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Review

Lessons for general vaccinology research from attempts to develop an HIV vaccine

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ABSTRACT

In the past when large investments have been made in tackling narrow scientific challenges, the enormous expansion in our knowledge in one small area has had a spill-over effect on research and treatment of other diseases. The large investment in HIV vaccine development in recent years has the potential for such an effect on vaccine development for other diseases. HIV vaccine developers have experienced repeated failure using the standard approaches to vaccine development. This has forced them to consider immune responses in greater depth and detail. It has led to a recognition of the importance of epitopic specificity in both antibody and T cell responses. Also, it has led to an understanding of the importance of affinity maturation in antibody responses and the quality of T cell responses in T cell-mediated immunity. It has advanced the development of many novel vaccine vectors and vehicles that are now available for use in other vaccines. Further, it has focused attention on the impact of research funding mechanisms and community engagement on vaccine development. These developments and considerations have implications for vaccinology more generally. Some suggestions are made for investigators working on other "hard-to-develop" vaccines.

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Contents

1. Introduction

At the turn of the 20th century Sir William Osler, the Father of Modern Medicine, said "He who knows syphilis knows medicine" [1]. At that time the many manifestations of "the great imitator" required syphilis to be part of the differential diagnosis of most illnesses. Today, because the treatment and prevention of HIV infection touches upon so many different aspects of the practice of medicine it could be said that "Those who know HIV/AIDS know medicine." Also, the urgency of this new pandemic and powerful advocacy of its at-risk populations has led to a very substantial biomedical research investment in a single disease. As in

Abbreviations: CAR-T, chimeric antigen receptor T cells; BNAbs, broadly neutralizing antibodies; BCR, B cell receptor; TfH, T follicular helper cell; TfR, T follicular regulatory cell; ADCC, antibody-dependent cellular cytotoxicity.

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the past, when there have been large investments in tackling narrow scientific challenges, the enormous expansion in our knowledge in one small area is having a spill-over effect[1] on research and treatment of other diseases. Among these are major advances in drug development for Hepatitis C modeled on AIDS drug development [2], and CAR-T cells using lentiviral vectors for treating cancer [3].

While the development of an effective HIV/AIDS vaccine has not yet been accomplished, the deeper understanding of the obstacles, gained from the research effort, has the potential to transform the way scientists think about vaccine development more generally. There are two classic approaches to vaccine development: (1) killed pathogen and (2) live-attenuated vaccines. Research in the 1970s added recombinant protein vaccines. These approaches are where HIV/AIDS vaccine development started. A recombinant HIV-1 envelope protein vaccine (AIDSVAX) was tested in large scale clinical trials in the late 1990s and failed to demonstrate any protection. A whole-killed vaccine (the Salk AIDS vaccine, Remmune) was tested as a therapeutic vaccine in early phase clinical trials with similarly unsuccessful results. And after early promising results in nonhuman primate studies, a live-attenuated HIV vaccine approach has been abandoned because of safety concerns.[2]

This article will present the different approaches to vaccine development that have evolved for HIV-1, approaches informed by the recognition of obstacles to classical vaccine development approaches. Others view new approaches to vaccinology from the perspective of new technologies that can be used. These developments have been the subject of many recent reviews, most notably those of Dr. Rino Rippuoli [4,5]. While citing how new technologies are being applied in HIV vaccine development this article will review studies more from the perspective of the obstacles that they illuminate.

2. Antibodies and/or T cells?

An early question in vaccine development is whether the mechanism of vaccine protection will be an antibody response or a T cell response. Some say that an effective vaccine should elicit both types of responses. The problem for such vaccine development is that the vectors, adjuvants, or cytokine milieus that promote strong antibody responses are usually not the same that promote strong T cell responses. Thus, it may be difficult to optimize a candidate vaccine product for both types of responses at the same time. It is logical to optimize different modalities of immune responses separately to show they have some effect on infectivity, pathogen replication or pathogenesis independently before attempting to put them together in one syringe. In this way biomarkers of probable efficacy can be determined for each of the different immune responses independently such that the vaccine developer will know whether optimizing one is interfering with the other during early clinical trials before attempting an expensive, large scale efficacy trial. Similar independent development advice is given by the FDA for combination vaccine development with vaccines against different pathogens [6].

[1] The Manhattan Project gave us nuclear energy and radiation medicine; the moon race drove a generation of improvements in micro-circuitry, remote sensing, GPS, and computer technology; and the Human Genome Project has delivered remarkable advances in sequencing technology and promises new diagnostics, disease cures, and personalized medicine.

[2] An attenuated HIV-1 would insert itself into the vaccinated person's genome. Regulators worry about insertional mutagenesis causing cancer as well as the potential for recombination leading to reconstitution of a pathogenic virus.

3. Antibodies

a. Focus on epitopes

Despite the inability to induce broadly neutralizing antibodies with a vaccine, since the 1990s investigators have been able to isolate monoclonal broadly neutralizing antibodies (BNAbs) from the blood of some HIV-infected patients. Analysis of these BNAbs has shown they are directed at a limited set (5) of conserved epitopes on the HIV envelope surface glycoprotein (the CD4 binding site, a V2 loop glycan epitope, a V3 loop glycan epitope, the gp41/gp120 interface, and the membrane proximal region) [7]. This limited target space on the surface of the relatively large, 160,000 Dalton virus surface protein is explained by both the small amount of conserved amino acid sequence on the surface of this protein and extensive glycosylation shielding much of the surface of the protein from antibodies (fully half of the HIV-1 envelope protein is self-like sugar molecules). Also, these targets are partially buried in protein structure or glycan chain-formed crevices such that a restricted angle of approach further limits the number of different germline-encoded B cell receptors (BCRs) or antibodies that can access them. When the whole protein is presented to the host immune system more abundant variable and internal epitopes are more likely to elicit an antibody response than the rarer and less accessible BNAb targets. Thus, HIV vaccine developers have been forced to focus their attention on inducing antibodies against specific epitopes within the surface protein rather than immunizing with a whole antigen and letting the immune system react with what is easiest.

