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DNA Vaccine for AIDS a Myth or Reality?

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Abstract: One of the most elusive hurdles of the modern century has been the task of finding a cure for AIDS and although many strategies have been used in this pursuit one of the most common strategies is the development of a vaccine for AIDS. In this paper I have tried to present a comprehensive coverage of the various vaccines that are in development with the in depth focus on the DNA vaccine that is currently in the clinical trial on humans. The review begins with the brief description on what is AIDS and a short introduction about the HIV (Human Immunodeficiency Virus) the causative organism of this illness. The review then goes on to explain the molecular pathogenesis of the HIV and how it infects the Host cell. This is followed by the most common vaccine strategies that are employed and the various barriers that hinder the development of these vaccines with the special emphasis on the DNA vaccines currently in development and the science behind its development. The paper also points out few novel ideas especially the use of Nanobiotechnology in vaccine research for AIDS and how the vaccine specificity and efficacy could be improved. The paper concludes by summarizing the impact of such a vaccine in the society and its potential benefit to the mankind. Thus in the words of Anne, Princess Royal of Great Britain And Northern Ireland "It could be said that the AIDS pandemic is a classic own-goal scored by the human race against itself."

Key words: AIDS · pathogenesis · vaccine · nanotechnology

INTRODUCTION

AIDS (Acquired Immune Deficiency Syndrome) is a group of illness caused by HIV (Human Immunodeficiency Virus), which suppresses the immune system by attacking the CD4 T lymphocytes cells responsible for immunity against infections in the body. As a result of this the immune system is compromised due to which diseases that normally are not lethal (Opportunistic infections) becomes life threatening complications due to the inability of the immune system to ward off against these infections which ultimately results in the death of the individual.

The virus was first isolated in 1983 by three groups of scientists namely Luc Montagnier of Pasteur Institute in Paris, Robert Gallo at the National Cancer Institute in Maryland, United States and Virologist Jay Levy of University of California. Although they worked independently of each other they isolated the virus now referred to as HIV.

In 2005 an estimated 40.3 million people worldwide were living with HIV or AIDS of these 38 million are adults 17.5 million are women and 2.3 million are children. The World Health Organization (WHO) estimates that between 1981, when the first AIDS cases were reported and the end of 2005 (October); 25 million people had died from AIDS or HIV worldwide.

HIV (Human Immunodeficiency Virus):

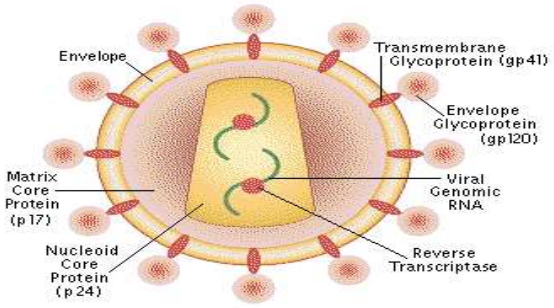

Fig. 1: HIV virus structure; Source: Nature Medicine

HIV is a retrovirus that belongs to the Lentivirus group. Unlike most viruses that infect humans the genetic material present in HIV is RNA. The virus has two copies of the single stranded RNA molecule. HIV uses the reverse transcriptase present in its genome to convert this RNA molecule to a functional DNA molecule with the help of the Host cell machinery, which then integrates with the host genome and begins to replicate along with the host cell DNA. The main target of HIV is CD4 T Lymphocytes (the cells responsible for the host cell immunity). Since HIV kills these immune cells or render them inactive, the host cell loses the ability to mount a significant immune response to the presence of the pathogen.

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The HIV binds to the CD4 molecule present in the immune cells with the help of a surface protein gp120 but it is inferred now that the presence of CD4 molecule alone will not enable the virus to bind to these cells it also needs additional molecules now referred to as chemokine receptors which are protein molecules present in the cell surface of immune cells. The two most common chemokine receptors are CXCR4, which is involved in the entry of the virus in to the host cell and another chemokine receptor used by the virus is CCR5. The scientists now conclude that there are many factors including Genetic, immunologic and environmental factors that play a role in the pathogenesis of the HIV [1, 2].

