

HIV/AIDS vaccine: rumors and insights on a T-cell-based vaccine

Fausto Titti[†] & Diana Stephanie Hammer

[†]Author for correspondence: Division of Experimental Retrovirology & Non-Human Primate Models, National AIDS Center, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome 00161, Italy ■ Tel.: +39 064 990 3325 ■ Fax: +39 064 990 3615 ■ fausto.titti@iss.it

Evaluation of: Liu J, O'Brien KL, Lynch DM *et al.*: Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. *Nature* 457(7225), 87–91 (2009). The generation of a vaccine against HIV/AIDS has turned out to be extremely challenging. In spite of enormous experience both in preclinical and clinical models, we still do not know what viral gene is essential for protective immunity, what the correlate(s) of protection are and what type of delivery system works better in term of safety, immunogenicity and efficacy. Recently, the STEP (double-blind, randomized trial HTVN502; Merck V520, protocol 023) vaccine failure in the scientific community raised the question as to whether a T-cell-based vaccine against HIV/AIDS is still feasible. Liu and colleagues demonstrated that a combination of serologically distinct adenoviral vectors expressing the SIV Gag protein elicited polyfunctional and broad T-cell responses that correlated with a durable but partial protection in macaques challenged with pathogenic SIV in the absence of homologous Env antigen (i.e., neutralizing antibodies). This, and other preclinical trials, are providing convincing evidence that T-cell-based vaccine strategies – while not able to elicit sterilizing immunity – could, however, be sufficient to control viral replication and lower viral transmission rate among individuals (secondary end point of HIV vaccine). These approaches, as long as they demonstrate their ability to elicit broad and antigen-specific polyfunctional activities in relevant animal models, have the potential to be studied further in clinical trials.

Objectives of the study

- To evaluate the safety and immunogenicity of heterologous and homologous rAd–Gag prime-boost regimens of immunization in rhesus macaques lacking Mamu-A*01 and Mamu-B*17 major histocompatibility class I alleles;
- To evaluate the protective efficacy of the vaccine regimen upon systemic challenge infection with SIVmac251.

Methods

- Immunization (intramuscularly, at weeks 0 and 24) of rhesus macaques with a combination of replication-incompetent E1/E3-deleted recombinant adenoviral vector (rAd)26 or rAd35, and rAd5 serologically distinct vectors expressing the SIV–Gag antigen;
- Intravenous challenge infection of vaccinees and of placebo-controlled monkeys with SIVmac251;
- Evaluation of virological outcome by quantitative evaluation of SIV RNA levels in plasma;
- Evaluation of immune responses: binding antibodies and antibody-dependant cell-mediated virus inhibition (ACDVI), cellular immune assays (ELISPOT, multiparametric intracellular staining) both before and after challenge.

Results

Effects of vector combination

- Heterologous prime-boost regimen elicited higher immune responses, both quantitative and qualitative, than the immune responses elicited by the homologous prime-boost regimen, probably owing to the absence of antivector responses.
- Heterologous prime-boost vaccination induced polyfunctional (IFN- γ /TNF- α /IL-2⁺) T cells and ADCVI antibodies.

Virological outcome & clinical progression

- Heterologous rAd26–Gag/rAd5–Gag prime-boost vaccination significantly controlled viral replication in vaccinees, both in the acute and in the chronic phase of the infection;
- Clinical progression of the infection was delayed in monkeys that received heterologous rAd26–Gag/rAd5–Gag prime-boost vaccinations.

Conclusion

- The heterologous rAd26–Gag/rAd5–Gag prime-boost vaccination elicited T-cell immune responses with augmented magnitude, breadth

and polyfunctionality (IFN- γ^+ /TNF- α^+ /IL-2 $^+$) that were associated with the partial control of viral replication and clinical benefits;

- ADCVI antibodies, detectable only after challenge, did not play a relevant role in the observed level of protection.

Discussion

A recent paper by Liu and colleagues raises the issue of feasibility of a T-cell-based vaccine for HIV/AIDS, reporting immunogenicity and efficacy data of a trial performed in the SIV-macaque model [1].

