HIV VACCINES: PRESENT SCENARIO AND FUTURE PROSPECTS

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Summary

AIDS, first described in early1980s, has reached the far corners of the globe and is the main cause of death throughout the world. Potential adverse effects of the present anti-HIV drugs, multidrug regimens leading to drug resistance and conferring mutations in the virus leading to more virulent forms have provoked the urgency for vaccine development. However, the development of an HIV vaccine faces formidable scientific challenges related to the high genetic variability in the virus, the lack of immune correlates of protection, lack of animal models and logistical problems associated with the conduct of multiple clinical trials. Yet, live vectored recombinant vaccines, replication incompetent viral vectors, subunit vaccines and DNA vaccines have paved the way for the development of a variety of novel and specific vaccine approaches. Researchers are currently exploring the uncharted immunologic terrain which could also prove invaluable. Although they have turned the virus inside-out and carefully detailed how it destroys the immune system, it is yet to unravel which immune responses can fend off an infection indicating, AIDS vaccine research for more than a decade is "flying without a compass."

Keywords: (AIDS) Acquired Immunodeficiency syndrome, (CTLs) Cytotoxic T-lymphocytes, (HIV) Human Immunodeficiency Virus, Vaccines

INTRODUCTION

Although HIV transmission is in theory largely preventable, in practice, without the development of an effective vaccine, HIV will continue to infect millions throughout the world. According to recent United Nations AIDS estimates at least 39.5 million (34.1–47.1 million) people were living with HIV in 2006 which includes 37.2 (32.1–44.5 million) adults, 17.7 million (15.1–20.9 million) women and 2.3 million (1.7–3.5 million) children less than 15 years of age. Around 4.3 million (3.6–6.6 million) people were newly infected with HIV in 2006 and the death toll was 2.9 million (2.5–3.5 million)(1).

The current HIV therapy has many drawbacks **(**2, 3),such as:

- No ready access to antiretroviral treatment (ART).
- Potential adverse effects of the anti-HIV drugs.
- Extensive drug-drug interactions.
- High cost.
- Emergence of drug resistance.

An alternative objective of AIDS vaccine is to prevent disease rather than infection. Vaccine-induced protection against HIV disease could be achieved by:

- Complete protection from infection (sterile immunity)
- Clearance of virus and infected cells (abortive infection)
- Persistent infection without disease

Because HIV may induce AIDS even after a long asymptomatic period, the desired goal of most AIDS vaccine trials to date has been to induce sterile immunity. Sterile immunity may be difficult to achieve, however, as most existing viral vaccines prevent disease and do not prevent infection(4). An alternative goal for AIDS vaccine development might therefore be to induce immune responses that would contain HIV replication, resulting in a prolonged (perhaps indefinite) asymptomatic period, similar to long-term nonprogressors(5, 6)**.**

CELLULAR AND MOLECULAR BIOLOGY OF HIV

The complete understanding of the cellular structure of HIV is significant for the vaccine design. HIV is a member of the Lentivirinae subfamily of Retroviruses. Retroviruses belong to the diverse family of enveloped RNA viruses. They are typically 100nm in diameter and contain 2 single strands of RNA, which permits recombination between the strands. The genome is 10kb in size and contains 3 major genes-*gags; pol* and *env.* HIV virion measures 80nm to 130 nm in diameter and has a unique 3 layered structure. It contains an inner conical structure and 72 external spikes formed by the 2 major viral envelope proteins gp120 and gp41. Also it contains gp160-precursor of envelope glycoproteins.These gp120 and gp41 are assembled into a trimeric complex(7).

The HIV lipid bilayer consists of a number of host proteins including the Class 1 and 2 MHC antigens, acquired during virion budding. The viral core contains core proteins, the p24 capsid proteins (phosphorylated), p17 matrix protein and the p7, p6 nucleocapsid proteins, each of which is proteolytically cleaved from a 55kDa Gag precursor by the HIV-1 protease. The capsid protein forms the chief component of the inner shell of the virion, where as the matrix protein is associated with inner surface of the lipid bilayer and probably stabilizes the external and internal components of the virion. The p7 nucleocapsid protein binds directly to the genomic RNA through a zinc finger

structural motif and together with p6 nucleocapsid forms the retroviral core. It also contains 2 copies of single stranded HIV1 genomic RNA that are associated with the various preformed viral enzymes including reverse transcriptase, integrase and protease(8). (fig-1)

