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Review article

HIV-vaccines: lessons learned and the way forward

Jean-Louis Excler

International AIDS Vaccine Initiative, New York, NY 10038*, USA*

A safe and efficacious preventive HIV vaccine, as part of a comprehensive prevention program, remains among the highest public health priorities. It would be the best tool that could reduce the spread of HIV significantly in the long run. Current AIDS vaccine candidates are unable to induce neutralizing antibodies against primary HIV isolates or only to a very limited and narrow extent, representing a major obstacle in the development of an efficacious HIV vaccine. Clinical efforts have mainly focused on T-cell vaccines such as DNA and various recombinant vectors alone or in prime-boost regimens. The Merck Ad5 vaccine not only failed to show efficacy but also was associated with increased risk of HIV acquisition in vaccinees in a Phase IIb trial. While gp120 alone was not efficacious, the ALVAC prime and gp120 boost regimen showed 31% efficacy in a Phase III trial in Thailand. These contrasting results illustrate the limitations of available laboratory assays to assess the vaccine-induced immune responses and the lack of understanding of immune correlates of protection. Efforts should therefore focus on developing vaccine candidates inducing broadly neutralizing antibodies. Similarly, new vector strategies such as replicating vectors should be explored to induce strong and broad T-cell responses in the systemic and mucosal compartments. Innovation in immune assay development and testing algorithms is critically needed. The standardization of more relevant and predictive non-human primate models for immunogenicity and efficacy studies will contribute to better and faster vaccine assessment. HIV vaccine development requires innovative ideas and a sustained long-term commitment of the scientific community, civil society, politicians, and donors and participants for clinical research.

Keywords: Clinical trial, efficacy, HIV, Thailand, vaccine

In 2008, UNAIDS estimated that 2.7 million people worldwide became newly infected with HIV and 2.1 million people died from AIDS. The total number of people living with HIV is estimated to be near 33.4 million people, with almost two-thirds living in Africa. In Asia and the Pacific, 4.7 million adults and children are estimated to be living with HIV [1] Despite tremendous efforts in HIV prevention, Thailand being on the forefront [2, 3], the epidemic is still rampant. The development of a safe and efficacious preventive HIV vaccine, as part of a comprehensive prevention program, remains among the highest public health priorities. It would be the best long-term tool that could reduce the spread of HIV significantly.

Correspondence to: Dr. Jean-Louis Excler, Senior Director, Medical Affairs, International AIDS Vaccine Initiative, 110 William Street, 27th Floor, New York, NY 10038, USA. E-mail: jlexcler@iavi.org

Despite the enormous efforts deployed by the international scientific community over the past 20 years, the discovery of a preventive HIV vaccine has remained elusive. This is mainly because of the unprecedented scientific and non-scientific obstacles (**Table 1**) presented by the virus [4-7]. The immune correlates and the quality and magnitude of the immune response needed to confer protection against HIV infection are still unclear [8].

The efficacy of most currently licensed viral vaccines in public health use is correlated with neutralizing antibodies against the infectious agent. The first HIV vaccine approaches investigated different forms of the HIV envelope protein. Unfortunately, these products failed to protect volunteers in two phase III efficacy trials [9, 10]. Current AIDS vaccine candidates are unable to induce neutralizing antibodies against primary HIV isolates or only to a very limited and narrow extent

Table 1. Scientific and non-scientific obstacles to the development of an HIV vaccine.

- Extensive viral subtype and sequence diversity
- Early establishment of latent viral reservoirs
- Narrow window of opportunity for the immune system to clear initial infection
- Immune correlates of protection unclear to unknown
- Viral escape of humoral and cellular immune responses
- Conserved antibody targets on the outer envelope protein are hidden
- Lack of a predictive animal model
- Limited interest of the pharmaceutical industry
- Long term sustained commitment from politicians and donors

[11], representing a major obstacle in the development of an effective HIV vaccine [12].

Natural history studies of HIV infection provided growing evidence of the role of T cells in the control of disease progression [13, 14]. The immune response elicited by a successful vaccine likely will require both antibodies and T cells that recognize, neutralize, and/or inactivate diverse strains of HIV, and that reach the site of infection before infection becomes irreversibly established [15]. Given the hurdles of eliciting broadly neutralizing antibodies (bNAb), the focus of HIV vaccine development turned to evaluating T-cell vaccines capable of reducing viral replication after infection as an intermediate step until bNAb-inducing immunogens are identified. The control of viral replication could conceivably slow the rate of disease progression and/or reduce transmission of HIV from the infected vaccinee to his/her partner [16]. Indeed, several non-human primate (NHP) challenge studies demonstrated that vaccine candidates that elicited T-cell responses enabled animals to better control viral replication after challenge with a pathogenic virus [17-19]. However, the inclusion of envelope in some of these vaccines, which leads to antibody induction, and the use of challenge strains that were homologous to the vaccine inserts suggest that most of these studies were not a stringent test of the T-cell vaccine concept [20].