Despite more than 20 years of efforts, the search for an effective HIV vaccine still continues to remain extremely challenging. The best solution to tackle the HIV-1 pandemic is the development of an immunity that is able to prevent HIV-1 entry, replication and dissemination (i.e., sterilizing immunity). So far, the sterilizing immunity approach, based on the induction of potent and broadly neutralizing antibodies to Env, has failed for a number of reasons, depending on the variability of Env (monomeric vs trimeric, glycosylation) [2] and the presence of host contaminants on the HIV envelope [3,4]. The failure of VaxGen's (CA, USA) AIDSVAX HIV vaccine based on immunization only with the gp120 protein, which did not prevent HIV infection nor affect viremia level in participants that got HIV infection after immunization, well reflect this complex scenario [5,6].

Thus, a T-cell-based vaccine was thought to have the potential to efficiently control viral replication to minimize the risk of transmitting the infection to a new host (secondary end point). Nevertheless, the disastrous results of the screening-test-of-concept efficacy study (STOC) STEP (double-blind, randomized trial HTVN502; Merck V520, protocol 023) Phase IIb trial raised the question whether T-cell-based HIV vaccines are still a viable option for preventing the disease. Patients at a high risk of acquiring HIV were either immunized three-times intramuscularly with the replication-deficient rAd-5 expressing the Gag, Pol and Nef proteins of HIV, or received a nonrecombinant Ad5 placebo. The aims of the STEP trial were to evaluate whether this vaccine approach could prevent HIV infection (infection end point) or to control viral replication in vaccinees who eventually become infected [7]. Since the *env* gene was not included within the vaccine formulation, this approach focused on the generation of T-cell immunity. It turned

out that some vaccinees, while in the clinical trial, acquired HIV infection demonstrating enhanced susceptibility to HIV, and viral loads were not controlled by the vaccine regimen. To explain this enhanced susceptibility, hypotheses were generated based on:

- The high immune responses (i.e., high cell-activation status) either to the rAd5 vector and/or immune responses to HIV immunogens (Gag, Pol and Nef);
- Vaccines that boosted the number of CCR5/CD4 $^+$ T cells, providing additional target cells for HIV-infected individuals, particularly in those having pre-existing immunity to rAd5 [8].

To overcome this issue, Liu and colleagues, explored the safety, immunogenicity and efficacy of the homologous and heterologous prime-boost regimen of vaccination in the macaque model by using two different rAd vectors: rAd26 (serotype subgroup D) or rAd35 (serotype subgroup B), and rAd5 (serotype subgroup C). The rAd35 and rAd26 vectors share the usage of the human and simian CD46 receptor, although the usage of other cellular receptors by rAd26 cannot be excluded, while rAd5 uses the coxsackievirus and adenovirus receptor. Rhesus monkeys were inoculated intramuscularly with rAd26–Gag prime/rAd5–Gag boost, rAd35–Gag prime/rAd5–Gag boost or rAd5–Gag prime/rAd5–Gag boost. To evaluate whether the different vaccine regimens (heterologous vs homologous) could have had an impact on the immunological outcome, particular attention was focused on the type and breadth of the vaccine-induced immune responses. Monkeys that received homologous vaccines (rAd5–Gag prime/rAd5–Gag boost) had a frequency of IFN- γ ELISPOT responses (primarily CD8 $^+$) that, at week 2, were quantitatively similar to that observed in other groups. Nevertheless, and as expected, these responses were not boosted by the second inoculum of rAd5–Gag, probably owing to the antivector responses. By contrast, monkeys that were primed with either rAd26 or rAd35 were efficiently boosted by the rAd5–Gag vector, confirming the fact the rAd25 and rAd35 are indeed different serotypes.

Shifting to the quality and breadth of the immune responses, Liu and colleagues outlined that these were dependent on the heterologous rather than homologous regimen of immunization. Thus, in contrast to what was observed in the STEP study, the number of detectable Gag epitopes was higher in rAd26/rAd5 than in the rAd35/rAd5 and rAd5/rAd5 regimens of immunization. Similarly, larger proportions of

poly- (IFN- γ /TNF- γ /IL-2⁺) or mono- (IL-2⁺) functional CD4⁺ and CD8⁺ T-cell subsets were observed according to the same hierarchy.