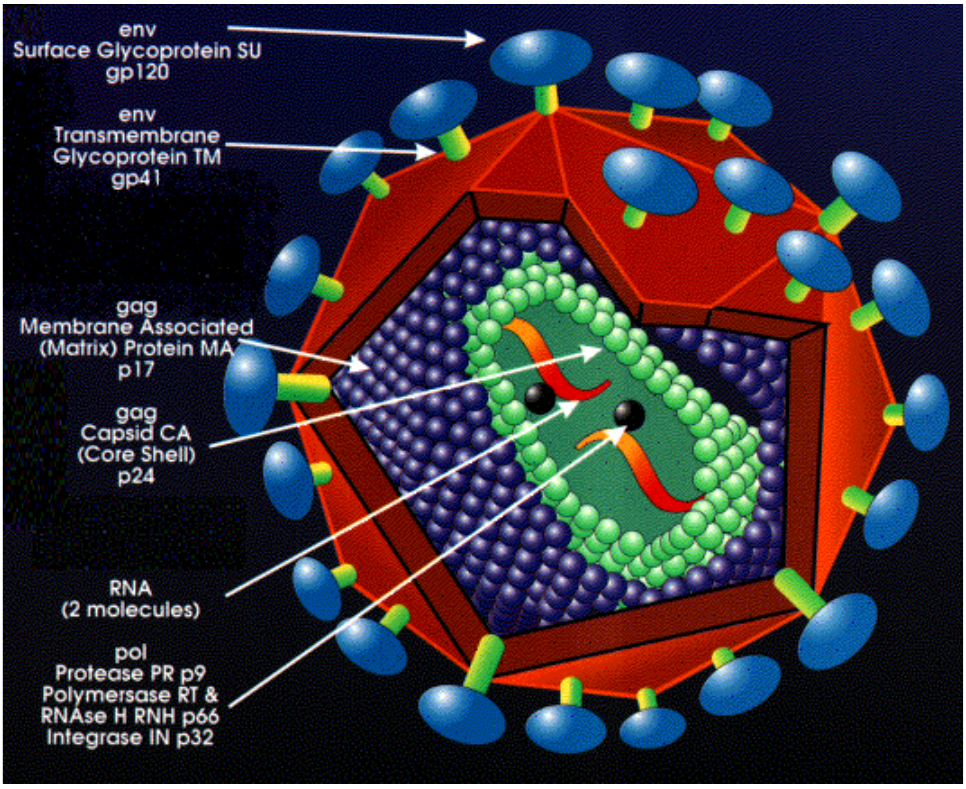

Figure 1: structure of HIV

PATHOGENESIS

It involves the attachment of viral protein gp120 to the host cell surface receptor CD4 leading to a conformational change in gp120 and the receptor followed by displacement of gp120.As a result of this process, proteolytic cleavage of V3 loop or its envelope counterpart occurs. Then it involves the interaction of the fusion domain of HIV (gp41) with a fusion receptor on the host cell surface leading to viral fusion with the host cell membrane. This is followed by entry of viral RNA associated with core and polymerase proteins into the cytoplasm. The double stranded DNA (cDNA) is made from a viral RNA by reverse transcription involving the reverse transcriptase enzyme. This proviral cDNA is then transported into the cell nucleus, where it integrates into the host cell chromosome DNA.Viral mRNA and genomic RNA are produced from the integrated proviral DNA. The extent of this production is dependent on the expression of HIV regulatory genes (*tat, rev, nef, vpr*). Viral proteins are produced from full length and spliced mRNA and viral genomic RNA is incorporated into the capsid forming at the cell membrane. *Gag* and *gag-pol* polyproteins are processed via protease at the cell surface or in budding virions. Finally, the viral capsid buds through the cell membrane incorporating both the processed viral envelope glycoprotein and some host cell proteins present on the host cell surface. This final stage may involve the function of the viral *vif* gene (8).

Table 1: GENES AND GENE PRODUCTS OF HIV-1 & 2 (6,7)

GENE	PROTEINS	FUNCTIONS/PROPERTIES
Gag	p17 p24 p6 p7	Matrix protein: interacts with gp41 Core protein Core protein; binds to *Vpr* Nucleocapsid: binds to RNA
Pol	Protease Reverse transcriptase Integrase	Proteolytic cleavage of *gag* and *pol* Polymerase and Rnase H activity Integration into chromosome
Env	gp120 gp41	Envelope: viral entry into cell Transmembrane protein: cell fusion
Vif	Virion infectivity protein	Efficient cell free transmission
Vpr	Viral protein R	Enhances viral replication in primary cells, Virion associated protein:g2/M phase arrest: nuclear localization
Tat	Transactivator of transcription	Major viral transactivator
Rev	Regulator of expression of virion protein	Enhances expression of unspliced and singly spliced RNA's
vpu #	Viral protein U	Enhances virion releases from cells: downs regulates CD4
Nef	Negative regulator factory	Inhibits or enhances viral replication depending on strain and cell type. Downregulates CD4; MHC-I.
Vpx ##	Virion protein X	Packaged into virion

HIV-1 only
HIV-2 only.