"Original antigenic sin" is a phenomenon described for influenza infection where exposure to a second, different strain of the virus results primarily in boosting the antibody response to the first strain encountered, to the detriment of an effective antibody response to the new strain [8]. Because HIV vaccine developers have rarely obtained a neutralizing antibody response to even the first strain on vaccination, there has been little worry about original antigenic sin. However, hypotheses of mechanisms underlying original antigenic sin may inform a key difficulty in HIV vaccine development. Prior exposure to the first influenza antigen causes the proliferation of responding specific B cells to create a much larger pool of memory B lymphocytes that recognize the first antigen than the naïve B cells that may bind the new antigen better [9]. In competition for limited amount of antigen the larger pool of cells is more readily activated and thus the antibodies produced reflect the earlier immune response. Perhaps, in the case of the limited HIV envelope target epitopes with restricted access, there are rarely enough naïve B cells with the right BCR to out compete the vastly larger number of naïve B cells that can respond to the large number of easily accessible variable or internal epitopes in the large amount of denatured envelope protein circulating in infected persons or presented as immunogens in earlier HIV vaccination attempts.

b. Use of structural biology tools to map and define conformational and difficult to access epitopes

Epitopes localized to the outer surface of antigens are frequently on amino acid loops and easily defined as discrete sequences of 5–6 amino acids in length (i.e. linear epitopes). Other epitopes may be composed of amino acids quite distant in the primary sequence which come into proximity as the protein folds. Some of the broad neutralization target epitopes recognized by BNAbs isolated from HIV-infected patients are such "conformational" epitopes. The analysis of such epitopes has been enabled by advances in structural biology which has revealed important details of these epitopes that have facilitated immunogen design [10]. Some epitopes have been

constructed as scaffolded minimal epitopes once actual conformations were known [11,12]. Detailed knowledge of the native envelope trimer has also allowed the construction of more stabilized trimers (by informing placement of amino acid mutations that can then form intramolecular bonds to stabilize the three-dimensional structure of the protein) that express fewer non-neutralizing, denatured protein epitopes forcing the immune response to focus on BNAb targets [13]. Stabilization of the overall protein structure may also provide critical stabilization of the conformation of conformational epitopes as well as stabilization of access to epitopes buried in protein crevices. Furthermore, detailed structural analysis of BNAb binding to envelope has guided subtle mutation of envelopes to allow for the binding of antibodies with intermediate binding affinity to select for critical antibody mutations in the process of affinity maturation [14].

c. Affinity maturation may be crucial to vaccine activity

The monoclonal BNAbs isolated from HIV-infected patients themselves have become the subject of research. They are unusual in several ways.[3] One distinct difference is that they appear to have undergone significantly more somatic hypermutation than antibodies formed in response to other pathogens [16]. If this is required for BNAbs, investigators reasoned it could explain the difficulty inducing such antibodies with the usual regimen of 3–4 immunizations, which would only drive a limited amount of the re-entry to lymph node germinal centers required for such extensive antibody mutation. Several reasons are hypothesized to explain the necessity for such extensive somatic hypermutation. They are: sparsity of surface antigen, less epitope exposed, mutations requiring multiple base changes, framework mutations, and antibody-envelope co-evolution (Table 1).

Some of the reasons discussed in Table 1 are associated with the extreme mutability of HIV-1. After antibody is elicited that can neutralize virus by binding to a BNAb target, mutations occur in the epitope, or surrounding areas, that hinder antibody binding. Individuals eventually develop BNAb responses because somatic hypermutation allows antibody to co-evolve with the virus envelope protein facilitating continued high affinity recognition of the evolving epitope and/or continued access to a partly buried, conserved epitope [21]. Such co-evolution results in an antibody with enough breadth of epitopic recognition to neutralize many HIV-1 isolates. How to induce such breadth with a vaccine which usually contains only a single or small number of antigens is problematic. Some HIV vaccine developers are exploring several types of sequential immunization schemes [22]. All schemes start with a priming immunogen (sometimes a scaffolded minimal epitope) with high affinity for the rare, unmutated germline antibody gene. Some schemes select boost immunogens by analysis of B cell antibody and envelope lineages isolated from HIV-infected patients who have developed BNAbs. Envelope variants with high binding affinity for intermediate antibodies in the lineage are used as boosting immunogens until BNAb activity is obtained [15]. Other schemes rely on structural analysis to design intermediate boosting immunogens that select for critical mutations in BNAb evolution [23]. Also, there is renewed interest in the TfH and TfR cell responses that promote and regulate the germinal center response where affinity maturation occurs. These complicated vaccination schemes are for proof of concept. Once BNAbs can be induced with any of these complicated schemes it will become an engineering problem to combine multiple immunogens into single shots using optimal adjuvants and rapid and delayed release materials. Another approach has been to treat the extremes of variation in specific BNAb target epitopes as essentially different epitopes for a multivalent vaccine. Thus, instead of inducing antibody maturation to accommodate multiple variations in an epitope, computational analysis has been used to establish viral envelope sequence signatures associated with differing extremes in sensitivity to the same BNAb. It has been found that immunization with combinations of envelope immunogens constructed to cover several signatures will induce greater breadth in a poly-specific antibody response than single immunogen constructs [24]. How many variants will be needed to give enough breadth to protect against a large percentage of the HIV strains circulating in the population is unclear; but this approach should not require as much somatic hypermutation as attempting to induce monospecific BNAbs.

d. Tolerance regulation may interfere with broadly neutralizing antibody induction

It was noted fifteen years ago that some of the isolated BNAbs were highly polyreactive, even reacting with multiple host antigens [25]. It is not surprising that the CD4 binding site which may be self-like enough to interact with CD4, which is a host protein, elicits some autoreactive response. In one case (the 2F5 BNAb) the HIV-1 envelope epitope's amino acid sequence is found in the human genome [26] which also makes autoreactivity unsurprising. However, most cases of autoreactivity are directed at epitopes of intracellular enzymes with no known relation to the virus [27]. Nevertheless, studies in mouse strains knocked-in with several BNAb antibody genes and their unmutated germline ancestors verify that production of HIV-1 BNAbs of multiple specificities is restricted by different levels of self-tolerance controls [28]. It may be the case that more antibody lineages are under tolerance restrictions than commonly thought. The explanation for seeing it so frequently with HIV-1 could then be that the extremely limited number of germline antibody genes for HIV-1 BNAbs forces the use of such tolerance-restricted genes while for normal infections or vaccines, where many more germline antibody genes are responsive, the immune response just uses the readily available, less-restricted antibody lineages. This clearly causes problems for specific antibody induction by vaccination, so some HIV vaccine developers are investigating transient immunomodulation techniques to get around tolerance controls to enhance immunity [29].

e. Fc/biological functions: pay attention to the other end of the antibody

In 2007 it was recognized that sequences in the Fc region of an HIV-1 BNAb influence its protective capacity in BNAb passive transfer studies [30]. This is less important when high affinity neutralizing antibodies are studied [31]. However, as it may be difficult to induce high affinity antibodies, anything that enhances the protective capability of lower affinity BNAbs may enhance vaccine efficacy. Also, in 2009 the USMHRP-Thailand Ministry of Public Health co-sponsored RV144 trial demonstrated modest efficacy (31%) in prevention of acquisition of HIV infection with a vaccine regimen that induced little if any neutralizing antibodies or cytotoxic T cell activity [32]. *Post hoc* analysis of correlates of risk suggested that non-neutralizing antibodies binding to HIV-1 envelope protein might have mediated the protection observed [33]. The Fc region of these antibodies must have mediated some biological function, other than direct virus neutralization, that was able to prevent establishment of infection. A clearer answer to the mechanism of protection in this trial should be obtained from analyses of samples from a follow up study (HVTN 702) which will be unblinded in 2021. Work is ongoing on refining assays for multiple

[3] Most monoclonal HIV BNAbs have one or more of the following uncommon characteristics: a high number of somatic mutations, restricted V_H gene usage, long heavy chain CDR3 regions, or poly- or auto-reactivity [15].