The different phases of HIV life cycle are

- Attachment
- Fusion
- Reverse Transcription
- Second strand synthesis & RNAse degradation
- Viral integration
- Transcription & translation
- Expression of protein
- Assembly of matured virions [3].

The HIV has three important genes that are responsible for the pathogenesis and are most commonly used in the vaccine design they are-

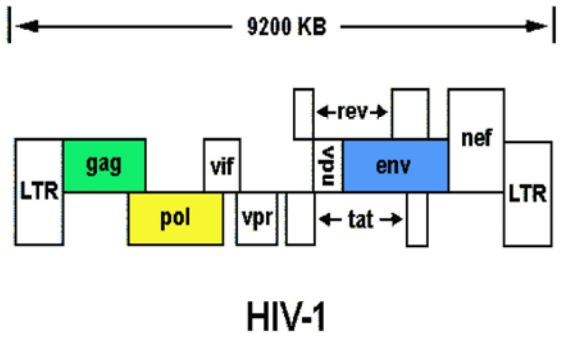

Fig. 2: The genome of the human immunodeficiency virus (type 1); Source: Nature Medicine

Gag: This gene codes for the nucleocapsid protein (24 kd protein) present in the virus

Pol: This gene codes for three enzymes namely

- Reverse Transcriptase (Used to convert single stranded RNA into DNA),
- Integrase, the enzyme responsible for the integration of viral gene into the host genome
- HIV Protease, this enzymes cleaves the newly synthesized protein molecule by the host cell (gp161) into proteins of specific sizes namely gp120 and gp41

Envelope: This gene codes for the envelope region of the virus through which the virus attach itself to the host cell [3].

The other genes, which play a vital role in the pathogenesis, are

Tat (Trans activator of transcription): This gene is responsible for the production of tat protein, which helps, in the active transcription of HIV DNA. It also helps in the evasion of HIV cells from the immune cells and causes the apoptosis (cell death) of uninfected T-cells [3].

Rev (Regulator of virion): This codes for rev protein that helps in the transport of RNA from the nucleus to the cytoplasm, which prevents the cleavage of the viral RNA by the Nucleases in the host cell [3].

Nef (Negative regulatory factor): This codes for nef protein, which helps in the activation of T cells and causes the persistent infection [3].

Vif (viral infectivity factor): This codes for vif, a 23 kd protein responsible for viral replication it has other function that are yet to be understood fully [3].

The other genes present are *vpr (viral protein R)*, *vpu (viral protein U)* and *tev* [3].

Pathogenesis: The infection occurs when the HIV enters the human and reaches the lymphoid tissues. The virus has a surface protein gp120, which binds to the CD4 present in T-lymphocytes and the macrophages (immune cells). Then another protein gp41, which is connected to the gp120, helps in the fusion of the envelope of HIV to the host cell. Then the viral RNA enters the host cell where the enzyme Reverse Transcriptase present in the virus converts the RNA into a single stranded DNA followed by a complementary second strand synthesis. This double stranded DNA is then integrated into the host chromosome by a second enzyme present in the virus namely Integrase. Once the viral DNA is integrated the host cell begins to replicate the viral DNA along with its own DNA. This is then transcribed into an mRNA, which is then translated into a protein molecule (gp160). The gp160 molecules are then cleaved by the enzymes present in the virus to gp120 and gp41 and these along with the genetic material form new virions, which then

take up a part of the cell membrane along with the envelope and these virions then infect other cells.

VACCINE RESEARCH

Vaccine is a compound that is injected into the patient to create a protective immune response so when the disease-causing agent enters the host cell, the host cell can defend itself against the infection effectively due to the immediate immune response.

The main reason behind the development of vaccines for HIV is that the antiretroviral drugs that are currently in the market are expensive also the toxicity of these drugs towards the host cell is very high and more importantly the HIV strains have become resistant to theses drugs rendering them ineffective [4]. There are different types of vaccines that are being developed to combat against AIDS but so far none of them have succeeded in that goal of total control of AIDS. Currently there are many vaccines that are showing a lot of promise some of which include:

- Recombinant protein vaccine
- Live Virus vaccine
- Peptide vaccine
- DNA vaccine
- Virus like particle vaccine (Pseudovirion vaccine)

Recombinant protein vaccine: In this type of vaccine the larger proteins present in the HIV are used as the immunizing agents to elicit an immune response by the host cell. This vaccine makes use of recombinant proteins such as gp120, gp141 and 160.