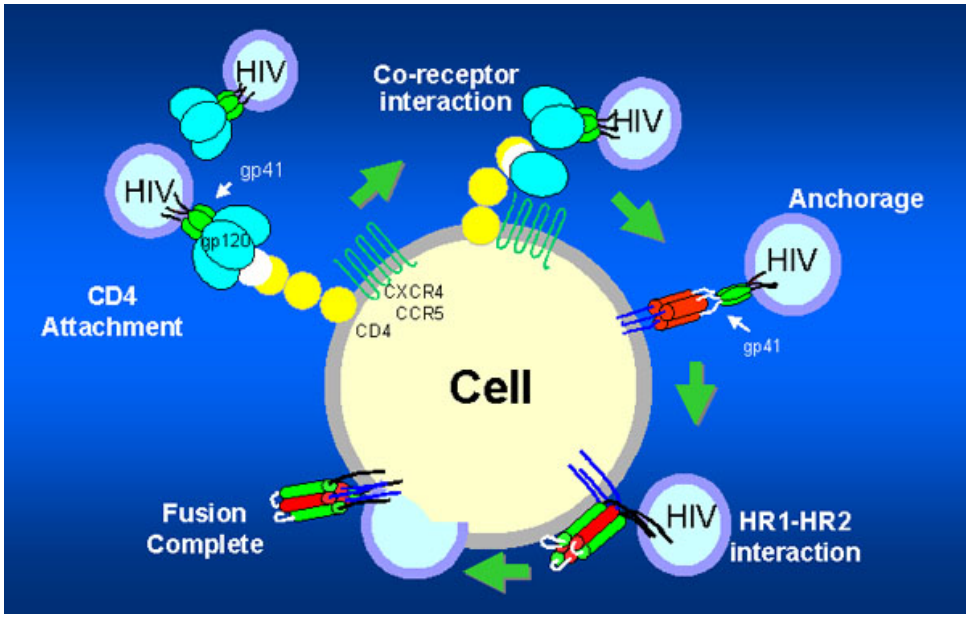

Figure 2: Interaction of HIV and CD4 molecule.

CHALLENGES AND OBSTACLES IN VACCINE DEVELOPMENT

Obstacles to the development of an effective AIDS vaccine include factors related to the biology of HIV-1 infection and practical realities of developing and testing an AIDS vaccine. The molecular and cellular entities of HIV allow it to escape from the host immune system and this presents a major challenge for the designing of an effective vaccine (9).

ANTIGENIC VARIATION

Intense genetic variations occur in the HIV gene sequences and thus in the viral antigens. There is no doubt that new antigenic variants of HIV constantly arise during the long course of AIDS because of the low fidelity of reverse transcription. The mutation rate of HIV-1 is high because reverse transcriptase lacks a proof reading domain thereby allowing a great deal of sequence variation in viral nucleic acids. Mutations occur during viral replication giving rise to virulent forms of the virus with increased cytopathic capacity and altered cell tropisms(10, 11). The V3 loop of the HIV1 envelope contains GP-GRA amino acid sequences that encode major antigens reflecting the variability. A competent vaccine is required to induce immune responses against neutralizing epitopes from diverse isolates. Among diverse HIV-1 isolates, there are five to six amino acids at the tip of the V3 loop, where comparatively fewer mutations are identifiable (conserved region). Such a conserved neutralizing epitope may allow vaccination against multiple strains(12).

INTEGRATION OF VIRAL DNA

The immune system is unable to detect cells infected with a retrovirus as the viral DNA remains integrated into host cell DNA, but not expressed. Integration of the viral DNA with the host cell DNA is catalyzed by the enzymes reverse transcriptase and integrase, which makes double stranded DNA copy of the viral RNA genome which integrates into the chromosomal DNA of the

host cells. In this pro-viral form, the viral genome remains in a "latent" state of expression in which the viral RNA and protein synthesis is limited (13-15).

INFECTION AND DESTRUCTION OF CRUCIAL CD4 CELLS

CD4 molecule present on the surface of the T-helper cells provides the high affinity receptor for HIV cell binding (16). But the binding is not successfully complete unless a co-receptor is present on the cell surface in addition to CD4. For T-cells it is CXCR4 (fusin) or CXCR5 and for macrophages the co-receptor is CCR5.These co-receptors are transmembrane receptors containing a N-terminus extra cellular domain, seven membrane spanning regions and a C-terminus intracellular signaling domain that interacts with G-proteins. Activated CD4+ lymphocytes, the key cells targeted by HIV-1, bear both CCR5 and CXCR4[17]. Binding of CD4 to HIV-1 envelope gp120 induces conformational rearrangement in the envelope complex facilitating recognition of the CCR5 chemokine co-receptors and consequent viral entry into the cell. Zhang *et al* defined the mechanism of virus docking and cell fusion by revealing that CD4 induced conformational changes in the equilibrium state of gp120 lead both to the movement of V1/V2 loops and to the conformational rearrangement in the gp120 core structure and that both results in the greater exposure of the co receptor binding epitope in gp120 (18).

CyclophilinA, a cellular protein, involved in the receptor binding process, emerges out from HIV envelope membrane and sticks to heparan molecules (CD147) on the cell surface thus, facilitating HIV-1 to attach to new target cells even before the interaction of gp120 and CD4 cells. It is involved in T cell activation and functions as a "molecular chaperone" protein that helps other proteins to fold into their correct conformation (19-20).