Table 1
Hypotheses explaining a requirement for extensive somatic hypermutation.

1. Sparsity of surface antigen [17,18] There are only 10–15 envelope trimers on the surface of any HIV-1 virion. Because of the distance between sparsely distributed trimer molecules an HIV-specific antibody is not able to use both its antigen binding arms (Fabs) to bind, bivalently, to the virus surface as is possible for antibodies binding to the surface of more densely coated pathogens. Higher binding affinity for the epitope is required of anti-HIV neutralizing antibodies to compensate for the decreased avidity. This higher affinity is achieved by affinity maturation or the evolution of a tighter fit of the antibody paratope for the epitope. The distance between trimer spikes may also explain why whole-killed HIV-1 is a poor inducer of neutralizing antibodies as the distant antigens would have difficulty engaging multiple B cell receptors to activate naïve B cells.
2. Less epitope exposed A difficult angle of approach to the epitope for antibodies and B cells may also necessitate affinity maturation. When the neutralization epitope is buried in a protein cleft or at the bottom of a well formed of glycan chains less of the epitope will be exposed for binding within the paratope than would occur with a surface-exposed epitope; less contact between the paratope and the epitope logically will result in lower affinity of binding.
3. Mutations requiring multiple base changes Analysis of the evolution of HIV BNAbs in mice knocked in for the germline genes from which those antibodies developed has shown that some of the mutations critical to high affinity binding are difficult to obtain mutations either not in mutational hotspots or requiring multiple base changes [19]. Such mutations may only occur as a side-effect of a large amount of mutational activity.
4. Framework mutations Enhancing access to a buried epitope may require greater flexibility in the antibody to engage the walls of the crevice, especially as those walls evolve with envelope protein mutation. Such flexibility is acquired by mutating framework regions of the antibody which are not hotspots for somatic hypermutation [20]. Mutations there may also only occur as an accidental result of a large amount of mutational activity.
5. Antibody-envelope co-evolution After antibody is elicited that can neutralize virus by binding to a BNAb target, mutations occur in the epitope, or surrounding areas, that hinder antibody binding. Somatic hypermutation allows antibody to co-evolve with the virus envelope protein facilitating continued high affinity recognition of the evolving epitope and/or continued access to a buried, conserved epitope [21].

Fc-mediated antibody functions [34] as well as development of multivariate analyses (systems serology) to characterize the contributions of multiple different Fc-mediated antibody functions [35]. As with neutralizing antibodies, the epitopic specificity of functional non-neutralizing is important, although the epitopes may differ from the neutralizing epitopes as different envelope epitopes may be presented on the surface of infected cells [36].

f. Implications for other vaccines

The scientists who aim to develop vaccines against **families of viruses** will be forced to focus on specific conserved epitopes. A similar focus on specific epitopes has already developed among the universal **influenza** vaccine developers because only a limited amount of the hemagglutinin surface is conserved [37]. Both groups are focusing on a smaller number of epitopes than HIV vaccine developers. Their chances of success will be improved if they have more epitopes to target. Improved monoclonal antibody isolation technology, coupled with FACS sorting of B memory cell populations has allowed HIV vaccine scientists to obtain monoclonal BNAbs from patients with barely detectable levels of BNAb activity [38]. This has been valuable for target epitope characterization, and it could be a useful supplement to other vaccine developers who could screen multiply infected people to discover more epitopic targets for BNAb attack. Likewise, vaccine developers for diseases where antigenicity evolves or adapts to the host over the course of chronic infection, such as **syphilis**, **malaria**, and **African trypanosomiasis**, might be able to use this approach to identify rare BNAb epitopes amid the noise of unhelpful antibody responses to variable or strain-specific epitopes.

A combination of envelope protein diversification, sparsity of surface antigen, less epitope exposure, mutations requiring multiple base changes and hard-to-get framework mutations probably explains the need for the extensive somatic hypermutation in affinity maturation observed for BNAbs against HIV. Other vaccine developers may not encounter so many obstacles, but all working with sparsely distributed (**syphilis**) or difficult to access surface (universal **influenza**) epitopes are advised to isolate and sequence neutralizing antibodies to their pathogen to determine if affinity maturation will be required. If more than minimal somatic hypermutation is detected be prepared to investigate immunization strategies and adjuvants that promote affinity maturation.

HIV vaccine scientists are developing scaffolded, minimal epitope, stabilized immunogens, sequential and combination immunization strategies to get around the limited germline gene, diversity, and affinity maturation obstacles to HIV vaccine development. They have already applied the scaffolded epitope approach [39] and the stabilization approach [40] to **RSV** vaccine development with good result. Other vaccine developers may benefit from some of these approaches. For example, **Alzheimer's disease** vaccine developers have noted that their target is an aberrant conformational epitope in a normal host protein that has folded improperly. However, their vaccine attempts have been complicated by adverse T cell responses to other parts of the protein immunogens they have used in vaccinations [41]. Perhaps using a scaffolded minimal B cell epitope [11] would be a safer approach. And all vaccinologists experiencing difficulty inducing neutralizing antibody responses with a surface antigen identified as a good target by antibodies from recovering patients should perform structural studies to determine whether structural stabilization is required to maintain the conformation or access to the epitope.

Tolerance controls interfering with vaccination may be a special problem for HIV-1 vaccine developers. However, if the use of a limited number of germline antibody genes explains tolerance restrictions for many HIV BNAbs then other vaccine developers targeting rare germline genes may also encounter tolerance problems in vaccine development. They are advised to screen rare broadly neutralizing monoclonal antibodies against fixed host cells and/or a host proteome array for autoreactivity early in development to rule out a tolerance problem.

Lastly, attention is rarely focused on Fc region mediated functions until *post hoc* efficacy correlates analyses suggest their importance. This was the case with HIV vaccine development and the RV144 clinical trial. However, there are some situations where the need for Fc-mediated functions such as ADCC are predictable. Such is the case of **malaria** where an important proliferative phase of development occurs inside red blood cells which lack the MHC antigens needed to present pathogen epitopes to cytotoxic T cells. ADCC could facilitate antibodies in the killing of early infected red blood cells stemming parasite proliferation and disease.