The goal of a vaccination regimen designed to induce cell-mediated immune (CMI) responses should therefore be to reduce the plasma viral load at set point and preserve memory CD4+ lymphocytes. Clinical efforts have mainly focused on CMI-inducing vaccines such as DNA and various recombinant vectors alone or in prime-boost regimens [21-24] (**Table 2**). In human trials, the response to HIV genes inserted into poxvirus and adenovirus vectors with or without DNA prime has been variable in frequency, magnitude, and breadth [25-29]. Such regimens at various degrees have conferred protection in the SHIV and SIV challenge macaque models [30-33].

One of these vectors, the replication-incompetent Merck rAd5 HIV vaccine was tested in two phase IIb 'test-of-concept' studies [34] to determine whether HIV-1-specific CMI responses induced by the vaccine would prevent HIV-1 infection or would reduce viral loads after acquisition of HIV infection by vaccinees. The STEP study (HVTN 502) enrolled 3,000 subjects in the Americas, the Caribbean, and Australia [35, 36], while the Phambili study (HVTN 503) was a parallel 3,000-subject study in South Africa [37]. HVTN 502 was unexpectedly terminated at the first planned interim analysis when the Data and Safety Monitoring Board declared futility in achieving the study primary endpoints. Moreover, in subjects with pre-existing Ad5-specific neutralizing antibody titres, a greater number of HIV-1 infections occurred in vaccinees than in placebo recipients. Although the biological basis for this observation remains unclear, these data suggest that vaccination with rAd5 vectors may be associated with an increased risk of HIV-1 acquisition in this subgroup. Post-hoc multivariate analysis further suggested that the greatest increased risk was in men who had pre-existing Ad5-specific neutralizing antibodies and who were uncircumcised.

Although the Merck Ad5 vaccine failed to show efficacy, the trial demonstrated that the current SHIV NHP challenge model is misleading and inadequate for evaluating T-cell vaccines, while the SIV challenge model seems more predictive [20]. Other conclusions were that immunity to vectors, including at the tissue level, should be evaluated in future clinical studies [25] and that smaller efficacy trial designs can yield valuable information to guide future efforts [38]. The design of parallel NHP and clinical studies for a more direct comparison of the results would help identifying and validating the best predictive NHP model(s) for immunogenicity and efficacy.

Table 2. Vaccine concepts currently in clinical *or preclinical* development.

- Soluble subunits and peptides with or without adjuvant
- DNA administered by needle injection or by electroporation
- Non-replicating vectors:
  - ALVAC, fowlpox, MVA, NYVAC, Vaccinia Tiantan, Adenovirus [5, 6, 26, 35]
- Prime-boost regimens:
  - Vector + subunit, DNA + subunit, DNA + vector, vector + vector
- *Replicating vectors:*
  - Vaccina Tiantan, *Measles, Sendai, CMV, Ad4, Ad7, VSV, NDV, CDV, YF, HSV, Reo, VEEV*

New areas for innovation and improvement

- Design and synthesize antigens capable of inducing broadly neutralizing antibodies
- Refine and develop new immune assessment tools:
  - ADCC, ADCVI
  - Lymphoproliferation
  - Antibody avidity
  - *In-vitro* viral inhibition
  - Mucosal humoral and cell-mediated immune response
  - Transcytosis
- Determine more relevant NHP challenge models
- Explore mucosal immune response in NHP and human vaccine studies
- Conduct NHP studies parallel to vaccine clinical trials

ALVAC: recombinant canarypox vector, NYVAC: attenuated non-replicating vaccinia vector, MVA: modified vaccinia Ankara (non-replicating vaccinia-derived virus), CMV: cytomegalovirus, Ad: adenovirus, VSV: vesicular stomatitis virus, CDV: canine distemper virus, YF: yellow fever virus - HSV: herpes simplex virus type 1, Reo: reovirus, VEEV: venezuelan equine encephalitis virus, ADCC: antibody-dependent cellular cytotoxicity, ADCVI: antibody-dependent cell-mediated virus inhibition, NHP: non-human primate.

The long awaited breaking news in HIV vaccine development came recently from Thailand. The results of a Phase III (RV144) conducted by the Thai Ministry of Public Health in collaboration with a team of leading Thai and US researchers and coordinated by the US Military HIV Research Program, were recently released [39, 40]. The trial tested a prime-boost regimen consisting of priming with ALVAC-HIV (vCP1521), a live, recombinant, non-replicating canarypox viral vector encoding subtype B gag/pro and subtype E env gp120 linked to a portion of the subtype B gp41 domain and boosting with AIDSVAX gp120 B/E, a genetically engineered soluble gp120 Env protein with equal concentrations of the subtype B and E gp120 antigen. Phase I/II trials were previously conducted showing the vaccine was safe and well tolerated and immunogenic [41-43]. AIDSVAX gp120 B/E previously tested alone in a Phase III trial in Thailand showed no efficacy [44]. RV144 enrolled 16,402 Thai volunteers. The co-primary endpoints were prevention of HIV infection and reduction in plasma RNA viral load. Although a criticized trial [45], RV144 results showed that the vaccine regimen is safe and 31.2 % efficacious in preventing HIV infection with no effect on RNA viral load. While this is a modest level of efficacy, it represents a major step forward for HIV vaccines, providing the first evidence that a safe and effective preventive HIV vaccine is possible. Additional research is needed to better understand how the regimen reduced study volunteers' risk of HIV infection to help guiding further improvements.