EVADING ATTACK FROM NEUTRALIZING ANTIBODIES

HIV has developed sophisticated mechanisms to dodge immune attack, shrouding its surface protein in sugars (gp120 is heavily glycosylated) to hide vulnerable sites from antibodies and producing proteins that thwart production of other immune warriors. Wyatt *et al* described the spatial organization of conserved neutralization epitopes on gp120, using epitope maps along with the X-ray crystal structure of a ternary complex that includes a gp120 core, CD4 and a neutralizing antibody. A large fraction of the predicted accessible surface of gp120 in the trimer is composed of variable heavily glycosylated core and loop structures that surround the receptor binding regions (21, 22).

ESCAPE FROM ANTIVIRAL CYTOKINES

The interchange of viral phenotype from M- tropic (CCR5 using) to T -tropic (CXCR4 using) during late disease indicates the escape from the inhibitory effects of CCR5 binding chemokines-RANTES, MIP-1α and MIP-1β (10).

NEUROTROPISM OF HIV

Infection of cells in immune privileged sites is another main mechanism underlying HIV evasion of host immunity .This immunopriveleged sites are relatively insulated from the systemic immune defense mechanisms where the CTLs have poor penetration and reduced ability to clear infected cells, such as the CNS and microglia in the brain[3].

LACK OF ANIMAL MODEL

Chimpanzees are the only animals that are susceptible to HIV. Not only the supply is scarce but also chimpanzees develop viremia and antibodies, they do not develop immunodeficiency. The SIV macaque model of Simian AIDS does develop disease and helpful for vaccine design studies (10- 23).

THE PROCESS OF DEVELOPMENT OF VACCINES

- **FIRST GENERATION APPROACHES**- Enveloped glycoprotein antigens.

Initial approaches were based on enveloped glycoprotein gp120 due to their functional importance for virus attachment and entry and as primary targets for neutralizing antibodies. CHO- derived rgp120(r = recombinant) emerged as the most immunogenic of early subunit vaccines (24-25). The disadvantages of this approach was that the serum antibody titers had short half life (<6 months) and when boosted, the peak level reaches only after third or fourth injections. Monomeric gp120 elicits antibody and CD4+ T cell responses, which are very type specific failing to neutralize commonly transmitted primary R5 HIV-1 isolates (26).

- **SECOND GENERATION APPROACHES**- Recombinant pox virus vectors

Pox virus vectors were used to express *gag* which induced the production of pseudo virus from infected cells (27). Canary pox was the recombinant pox virus vector evaluated in the clinical trials in mid 1990s.Canary pox is grown and manufactured in chicken embryo fibroblasts (CEF) but is replication incompetent in mammalian cells. Hence, it is used to deliver its recombinant gene to the cytoplasm, providing the machinery necessary for gene expression but unable to propagate itself to cause primary disease in the host cell. (28-29). The construct included a series of products that expressed gp160 (vCP125) gp120,gp41(transmembrane), *gag*, proteases(vCP205),vCP205 plus selected epitopes from *nef* and *pol* (vCP300),vCP300 plus E3L and K3L genes from Vaccinia that inhibit dsRNA and interferon inducible protein kinase and that inhibited the apoptosis of the infected cell(vCP1452). The data from above studies revealed that canary pox vectors are well tolerated at doses above 10^7pfu (plaque forming unit). The HIV specific antibody response after recombinant canary pox immunization alone is weak, but subsequent boosting with recombinant envelope subunit protein induces HIV specific antibody titers of the same or higher magnitudes (32)**.**

- **THIRD GENERATION APPROACHES**- More potent gene delivery vehicles along with DNA vaccination.

These studies utilized either recombinant modified Vaccinia Ankara (rMVA) or replication incompetent recombinant adenovirus (rAd) alone or in combination with DNA immunization. The combination of DNA priming followed by MVA or rAd boosting is a potent vaccine regimen for inducing high levels of HIV specific CD8+ CTL responses (30-31). The “prime boost” approach involves the administration of the same antigen in two different vectors given consecutively. Exposure to the antigen in the first vector “primes” the immune response; re-exposure to the same antigen in the second vector “boosts” the response .This approach can provide HIV proteins in non HIV- viral vectors to induce a cytotoxic cellular immune response; thus avoiding attenuation of boost effect from sequential use of the same viral vector. It is also used to augment the response to potentially convenient vaccines such as DNA plasmid products which themselves are not satisfactorily immunogenic. This approach stimulates various immune responses resulting in an

effectual combination of cellular and antibody responses elicited by a single vaccine[33]. The rAd vectors are produced by inserting the recombinant genes of interest in the E1 gene cassette of the adenovirus genome and by producing the rAd particles in a cell line that constitutively expressed complementary E1 genes (32).

