruses. AIDS Research and Human Retroviruses, 14 (Suppl 3): S265-70.

Subba-Rao, S., K. Limpakarnjanarut and T. Mastro, 1998. HIV type 1 in Thailand 1994-1995: persistence of two subtypes with low genetic diversity. AIDS Research and Human Retroviruses, 14: 319-27.

Ulmer, J.B., J.J. Donnelly and S.E. Parker, 1993. Heterologous protection against influenza by injection of DNA encoding a viral protein. Sci., 259: 1745-49.

van-der-Groen, G., P.N. Nyambi, E. Beirnaert, D. Davis, K. Fransen, L. Heyndrickx, P. Ondoa, G. Van-der-Auwera and W. Janssens, 1998. Genetic variation of HIV type1: relevance to interclade variation to vaccine development. AIDS Research and Human Retroviruses, 14 (Suppl 3): 211-21.

Vasudevachari, M.B., K.W. Uffelman, J. Kovacs, C. Yen, H.C. Lane and N.P. Salzman, 1992. Envelope-specific in the antibodies in the saliva of individuals vaccinated with recombinant HIV-1 gp160. J. AIDS, 5: 818-821.

Wahren, B., J. Hinkula, E. Ljungdahl-Stahle, C. Borrebaeck, S. Schwartz and H. Wigzell, 1995. Nucleic acid vaccination HIV regulatory genes DNA vaccines, a new era in vaccinology. Ann. New York Acad. Sci., 772: 278-81.

Walker, R.I., 1994. New strategies for using mucosal vaccination to achieve more effective immunization. Vaccine, 12: 387-400.

Wang, B., K. Dang, M.G. Agadjanyan, V. Srikantan, F. Li, K.E. Ugen, J. Boyer, M. Merva, W.V. Williams and D.B. Weiner, 1997. Mucosal immunization with a DNA vaccine induce immune responses against HIV-1 at mucosal site. Vaccine, 15: 821-825.

Wang, B., J.J. Boyer, V. Srikantan, L. Coney, R. Carrano, C. Phan, M. Merva, K. Dang, M. Agadjanyan, L. Gilbert, K. Ugen, V. W. Williams and D. B. Weiner, 1993a. DNA inoculation induces neutralizing immune responses against human immunodeficiency virus type 1 mice and nonhuman primates. DNA Cell Biol., 12: 799-805.

Wang, B., E.K. Ugen, V. Srikantan, M. Agaduanyan, K. Dang, Y. Refaeli, A.L. Sato, J. Boyer, W.V. Williams and D.B. Weiner, 1993b. Gene inoculation generates immune responses against human immunodeficiency virus type 1. Pro. Nat. Acad. Sci. USA., 90: 4156-60.

Wasi, C., B. Herring and S. Rakthan, 1995. Determination of HIV-1 subtypes in injecting drug users in Bangkok, Thailand, using peptide-binding enzyme immunoassy and heteroduplex mobility assay: evidence of increasing infection with HIV-1 subtype E. AIDS., 9: 843-9.

Weissburg, R.P., P.W. Beramn, J.L. Cleland, D. Eastman, F. Farina, S. Frie, A. Lim, J. Mordenti, T.T. Nguyen and M.R. Peterson, 1995. Characterization of the MN gp120 HIV-1 vaccine: antigen binding to alum. Pharmaceutical Res., 12: 1439-46.

Wnag, J., C.M. Lyles, C. Beyrer, D.D. Celentano, D. Vlahob, C. Natpratan, R. Markham, C. Khambunruang, K. Nelson and X.F. Yu, 1998. Diversification of subtype E human immunodeficiency virus type 1 env in heterosexual seroconverters from Northern Thailand. J. Infectious Dis., 178: 1507-11.

Wolff, J., R. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani and P. Feigner, 1990. Direct gene transfer into mouse muscle ***in vivo***. Sci., 247: 1465-68.

Yu, X.F., Z. Wang and C. Beyrer, 1995. Phenotypic and genotypic characteristic of human immunodeficiency virus type 1 from patients with AIDS in Northern Thailand. J. Virol., 69: 4649-55.