Anti-HIV therapy: pipeline approaches and future directions

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Abstract

Human immunodeficiency virus (HIV), with about 30 million deaths and double infections (in developing countries), is an open challenge today for global scientists. Developing safe and effective measurements against it has become the prime need of hour. Though, putting it at health priority, various efforts like chemotherapy, vaccines and others are attempted globally over last decade. Consequently, highly active antiretroviral therapy was introduced but fails to completely block the viral replication due to drug resistance and various other severe side effects. The antigenic variability and lack of appropriate experimental models is the major obstacle in the development of an ever effective treatment against HIV. However, to overcome the present hurdles and to emerge a preventive HIV vaccine efforts at various platforms are done. A renewed, coordinated research, preclinical studies, clinical trials together with sufficient long term scientific and commercial commitments are made. Few of the therapeutic efforts viz. RNA interference (RNAi) based replication arrest of HIV, viral enzymes' inhibitors, nanotechnology based HIV control and various preclinically trialed vaccines are reviewed in this paper. Also, the observed toxicity of existing therapeutic regimen, key challenges and future prospects for the development of better tolerated prophylactic HIV-1 vaccine are discussed.

Key words: HAART, HIV-1 vaccine, RNAi, HIV enzyme inhibitors.

1. Introduction

HIV is a silent killer of human beings. People with contagion of HIV may have no symptoms for about 10 years, but tend to surpass the infectivity to others (Piot et al., 2007; Del Rio et al., 2009; Sterling et al., 2009). Since, it lysogenically invades and hides itself into the host cell's genome therefore it may remains asymptomatic for long time. Nevertheless, by integrating and mutating the genome further it disables the body's defense system through infecting the T_H, Macrophages and Dendritic cells (Cunningham et al. 2010). Structurally the HIV is an enveloped RNA virus containing 72 little projections of glycoprotein 120 and 41 on cell surface. Also, protein *p17* lies beneath the matrix and bullet shaped viral core made from protein *p24* is found around the genetic material (Nielsen et al., 2005).

The cellular receptors CD4 and chemokine receptors CCR5 or CXCR4 expressed on Macrophages and TH cells' surface are targeted by HIV-1 to bind with gp120 followed by conformational change in gp41 for fusion of virus-cell membrane to host cell and entry of nucleocapsid into cytoplasm **(**Eckert et al., 2001; Platt et al., 2005; Ray et al., 2006**).** Then virally encoded enzyme Reverse Transcriptase (RT) converts the viral RNA genome into proviral DNA **(**Coffin et al., 1997**),** which on entering into nucleus gets covalently integrated with the genome of host cell by the activity of another virally encoded enzyme Integrase (IN) **(**Mitchell et al.**,** 2004; Scherdin et al, 1990; Schroder et al, 2002**).** Now it serves as template for viral transcription **(**Coffin et al, 1997). Consequently, the expressed core proteins processed through viral proteases into morphologically changed structure **(**Coffin et al. 1997; Zhu et al, 1993**)** to produce new viral progeny and augmenting the infection.

The latent ability of HIV to hide itself into host cell and to bring variations in its genetic composition has made it difficult for human defense system to find and attack on it (Chun et al. 1999). Hence, the type of treatment imperatively needed is, which could only kill the virus without affecting host cells. Though, involving HAART and some other antiviral substances viz. interferon have been effective in preventing virus from budding. Moreover, scientists are exploring the preventive role of viral enzymes, protein molecules and RNA interference as well. Above all, development of simplest, safest, and more effective vaccine which could interfere the viral interactions with host body in any way is also attempted.

2. Targeting the HIV and host cell interaction

Since, it is tough to get rid off from virus once entered in host body therefore blockade of entry of HIV while interacting with its surface glycoprotein to CCR5 and CXCR4 chemo receptors of CD4+ and Macrophage cells, are endeavored. Consequently, an antagonist TAK-779 specific to CCR5 was introduced to compete with gp 120 during HIV infection (Dragic et al. 2000). Moreover, some other inhibitors like anti-CD4-IgG_2, PRO 140 and T-20, inhibiting to HIV-1 attachment, CCR5 usage and fusion respectively, have undergone for testing with diverse primary cells types that represent the major targets for both infection in vivo and for the inhibition of trans infection of target cells by virus bound to dendritic cells. (Ketas et al. 2003). A small compound *BMS-378806* had been discovered that bind with gp120 of HIV-1 and block its entry into host cells by inhibiting interactions between gp120 and CD4+ receptor molecule (Lin et al. 2003). This can be orally administered in combination with other drugs to attain utmost anticipation of HIV-1 infection (Kadow et al. 2006). Besides this, a tetravalent CD4-immunoglobulin protein, PRO 542 (CD4-immunoglobulin G2) has also been developed which similarly bind to gp120 and blocks the attachment and entry of virus into CD4+ cells (Jacobson et al. 2004). It has demonstrated good antiviral activity by reducing viral RNA levels without any significant toxicity in tested candidates. Interestingly, its greater antiviral effects were observed among advanced HIV-1 patients. (Jacobson et al., 2004). Meanwhile some other gp 120 inhibitors potent to prevent its conformational changes during infection were also developed.