Table 2. Ongoing Trials: Phase II

Protocol Number	Status as of February 2007	Class	Producer	Product	Adjuvant
HVTN 502/Merck 023 (Step) (n=3000)	Enrolling at clade B sites	Nonreplicating adenoviral vectors (clade B Gag-Pol-Nef)	Merck	MRKAd5 trivalent	
HVTN 204 (n=480)	Enrolling	DNA plasmids (clade B Gag, Pol, Nef; clade A,B,C Env)	NIH VRC	VRC-HIVDNA-016	
HVTN 503 (n=3000) Prime	Enrolling	Nonreplicating adenoviral vectors (clade B Gag-Pol-Nef)	Merck	MRKAd5 trivalent	
HVTN 503 (n=3000) Boost	Enrolling	Nonreplicating adenoviral vectors (clade B Gag-Pol; clade A,B,C Env)	NIH VRC	VRC-ADV-014	

Table 3. Ongoing Trials: Phase I

Protocol Number	Status as of February 2007	Class	Producer	Product	Adjuvant
HVTN 042/ANRS VAC19 (n=174) prime	Closed to accrual	Canarypox vector (clade B Env, Gag, Pro, RT, Nef)	Sanofi Pasteur	ALVAC vCP1452	
HVTN 042/ANRS VAC19 (n=174) boost	Closed to accrual	Lipopeptides (poly-epitopic: clade B Gag, Pol, New)	Sanofi Pasteur/ ANRS	LIPO-5	
HVTN 049 (n= 96) prime	Closed to accrual	DNA plasmids (clade B Gag, Env)	Chiron	Gag and Env DNA/PLG microparticles	PLG

HVTN 049 (n=96) boost	Closed to accrual	Protein subunit (clade B Env)	Chiron	Oligomeric, V2-deleted HIV gp140 SF-162	
HVTN 050/Merck 018 (n=435)	Closed to accrual	Nonreplicating adenoviral vector (clade B Gag)	Merck	MRKAd5 HIV-1 Gag	
HVTN 063 (n=156) prime	Enrolling	DNA plasmids (clade B Gag)	Wyeth	GENEVAX gag-2962	DNA plasmid cytokine (IL-15) + bupivacaine
HVTN 063 (n=156) boost	Enrolling	Peptides (polyepitopic: clade B Env, Gag, Nef)	Wyeth	Wyeth multiepitope CTL peptide vaccine	RC529-SE, GM-CSF

Table 4. Planned Trials: Phase I

Protocol Number	Anticipated Start Date	Class	Producer	Product	Adjuvant
HVTN 066 (n=132)	2008	VEE vectors (clade C Env, Gag-Nef-Pol)	AlphaVax	AVX-201	
HVTN 067 (n=108)	Q2 2007	DNA plasmid (polyepitopic: Gag, Pol, Vpr, Nef, Rev, Env)	Pharmexa-Epimmune	EP HIV-1233	
HVTN 071 (n=60	Q2 2007	Nonreplicating adenoviral vectors (clade type 5 B Gag, Pol, Nef)	Merck	MRKAd5	
HVTN 072 (n=204)	Q3 2007	DNA plasmids (clade A Env)	NIH VRC	VRC-HIVDNA-044	

Table 5. Completed Trials

Protocol Number	Start Date	Class	Producer	Product	Adjuvant
HVTN 041 (n=84)	2003	Protein (clade B Nef-Tat fusion protein + clade B Env subunit)	GlaxoSmithKline	NefTat + gp120W61D	AS02A
HVTN 040 (n=48)	2005	VEE vector (clade C Gag)	AlphaVax	AVX-101	

HVTN 048 (n=42)	2005	DNA plasmid (poly-epitopic: Gag, Pol, Vpr, Nef, Rev, and Env)	Pharmexa-Epimmune	EP HIV-1090	
HVTN 056 (n=96)	2006	Peptides (poly-epitopic: clade B Env, Gag, Nef)	Wyeth	Wyeth multiepitope CTL peptide vaccine	RC-529-SE, GM-CSF
HVTN 059 (n=96)	2006	VEE vector (clade C Gag)	AlphaVax	AVX-101	DNA plasmid cytokine (IL2-Ig)
HVTN 044 (n=70)	2006	DNA plasmids (clade B Gag-Pol-Nef; clade A,B,C Env)	NIH VRC	VRC-HIVDNA-009	

FUTURE CONCEPTS IN THE PIPELINE

- **DNA VACCINE STRATEGY**

In addition to the use of DNA for priming individuals before boosting with recombinant virus vectors, DNA plasmids encoding HIV derived genes are used as primer for subsequent envelope subunit protein administration and are given along with plasmids encoding the IL-2-Ig fusion proteins as adjuvant. The antigen is presented by both MHC class I and class II molecules, stimulating various immune responses. They induce cytotoxic T-cell responses which are of relatively low magnitude at least in absence of adjuvants. In monkey studies boosting with viral vectors expressing the same SIV antigens encoded in a DNA priming sequence showed an increased magnitude of CD8 responses (33-34).