4. T cells

a. Diversity

One major problem confronting the T cell-based HIV-1 vaccine developer is diversity, both human MHC diversity and viral diversity. Because of diversity in MHC different people will not present the same epitopes within the virus sequence to T cells. Thus, T cell responses to any given antigen are highly variable in the human population. Additionally, extreme virus sequence diversity in HIV-1 makes it even more difficult to rely on a vaccine, which necessarily will only contain a small amount of that diversity, to induce T cells responses that will provide broad protection in a large human population to the diverse population of viruses to which they are exposed. Taken together the great sequence diversity of HIV-1 and human population diversity in antigen presentation suggests that the identification of the most effective subset of target virus amino acid sequences may simplify the construction of a broadly applicable vaccine.

b. Epitopic specificity

CD8 T-cell anti-viral immune responses measured in the laboratory are not all equally effective at killing infected cells or suppressing virus proliferation to control viral load. Sometimes peptide epitopes which can be synthesized and recognized by an *in vitro* ELISPOT assay, which enumerates T cells that detect specific epitopes [42], are not efficiently processed or presented *in vivo*. In cases of pathogen antigens present in small concentrations the presentation of recognizable epitopes on the surface of the infected cell may be overwhelmed by pathogen epitopes made in much larger amounts. In other situations, escape from different responses by mutation of specific target epitopes may have very different fitness costs for the virus which will be reflected in different levels of control of viremia [43]. If the response to "easy to escape from" epitopes is immunodominant that may divert the immune response from more useful subdominant epitopic responses. In yet other cases what may be effective epitopic targets in some virus isolates may not be present in other virus strains or may not be presented by the polymorphic antigen presenting molecules of other individuals. Simply getting a response to a large enough diversity of epitopic targets as measured by an ELISPOT assay may not suffice for effective control of infection. It is believed that an effective vaccine will require focusing the immune response on the specific epitopes most commonly and efficiently presented on the surface of infected cells and those from which escape imposes the greatest fitness cost. Competing theories of how to select the optimal virus antigen target epitopes have been proposed. These theories are the consensus, mosaic, and conserved sequence approaches, and the networked epitope, functional epitope, and critical function epitope approaches (Table 2).

c. T cell quality

Other questions arise about the qualities of the T cells needed to be effective in a vaccine. The licensed vaccines where T cell responses are believed to play a role in efficacy are live-attenuated vaccines where developers have not had to reason out needed T cell quality because it was determined by innate immune responses to the attenuated pathogen which are like the unattenuated pathogen. However, vaccine developers using quite different viral or bacterial vectors to deliver pathogen antigens must worry if different innate responses to the vector will set up the body to deliver the correct quality of T cell response needed to control the new pathogen. Recently a vaccine vectored in an attenuated cytomegalovirus demonstrated some unusual efficacy in a nonhuman primate model for HIV. It appears that the underlying mechanism of protection may be CD8+ T cell recognition of virus epitopes presented by MHC-E or class II MHC instead of class I MHC presented epitopes [51]. This unusual presentation to CD8+ T cells derives from the unique vector in which the virus antigens were delivered. Another factor in the effectiveness of cytotoxic T cells is T cell avidity. In addition to the TCR-peptide-MHC interaction, T cell avidity is influenced by CD8 co-receptor expression and intracellular signaling molecules [52]. It has been noted that cytotoxic T cells induced by antigen in some vectors may have greater avidity for their targets than the same specificity of T cells induced by other vectors. Thus, it has been observed that HIV antigens vectored in fowlpox, which presents antigens via lung antigen presenting cells, induces cytotoxic T cells with greater avidity than CTL against the same antigen presented in other ways [53].

Another question related to quality is the location of the T cell response; reactive T cells may be needed in different places depending upon the transmission pathway and pathogenesis of

Table 2
Competing optimal T-cell epitope theories.

1. Consensus approach [44]
Use consensus or ancestor sequences of the virus proteins to minimize the sequence differences between vaccine constructs and the virus in circulation to which people are exposed. This is suited to situations where sufficiently broad and potent coverage of sequence diversity will enable T cell-mediated control of the infection before escape from the immune response can occur.

2. Mosaic approach [45]
A small set (2–4) of "mosaic" proteins, assembled from fragments of natural sequences via a computational optimization method, is proposed to cover most virus sequence diversity. This also is suited to a situation where broad coverage is the goal.

3. Conserved sequence approach [46,47]
Building on the observation that greater viremia control correlates with CD8+ cell recognition of epitopes in protein sequences that showed little variability (suggesting these immune responses might be more difficult to escape from because the sequence of the protein in that place was more critical to virus replication) vaccine designers use immunogen constructs that only contain the most conserved regions of the virus protein to focus the immune response.

4. Networked epitope approach [48]
Assuming epitopes with the greatest fitness cost for escape are a subset of conserved epitopes (those epitopes containing amino acid residues interacting with the largest number of other residues in the protein three-dimensional structure; i.e. highly "networked" amino acids) the vaccine developer analyzes pathogen protein structure to identify such residues then builds a vaccine immunogen from the small sequences containing those residues.

5. Functional epitope approach [49]
Investigators who have analyzed HIV-infected individuals to determine which epitopes are recognized by those that appear to control viremia best (reasoning that these are difficult to escape from epitopes) have formulated vaccine immunogens with just those epitopes.

6. Critical function epitope approach [50]
This approach targets the conserved sequences around essential virus protein protease cleavage sites reasoning that mutational escape of these epitopes will debilitate virus maturation.

the disease. As a sexually transmitted disease some HIV vaccine developers have postulated that an effective HIV vaccine should induce tissue resident memory T cells in the mucosal portal of entry of the virus. They have experimented with vectoring HIV antigens in replicating vectors that target mucosal membranes [54]. While they have been successful at inducing high levels of such antigen specific T-cells they have not reported protection in animal models, perhaps because they have failed to focus the T cell response on critical epitopes.

d. Implications for other vaccines

Developers of vaccines against most single pathogens should not have a problem with pathogen sequence diversity. However, sequence diversity may be a problem for universal influenza vaccine developers or developers of vaccines against families of viruses hoping to add a cytotoxic T cell component to antibody-based protection, as well as developers of T cell-based vaccines against parasites with extreme strain variability such as ***Theileria parva*** [55]. Unfortunately, studies of the different approaches to selecting optimal T cell epitopes for an HIV vaccine have not advanced far enough to inform developers of the very best strategy for other vaccines with epitope selection problems.

5. Vaccine vectors

a. HIV vaccine developments

Some vectors are organisms themselves like viruses or bacteria used to deliver the sequence of a protein immunogen (Table 3). The selection of such vectors is dependent upon certain factors: absence of pre-existing vector-specific immunity, nature of illness associated with the wild type vector or assurance of replication incompetence, tissue tropism, and manufacturability. Other vectors are biological information encoding molecules (DNA or mRNA) that encode the immunogen gene sequence for delivery into cells. These have a good safety profile, are relatively easy to manufacture, extremely stable and do not suffer from pre-existing vector immunity problems.