Advancements of entry inhibitors is the latest trend in the progression of antiretroviral compounds, a fusion inhibitor *Efuvirtide* is the first to get approval in this regard (Briz et al. 2006), and several other are near to clinical approach and hopefully soon be a part of therapeutic treatment.

3. Impeding the HIV replication by viral enzyme inhibition

Current HIV-1 treatments given are the combination of drugs targeting to Reverse Transcriptase (RT), Protease (PR) and Integrase (IN). At present, 22 compounds have been formally approved by the US Food and Drug Administration for the treatment of HIV

infections (De Clercq, 2009). Since, RT, PR and IN are essential enzymes for viral replication hence their arrested expression is thought as better alternative way to stop viral growth in host cell (Andréola et al. 2002). The combination of these enzyme inhibitors is found capable to reduce viral load for extended duration and slowing the disease progression as well (Reeves et al. 2005).

In addition to above, following other events of HIV replication cycle are also considered as potential targets for chemotherapeutic interventions (De Clercq 2002).

(i) ***Viral adsorption***; through binding to the viral envelope glycoprotein gp120 (polysulfates, polysulfonates, polycarboxylates, polyoxometalates, polynucleotides, and negatively charged albumins)

(ii) ***Viral entry***; through blockade of viral co-receptors CXCR4 (i.e., bicyclam (AMD3100) derivatives) and CCR5 (i.e., TAK-779 derivatives)

(iii) ***Virus–cell fusion***; through binding to the viral envelope glycoprotein gp41 (T-20, T-1249)

(iv) ***Viral assembly and disassembly***; through NCp7 zinc finger-targeted agents (2,2′-dithiobisbenzamides (DIBAs), azadicarbonamide (ADA))

(v) ***Proviral DNA integration***; through integrase inhibitors such as 4-aryl-2,4-dioxobutanoic acid derivatives

(vi) ***Viral mRNA transcription***; through transcription inhibitors (flavopiridol, fluoroquinolones**)**.

However, regular doses of these drugs often cause mild side effects and sometimes even serious also which can impact on health or quality of life. The abuses of them vary from appetite loss, fatigue, insomnia, diabetes, nephrotoxicity, lactic acidosis, hepatotoxicity, pancreatitis, hypercholesterolemia, diarrhea, peripheral neuropathy (nerve damage), lipodystrophy to effect on central nervous system (Ian, 2005).

4. Inhibition of HIV infection by RNA interference

RNAi, with its interfering ability is become a hot point for current research approaches. It was first observed in lower organisms such as plants, C. elegans, Drosophila and Protozoans,

in which double stranded RNA (dsRNA) are digested into short double-stranded interfering RNA (siRNA) which then combine with endonucleases and form complexes to target homologous mRNA and degrade them (Bennasser, 2005).

In order to use RNAi as therapeutic tool against HIV-1 work is done by Jacque et al. 2002 and reported that duplexes of 21-23bp in length of plasmid-derived siRNA could target HIV-1 genome so that HIV-1 replication in early and late stages can be inhibited and viral cDNA intermediates cannot be formed. Therefore, RNAi is thought a better tool to use against HIV multiplication in infected cells especially when its genome is also the RNA (Rossi et al. 2002).

5. Active immunization by therapeutic vaccines

The concept of therapeutic immunization against HIV-1, as an alternative to HAART, was brought into focus by the polio vaccine pioneer Jonas Salk in 1987. Since, it allow less medication and scheduled holiday treatment therefore scientific workforce of nearly entire world involved to crack the mystery and to develop an ever effective and safer vaccine against HIV-1. Million of science brains employed to understand the various mechanism viz. cellular and molecular regulation of viral gene expression, viral entry into host cell, HIV replication, development of improved HIV envelope immunogens, assembly of viruses, and others for developing the HIV-vaccine. As a result, several attempts were executed as vaccines and clinically trialed but only few of them got bit success, are reviewed below. Furthermore the unique qualities needed to be an ideal HIV vaccine and major obstacles in its development are also discussed.

