



Review

Rethinking Globally Relevant Vaccine Strategies to Human Immunodeficiency Virus Type-1

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Abstract. According to the latest UNAIDS figures for 1999 there were an estimated 30.6 million people living with HIV-1, with 16000 new HIV infections per day. The only global strategy of combating new HIV infections is to make a vaccine that is affordable to developing countries, where greater than 90% of new infections occur, and that has enough efficacy to interrupt high rates of transmission. This review critically examines: 1) important immune parameters that should be considered which will allow an understanding of preventative vaccine design and 2) the mechanisms underlying immune destruction during HIV-1 infection that will facilitate design of therapeutic vaccines. A realistic goal of a preventative vaccine is to elicit protective immune responses in vaccinees that would prevent HIV-1 from replicating extensively in the host. Components of protective immunity are thought to include neutralizing antibodies (NAB) and cytotoxic T lymphocytes (CTL). Rethinking vaccine strategies has to take into account that HIV-1 vaccines must elicit primary cellular and humoral immunity via dendritic cell and Langerhan cell priming. It is only under these conditions that boosting immunity with subsequent vaccinations will allow high enough CTL effector cells and NAB titres to impede or to prevent HIV-1 replication. Success of therapeutic vaccine strategies, has to take into consideration the pathology of persistent immune stimulation by chronic HIV-1 infection. To re-stimulate immunity and re-direct immune responses, chronic immune stimulation by HIV-1 has to be alleviated by reducing high levels of viral antigen presentation by suppressing virus with antiretroviral agents. Such treatment courses may only have to be transient, long enough for immunity to respond to an immunogenic stimulus. Short-course drug therapy may then be an affordable option for many countries already carrying a high burden of HIV-1/AIDS.

Key words: vaccines; protective immunity; cytotoxic T lymphocytes; neutralizing antibodies; dendritic cells; antiretroviral drugs.

Introduction

HIV-1 and AIDS have reached epidemic proportions in many regions of the globe. According to the latest UN AIDS figures for 1999, there are now an estimated 30.6 million people living with HIV-1 and the estimated cumulative total of deaths from AIDS is approximately 12 million²⁰. It has been calculated that

there are 16 000 new HIV infections per day, where one tenth of these are in children²⁰ infected either through intrauterine, intrapartum/early postpartum (0–2 days) and late postpartum (3–5 months) infection⁴⁸. The only global strategy for combating new HIV infections is to make a vaccine that is affordable to developing countries, where more than 90% of new infections occur. After almost 18 years of intensive research,

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many strides have been made in understanding HIV-1 transmission, its life cycle and the genes that appear to be vital for successful replication. This information has been welded to formulate expensive novel drug strategies that have allowed some of the world's infected individuals to be successfully treated. Although drug therapeutic approaches have taken precedence over vaccine research over the past few years, the narrowly focused approach used to strategize antiretroviral drug development has often been translated to vaccine research. This "virological translation" has resulted in a narrow and highly focused vaccine agenda which has based candidate vaccines on laboratory HIV-1 strains originally derived from infected gay men in the USA, Europe or Australia and developed around a minor subtype of HIV-1. It thus may not be surprising that promising vaccine candidates have been lacking and results to date have caused vaccine researchers to rethink previous limited approaches. Vaccine research is no longer solely taking its cue from the virology of the HIV-1 genome, but is now paying more attention to immune responses in infected individuals that may determine protection versus pathology. The current scientific vision is beginning to broaden and encompass the global issues of HIV-1 variation, modes of transmission and human genetic variation. This approach will, hopefully, increase the probability that vaccine candidates are more globally relevant.

The aim of this review is two-fold: 1) to critically examine some of the important scientific parameters that should be considered when understanding preventative vaccine design and 2) to understand the mechanisms underlying immune destruction during HIV-1 infection that may facilitate design of therapeutic vaccines.

The Greater Part of the HIV-1 Burden Rests on Countries that Cannot Afford Highly Active Antiretroviral Therapy

The effectiveness of highly active antiretroviral therapy (HAART), which consists of protease inhibitors, nucleoside-analogue and non-nucleoside analogue reverse transcriptase inhibitors, has had a pronounced influence on the decline in the incidence of opportunistic infections, mortality and morbidity in the USA^{38, 43} and Europe³⁶. The most likely explanation for this lies with the combined effect of reverse transcriptase and protease inhibitors that can effect HIV suppression at any stage of infection^{19, 28, 38} and allow host immunity to be restored and stabilized^{2, 4, 17, 24, 25, 30}. The high

