STRATAGIES FOR DEVELOPING VACCINES FOR HIV/AIDS. (REVERSE VACCINOLOGY)

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ABSTRACT: Acquired Immuno Deficiency Syndrome or AIDS was first recognized in the early 1980's. Although, with the use of antiretroviral medication, the life expectancy of an HIV infected patient can be elongated temporarily, but there is neither a known cure nor a vaccine for AIDS. This paper shows the approaches undertaken in developing a preventive mechanism against HIV by using epitope driven vaccines which target the global variability of HIV. For this purpose computer driven methods are adapted i.e., Insilco methods for increasing the efficiency of vaccines like Epi-Assembler and Vaccine. Use of broadly reactive and neutralizing antibodies also potentially counteracts HIV-1 diversity. HIV-1 Virus like Particles (VLPs) consists of viral gag proteins which self assemble into particular structures analogous in size, morphology to immature HIV-1 particles. As a non-infectious, replication deficient particles, the VLPs are much safer efficient in developing vaccines when compared to traditional vaccines.

INTRODUCTION

The need for an efficient and an effective defense mechanism against AIDS has never been greater as it is now. Although, with the use of antiretroviral medication, the life expectancy of an HIV infected patient can be elongated temporarily, but there is neither a known cure nor a vaccine for AIDS. This paper reviews the progress and current approaches undertaken in developing a preventive mechanism for HIV, and thereafter suggests a way to confront the issue by focusing on various vaccine development approaches for HIV, specifically concentrating on the treatment of HIV-1 (Figure 1).

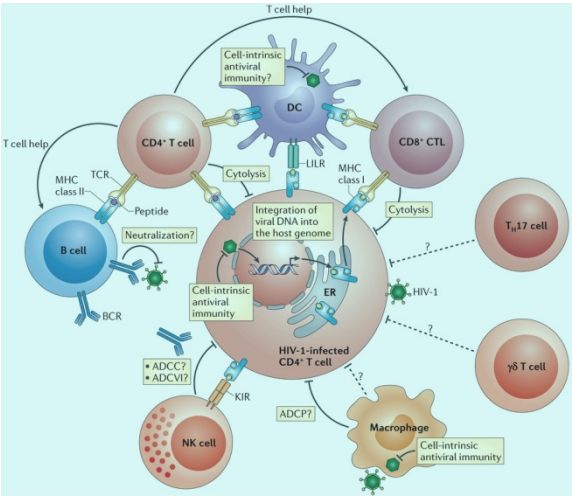

Fig-1:Replication HIV model

HUMAN IMMUNODEFICIENCY VIRUS

The **human immunodeficiency virus** (**HIV**) is a lentivirus (a subgroup of retrovirus) that causes the acquired immunodeficiency syndrome(AIDS), a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype.Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells.HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms, including apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

Group: Group VI (ssRNA-RT)
Order: Unassigned
Family: Retroviridae
Subfamily:Orthoretrovirinae
Genus: Lentivirus

Fig:2 HIV binding CD4 cells

Features of HIV-1 that helps in escaping immune detection

- Surface Env protein escapes antibody recognition as it possesses variable loops, N-linked glycosylation, conformational flexibility and presence of glycan shield over highly immunogenic epitopes.
- Persistently replicates in the infected individual and keeps on attacking CD 4+ T cells.
- Rapidly mutates during infection and thus escapes immune recognition and have extensive viral clade and sequence diversity.
- Persist indefinitely as latent proviral DNA, capable of replication in individuals at a later time.

Types of HIV virus

HIV is a highly variable virus which mutates very readily. This means there are many different strains of HIV, even within the body of a single infected person.

Based on genetic similarities, the numerous virus strains may be classified into types, groups and subtypes.

There are two **types** of HIV: HIV-1 and HIV-2. Both types are transmitted by sexual contact, through blood, and from mother to child, and they appear to cause clinically indistinguishable AIDS. However, it seems that HIV-2 is less easily transmitted, and the period between initial infection and illness is longer in the case of HIV-2.