Data suggest that viral vectors, depending upon their target cells for infection as well as a multiplicity of pattern recognition and activating factors, can actively recruit elements of innate immunity to create a cytokine milieu which can influence immune responses. Most attention has focused on non-replicating viral vectors for safety concerns. However, replication-competent virally-vectored vaccines are potentially more immunogenic than replication-incompetent vectors because they may replicate in tissues to levels that exceed the total dose of replication-incompetent vectors. Also, replication-competent vectors are more easily manufactured. Novel replicating vectors with diverse biological properties are being explored to increase or prolong expression of the HIV-1 envelope protein or other HIV antigens, and to direct antigen expression to mucosal surfaces [68,69]. While much optimism surrounds the field of replicating vaccine vector development, at present safety concerns make the regulatory hurdles for testing replicating vectors higher. Earlier in HIV vaccine development the use of bacterial vectors was also explored; but work in this area has not progressed as much as viral vectors.

HIV vaccine investigators are also using information encoding molecules for vaccine development. DNA plasmid products have been a major focus because, like viral vectors they cause vaccine antigens to be made inside host cells to induce cellular immune responses. Unfortunately, early generations of DNA plasmid vaccines with HIV antigen inserts were not very immunogenic in people. Co-administration of DNA vaccines with cytokines and electroporation appears to give more robust T-cell responses at lower vaccine doses, and with fewer vaccinations [66]. Investigators are also experimenting with a strategy to direct vehicle-induced vaccine immune responses toward mucosal portals of HIV entry by supplementing DNA vaccines with mucosal chemokines [67]. More recently HIV vaccine developers have been exploring mRNA for delivery of HIV antigens [70], especially for the delivery of expensive to manufacture envelope protein. Advances in nanoparticle delivery systems and optimization of mRNA sequence to avoid activating degradation mechanisms and innate immune system sensors have made mRNA a very attractive vaccine vector. Also, various molecular modifications have made mRNA more stable and highly translatable. Cytokine and immunomodulatory molecules can be encoded in mRNA and delivered at the same time allowing for the modulation of the quality of the immune response.

Table 3
Viral, bacterial and gene sequence vectors used for candidate HIV vaccine development.

1. Non-replicating viral vectors [56]
- Canarypox (ALVAC)
- Vaccinia vectors (NYVAC and MVA)
- Fowlpox
- Adenoviruses
- Rhabdoviruses
- Alphaviruses

2. Replicating viral vectors [57]
- Vesicular Stomatitis virus (VSV)
- Cytomegalovirus (CMV)
- Adenoviruses
- Poxviruses
- Canine Distemper virus [58]
- Sendai virus [59]
- Yellow Fever virus [60]
- Measles virus [61]
- Rubella virus [62]

3. Bacterial vectors
- Salmonella [63]
- Listeria [64]
- BCG [65]

4. Information encoding biomolecules
- Plasmid DNA [66]
- mRNA [67]

b. Implications for other vaccines

HIV vaccine investigators have put much effort into developing novel vaccine vectors for delivery of vaccine antigens. Vaccine vector developers interested in other vaccines have frequently used HIV-1 antigens as models because of the greater availability of HIV vaccine development funding. This is especially true for those interested in T cell functions in protection because these systems will express antigens inside cells which is necessary to induce T cell responses; but even some antibody-based vaccine designers are looking at these delivery systems to bypass expensive, time-consuming and often complicated protein vaccine manufacturing problems. Investigators developing vaccines for other pathogens such as **Ebola** [71], **tuberculosis** [72], and **Zika** [73,74] have already made use of vectors developed for HIV vaccines.

6. Research support

a. Research structure and funding

Until this point, the discussion in this review has focused on specific scientific obstacles in HIV vaccine development. However, sometimes the structure of funding scientific research can present an obstacle to performing needed research. The basic mechanism for biomedical research funding at the NIH is the unsolicited

5-year R01 research grant. This funding mechanism is superb for supporting investigator-initiated, hypothesis-driven basic research. For small scale, highly speculative research designed to obtain preliminary data to compete for an R01 grant there are smaller 2-year R21 or R03 mechanisms. However, a gap exists in funding for large scale, data-landscape developing work sometimes needed to prepare a field for hypothesis-testing research [75].

Responding to an international group of senior HIV vaccine investigators [76] the NIAID launched the Center for HIV/AIDS Vaccine Immunology (CHAVI) a large budget, multi-disciplinary, extramural effort in HIV vaccine development in 2005. CHAVI was tasked with investigating early HIV-1 infection to discover why the early host immune responses were unable to contain and control infection. Although the award competition attracted investigators anxious to design and test candidate HIV vaccines, they were constrained to detailed exploration of early immune responses to infection in order to establish a richer database to facilitate themselves and others formulating better hypotheses to test in vaccine development. Key to this exploration was recruitment of a cohort of individuals extremely early in infection to be followed through development of early immune responses and early stages of viral control. Facilitated by the development of a novel strategy for diagnosis of acutely infected individuals [77] CHAVI assembled, and followed with intense sampling, a cohort of more than 300 acutely infected subjects. This led to the clear establishment of the cytotoxic T cell response as a determinant of early virus control [43], as well as the crucial role of affinity maturation in the development of broadly neutralizing antibody responses [78], the co-evolution of virus envelope and antibody [21], the B cell lineage-based design hypothesis for immunization [15], and the possible contribution of non-neutralizing antibodies to protection [33]. After seven years the award was recompeted as the Center(s) for HIV/AIDS Vaccine Immunology and Immunogen Discovery (CHAVI-ID) because it was felt that enough understanding had been developed to move into vaccine immunogen discovery and design. Much of the perspective discussed in this article comes from the work of CHAVI and CHAVI-ID investigators.

b. Community engagement

Another lesson learned from HIV vaccine development is the importance of community engagement. This is not just a matter of recruitment for clinical trials. The HIV-infection at-risk Men Who Have Sex with Men (MSM) population in the United States was pivotal in motivating all types of HIV/AIDS research. The rapid pace of antiretroviral drug development would not have occurred without the push for mobilization of resources from this community. HIV vaccine development has also benefited from the mobilization of the large amount of resources scientific investigators have needed to pursue the work. But mobilization of resources is not the whole story. Many HIV vaccine investigators have friends or family who have been infected and some who have died. Their suffering and their struggles have motivated the field and continue to inspire it.

c. Implications for other vaccines

The large funding approach of CHAVI is not needed for most vaccine development. However, fields where scientists have been struggling for a long time with at best marginal results and where the public health need is great might benefit from a CHAVI-like effort in assembling a crucial clinical cohort for intensive sample collection and immunologic study to develop the data-landscape for better hypothesis formulation. The fields of **tuberculosis**, **malaria**, and **syphilis** vaccine development stand out as areas funders should consider for such an effort.

Also, the importance of community in vaccine development is not a new story. In the 1940 s and 1950 s the effort at that time to develop a **polio** vaccine was similarly spurred by community engagement – the March of Dimes [79]. The bigger the scientific challenge the more certain it is that a long-term commitment will be needed and will only benefit from dedicated community support.