5.1 Vaccines

5.1.1 Whole Inactivated HIV Vaccines

Remune, a purified and inactivated HIV-1 particle is used as vaccine and found able to give HIV-specific cell-mediated immune responses in 48% of AIDS patients (Egan, 2005). The greater effect of it was shown in the people who have undergone clinical treatment over the period of 6 years for strengthening the immune responses (Egan, 2005). However, conclusion

regarding Remune treatment efficacy could not be made because of the lack of control of patients who did not received vaccine trials. But it had proved the immune potency in adults who were given Remune alone, or in combination with antiretroviral therapy (Egan, 2005).

5.1.2 *Virus-like Particle Vaccines*

According to a study done by Lindenburg et al., in 2002, some HIV structural proteins like Gag were reported capable of assembling themselves into virus-like particles, when expressed alone and elicit HIV-specific immune responses in animals without HIV-1. However, as a therapeutic vaccine they failed to reveal clinical benefits.

5.1.3 Recombinant Protein Vaccines

In year 1991, augmentation of active immunization against HIV-1 with molecularly engineered, or recombinant, viral proteins was also attempted. In this study, a vaccine *(VaxSyn)* comprised of recombinant HIV-1 gp160 was tested on untreated 30 individuals infected with HIV-1. Encouragingly, 63% individuals, received 6 doses of vaccine, were developed immunity in its response at initial level of trial (Tsoukas et al., 1998). However, larger efficacy trial on 278 healthy, untreated HIV-1 infected individuals did not provide any clinical benefit. Also, any alteration in $CD4^+$ cell loss, HIV-1 viral load, progression to AIDS-defining illness, time to initiation of HAART, or time to death was not observed (Tsoukas et al., 1998). Conclusively, *VaxSyn gp160* was found lesses effective than antiretroviral monotherapy on clinical markers of disease progression (Tsoukas et al., 1998).

5.1.4 Peptide Vaccines

A number of synthetic viral peptides (fragments of entire viral proteins) have also been tested as potential therapeutic vaccines against HIV-1. In these approaches, specific viral sequences such as NK receptors' complementary region and area responsible to generate HIV neutralizing antibody are often targeted to generate immune response. One such HIV envelope-derived peptide vaccine C4-V3 consisting of four synthetic peptides has been tested for its efficacy. Each unit of vaccine contains viral regions known to stimulate cellular immune responses and to generate HIV-neutralizing antibody responses as well (Egan,

2005). In trial observations, after five injections of vaccine, about 50% recipient populations were found containing vaccine-specific immune responses and increased ability to neutralize laboratory-grown HIV-1 as well (Bartlett et al., 1998). It appeared to be safe and immunopotent. Clinical measures of HIV infection did not change in patients who received the peptide vaccine relative to individuals vaccinated with the placebo (Bartlett et al., 1998). Other similar vaccines like Vacc-4x, developed by Norwegian biotechnology company *Bionor Immuno*, is currently being tested in an ongoing clinical trial in Norway.

5.1.5 *Vaccines Delivered through Viral Vectors*

Currently, live viral vectors based vaccines (gene carriers) are efficiently being used to deliver enclosed HIV genes to host cells where they mimic like natural HIV infection and behave like potential prophylactic HIV-1 vaccines. To deliver vaccines using Canary pox virus (ALVAC), Adenovirus type 5 (Ad5), Adeno-Associated Virus (AAV), Venezuelan Equine Encephalitis Virus (VEE), Vesicular Stomatitis Virus (VSV) and Modified Vaccinia Virus Ankara (MVA) as vector are under evaluation (Egan, 2005).

5.1.6 *Plasmid DNA Vaccine*

In 1990, after demonstration of plasmid DNA expression in mouse muscle cells and eliciting vaccine-specific immune responses, many DNA vaccines against viral, bacterial, parasites infections were evaluated in animals and human trials and found to be well-tolerated. Considering these pioneering studies, the first human trial of a DNA-based vaccine carrying HIV-1 *env* and *rev* genes for the treatment of HIV-1 infection was attempted in 1998 at University of Pennsylvania (MacGregor et al., 1998) to evaluate the safety and immunopotency of a plasmid DNA vaccine. Though, vaccine was safe and well-tolerated but only marginal increase in anti-env immune responses was elicited with no measurable beneficial effect on clinical measures of HIV infection.