The way forward

The development of immunogens that elicit bNAb remains a high priority research goal. All of the known bNAb (4E10, 2F5, b12, 2G12) provide protection in the best available primate models and therefore are considered to be the types of antibodies that should be elicited by a vaccine [46]. Unfortunately, these antibodies recognize conserved recessed viral epitopes that have so far failed to elicit broadly neutralizing responses when incorporated into a diverse range of immunogens [47, 48]. In addition, bNAb 2G12

and 2F5 do not neutralize HIV-1 clade C viruses as the epitope for these antibodies are absent in these viruses. Broadly neutralizing antibodies that develop over time in some HIV-1–infected individuals, define critical epitopes for HIV vaccine design. Using a systematic approach, researchers from IAVI and Scripps Research Institute and two biotechnology companies, Monogram Biosciences and Theraclone Sciences, examined neutralization breadth in the sera of about 1800 HIV-1-infected individuals, primarily infected with non-clade B viruses, and have selected donors for monoclonal antibody (mAb) generation [49]. They used a high-throughput micro-neutralization screen of antibody-containing culture supernatants from approximately 30,000 activated memory B cells from a clade A-infected African donor to isolate two broad and potent mAbs (PG9 and PG16) that target a broadly neutralizing epitope. This epitope is expressed on trimeric Envelope protein and spans over conserved regions of variable loops of the gp120 subunit. The results provide a framework for the design of new vaccine candidates eliciting bNAb responses [50]. Long-lasting neutralizing activity in serum of monkeys has been induced by vaccination with an adeno-associated virus gene transfer vector expressing antibodies or antibody-like immunoadhesins with predetermined SIV specificity and complete protection against intravenous SIV challenge has been observed. SIV-specific molecules are endogenously synthesized in myofibers and passively distributed to the circulatory system. This strategy bypasses the adaptive immune system and holds considerable promise as a unique approach to an effective HIV vaccine [51].

Novel vector approaches may be limited by pre-existing or vaccine-induced anti-vector immune responses that may blunt vaccine-induced T-cell responses [43, 52, 53]. A vector that undergoes only a single round of replication failed to provide protection against challenge with pathogenic SIV, in contrast to the robust protection conferred with live-attenuated SIV. This has prompted researchers to develop replicating vectors (**Table 2**) [54]. New vaccination regimens such as heterologous prime-boost regimens, aiming at increasing the breadth and magnitude of the CMI response and targeting the mucosal compartment should be intensely pursued [55].

The STEP and the Thai efficacy trials illustrate well the limits and therefore urgent need to revisit the concepts for immune protection and the laboratory assays available for assessment of vaccine immunogenicity [56, 57]. Elispot assays and intracellular cytokine analysis should no longer be the only tools used. The development and validation of additional assays measuring lymphoproliferation, mucosal responses, cytotoxic capacity, in-vitro CD8-mediated viral inhibition [59, 60], or other immune functions such non-neutralizing antibody avidity and functionality through antibody-dependent cytotoxicity (ADCC) and antibody-dependent cell-mediated viral inhibition (ADCVI) [43, 61] may provide a more robust indication of functional antiviral activity. A particular consideration should be given to the exploration of the vaccine-induced mucosal response.

As a consequence of lowering HIV incidence rates due to scaling up of prevention strategies, vaccine efficacy trials will need to be multicenter and/or multinational in order to reach the sample size and endpoints needed at the final analysis stage. Incidence cohort studies should therefore be expanded to meet the need for efficacy testing of new vaccine candidates in the pipeline [62-64]. HIV vaccine and other new prevention technologies such as pre-exposure prophylaxis and microbicides deserve studies exploring their possible synergistic effect [65, 66].

Conclusion

Despite the obstacles that HIV presents to vaccine researchers, the historic success of vaccines argues that HIV vaccine research must be continued and accelerated [67]. The recent results of the RV144 efficacy trial open promising avenues and will boost the efforts of the scientific community. The shift in research focus to less product evaluation and more vaccine discovery research will nevertheless require a sustained clinical research infrastructure in linking funding of sites to clinical research activity. It will also require innovative ideas and a sustained long-term commitment of the scientific community, civil society, politicians and donors and study participants.

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