7. Conclusions

HIV vaccine developers have experienced repeated failure using the standard approaches to vaccine development that have worked for so many other pathogens. This has forced them to consider immune responses in greater depth and detail than other vaccinologists. It has led to a recognition of the importance of epitopic specificity in both antibody and T cell responses. Also, it has led to an understanding of the importance of affinity maturation in antibody responses and the quality of T cell responses in T cell-mediated immunity. It has advanced the development of many novel vaccine vectors and vehicles that are now available for use in other vaccines. Further it has focused attention on the impact of research funding mechanisms and community engagement in vaccine development. Scientists attempting to make vaccines against other pathogens will probably not encounter the many difficulties that HIV vaccine scientists have encountered. Yet they may encounter some of the same obstacles. Recognition and understanding these obstacles are contributions HIV vaccine development makes to vaccinology in general. Leading HIV scientists believe that the only way to truly end this epidemic is with a vaccine [80]. HIV vaccine investigators are determined to make an effective HIV vaccine. However, new strategies and technologies will be needed. The determination exists among HIV vaccine scientists to develop these new strategies and technologies. These new strategies and technologies will contribute even more to the science of vaccinology.

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Conflicts of interest

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References

[1] Sefton AM. The great pox that was...syphilis. J Appl Microbiol 2001;91(4):592–6.
[2] Schwetz TA, Fauci AS. The extended impact of human immunodeficiency virus/AIDS research. J Infect Dis 2019;219(1):6–9. https://doi.org/10.1093/infdis/jiy441.