SYNTHETIC PEPTIDES

Peptides are also used as immunogens because they are safe, prepared in large quantities, relatively inexpensive, free of contaminating host materials, induction of specific immune response and multiple epitopes with required immunogenic properties formulated solely. The disadvantage of this approach is the narrow size of the antigenic sites and low immunogenicity of peptides as that of complex antigens (32). The various approaches utilized to produce peptide immunogens as candidate AIDS vaccines are:

- Peptides based on the V3 loop sequence from one or multiple strains and covalently linked to an oligolysine backbone formulated with alum, which was not immunogenic in humans (35).

- To synthesize a hybrid linear peptide consisting of helper T-cell, B-cell, CTL epitopes formulated in incomplete Freund's adjuvant and was found immunogenic but caused sterile abscess in some subjects, hence the trial was stopped immediately (36).
- Peptides based on the highly conserved caveolin-I binding domains of HIV-1 gp41 in vaccine candidates. But the antibodies against the caveolin-1 binding domain are rare in

many HIV infected individuals, due to the presence of the hyper variable immunodominant epitopes (37).

VECTOR VACCINE STRATEGY

1. LIVE RECOMBINANT VIRUS VECTORS

Adenovirus vectors are used mostly because of the feasibility of oral delivery with gut replication, induction of systemic and mucosal immunity after a single oral dose and can be used safely for the prevention of respiratory diseases. Other viral vectors which are used are polio, influenza, rhinovirus and hepatitis B chimeras (38).

The advantages of using viral vectors lies in the fact that immunogens can be presented in the course of natural infection, antigens processed and presented in the form of MHC class-I molecules leading to CTL responses, multiple antigens may be simultaneously expressed, administration routes are simple and production and delivery inexpensive. The major limitations of these viruses are the amount of foreign genome that can be accommodated and the virulence properties of the parent vector (32).

2. LIVE RECOMBINANT BACTERIAL VECTORS

Attenuated *Salmonella* has been used as a vaccine vehicle for delivering recombinant HIV antigens or recombinant plasmid DNA. *Listeria monocytogenes* is another potential vector. They possess the similar advantages as that of a live virus vector but the post translational processing of the viral protein differs from that of mammalian processes and the induction of MHC class I restricted CTL responses are less efficient (39).

3. REPLICATION INCOMPETENT VIRUS BASED VECTORS.

The advantages of these vectors lie in their unique abilities to target specialized antigen presenting cells. A vaccine expressing gag from HIV-1 clade C isolate in *Venezuelan equine encephalitis* (VEE) virus is prepared and sent for clinical testing (40). Adeno-associated virus and replication defective *Herpes simplex* virus are also the favored approaches (41).

4. REPLICATION DEFECTIVE FUNGI VECTORS

Heat killed recombinant *Saccharomyces cerevisiae* represents a novel vaccine strategy for the delivery of the HIV vaccine antigens. Whole recombinant *Saccharomyces cerevisiae* yeast expresses HIV-1 antigens and potently induces antigen-specific, CTL responses, in vaccinated animals. Interactions between yeast and Dendritic cells(DC) led to DC maturation, IL-12 production and the efficient priming of MHC class I- and class II-restricted, antigen-specific T-cell responses. Yeast exerted a strong adjuvant effect, augmenting DC presentation of exogenous whole-protein antigen to MHC class I- and class II-restricted T cells (42).

NEW SUBUNIT PROTEIN VACCINE STRATEGY

The product from Chiron Corporation derived from primary HIV isolate SF162 is based on V2 deleted gp140 envelope construct and ready for clinical trials. There are also other groups pursuing methods to present a “receptor-triggered” envelope conformation to the immune system. Recently, a construct containing CD4-gp120-*env* single chain fusion protein has shown to elicit neutralizing

antibody response to some extent in mice and Rhesus macaques (43). Another vaccine approach involved the use of tat protein as an immunogen.Glaxo-Smithkline has developed a nef-tat fusion protein in combination with a monomeric rgp120 protein, evaluated in clinical trials (44).

MONOCLONAL ANTIBODY APPROACH

Antibodies that bind to epitopes overlapping the CD4-binding site (CD4bs) on gp120 can prevent HIV entry by competing with cell-associated CD4; their ability to outcompete CD4 is a major determinant of their neutralizing potency and is proportional to their avidity. Because CD4bs is highly conserved, it is reasonable to hypothesize that antibodies closely mimicking CD4 could exhibit relatively broad cross-reactivity and a high probability of preventing the emergence of resistant viruses. This approach was taken up by structurally imitating the CD4 molecule by a cross reactive HIV-1 neutralizing antibody with complementarity determining regions (CDRs) i.e.CDR-H2 and H3 possessing unique motifs. Prabhakaran *et al* carried out a study on the crystal structure of Fab m18 at 2.03A resolution, revealing the unique conformation of heavy chain CDR2 and 3 (H2&H3).It shows striking similarity to the Ig CDR2 like C'C'' region of the CD4 first domain D1, involved with the binding of CD4 to gp120.Docking simulations suggested significant similarity between the m18 epitopes and the CD4 binding site (CD4bs) on gp120.Thus, vaccine immunogens can be based on the m18 epitope structure (45).