Overall, the low immune responses obtained in the rAd35–Gag prime/rAd5–Gag boost regimen as compared with those quantified in the rAd26–Gag prime/rAd5–Gag boost group is not surprising and could likely be due to the immunogenic potential of this vector or to the induction of antivector responses. Indeed, data in mice support the evidence of cross-reactivity between rAd5 and rAd35 generating specific neutralizing antibody (NAb) responses after heterologous prime-boost regimens of vaccination [9]. In a seroprevalence study performed in sub-Saharan Africa, 48% of the population was found positive for rAd5 with anti-rAd5 NAb titers greater than 1:1000, and 17–22% were found positive for rAd35 and rAd26, respectively, demonstrating NAb titers greater than 1:1000 from 0–2% [10]. Taken together, these data raise relevant questions about the use of the rAd vector, even in a heterologous prime boost-regimen of vaccination. Overall, this issue is not limited to the rAd vector but could be expanded to other viral and bacterial vectors where pre-existing immunity can easily be found in the human population (e.g., Herpes, Modified Vaccinia Ankara strain and Salmonella). A wide range of viruses have been or are going to be investigated for their ability to express heterologous protein(s), to target specific antigen-presenting cells, stimulate innate immunity and induce/expand antiviral responses against the vaccine antigen(s) *in vivo*. Each viral vector has its own unique biological characteristics, but so far none of them have proven to be an ideal candidate as a vaccine vehicle for HIV-1. To overcome the risks of detrimental antivector responses, a suggestion could be the usage of heterologous dual- or triple-modality of prime-boost regimens of vaccine administration with nonrelated vectors. Of course, these modalities of immunization, for a number of reasons, appear to be less practical, more expensive and may not encounter the business strategies of private vaccine-dedicated companies. The results should be a balance between cost and benefit.

Shifting to the quality of the immune response generated by the different prime-boost regimens of vaccination and on their efficacy upon intravenous infection with pathogenic SIV, Liu and colleagues demonstrated that only rAd26–Gag prime/rAd5–Gag boost regimens elicited T-cell responses that correlated with diminished plasma viremia levels and better clinical outcome. Interestingly:

- Monkeys that did not control SIV replication (rAd5/rAd5; rAd35/rAd5) had high proportions of Gag-specific monofunctional IFN- γ CD4⁺ and CD8⁺ T cells;
- The polyfunctional IFN- γ /TNF- α /IL-2⁺ cells were present to a certain degree in the rAd35/rAd5 group that, however, did not exhibit reduction of viremia level;
- In the rAd26/rAd5 group, the frequency of trifunctional cells was higher than in the rAd35/rAd5 group, ranging from approximately 17–28%, depending on the lymphocyte subset analyzed and correlating with the significant reduction of viremia both in the acute phase and at the set point;
- Control of viral replication appeared to be associated with the high number of responses to various Gag epitopes detected during immunization in the rAd26/rAd5 group, while it was reduced in the other groups.

There is growing evidence indicating that polyfunctional CD4⁺ and CD8⁺ T cells are associated with a better virological outcome. In natural infection, HIV-infected nonprogressors, as compared with progressors, maintain highly functional polyspecific CD8⁺ cells [11]. Interestingly, the fact that polyfunctional T cells represent a hallmark of nonprogression in patients infected with HIV-2 [12], which is less pathogenic than HIV-1, underscores the relevance of these responses in the evaluation of vaccine efficacy. However, a distinct scenario is far from being clearly designed and further efforts are needed. From these studies, the question emerged as to whether the IFN- γ responses, as measured by the ELISPOT assay, could be a useful parameter to evaluate vaccine efficacy [13]. In fact, the general experience in the macaque field suggests that the high number of IFN- γ spots does not always correlate with protective immunity. Rather, this assay was suggested to be used to evaluate vaccine immunopotency [14,15].