[3] Milone MC, O'Doherty U. Clinical use of lentiviral vectors. Leukemia 2018;32 (7):1529–41. https://doi.org/10.1038/s41375-018-0106-0.
[4] Rappuoli R. Reverse vaccinology. Curr Opin Microbiol 2000;3(5):445–50.
[5] Rappuoli R, Bottomley MJ, D'Oro U, Finco O, De Gregorio E. Reverse vaccinology 2.0: human immunology instructs vaccine antigen design. J Exp Med 2016;213 (4):469–81. https://doi.org/10.1084/jem.20151960.
[6] FDA guidance for Industry. Guidance for the evaluation of combination vaccines for preventable diseases: production, testing and clinical studies. US Dept HHS FDA CBER; 1997: April. Docket No. 97N-0029. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM175909.pdf.
[7] Burton DR, Hangartner L. Broadly neutralizing antibodies to HIV and their role in vaccine design. Annu Rev Immunol 2016;34:635–59. https://doi.org/10.1146/annurev-immunol-041015-055515.
[8] Fazekas deSt.Groth S, Webster RG. Disquisitions on original antigenic sin. J Exp Med 1966;124(3):331–45.
[9] Vatti A, Monsalve DM, Pacheco Y, Chang C, Anaya J-M, Gershwin ME. Original antigenic sin: a comprehensive review. J Autoimmun 2017;83:12–21. https://doi.org/10.1016/j.jaut.2017.04.008.
[10] McLellan JS, Chen M, Leung S, Graepel KW, Du X, Yang Y, et al. Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody. Science 2013;340(6136):1113–7. https://doi.org/10.1126/science.1234914.
[11] Azoitei ML, Ban YE, Julien JP, Bryson S, Schroeter A, Kalyuzhniv O, et al. Computational design of high-affinity epitope scaffolds by backbone grafting of a linear epitope. J Mol Biol 2012;415(1):175–92. https://doi.org/10.1016/j.jmb.2011.10.003.
[12] Azoitei ML, Correia BE, Ban YE, Carrico C, Kalyuzhniv O, Chen L, et al. Computation-guided backbone grafting of a discontinuous motif onto a protein scaffold. Science 2011;334(6054):373–6. https://doi.org/10.1126/science.1209368.
[13] Sanders RW, Vesanen M, Schuelke N, Master A, Schiffner L, Kalyanaraman R, et al. Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. J Virol 2002;76(17):8875–89.
[14] LeBranche CC, McGuire AT, Gray MD, Behrens S, Zhou T, Sattentau QJ, et al. HIV-1 envelope glycan modifications that permit neutralization by germline-reverted VRC01-class broadly neutralizing antibodies. PLoS Pathog 2018;14 (11):e1007431. https://doi.org/10.1371/journal.ppat.1007431.
[15] Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. Nat Biotechnol 2012;30 (5):423–33. https://doi.org/10.1038/nbt.2197.
[16] Xiao X, Chen W, Feng Y, Dimitrov DS. Maturation pathways of cross-reactive HIV-1 neutralizing antibodies. Viruses 2009;1(3):802–17. https://doi.org/10.3390/v1030802.
[17] Klein JS, Bjorkman PJ. Few and far between: how HIV may be evading antibody avidity. PLoS Pathog 2010;6(5):e1000908. https://doi.org/10.1371/journal.ppat.1000908.
[18] Schiller J, Chackerian B. Why HIV virions have low numbers of envelope spikes: implications for vaccine development. PLoS Pathog 2014;10(8): e1004254. https://doi.org/10.1371/journal.ppat.1004254.
[19] Wiehe K, Bradley T, Meyerhoff RR, Hart C, Williams WB, Easterhoff D, et al. Functional relevance of improbable antibody mutations for HIV broadly neutralizing antibody development. Cell Host Microbe 2018;23(6):759–65. https://doi.org/10.1016/j.chom.2018.04.018.
[20] Ovchinnikov V, Louveau JE, Barton JP, Karplus M, Chakraborty AK. Role of framework mutations and antibody flexibility in the evolution of broadly neutralizing antibodies. Elife 2018;7:e33038. https://doi.org/10.7554/eLife.33038.
[21] Liao H-X, Lynch R, Zhou T, Gao F, Alam SM, Boyd SD, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature 2013;496 (7446):469–76. https://doi.org/10.1038/nature12053.
[22] Burton DR. Advancing an HIV vaccine; advancing vaccinology. Nat Rev Immunol 2019;19(2):77–8. , http://www.nature.com/articles/s41577-018-0103-6. https://doi.org/10.1038/s41577-018-0103-6.
[23] Jardine JG, Sok D, Julien JP, Briney B, Sarkar A, Liang CH, et al. Minimally mutated HIV-1 broadly neutralizing antibodies to guide reductionist vaccine design. PLoS Pathog 2016;12(8):e1005815. https://doi.org/10.1371/journal.ppat.1005815.
[24] Haynes BF, Moody AM, Verkoczy L, Kelsoe G, Alam SM. Antibody polyspecificity and neutraliziation of HIV-1: a hypothesis. Hum Antibodies 2005;14(3–4):59–67.
[25] Bricault CA, Yusim K, Seaman MS, Yoon H, Theiler J, Giorgi EE, et al. HIV-1 neutralizing antibody signatures and application to epitope targeted vaccine design. Cell Host Microbe 2019;25(1):59–72. https://doi.org/10.1016/j.chom.2018.12.001.
[26] Yang G, Holl TM, Liu Y, Li Y, Lu X, Nicely NI, et al. Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. J Exp Med 2013;210(2):241–56. https://doi.org/10.1084/jem.20121977.
[27] Liu M, Yang G, Wiehe K, Nicely NI, Vandergrift NA, Rountree W, et al. Polyreactivity and autoreactivity among HIV-1 antibodies. J Virol 2015;89 (1):784–98. https://doi.org/10.1128/JVI.02378-14.
[28] Verkoczy L, Alt FW, Tian M. Human Ig knockin mice to study the development and regulation of HIV-1 broadly neutralizing antibodies. Immuno Rev 2017;275(1):89–107. https://doi.org/10.1111/imr.12505.
[29] Kelsoe G, Haynes BF. Host controls of HIV broadly neutralizing antibody development. Immunol Rev 2017;275(1):79–88. https://doi.org/10.1111/imr.12508.
[30] Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, et al. Fc receptor but not complement binding is important in antibody protection against HIV. Nature 2007;449(7158):101–4. https://doi.org/10.1038/nature06106.
[31] Parsons MS, Lee WS, Kristensen AB, Amarasena T, Khoury G, Wheatley AK, et al. Fc-dependent functions are redundant to efficacy of anti-HIV antibody PGT121 in macaques. J Clin Invest 2019;129(1):182–91. https://doi.org/10.1172/JCI122466.
[32] Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009;361(23):2209–20. https://doi.org/10.1056/NEJMoa0908492.
[33] Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 2012;366(14):1275–86. https://doi.org/10.1056/NEJMoa1113425.
[34] Huang Y, Ferrari G, Alter G, Forthal DN, Kappes JC, Lewis GK, et al. Diversity of antiviral IgG effector activities observed in HIV-infected and vaccinated subjects. J Immunol 2016;197(12):4603–12. https://doi.org/10.4049/jimmunol.1601197.
[35] Alter G, Dowell KG, Brown EP, Suscovich TJ, Mikhailova A, Mahan AE, et al. High-resolution definition of humoral immune response correlates of effective immunity against HIV. Mol Syst Biol 2018;14(3):e7881. https://doi.org/10.15252/msb.20177881.
[36] Castillo-Menendez LR, Witt K, Espy N, Princiotto A, Madani N, Pacheco B, et al. Comparison of uncleaved and mature human immunodeficiency virus membrane envelope glycoprotein trimers. J Virol 2018;92(12):e00277–e318. https://doi.org/10.1128/JVI.00277-18.
[37] Neu KE, Dunand CJH, Wilson PC. Heads, stalks and everything else: how can antibodies eradicate influenza as a human disease? Curr Opin Immunol 2016;42:48–55. https://doi.org/10.1016/j.coi.2016.05.012.
[38] McCoy LE, Burton DR. Identification and specificity of broadly neutralizing antibodies against HIV. Immunol Rev 2017;275(1):11–20. https://doi.org/10.1111/imr.12484.
[39] McLellan JS, Correia BE, Chen M, Yang Y, Graham BS, Schief WR, et al. Design and characterization of epitope-scaffold immunogens that present the motavizumab epitope from respiratory syncytial virus. J Mol Biol 2011;409 (5):853–66. https://doi.org/10.1016/j.jmb.2011.04.044.
[40] McLellan JS, Chen M, Joyce MG, Sastry M, Stewart-Jones GBE, Yang Y, et al. Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. Science 2013;342(6158):592–8. https://doi.org/10.1126/science.1243283.
[41] Marciani DJ. A retrospective analysis of the Alzheimer's disease vaccine progress – the critical need for new development strategies. J Neurochem 2016;137(5):687–700. https://doi.org/10.1111/jnc.13608.
[42] Flaxman A, Ewer KJ. Methods for measuring T-cell memory to vaccination: from mouse to man. Vaccines 2018;6(3):E43. https://doi.org/10.3390/vaccines6030043.
[43] Goonetilleke N, Liu MK, Salazar-Gonzalez JF, Ferrari G, Giorgi E, Ganusov VV, et al. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J Exp Med 2009;206(6):1253–72. https://doi.org/10.1084/jem.20090365.
[44] Gaschen B, Taylor J, Yusim K, Foley B, Gao F, Lang D, et al. Diversity considerations in HIV-1 vaccine selection. Science 2002;296(5577):2354–60. https://doi.org/10.1126/science.1070441.
[45] Fischer W, Perkins S, Theiler J, Bhattacharya T, Yusim K, Funkhouser R, et al. Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. Nat Med 2007;13(1):100–6. https://doi.org/10.1038/nm1461.
[46] Kunwar P, Hawkins N, Dinges WL, Liu Y, Gabriel EE, Swan DA, et al. Superior control of HIV-1 replication by CD8+ T cells targeting conserved epitopes: implications for HIV vaccine design. PLoS ONE 2013;8(5):e64405. https://doi.org/10.1371/journal.pone.0064405.
[47] Rosario M, Bridgeman A, Quakkelaar ED, Quigley MF, Hill BJ, Knudsen ML, et al. Long peptides induce polyfunctional T cells against conserved regions of HIV-1 with superior breadth to single-gene vaccines in macaques. Eur J Immunol 2010;40(7):1973–84. https://doi.org/10.1002/eji.201040344.
[48] Gaiha GD, Rossin EJ, Urbach J, Landeros C, Collins DR, Nwonu C, et al. Structural topology defines protective CD8+ T cell epitopes in the HIV proteome. Science 2019 [in press].
[49] Mothe B, Hu X, Llano A, Rosati M, Olvera A, Kulkarni V, et al. A human immune data-informed vaccine concept elicits strong and broad T-cell specificities associated with HIV-1 control in mice and macaques. J Transl Med 2015;13:60. https://doi.org/10.1186/s12967-015-0392-5.
[50] Luo M, Capina R, Daniuk C, Tuff J, Peters H, Kimani M, et al. Immunogenicity of sequences around HIV-1 protease cleavage sites: potential targets and population coverage analysis for a HIV vaccine targeting protease cleavage sites. Vaccine 2013;31(29):3000–8. https://doi.org/10.1016/j.vaccine.2013.04.057.
[51] Fruh K, Picker L. CD8+ T cell programming by cytomegalovirus vectors: applications in prophylactic and therapeutic vaccination. Curr Opin Immunol 2017;47:52–6. https://doi.org/10.1016/j.coi.2017.06.010.
[52] von Essen MR, Kongsbak M, Geisler C. Mechanisms behind functional avidity maturation in T cells. Clin Dev Immunol 2012;2012:163453. https://doi.org/10.1155/2012/163453.