5.1.7 Antigen-pulsed Dendritic Cells

Dendritic cells (DCs), being key inducer of cellular immune response, are often used to load protein or peptide to which an immune response is desired, and infused back into the patient.

After finding it quite successful against cancer, its ability to augment HIV-specific immune responses is also been tested. In a pilot study, nine infusions of HIV-1 protein/peptide pulsed DCs in HIV-1-infected individuals were observed safe, well tolerated and immunopotent (Kundu, 1998). However, larger placebo-controlled trials are required to determine if the use of antigen-loaded DCs can produce a measurable clinical benefit.

5.1.8 *CTL-based Vaccines*

In these approaches HIV-specific CD4+ and CD8+ T cells are stimulated with prepared gene-based immunogens that are modified to improve protein expression and immunogenicity to arrest the HIV infection (Nabel et al., 1987). This is done through insertion of HIV cDNAs into relevant plasmids possessing replication-defective forms of adenoviruses and poxviruses. Therefore, provide great flexibility in identification of immunogens that can induce broad and potent CTL immune responses. Moreover, several mutants of gp160 and fusion intermediates' analogues are also developed which can illicit CTL responses and enhanced antibody responses as well (Nabel et al., 1987)

5.2 ***What can be an ideal HIV vaccine?***

An agent potent to ultimately grant the sterilizing immunity by eliciting the production of broadly cross-reactive neutralizing Abs, block viral entry to host cell and target viral replication, can be an ideal prophylactic or therapeutic HIV vaccine. But weighty stones like poor accessibility of conserved receptor-binding sites, extensive glycosylation and antigenic variation of gp120 (Preston, 1997) are blocking the path to generate specific Abs eliciting vaccines. Hence, unable to target unique complex epitopes and trimeric fusion intermediates for efficient inhibition of virus binding and entry into host cell.

5.3 ***Major roadblocks in HIV vaccine development***

Though plenty of attempts are made for developing safe and effective vaccine, and few are under trials but could have not been proved successful due to complex structure, life cycle and high mutation rate of HIV-1 which facilitate it for drug resistance and immune escape.

So far, 12 known HIV-1 genetic subtypes rapidly diversified to yield intersubtype recombinants are identified existing in humans (Korber et al. 2001; McCutchan, 2000).

Besides them, thermostably concealed conserved receptor- and coreceptor-binding sites on viral envelope (Myszka et al. 2000), hidden glycoprotein (Kwong et al. 1998), conformational flexibility of gp120 (Kwong et al. 2002), high error rate in reverse transcription (Preston, 1997), diminished (Finzi et al. 1998) and variant (Ho et al. 1995) protein expression to get escaped from immune recognition (Evans et al. 1999; Parren et al. 1999) and several yet to discover factors are stretching the goal of HIV vaccine development farther from reach.

6. Nanotechnology based approaches

Recently, after successful implementation of nanotechnology-based drug delivery system against cancer (van Vlerken et al., 2006) the polymer-based nanoparticles, liposomes, nano-emulsions, dendrimers and micellar technologies (Amiji et al. 2006) are being used in AIDS also to overcome several anatomical & physiological barriers and transport therapeutic agents locally at the site of HIV. For example, Saquinavir, a member of protease inhibitor class, potent to inhibit protease-mediated cleavage of *gag* and *pol* polyproteins in HIV genome, is encapsulated efficiently in biodegradable hydrophobic polymeric nanoparticles, made with poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) (Shah et al. 2006) and therefore finally preventing the post-translational processing required for maturation and spreading of virus.

7. Conclusions

Yield of more and more mutated viruses and depletion of host's initial immune response has made the HIV harder nut to crack. A therapeutic vaccine, able to overcome the hide and latency of virus and elicit de novo cellular immunity and virus-neutralizing antibody responses is the need of hour. Though, certain vaccine candidates in recent non-human primate experiments like combination of DNA vaccines with potent immunomodulators and improved viral vectors (such as recombinant vesicular stomatitis virus or adenovirus), multi-

epitope peptide vaccine and some other have shown the promising effects but few more honest approaches involving the recent technologies are urgently needed to win the race.

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