Live virus vaccine: This vaccine makes use of live virus which are not virulent these viruses are modified to carry some of the HIV genes in their genome and injected into the host cell where these viruses begin to express the HIV proteins which results in a immune response by the host cell. Since these viruses are not virulent they are not able to cause infection to the host cell. But the use, of these vaccines is limited as they are considered to be a potential threat since they are alive. Ex Adenovirus is commonly used as a vector in this type of vaccine development [4].

Peptide vaccine: Peptide vaccine makes use of small surface proteins present in the HIV as the immunizing agent. These peptide molecules mimic the real proteins present in the HIV and induce an immune response in the host cell.

Virus like particle vaccine (Pseudo virion vaccine): In this type of vaccine a virus which looks alike and expresses some of the proteins of HIV is used as a the immunizing agent but this does not cause infection as it does not express all the proteins expressed by HIV [4].

Having implored all these vaccine strategies the most promising strategy currently in Development is the naked DNA vaccine.

DNA Vaccine

DNA vaccine development began in the early 1990 and has been a successful strategy in developing vaccines against most viruses and this is the strategy that is showing more promise in the development of vaccine for HIV.

The principle behind the DNA vaccine is using a naked DNA which contains the coding region of some of the proteins present in the HIV and when injected into the host system this DNA enters the host cell and expresses these proteins which are then presented to the MHC1 and MHCII molecules that helps in inducing an immune response against these proteins so that the memory cells of the immune system recognize these proteins and mount an overwhelming immune response against HIV when it enters the host cell, so that the virus is neutralized immediately even before it reaches the Lymphoid system and establishes itself [4, 5].

The minimum requirements of any DNA vaccines are the following:

- Plasmid DNA backbone
- Inserted Viral Gene
- Ori Region (Origin of replication for the Plasmid to replicate inside the host cell)
- Antibiotic resistance gene

The DNA is administered by injecting the needle either intramuscularly or into the skin. The efficacy of this type of vaccine depends upon the promoter sequence that is used. Usually the promoter region that is commonly employed is derived from Cytomegalo Virus (CMV). Other promoter region used is the promoter from Adenovirus but the efficacy level is low when compared to the promoter from CMV.

The DNA vaccine is designed in such a way that it contains only few genes of the virus and these genes expresses only some of the proteins that are involved in infection and which are not virulent or disease causing. Usually a typical DNA vaccine against HIV contains either individual genes or the combination of genes such as gag, pol, env, tat, rev, nef, but never is a DNA vaccine designed with all the genes, since such a DNA will become a potential vehicle to transmit infection [4, 5].

There are many different DNA vaccines that are currently in trial these vaccines use either gag-pol combination or gag-env