The influence of T-cell response on plasma viral loads could not be explained by the mere quantification of their magnitude and breadth. In a recent study performed in untreated but infected African patients, Kiepiela *et al.* demonstrated that of the HIV proteins targeted (practically all HIV proteins), only the increasing breadth of the Gag-specific responses was associated with a significant reduction of viral load independent of particular HLA type [16]. By contrast, although the Gag peptides were reported

to be the most targeted, their association with reduced viral load remains controversial, probably owing to the different population group studied [17]. Nevertheless, as a proof-of-concept, it remains that the broader the T-cell response is to a given antigen, the higher the potential association with decreased viremia is.

In the paper of Liu and colleagues, the relevant issue was the description of the antiviral non-neutralizing (nonconventional) anti-HIV antibodies. After challenge, all vaccinees exhibited a more rapid appearance of anti-Gag antibodies with ADCVI activity as compared with the control group. Although, in this setting, the correlation of such responses with the ability to control viral replication could not be demonstrated, the study of nonconventional antibody response is nevertheless of great importance for a T-cell-based vaccine. These antibodies can either block the virus and kill HIV-infected cells by antibody-dependent cellular cytotoxicity (ADCC) or ADCVI in the case where NAb are either undetectable or at low titers during the acute infection. Indeed, priming of chimpanzees with repeated inoculation of a replication-competent rAd-SIV vector, in spite of the presence of antivector responses, was able to increase the breadth of CD8⁺ immune responses and induced non-neutralizing antibodies with ADCC and ADCVI activities that correlated with an improved protection upon mucosal challenge with SIVmac251 [18].

Finally, but not less important, the crucial point is the route used to challenge the monkeys and the homology between vaccine antigen and SIV strain. Liu and colleagues used the intravenous route to test the efficacy of their vaccine approach and the differences between vaccinees (rAd26-Gag/rAd5-Gag) and control, although statistically significant, are not that promising. It is likely that, by using a mucosal route such as intrarectal or a heterologous challenge virus, such a level of protection could vanish. Experimental infection via the mucosal route is by far much more representative of the natural infection than the systemic route.

Future perspective

In spite of enormous experience, both in preclinical and clinical models, we do not yet know what viral gene is essential for protective immunity, what are the correlate(s) of protection and what type of delivery system works better in terms of safety, immunogenicity and efficacy. As for many common infectious agents, HIV gains access to the host through mucosal membranes, both in

the horizontal and vertical modalities of transmission. In spite of the fact that the mucosal cells present at the port of entry are not considered among the preferential targets for HIV infection, HIV can clearly overcome the first line of immune defence and establish a productive infection via different mechanisms [19].

A T-cell-based vaccine truly capable of fighting the virus at the port of entry and minimizing/halting viral replication and dissemination should:

- Induce polyfunctional and broad CD4⁺ and CD8⁺ T-cell responses;
- Generate and maintain a large pool/collection of antigen-specific memory T and B cells;
- Elicit nonconventional antibodies able to bind to complement proteins or Fcγ receptors on effector cells, thereby promoting complement-mediated lysis of viral particles and ADCC.

The failure of the STEP trial could not be a reason for the total dismissal of a T-cell-based vaccine. As Liu and colleagues demonstrated, a T-cell-based vaccine is a feasible approach, although questions are raised concerning the usage of rAd vectors. Clearly, Gag alone is not sufficient to elicit adequate levels and specificity of protective immunity at the port of entry. Thus, the inclusion in a vaccine formulation of nonstructural genes, such as *rev*, *tat* and *nef*, is an emerging approach in this field. Rev, Tat and Nef proteins are expressed early after infection, are essential for virus replication, are found extracellularly, are immunogenic, and exert multiple dysregulatory effects on bystander cells aimed at facilitating immune-cell recruitment and activation, cytotoxic T-lymphocyte (CTL) evasion, increase of viral infection, and transmission and dissemination from the mucosal sites. Tat, Rev and, to a lesser degree, Nef proteins are highly conserved in their immunodominant regions encompassing B and T (including CTL) epitopes, a very desirable feature for the generation of a vaccine that has viral variability. Indeed, experimental evidence in macaque models indicate that a vaccine approach also containing these nonstructural genes was able to control, down to undetectable levels, viral replication upon mucosal challenge with pathogenic SIV in the absence of detectable conventional neutralizing antibodies [20,21]. These data further confirm the viability of a T-cell-based vaccine.