[53] Trivedi S, Jackson RJ, Ranasinghe C. Different HIV pox viral vector-based vaccines and adjuvants can induce unique antigen presenting cells that modulate CD8 T cell avidity. Virology 2014;468–470:479–89. https://doi.org/10.1016/j.virol.2014.09.004.
[54] Masopust D. Developing an HIV cytotoxic T-lymphocyte vaccine: issues of CD8 T-cell quantity, quality and location. J Intern Med 2009;265(1):125–37. https://doi.org/10.1111/j.1365-2796.2008.02054.x.
[55] Nene V, Svitek N, Toye P, Golde WT, Barlow J, Harndahl M, et al. Designing bovine T cell vaccines via reverse immunology. Ticks Tick Borne Dis 2012;3(3):188–92. https://doi.org/10.1016/j.ttbdis.2011.12.001.
[56] Johnson JA, Barouch DH, Baden LR. Nonreplicating vectors in HIV vaccines. Curr Opin HIV AIDS 2013;8(5):412–20. https://doi.org/10.1097/COH.0b013e328363d3b7.
[57] Stephenson KE, D'Couto HT, Barouch DH. New concepts in HIV-1 vaccine development. Curr Opin Immunol 2016;41:39–46. https://doi.org/10.1016/j.coi.2016.05.011.
[58] Zhang X, Wallace O, Wright KJ, Backer M, Coleman JW, Koehnke R, et al. Membrane-bound SIV envelope trimers are immunogenic in ferrets after intranasal vaccination with a replication-competent canine distemper virus vector. Virology 2013;446(1–2):25–36. https://doi.org/10.1016/j.virol.2013.07.012.
[59] Nyombayire J, Anzala O, Gazzard B, Karita E, Bergin P, Hayes P, et al. First-in-human evaluation of the safety and immunogenicity of an intranasally administered replication-competent Sendai virus-vectored HIV type 1 gag vaccine: induction of potent T-cell or antibody responses in prime-boost regimens. J Infect Dis 2017;215(1):95–104. https://doi.org/10.1093/infdis/jiw500.
[60] Yu JS, Liao HX, Pritchett J, Bowman C, Vivian C, Parks R, et al. Development of a recombinant yellow fever vector expressing a HIV clade c founder envelope gp120. J Virol Methods 2017;249:85–93. https://doi.org/10.1016/j.jviromet.2017.08.012.
[61] Stebbings R, Li B, Lorin C, Koutsoukos M, Fevrier M, Mee ET, et al. Immunogenicity of a recombinant measles HIV-1 subtype c vaccine. Vaccine 2013;31(51):6079–86. https://doi.org/10.1016/j.vaccine.2013.09.072.
[62] Virnik K, Nesti E, Dail C, Hockenbury M, Ni Y, Felber BK, et al. Expression of complete SIV p27 gag and HIV gp120 engineered outer domains targeted by broadly neutralizing antibodies in live rubella vectors. Vaccine 2017;35(24):3272–8. https://doi.org/10.1016/j.vaccine.2017.04.047.
[63] Chin'ombe N, Ruhanya V. Recombinant salmonella bacteria vectoring HIV/AIDS vaccines.. Open Virol J 2013;7:121–6. https://doi.org/10.2174/1874357901307010121.
[64] Sciaranghella G, Lakhashe SK, Ayash-Rashkovsky M, Mirshahidi S, Siddappa NB, Novembre FJ, et al. A live attenuated Listeria monocytogenes vaccine vector expressing SIV gag is safe and immunogenic in macaques and can be administered repeatedly. Vaccine 2011;29(3):476–86. https://doi.org/10.1016/j.vaccine.2010.10.072.
[65] Hart BE, Asrican R, Lim SY, Sixsmith JD, Lukose R, Souther SJ, et al. Stable expression of lentiviral antigens by quality-controlled recombinant Mycobacterium bovis BCG vectors. Clin Vaccine Immunol 2015;22(7):726–41. https://doi.org/10.1128/CVI.00075-15.
[66] Kalams SA, Parker SD, Elizaga M, Metch B, Edupuganti S, Hural J, et al. Safety and comparative immunogenicity of an HIV-1 DNA vaccine in combination with plasmid interleukin 12 and impact of intramuscular electroporation for delivery. J Infect Dis 2013;208(5):818–29. https://doi.org/10.1093/infdis/jit236.
[67] Kutzler MA, Wise MC, Hutnick NA, Moldoveanu Z, Hunter M, Reuter M, et al. Chemokine-adjuvanted electroporated DNA vaccine induces substantial protection from simian immunodeficiency virus vaginal challenge. Mucosal Immunol 2016;9(1):13–23. https://doi.org/10.1038/mi.2015.31.
[68] Barouch DH, Picker LJ. Novel vaccine vectors for HIV-1. Nat Rev Microbiol 2014;12(11):765–71. https://doi.org/10.1038/nrmicro3360.
[69] Parks CL, Picker LJ, King CR. Development of replication-competent viral vectors for HIV vaccine delivery. Curr Opin HIV AIDS 2013;8(5):402–11. https://doi.org/10.1097/COH.0b013e328363d389.
[70] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines – a new era in vaccinology. Nat Rev Drug Discov 2018;17(4):261–79. https://doi.org/10.1038/nrd.2017.243.
[71] Watle SV, Norheim G, Rottingen JA. Ebola vaccines – where are we? Hum Vaccin Immunother 2016;12(10):2700–3. https://doi.org/10.1080/21645515.2016.1217372.
[72] Hansen SG, Zak DE, Xu G, Ford JC, Marshall EE, Malouli D, et al. Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine. Nat Med 2018;24(2):130–43. https://doi.org/10.1038/nm.4473.
[73] Emanuel J, Callison J, Dowd KA, Pierson TC, Feldmann H, Marzi A. A VSV-based Zika virus vaccine protects mice from lethal challenge. Sci Rep 2018;8(1):11043. https://doi.org/10.1038/s41598-018-29401-x.
[74] Pardi N, Hogan MJ, Pelc RS, Muramatsu H, Andersen H, DeMaso CR, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. Nature 2017;543(7644):248–51. https://doi.org/10.1038/nature21428.
[75] Nabel GJ. The coordinates of truth. Science 2009;326(5949):53–4. https://doi.org/10.1126/science.1177637.
[76] Klausner RD, Fauci AS, Corey L, Nabel GJ, Gayle H, Berkley S, et al. The need for a global HIV vaccine enterprise. Science 2003;300(5628):2036–9. https://doi.org/10.1126/science.1086916.
[77] Pilcher CD, Price MA, Hoffman IF, Galvin S, Martinson FE, Kazembe PN, et al. Frequent detection of acute primary HIV infection in men in Malawi. AIDS 2004;18(3):517–24.
[78] Moody MA, Yates NL, Amos JD, Drinker MS, Eudailey JA, Gurley TC, et al. HIV-1 gp120 vaccine induces affinity maturation in both new and persistent antibody clonal lineages. J Virol 2012;86(14):7496–507. https://doi.org/10.1128/JVI.00426-12.
[79] Larsen D. The march of dimes and polio: lessons in vaccine advocacy for health educators. Am J Health Ed 2012;43(1):47–54.
[80] Fauci AS, Marston HD. Ending AIDS – is an HIV vaccine necessary? N Engl J Med 2014;370(6):495–8. https://doi.org/10.1056/NEJMp1313771.