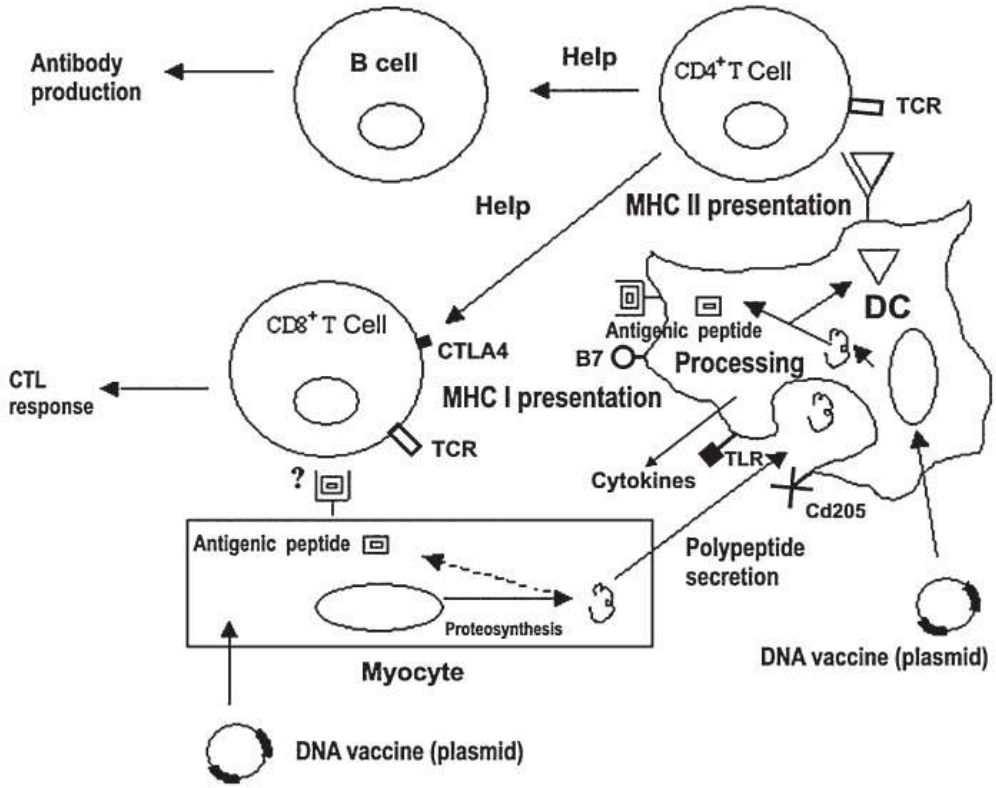

Fig. 3: The possible Pathway Antigen presentation following DNA immunization (Modified for HIV)
Source: Rev. Med. Virol. 2005; 15: 303-325

combination and they are fairly successful in testing with non-human primates. The vaccinated animals produced a large quantity of neutralizing antibodies when infected with the HIV strain. This is a relatively new phenomenon since most of the DNA vaccines do not produce significant amount of neutralizing antibodies. In addition to this they also induced cell mediated immunity by inducing the production of CTL (Cytotoxic T-Lymphocyte) these cells kill the cells infected with the viruses in the process destroying the viruses, which usually escape the neutralizing antibodies. But the response to the virus waned with time and some of the experimental animals died due to the virus but however the vaccine was successful in eliciting an immediate effective immune response [5 - 7].

To induce effective T-cell immunity usually DNA vaccine is administered along with Recombinant Viral carrier vaccine vectors such as Vaccinia, Fowlpox, Canarypox and Adeno viruses. This method has been demonstrated to be highly successful in primates [7, 8].

Advantages: The various advantages of DNA vaccine over other vaccine types are:

- When delivered properly the efficacy is much more than normal vaccines [6].
- The specificity of the vaccine is also high.
- Induces an immediate immune response at the entry of the pathogen.
- Can induce both Humoral and cell mediated immune response.
- Has been highly successful in primates when compared to other types of vaccines.
- Can also induce significant production of Cytotoxic T-Lymphocytes when compared to other types of vaccines [7, 8].

In addition to these properties the vaccine also offers the following advantages:

- The DNA vaccine is inexpensive when compared to other types of vaccines.
- It is highly stable.
- It can be transported easily.

Problems encountered: The various problems encountered in developing the DNA vaccine for HIV are:

- **The mutation rate in the virus:** HIV unlike the other viruses undergoes mutation so rapidly that the host cell

fails to recognize the virus after immunization. As a result after a small latency period when the old strain of virus is killed by the immune system the new strains begins to slowly dominate and take control [9].

- **High error of replication:** The HIV transcriptase is a high error redundant system i.e. it incorporates many errors in the DNA strand that is encoded by the virus. As a result of this new strains of viruses are produced, which do not respond to the immune system [8].

- **High replication rate:** Another crucial factor in the design of vaccine is the high replication rate. The virus divides so rapidly that it is difficult for the immune system to completely destroy all the viruses [8].

- **Integration of viral DNA:** the probability of viral DNA integrating with chromosomal DNA is high in case of retrovirus when compared to DNA from other viral DNA vaccines.

- **The virus also shows some genetic dependence** i.e. some people are more likely to be susceptible for HIV than others which poses a problem for the vaccine development in that it has to be developed genotypically according to the population group [8].