Since early events in virus-host interaction at the port of entry are critical for the establishment of infection, a better understanding of the mechanisms occurring during HIV

transmission is crucial for the rational design and development of new preventive and therapeutic HIV/AIDS vaccines. In this respect, further insights are needed on how Rev, Tat or Nef affect viral infectivity and tropism, modulate HIV trafficking across mucosal surfaces, drive recruitment of target cells and induce immunodysregulation at the port of entry, and how these events are related to the progression to AIDS.

Finally, to meet this unprecedented challenge, a close synergy between highly qualified scientists from public and private sectors is critical to maximize the chances of success. In this respect, the constitution of consortia represents a necessary step aimed at facilitating national and international partnership(s) for the development

of joint programs. They may provide the HIV field with the critical mass necessary to speed up vaccine development and testing.

Financial & competing interests disclosure

The content of the present manuscript reflects the personal position of the authors and does not necessarily reflect the official position of the National AIDS Center. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

The authors thank P Sergiampietri for editorial assistance in the preparation of this manuscript. No other writing assistance was utilized in the production of this manuscript.

Executive summary

- A T-cell-based vaccine still remains a viable vaccine approach.
- As HIV transmission occurs through the mucosal surface, vaccine approach should target these tissues, even if the antigen is administered via the systemic route.
- A heterologous prime-boost regimen of immunization should be used so that the potential risks associated with antivector response are avoided.
- The search for and identification of immune responses correlating with protection (antigen-specific polyfunctional T cells, nonconventional antibodies and responses of innate immunity) have to be expanded in a relevant animal model.
- To test vaccine efficacy, the mucosal rather than systemic route should be more representative of the natural infection.
- Gag alone is not sufficient to elicit protective immunity. Nonstructural genes are required to improve the quality and breadth of the immune responses, in particular at the mucosal level.
- A better understanding of virus–host interactions at the mucosal level is needed.

Bibliography

Papers of special note have been highlighted as:

- of interest
 - of considerable interest
1. Liu J, O'Brien KL, Lynch DM *et al.*: Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. *Nature* 457(7225), 87–91 (2009).
 2. Karlsson Hedestam GB, Fouchier RA, Phogat S, Burton DR, Sodroski J, Wyatt RT: The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. *Nat. Rev. Microbiol.* 6(2), 143–155 (2008).
 3. Kolegraft K, Bostik P, Ansari AA: Characterization and role of lentivirus-associated host proteins. *Exp. Biol. Med. (Maywood)* 231(3), 252–263 (2006).
 4. Morita E, Sundquist WI: Retrovirus budding. *Annu. Rev. Cell Dev. Biol.* 20, 395–425 (2004).
 5. Horton H, Havenar-Daughton C, Lee D *et al.*: Induction of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses in HIV vaccine trial participants who subsequently acquire HIV-1 infection. *J. Virol.* 80(19), 9779–9788 (2006).
 6. Jones NG, Decamp A, Gilbert P, Peterson ML, Gurwith M, Cao H: AIDSVAx immunization induces HIV-specific CD8⁺ T-cell responses in high-risk, HIV-negative volunteers who subsequently acquire HIV infection. *Vaccine* 27(7), 1136–1140 (2008).
 7. Buchbinder SP, Mehrotra DV, Duerr A *et al.*: Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the STEP Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* 372(9653), 1881–1893 (2008).
 8. McElrath MJ, De Rosa SC, Moodie Z *et al.*: HIV-1 vaccine-induced immunity in the test-of-concept STEP study: a case-cohort analysis. *Lancet* 372(9653), 1894–1905 (2008).
 9. Barouch DH, Pau MG, Custers JH *et al.*: Immunogenicity of recombinant adenovirus serotype 35 vaccine in the presence of pre-existing anti-Ad5 immunity. *J. Immunol.* 172(10), 6290–6297 (2004).
 10. Abbink P, Lemckert AA, Ewald BA *et al.*: Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J. Virol.* 81(9), 4654–4663 (2007).
 11. Betts MR, Nason MC, West SM *et al.*: HIV nonprogressors preferentially maintain highly functional HIV-specific CD8⁺ T cells. *Blood* 107(12), 4781–4789 (2006).
- **Along with [12], clear evidence that polyfunctional CD4⁺ and CD8⁺ cells are associated with the robust control of viral replication in the study of vaccine-induced protective immunity in nonprogressive HIV-1 and HIV-2-infected patients.**
 - 12. Duvall MG, Precopio ML, Ambrozak DA *et al.*: Polyfunctional T-cell responses are a hallmark of HIV-2 infection. *Eur. J. Immunol.* 38(2), 350–363 (2008).
 - **Along with [12], clear evidence that polyfunctional CD4⁺ and CD8⁺ cells are associated with the robust control of viral replication in the study of vaccine-induced protective immunity in nonprogressive HIV-1 and HIV-2-infected patients.**