CONCLUSION

Despite limited progress toward the goal of developing a safe and effective AIDS vaccine, several encouraging developments have taken place over the past several years. Although there is still considerable debate about the mechanisms of protective immunity, there is now an emerging consensus that a successful AIDS vaccine should be able to induce both CTL and antibody responses able to neutralize primary viral isolates. The National Institutes of Health have recently embarked on an expanded effort to accelerate AIDS vaccine development, which has emphasized development of novel vaccine approaches. Despite these encouraging advances, considerable challenges remain, and substantial progression must still be made to develop a safe and effective AIDS vaccine.

REFERENCES:

1. Global summary of the AIDS epidemic. [online]. 2006 [cited 2007 May27]. Available fromURL:http://data.unaids.org/pub/EpiReport/2006/02Global_Summary_2006_EpiUpdate_eng.pdf
2. Katzung B.G.Basic and clinical Pharmacology. 9th ed. Boston: McGraw Hill Publishers; 2007.
3. Meyer RA. Encyclopedia of Molecular Biology and Molecular Medicine. 2nd ed. Vol. 1.VCH Weinheim; 1996.
4. Murphy BR, Chanock RM. Immunization against virus disease. Fields Virology. 3rd ed. Philadelphia: Lippincott-Raven Publishers; 1996.
5. Cao Y, Qin L, Zhang L, et al. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. N. Engl. J. Med. 1995; 332:201-208.
6. Pantaleo G, Menzo S, Vaccarezza M, et al. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. N. Engl. J. Med.1995; 332:209-216.
7. Flint SJ, Enquist LW, Racaniello UR, Skalka A.M, Principles of Virology: Molecular biology, pathogenesis, and control of animal viruses.2nd ed. Washington: American Society for Microbiology; 2004.
8. Prescott Lansing M., Harley John P., Klein Donald A.6th ed. Boston: McGraw Hill Publishing House; 2005.

9. The Science of HIV Vaccine Development [online]. 2007[cited 2007 May 26, 2007]. Available from: URL http://hivinsite.ucsf.edu/InSite?page=kb-02-01-06#S1X, 27.
10. Goldsby RA., Kindt TJ, Osborne BA, Kuby J. Immunology. 5th ed. NewYork: Freewell publishers; 2003.
11. Brooks GF, Butel JS, Stephen AM: Jawetz, Melnick, and Adelberg's Medical Microbiology. 23rd edition. New York: Mc Graw Hill Publishers, 2004.
12. Cohen PT, Sande M A, Volberding PA.The AIDS Knowledge Base: A textbook on HIV disease from the University of California, SanFrancisco and San Francisco General Hospital. 2nd ed. Boston: Little Brown and Company; 1994.
13. Devita VT., Jr. Rosenburg SA., Helman S. AIDS Etiology, Diagnosis, Treatment and Prevention. 4th ed. Philadelphia: Lipincott-Raven Publishers; 1997.
14. Johnson WE, Desrosiers RC. Viral persistence: HIV's strategies of immune system evasion.Annu. Rev. Med. 2002; 53:499-518.
15. Blankson JN, Persaud D, Siliciano RF. The challenge of viral reservoirs in HIV-1 infection. Annu. Rev. Med. 2002; 53: 557-593.
16. Douek DC, et al. HIV preferentially infects HIV- specific CD4+ T-cells. Nature 2002; 417: 95-98.
17. Pier GB, Lyczak J B, Wetzler LM, Immunology, infection and Immunity.2nd ed. NewYork: American Society for Microbiology; 2004.
18. Zhang W, Canziani G, Plugariu C, et al. Conformational changes of gp120 in epitopes near the CCR5 binding site are induced by CD4 and a CD4 miniprotein mimetic. Biochem. J. 1999; 38(29): 9405-9416.
19. Sapphire A.C.S, Bobardt M.D, Gallay P.A. Host Cyclophilin-A mediates HIV-1 attachment to target cells via Heparans. EMBO Journal 1999; 18(23): 6771-6785.
20. Zander K, Sherman MP, Tessmer U, et al. CyclophilinA interacts with HIV-1 *vpr* and is required for its functional expression, J. Biol. Chem. 2003; 278(44): 43202-43213.
21. Wyatt R, Kwong PD, Desjardins E, Sweet RW, Robinson J, Hendrickson WA, Sodroski JG, The antigenic structure of the HIV gp120 envelope glycoprotein, Nature 1998;393(6686):705-11.
22. Kock M, Pancera M , Kwong PD, et al. Structure based targeted deglycosylation of HIV-1 gp120 and effects on neutralization sensitivity and antibody recognition. J. Virol. 2003; 313(2): 387-400.
23. Mascola JR, Stiegler G, VanCott TC. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat. Med. 2000; 6: 207-210.
24. Graham BS, Keefer MC, McElrath MJ, Gorse GJ. Safety and immunogenicity of a candidate HIV-1 vaccine in healthy adults: recombinant glycoprotein (rgp) 120- A randomized double blind trial. Ann. Intern. Med. 1996; 125: 270-279.
25. Belshe RB, Graham BS, Keefer MC et al.Neutralizing antibodies to HIV-1 in seronegative volunteers immunized with recombinant gp120 from the MN strain of HIV-1, NIAID AIDS Vaccine Trial Network. JAMA 1999; 272: 475-480.
26. Berman PW, Huang W, Riddle L, Gray AM, Wrin T. Development of bivalent (B/E) vaccines able to neutralize CCR5 dependent viruses from United States and Thailand. J. Virol. 1999; 265: 1-9.
27. Spearman P, Wang JJ, Heyden NV, Ratner L. Identification of Human immunodeficiency virus type-1 Gag protein domains essential to membrane binding and particle assembly. J. Virol. 1994; 68: 3232-3242.
28. Fang ZY, Fang ZY, Limbach K, Tartaglia J, Hammonds J, Chen X, Spearman P. Expression of vaccinia E3L and K3L genes by a novel recombinant canarypox HIV vaccine vector enhances HIV-1 pseudovirion production and inhibits apoptosis in human cells. J. Virol. 2001; 291: 272-284.
29. Belshe RB, Sheppard H, Stablein D, Self S, McNamara J, Frey S. Safety and immunogenecity of a canarypox vectored human immunodeficiency virus type I vaccine with or without gp120: a phase 2 study in higher and lower risk volunteers. J. Infect. Dis. 2001; 183: 1343-1352.
30. Amara RR, Francois V, John D et al. Control of a mucosal challenge and prevention of AIDS by a multi protein DNA /MVA vaccine. Science 2001; 292: 69-74.
31. Shiver JW, Lifson JD, Martin MA et al. Replication-incompetent adenoviral vaccine elicits effective anti immunodeficiency virus immunity. Nature 2002; 415: 331-335.
32. Levine Myron M, Kaper James B, Rappuoli Rino, Liu Margaret A, Good Michael F. New Generation Vaccines. 3rd ed. New York: Marcel Decker; 2004.