The strains of HIV-1 can be classified into four groups: the "major" group M, the "outlier" group O and two new groups, N and P. These four groups may represent four separate introductions of simian immunodeficiency virus into humans.

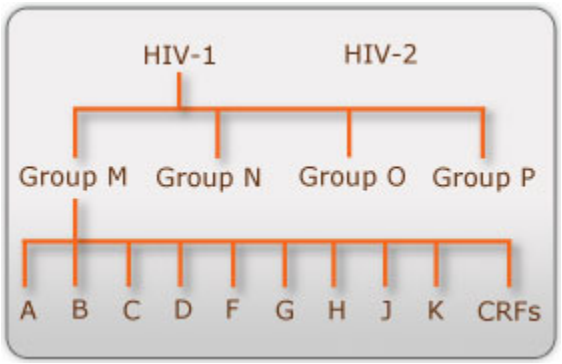

Fig:3HIV types, groups and subtypes

Group O appears to be restricted to west-central Africa and group N - a strain discovered in 1998 in Cameroon - is extremely rare. In 2009 a new strain closely relating to gorilla simian immunodeficiency virus was discovered in a Cameroonian woman. It was designated HIV-1 group P. 1 More than 90 percent of HIV-1 infections belong to HIV-1 group M and, unless specified, the rest of this page will relate to HIV-1 group M only.
Within group M there are known to be at least nine genetically distinct **subtypes** (or clades) of HIV-1. These are subtypes A, B, C, D, F, G, H, J and K.

Occasionally, two viruses of different subtypes can meet in the cell of an infected person and mix together their genetic material to create a new hybrid virus (a process similar to sexual reproduction, and sometimes called "viral sex"). Many of these new strains do not survive for long, but those that infect more than one person are known as "circulating recombinant forms" or **CRFs**. For example, the CRF A/B is a mixture of subtypes A and B.

The classification of HIV strains into subtypes and CRFs is a complex issue and the definitions are subject to change as new discoveries are made. Some scientists talk about subtypes A1, A2, A3, F1 and F2 instead of A and F, though others regard the former as sub-subtypes

HIV-1 is found to be genetically more diverse than HIV-2 in the envelope region comprising C2, V3 and C3 region. To confirm this further, statistical analysis was performed on HIV-1 and HIV-2 infected patients worldwide. Helena Barroso et al. took HIV-2 sequences from Portugal, belonging to group A, and the majority of HIV-1 sequences belonging to subtype B (75%) followed by subtypes G, C and F.

The time duration of initial infection to final stage in case of HIV-2 infection is much attenuated as compared to HIV-1 infection which quickly spreads throughout the host body. This difference in immunological response is expected to be associated with the lower state of immune activation in HIV-2, which may be related to the immunosuppressive activity of the C2-V3-C3 envelope region.

On comparing the amino acid diversity in the C2, V3 and C3 regions of HIV-1 and HIV-2 by Shannon's entropy studies confirmed that these regions are more variable in HIV-1 than in HIV-2. Entropy results also revealed the fact that HIV-1 possesses high entropy as compared to HIV-2 in each separate region. The region with higher mean entropy was C3 in both viruses for HIV-1 and also for HIV-2. V3 region was found to be the least entropic region

both in HIV forms. Amino acids with high entropy were principally found to be present in the C3 region of both viruses and there were more highly entropic amino acids in C3 in HIV-1 than in HIV-2 both in the Portuguese and Control datasets. It has been recently found that HIV-2 displays a faster evolutionary rate in the envelope gp125 and C2-V3-C3 region than HIV-1 in patients who are at an advanced stage of disease .