13. McKinnon LR, Ball TB, Wachihhi C *et al.*: Substantial inpatient differences in the breadth and specificity of HIV-specific CD8⁺ T-cell interferon- γ and proliferation responses. *J. Acquir. Immune. Defic. Syndr.* 49(2), 123–127 (2008).
14. Appay V, Douek DC, Price DA: CD8⁺ T cell efficacy in vaccination and disease. *Nat. Med.* 14(6), 623–628 (2008).
15. Boaz MJ, Hayes P, Tarragona T *et al.*: Measuring T-cell immunity in HIV vaccine clinical trials: PBMC and ELISPOT assay proficiency concordance in laboratories from 3 continents. *Clin. Vaccine Immunol.* (2008) (Epub ahead of print).
16. Kiepiela P, Ngumbela K, Thobakgale C *et al.*: CD8⁺ T-cell responses to different HIV proteins have discordant associations with viral load. *Nat. Med.* 13(1), 46–53 (2007).
17. Addo MM, Yu XG, Rathod A *et al.*: Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. *J. Virol.* 77(3), 2081–2092 (2003).
18. Hidajat R, Xiao P, Zhou Q *et al.*: Correlation of vaccine-elicited systemic and mucosal nonneutralizing antibody activities with reduced acute viremia following intrarectal simian immunodeficiency virus SIVmac251 challenge of rhesus macaques. *J. Virol.* 83(2), 791–801 (2009).
- **Reports that antibodies with ADCC and/or ADCVI activities elicited by a vaccine regimen of immunization are significantly associated with robust control of viral replication.**
19. Paiardini M, Frank I, Pandrea I, Apetrei C, Silvestri G: Mucosal immune dysfunction in AIDS pathogenesis. *AIDS Rev.* 10(1), 36–46 (2008).
- **Comprehensive review of the immune dysfunction(s) occurring at mucosal level during m HIV and SIV infection.**
20. Negri DR, Baroncelli S, Catone S *et al.*: Protective efficacy of a multicomponent vector vaccine in cynomolgus monkeys after intrarectal simian immunodeficiency virus challenge. *J. Gen. Virol.* 85(Pt 5), 1191–1201 (2004).
21. Wilson NA, Reed J, Napoe GS *et al.*: Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. *J. Virol.* 80(12), 5875–5885 (2006).

Affiliations

- Fausto Titti
Division of Experimental Retrovirology & Non-Human Primate Models, National AIDS Center, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome 00161, Italy
Tel.: +39 064 990 3325;
Fax: +39 064 990 3615;
fausto.titti@iss.it
- Diana Stephanie Hammer
Division of Experimental Retrovirology & Non-Human Primate Models, National AIDS Center, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome 00161, Italy
Tel.: +39 064 990 3083;
Fax: +39 064 990 3615;
diana.hammer@iss.it