33. Ertl HCJ. DNA Vaccines. Medical intelligence unit 35. New York: Kluwer academic/ Plenum publishers; 2003.
34. O'Hagan D, Singh M, Ugozzoli M et al. Induction of potent immune responses by cationic microparticles with adsorbed human immunodeficiency virus DNA vaccines. J. Virol. 2001; 75: 9037-9043.
35. Lambert JS, Keefer M, Mulligan MJ, et al. A phase I safety and immunogenecity trial of UBI microparticulate monovalent HIV-1 MN oral peptide immunogen with parenteral boost in HIV-1 seronegative human subjects. Vaccine 2001; 19: 3033-3042.
36. Gahery-Segard H, Pialoux G, Charmeteau B. Multiepitopic T and B cell responses induced in humans by a human immunodeficiency virus type-1 lipopeptide vaccine. J. Virol. 2000; 74: 1694-1703.
37. Hovanessian AG J, Briand E, Said, J et al. The caveolin-1 binding domain of HIV-1 glycoprotein gp41 is an efficient B cell epitope vaccine candidate against virus infection. Immunity 2004; 21: 617-627.
38. Crotty S, Christopher J M, Barbara L. Protection against Simian immunodeficiency virus vaginal challenge by using Sabin Poliovirus vectors. J. Virol 2001; 75: 7435-7452.
39. Lieberman J, Frankel FR, et al. Engineered *Listeria monocytogenes* as an AIDS vaccine. Vaccine 2002; 20: 2007-2010.
40. Caley IJ, Betts MR, Irlbeck DM, Davis NL, Swanstrom R, Frelinger JA, Johnston RE. Humoral, mucosal and cellular immunity in response to a human immunodeficiency virus type 1 immunogen expressed by a Venezuelan equine encephalitis virus vaccine vector. J. Virol. 1997; 71: 3031-3038.
41. Murphy CG, William TL, Robert EM, et al. Vaccine protection against simian immunodeficiency virus by recombinant strains of Herpes simplex virus. J. Virol. 2000; 74: 7745-7754.
42. Stubbs AC, Martin KS, Coeshott C et al. Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell mediated immunity. Nat. Med. 2001; 7: 625-629.
43. Schulke N, et al. Oligomeric and conformational properties of a proteolytically mature, disulfide stabilized human immunodeficiency virus type1 gp140 envelope glycoprotein. J. Virol. 2002; 76: 7760-7776.
44. Fanales-Belasio E, Cafaro A, Cara A et al. HIV-1 Tat based vaccines: from basic science to clinical trials. DNA Cell. Bio. 2002; 21:599-610.
45. Prabhakaran P, Gan J, Wu YQ, Zhang MY, Dimitrov DS, JI X. Structural mimicry of CD4 by a cross reactive HIV-1 neutralizing antibody with CDR- H2 and H3 containing unique motifs. J. Mol. Bio. 2006; 357(1): 82-99.
46. HVTN vaccine in development [online]. 2007[cited 2007 May 26]. Available from: URL: http://chi.ucsf.edu/vaccine/vaccines?page=vc-03-00.