VACCINES

Vaccines are the medical product designed to activate immune system of the recipient in order to control or prevent infection. In case of HIV-1, mostly preventive vaccines are being developed. These vaccines are designed to protect normal population from getting a HIV infection. Preventive vaccines are widely used to prevent diseases like the flu, chicken pox, measles, mumps, rubella, polio, and hepatitis A and B. Different types of preventive vaccines include subunit vaccines, recombinant vector vaccine, DNA vaccine.

PREVENTIVE HIV-1 VACCINE DESIGN

Due to the escape mechanisms against immune system and unique features of HIV-1, traditional vaccine designs were not of much success. These strategies have proved be successful against many other diseases and pathogens but failed to provide any positive result against HIV-1.

Types of Experimental HIV vaccines based on conventional approaches:

- Peptide vaccine
- Recombinant subunit protein vaccine
- Live vector vaccine
- DNA vaccine

Recent Advances

Due to the absence of a vaccine that can prevent HIV infection,stringent research was started to develop a vaccine that delays or prevents the progression of HIV infection which could be an important intervention to curtail the current global epidemic of HIV. Recently large scale clinical study- RV144 showed some modest response (31%) in reduction of HIV-1 infection [34]. RV144 vaccine consisted of live recombinant viral vector ALVAC-HIV-1, consisting proteins gag/pol/env as prime vaccine followed by booster injections carrying gp120 region (AIDSVAX B/E) as protein boost (Table 3). More than 16,000 people were subjected to trial of RV144 in Thailand. This vaccine targeted both humoral and cell mediated immune response and vaccine recipients exhibited antibody dependent cell mediated cytotoxicity responses . Another large scale clinical study, HVTN 505, also targeted the humoral and cellular immune responses. It consisted of multiclade HIV-1 DNA plasmid (EnvA, EnvB, EnvC, gagB, polB, nefB) followed by booster dose of recombinant adenovirus vector (Ad5 EnvA, EnvB, EnvC, gag/polB).

HIV VACCINE DEVELOPMENT BY COMPUTER AIDED DESIGN

In the year 2005, scientist came up with the approach of computer aided drug designing against HIV-1. They introduced the concept of designing epitope driven vaccines that target the global variability of HIV. The scientists developed computer driven methods for speeding up the process of vaccine development. These methods include the use of Epi-Assembler which derives “Immunogenic Consensus Sequence” (ICS) epitopes from multiple viral variants, and Vaccine CAD, which reduces junctional immunogenicity when epitopes are aligned in a string-of-beads format for insertion into a DNA expression vector.

In this research 20 consensus sequences were isolated from HIV-1 peptides that were found to be immunogenic. There immunogenicity was confirmed computationally by the Epi - Assembler. The core 9-mer contained in these consensus peptides was conserved in approximately 105 to 2250 individual HIV-1 strains. Nineteen of the twenty ICS epitopes (95%) evaluated in this study was confirmed in ELISpot assays using peripheral blood monocytes obtained from 13 healthy HIV-1 infected subjects. Twenty five ICS peptides (all 20 of the peptides evaluated in this study and 5 additional ICS epitopes) were then aligned in a pseudo protein string using “VaccineCAD”, an epitope alignment tool that eliminates immunogenicity created by the junctions between the epitopes. They reordered the construct to reduce the immunogenicity of the junctions between epitopes as measured by EpiMatrix, an epitope mapping algorithm. The reordered construct was also a more effective immunogen in vivo when tested in HLA-DR transgenic mice.

GENETIC DIVERSITY CONSIDERATIONS FOR VACCINE DESIGN

The genetic diversity of HIV-1 across the globe is a major challenge for developing an HIV vaccine. To facilitate immunogen design, it is important to characterize clusters of commonly targeted T-cell epitopes across different HIV

clades. For examining the conserved sequences present in cross clades, a team of scientist examined 39 HIV-1 clade C infected individuals for IFN-γ Gag-specific T-cell responses using five sets of overlapping peptides, two sets matching clade C vaccine candidates derived from trains from South Africa and China, and three peptide sets corresponding to consensus clades A, B, and D sequences . Previous results have shown that HIV-specific T-cells are cross-reactive among different HIV clades but with a preference for the infecting clade.The results from these studies demonstrated that within a single individual, some HIV peptides were exclusively recognized in the clade C sequence variants (CDu422 and CCH), whilst others were uniquely recognized in the clades B, A and D peptide variant. The recognition of clades B, A and D peptide variants and not the corresponding clade C peptide variants is of importance, as it demonstrates that using a single peptide reagent set leads to a considerable number of responses being missed when investigating T-cell immune responses.