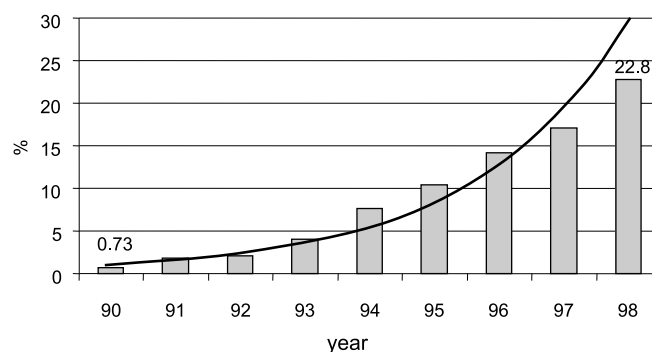


Fig. 1. Antenatal HIV-1 prevalence trends in South Africa from 1990 to 1998

cost of these drug cocktails however, makes them prohibitively expensive to many countries. South Africa is an example of a country that has the fastest global rate of HIV-1 infections (Fig. 1), with approximately 1800 new infections per day, with antenatal prevalence rates ranging from 6–33% across the country¹¹. The predominant subtype is C and the mode of transmission is through heterosexual contact²², which mirrors the major mode of spread throughout sub-Saharan Africa⁴⁸. The cost of treating the estimated 3.5 million HIV-1 South Africans²² with HAART is not a viable public health option, as reflected elsewhere in Africa, India and Asia.

Global Distribution of HIV-1 Subtypes

One of the possible confounding factors that may influence vaccine design is the genomic diversity found within HIV-1. Due to the inefficiency of viral reverse transcriptase, the HIV-1 genome mutates very readily with every life cycle. The resulting genetic variability has been extensively analyzed, mostly in the envelope region, and this has been the basis for characterizing HIV-1 into clades or subtypes, consisting of groups M (main) and O (outlier), where the majority of subtypes resides in group M, ranging from A through J⁵². In addition, the high rate of mutation causes genetic recombination between different subtypes. Most subtypes have been identified in central Africa, with subtypes A and D predominating along eastern Africa and C in southern Africa⁵². Subtype C HIV-1 constitutes the most predominant global strain, accounting for 54% of those infected⁵³. The recombinant subtype E is most common in south-east Asia and subtype B is the most predominant in Europe and North America, making up 16% of global infections⁵³. Much of the intensive research on HIV-1 has been performed on subtype B and vaccine strategies have historically focused on this rela-

tively minor global subtype. Whether HIV-1 subtypes have a major impact on vaccine development remains to be seen and the immediate goal will be to identify protein regions of homology between subtypes that evoke strong cellular and humoral immune responses. The ultimate global goal would be to develop a “universal” vaccine and it thus may be important to rethink matters concerning HIV-1 classification in the form of an immunological classification as opposed to subtypes. It is argued that neutralising antibodies may occur across clades, for example A, C and E but excluding B^{49, 56}. This immunologically stratified approach could be useful in scenarios where more than one clade is prevalent.

What Are the Vaccine Options?

Preventative vaccines

There is no doubt that the ultimate global public health intervention strategy is an HIV vaccine. What are the scientific issues concerning such a vaccine? The goal of a preventative vaccine is to elicit protective immune responses in vaccinees that would prevent HIV-1 from replicating extensively in the host. It is unlikely that any vaccine would evoke sterilizing immunity, but would rather halt disease at the very best, or slow disease at the very worst.

Protective immunity

Although no vaccine candidate has ever evoked neutralizing antibodies, these molecules are thought to be important in providing protection from virion entry into infectable cells²⁶. Even though neutralizing antibodies have been detected from infected individuals³⁷, it has been difficult to identify neutralizing antibody epitopes. Recent experimental data has shown that antibodies raised in mice immunized with “frozen” gp120-CD4-CCR5 fusion – competent proteins could neutralize a wide range of primary HIV-1 isolates across different subtypes²⁷. It is thought that the fusion – competent proteins express epitopes buried deep in gp120 that become transiently exposed upon fusion with CD4 and CCR5 (27). Revelation of these epitopes to the immune system is long enough to evoke neutralizing antibodies.

Cytotoxic T lymphocytes (CTL) are also considered crucial in preventing the spread of infectious virions from cell to cell³⁵. Most of the clinical data from natural infection and from those individuals highly exposed but

persistently seronegative^{45, 46} have provided strong evidence that HIV-1 can be controlled by CTL and that these cells can be protective. There is a wealth of evidence from mouse models that CTL are crucial for protection against lymphocyte choriomeningitis virus⁵⁰. Recent data using a simian immunodeficiency virus (SIV) model in monkeys showed that SIV viremia can be controlled by SIV-specific CD8⁺ T cells²⁹, strongly supporting a role for CD8⁺ CTL in controlling virus in larger outbred animal models. Peptide/MHC tetrameric molecules have allowed the antigen-specific nature of CD8⁺ T cells to be assessed directly¹ and have been used to study *ex vivo* the inter-relationship between CTL and HIV⁴¹. The use of peptide/MHC tetramers in a natural history study has shown that the frequency of antigen specific CD8⁺ T cells correlates inversely with HIV-1 viral load⁴¹ and that shortly after seroconversion there is a peak of these cells preceding the fall of plasma viremia⁴². These data are the first to show that antigen-specific CD8⁺ T cells recognizing two immunodominant epitopes are associated with control of HIV-1.