In addition to this there are also various other problems, which are inherent to development of DNA vaccine itself such as:

- Degradation of the DNA vaccine by host cell nucleases.
- Low availability of highly efficient delivery system for injecting the DNA vaccine.

FUTURE RESEARCH

The important area of research that is currently being investigated and the one that shows much promise is the use of Nanotechnology in the design of DNA vaccine for AIDS. Nanotechnology is the technology using particles of 10^{-9} (Nano) dimension. The materials used in this technology are referred to as Nanoparticles. These Nanoparticles have specific properties that make them indispensable in vaccine research. These particles are so small that they can easily enter the host cell machinery. This aspect is made use in the vaccine technology. Since the DNA molecule by itself is of Nano dimension it can be trapped inside an inert Nanoparticles such as liposome and this can then be introduced into the host cell [9].

Already silver Nanoparticle has been shown to interact with HIV-I strain and thereby inhibiting it from binding to the host cell [10, 11]. By using this technology along with the DNA vaccine two-way interacting novel drug delivery system could be developed where the Nanoparticle not only prevents the HIV from binding to the host cell but also delivers the DNA towards the target cell.

The advantages this technology provides are:

- Since the DNA is trapped inside the Nanoparticle it can easily enter the host cell.
- The injected DNA is prevented from degradation by Nucleases present in the host cell [12, 13].
- The DNA molecule also does not elicit an immune response as it is trapped inside the Nanoparticle hence it is prevented from inactivation by the host immune system [14].
- The specificity of the DNA vaccine system is also very high when compared to other systems currently in use.
- Also the Nanoparticle used to entrap the DNA, disintegrates as soon as it has accomplished its purpose hence no side reactions take place [9, 15].

BENEFITS TO SOCIETY

AIDS is not just a health issue it is a multifaceted problem having many implications such as social, economical and political issues and finding a Vaccine for prevention and treatment of AIDS has many benefits associated with it some of them are:

- Millions of lives could be saved especially the lives of innocent children who are at high risk of developing this infection from pregnant mothers carrying the virus in their blood.
- The main benefit of vaccine development is that prevention of the disease is possible which will one day led to eventual eradication of HIV like poliovirus.
- Development and administration of Vaccine is much cheaper and affordable by the developing world when compared to other retroviral therapies currently available.
- It could reduce the economic burden of most of the third world countries. As more and more people are infected by HIV the number of individuals contributing to the economic development of a country is decreased and by finding a cure this trend could be reversed.

Above all these factors by finding a cure for the disease we could be able to create a world with hope for the future generations and also we can weed out the social stigmata associated with the disease by providing these individuals a new life and a fresh beginning.

CONCLUSIONS

This review has tried to explain the development of DNA vaccine for prevention of AIDS and also the various problems encountered in the development of such a vaccine. The review also has focused on the advantages of such a vaccine and how the vaccine efficacy could be improved by using novel technologies such as Nanotechnology. The advantages of such a DNA vaccine are also compared to the other drug development strategies.

Already the DNA vaccine developed by Merck Frosst is currently in the first phase of the clinical trail on humans and has shown significant efficacy. Although till date no vaccine has given the efficacy level that is expected of them but DNA vaccine is certainly the future and the direction in which research should move on if we are to have a potent weapon in the fight against HIV.

There are millions of dollars that are put in the development of vaccines but the key to make sure that development proceeds at a much faster pace lies in relaxing the FDA and other government regulations, which is already currently underway so as to ensure that vaccines can be tested easily. Also it is important to involve all the countries in the fight against HIV and this can be achieved by cooperation in the field of technology transfer and resource sharing thus making a unified effort. The fact that has to be realized by every government if we are to succeed in this fight against AIDS is that it is not just a problem for few countries but it is a problem for the entire humanity. So we can no longer ignore this as a problem of third world and developing countries. Whatever has been the consequence of AIDS it has taught mankind one lesson and that is we are not after all invincible. Thus it would be apt to conclude in the words of Lewis Mumford, US Philosopher "However far modern science and techniques have fallen short of their inherent possibilities, they have taught mankind at least one lesson: Nothing is impossible".

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