These findings are of interest, since a previous study demonstrated no increase in epitope recognition when using the center of the tree(COT) and most common recent ancestor (MRCA) peptide sequences in addition to clade B consensus peptides, in a clade B HIV-infected population.

VIRUS LIKE PARTICLES (VLPS)

Recently a major approach came into light which focused to exploit the conserved regions of IV-1 proteins by overcoming viral immune evasion. gp120 consists of combination of conserved and variable domains. It have five conserved domain namely C1- C5 which are separated by variable domains- V1- V5 . The envelope(transmembrane) protein gp120 co-receptor inding site can also actas target. Inducing production of broadly reactive neutralizing antibodies could also potentially counteract HIV-1 diversity. Many antibodies like PG9, PG16 target even the conserved subdomain within the V1/V2 region. PGT 121- PGT123 and PGT 125-131 are potent enough to recognize the conserved base of V3 domain and interestingly antibodies against these regions are the one to be elicited first in HIV-1 infection. These potential solutions for HIV-1 vaccine development could be potentially realized by exploiting prophylactic HIV-1 virus-like particle (VLP) vaccines.

FEATURES OF VLP'S

- VLPs express multiple viral epitopes that stimulate a diverse set of immune responses, without many of the deleterious effects of a live-attenuated virus
- Potential for activating both endogenous and exogenous antigens processing pathways, leading to presentation of viral peptides by MHC class I and class II molecules
- VLPs may be more cost efficient than co-inoculating multiple single gene vaccines for future Phase I clinical trials

T Cell based vaccine

The previous vaccine attempts mainly focused on eliciting antibody response in patients, but now due to the failures or inefficiency of this approach, focus has been shifted to T cell based vaccines. These vaccines are expected to control virus replication rather than preventing infection because they target infected cells by recognizing the viral proteins presented on major histocompatibility complex (MHC) Proteins . Thus the main aim of these vaccines is to control viremia. A T cell based vaccine consists of epitopes of surface proteins and inside the virion. These DNA vaccines work by introducing the viral antigens to T cells through MHC complex class I and class II. The immunogenicity of these vaccines can be enhanced by new DNA delivery methods . Poxvirus, adenovirus and cytomegalovirus vectors are some of the vectors that can be used in T cell based vaccine Other than these replication incompetent adenoviruses have also been used that expresses HIV-1 immunogens and elicits CD4+ and CD8+ T cells response in recipient individual.

CONCLUSION

The basic idea behind this work is to develope effective potential vaccines which can fight against Human Immune Virus(HIV). The immune system employs mature defense mechanism which includes antibodies and numerous phagocytic cells. Early vaccine researches has mainly designed on the immune system competency by producing antibodies which would block HIV infection. However, such ideas failed in clinical trials because antibodies worked only against lab-cultured HIV, but not on wild strains of the HIV. As the results are not prooving enough and in need for the development of more sophisticated and quicker techniques regarding the same a promising remedy in this area is the use of reverse vaccinology approaches which includes various computational approaches for epitope prediction and their binding efficiency levels with broadly neutralizing antibodies. Following the prediction of a possible vaccine by in silico methods, their efficacy can be evaluated by IFN-γ ELISPOT assay,intracellular cytokine staining assay, virus neutralization assay, ELISA binding antibodies. Use of reverse vaccinology and immunoformatics in silico approaches will help in speeding up time for vaccine development which is cost effective.

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