It is unlikely that a preventative vaccine will induce sterilizing immunity and the goal of focussing on CTL will be to limit and abort productive HIV-1 replication in the event that neutralizing antibodies are not effective. Thus, the challenges facing the development of a preventative vaccine are to identify conserved HIV-1 proteins that would be consistently recognised which would allow the immune system to control viral replication. Usually, CTL responses to HIV-1 have been detected that are focused on single immunodominant epitopes³¹, mainly due to HIV-1 epitopes being preferentially restricted by certain HLA alleles. An example of an epitope in p17 Gag, which is preferentially restricted by HLA-A*0201 is SLYNTVATL¹⁵. In subtype B HIV-1 infections this epitope appears to be expressed in 80% of individuals¹⁶ and has been implicated in protective responses⁴⁰. It remains to be seen whether this epitope plays a role in non-B subtypes, although it is likely that the frequency of divergent HLA alleles in different geographic regions will be a major determinant of which epitopes of non-B subtypes will be immunodominant. To develop an immunogen that is relevant as a global vaccine against HIV-1, studies will have to be done which systematically and logically investigate the impact of host genetics on responses to HIV-1. In this regard, approaches to vaccine design have to focus more on human genetics and examination of host immune responses to HIV-1 in productively infected and exposed seronegative persons. Studies that have investigated CTL responses in exposed seronega-

tive prostitutes in Nairobi⁴⁶ have shown that several CTL epitopes are restricted by HLA-A*6802. These women have likely been exposed to subtype A HIV-1 and the data infer that CTL responses to defined epitopes may confer protection in these individuals. These approaches may offer a more strategized method towards basic preventative vaccine design. Contiguous to this epitope-based strategy would be the requirement to know the allele and gene frequencies of the class I and II HLA backgrounds of specific populations infected or at risk of HIV-1 infection. This may have to be by country or by distinct geographic regions. Recent detailed analysis has confirmed that specific haplotypes with maximal heterozygosity afforded long term non-progression and that specific alleles such as HLA B35 were associated with poorer outcome in HIV-1 infected individuals⁸. It is thus obvious that an extensive HLA data set is required for countries with HIV pandemics, most of which are in the “developing” world. At present such information is relatively scant or not sufficiently detailed for the rational choices of epitopes.

Immunological Rethinking

In order to generate good CTL responses to HIV proteins, efficient MHC class I presentation of HIV-1 antigens must be attained. There are two considerations for this to occur: 1) dendritic cell (DC) priming and 2) efficient delivery of antigens to the cytosol.

It is known that DC's play a vital role in the initial priming event of an immune response and their central role in facilitating CTL response has been shown^{39, 54}. One of the first events after HIV transmission through mucosa is the “seeding” of HIV to lymphoid structures, most notably the lymph nodes (LNs)¹⁸. This event is facilitated by dendritic cells and Langerhan cells residing at the mucosal interface that transport HIV to LNs³³, allowing the virus to interact with lymphoid structures crucial for immune initiation.

DC have the ability to engulf and process antigen at the site of immune insult and, by migrating through the lymphatics, can transport processed antigen to secondary lymphoid organs that provides the structural micro-environment allowing naïve T cells to be primed via T cell receptor engagement with cognate antigen on the DC cell surface. The resulting effector T cells are able to migrate from the LNs and interact with antigen in non-lymphoid tissue spaces. In terms of the ability of vaccine candidates to initiate fresh immune responses, it will be imperative to target DC and Langerhan cells

that would not only provide adequate systemic immunological priming, but also evoke mucosal immunity. It needs to be recognized, however, that mucosal immunity differs from systemic immunity. For example, the primary antibody response of the mucosal immune system is secretory (s) IgA as opposed to IgG in systemic immunity. Systemic immunity is relatively ineffectual in preventing entry of pathogenic organisms at the mucosal surface, so it is crucial to develop efficient local immune responses to HIV-1 at the site of transmission. The most studied example of mucosal associated lymphoid tissue is the Peyer's patches (PP), consisting of germinal centres where B cell division occurs and where a large proportion of sIgA can be found. In close proximity are defined T cell areas that provide CTL and T cell help. Moreover, within this mucosal lymphoid tissue are antigen-presenting cells including macrophages and dendritic cells³⁴. Thus, effective priming of DC and Langerhan cells at the mucosa by a vaccine candidate would most likely result in causing strong local cellular immune responses.

Efficient delivery systems of HIV-1 antigens to immunocompetent cells include either bacterial or viral vectors^{7, 9, 10, 32, 47}. Part of this rationale is to exploit the ability of other microorganisms to interact with natural cell receptors and deliver HIV-1 antigens more efficiently to MHC class I and II molecules. For example, the feasibility of using *Salmonella*⁵¹, *Listeria*³² and BCG²⁰ for the delivery of HIV-1 antigens is beginning to take prominence, where BCG may be particularly attractive because it has a long history of safety in humans and can be given at birth. Other viral vectors include the alphaviruses: Sindbis, Venezuelan equine encephalitis (VEE)⁷, Semliki Forest virus⁵ and modified vaccinia Ankara (MVA)⁴⁷. VEE is a most attractive vector as it has a tropism for Langerhan cells and DC⁷ and would thus allow priming of CTL responses both systemically and at the mucosa.

Therapeutic vaccines

What about therapeutic vaccine approaches, relevant for the 30 million people already infected with HIV-1? The track record of available therapeutic vaccines that can elicit new anti-HIV immune responses in infected individuals is not good. Attempts to boost specific immunity with recombinant gp120 or gp160¹⁴ or viral like particles^{21, 23} carrying specific HIV-1 proteins have not worked. There is perhaps a misconception that because HIV-1 infection induces an immunocompromised state, there is a need to reverse events by boosting immunity.

HIV-1 causes a chronic, persistent viral infection, with lymphoid structures being appropriated by HIV that disables initiation of new immune responses. HIV-1 can appropriate LN structures within the microenvironment of the germinal center (GC) by displacing antigens⁶ complexed with the follicular dendritic cell (FDC) network¹². This antigen displacement is thought to curtail immune responses to non-HIV antigens and viral antigen persistence additionally results in chronic stimulation leading to destruction of the lymphoid microenvironment and dissolution of the FDC network. Histologically, this is observed as a regression of the GC and disappearance of the FDC network⁴⁴. As an intact LN architecture is required for clonal expansion of effector T cells upon interaction with cognate antigens, disintegration of the LN microenvironment, therefore, impedes the development of new immune responses and leads to susceptibility to opportunistic infections. There is a loss of naive T cells due to thymic infection¹³ and along with lymphoid tissue destruction, the gross effect is CD4 decline and an immunocompromised state. Moreover, cells responsible for providing potential protective immune responses (B cells and CTL) either become non-functional or are deleted from the host armory due to persistent and chronic over-stimulation. It thus becomes imperative to devise strategies to restore immunity, but not necessarily by providing a stimulus that has already caused immune destruction. There can be no better immunogen than HIV itself and attempts to boost immunity with therapeutic vaccines underscores the lack of understanding of the processes leading to the immunopathogenic insult caused by HIV-1 infection.

Studies investigating immune reconstitution in response to HAART have provided evidence that potent suppression of HIV-1, leading to reduction of viral persistence, is required to curtail the immunopathogenic insult. A number of studies have shown the plasticity of the immune system to respond favorably to the removal of highly replicating virus²⁸, regardless of the stage of disease or previous failures of drug therapy¹⁷. Pronounced increases in numbers of memory and naive T cells have been observed⁴, accompanied by a return of the lymphoid architecture⁵⁵ and an improvement in the T cell repertoire. Herein lies a clue for therapeutic vaccine strategies. If the viral burden can be reduced, resulting in an alleviation of viral persistence, recovering T cells could then respond to a vaccine and the immune responses re-directed towards controlling HIV-1. Alternatively, a more controversial approach could be intermittent HAART, where rebounding replicating virus is thought to allow priming of restored

immunity³. The challenges facing a combined HAART and vaccine approach would be: a) to define the treatment regimen that reduces viral persistence at the lowest cost and b) to define the duration of treatment prior to administration of a vaccine candidate. If treatment consists of a short course of antiretroviral therapy, the goal would be to know when to stop drug administration. This may well be an option for countries that cannot afford to provide long-term drug treatment for infected individuals.

Conclusions

Although there have been major advances in therapeutic drug options for HIV-1 infected individuals, the development of vaccines as an affordable global public health option has been lacking. Only until very recently has funding become available for international vaccine research through agencies such as the International AIDS Vaccine Initiative and the US National Institutes of Health to explore imaginative ways of priming immunity to HIV-1. Bacterial and/or viral vectors may present HIV-1 proteins to the immune system that would be sufficient to prime both cellular and humoral immunity that may provide the necessary protection from disease, but not necessarily infection. Whilst it is important to continue to search for efficient delivery systems using various vector approaches as candidate vaccines, cognisance must also be taken of where the priming of immunological responses is done, i.e.: in secondary lymphoid structures. Targeting these structures with an efficient antigen delivery system would ensure that the immune system is appropriately primed enough to abort or prevent productive infection upon likely exposure to HIV-1.

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