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HIV vaccine design: insights from live attenuated SIV vaccines

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The International AIDS Vaccine Initiative has established a consortium to elucidate mechanisms of protection conferred by live attenuated simian immunodeficiency virus vaccines in monkeys. Here, the strategies defining key components of the protective immune response elicited by these vaccines are discussed.

Today, more than 20 years into the AIDS pandemic, the human immunodeficiency virus (HIV) vaccine pipeline remains inadequate. Vaccine candidates tested thus far in human efficacy trials have failed to prevent HIV infection or suppress the HIV viral load[1]. Candidates approaching human efficacy trials have shown some benefit in certain monkey models but not in others. There is considerable pessimism that these candidates will achieve more than limited success if any, as they have provided little or no protection from pathogenic simian immunodeficiency virus (SIV) challenge in monkeys[1]. The lack of protection is particularly disappointing, as the nonhuman primate trials have typically been designed and conducted as proof-of-concept studies aimed at demonstrating protection and have used conditions optimizing the potential for success[2]. For example, the challenge virus has been exactly matched in genetic sequence to the vaccine and the virus challenge has occurred at or near the peak of vaccine-induced immune responses, neither of which would be expected in real-world conditions. Even in such idealized conditions, the vaccine platforms tested so far have generally failed to confer protection, or at best have temporarily suppressed viral load by approximately 1 log (ref. 3). In fact, most vaccine trials so far in monkeys using SIVmac239, SIVmac251 or SIVsmE660 as the challenge virus have demonstrated little or no protection[2,4–6]. Those SIV strains were judiciously selected for challenge model development because they share many critical properties with primary isolates of HIV-1. Specifically, they are relatively resistant to antibody-mediated neutralization, use CCR5 as principal coreceptor for entry into cells, cause steady decreases in $CD4^+$ T lymphocyte numbers and mediate a progressive course of disease leading to AIDS.

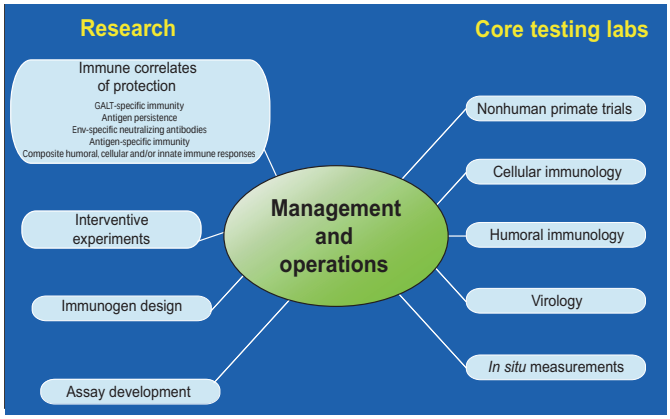

Figure 1 The IAVI consortium to elucidate mechanisms of protection by live attenuated SIV. These represent the research and core testing lab elements of the live attenuated consortium.

In contrast, use of live attenuated SIV viruses as vaccines has provided complete or near-complete protection from homologous challenge by those same wild-type SIV strains, thus providing a critical proof of principle for the feasibility of HIV vaccine development[7] (**Table 1**). Since the initial observations in 1992 of the protective efficacy of live attenuated SIV vaccines[8], the mechanisms for protection have remained undefined. Several lines of evidence have suggested that the protection is immune mediated[9]. Substantially better protection is obtained with homologous SIV challenge than with heterologous challenge[7]. In addition, protection is noted after a certain period of time has elapsed after vaccination, further supporting the idea of the involvement of specific anti-SIV immune responses.

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Table 1 Vaccine protection against pathogenic SIV in Indian rhesus macaques

Vaccine	Monkeys protected[a]	Refs.
Live attenuated: Δ*nef*	59 of 63	8,20–24
Live attenuated: Δ*3*	12 of 12	21
Live attenuated: Δ*5G*	3 of 3	25
Live attenuated summary	74 of 78 (95%)	
All other vaccine strategies[b]	18 of 256 (7%)[c]	2,4,6,26–46

Table includes published data from challenge experiments in Indian rhesus macaques using SIVmac251, SIVmac239, SIVmac32H, SIVsmE660 and SIVsmB670 by any route (intravenous, rectal, vaginal, oral or tonsillar); challenges with SHIV (simian-human immunodeficiency virus) or poorly pathogenic clones of SIV are excluded. [a]Data are presented as suppression of viral load of more than 3 logs and represent numbers of macaques strongly protected against disease progression, as assessed by plasma virus load measurements for at least 6 months and relative to challenge controls, compared with the number of vaccinated monkeys. In early papers published before plasma virus load assays were available, strong protection was judged by low or negative PCR results in peripheral blood mononuclear cells and/or failure of isolation of virus from peripheral blood mononuclear cells after challenge. [b]The 256 monkeys here are distributed among the following vaccine categories: DNA alone ($n = 11$), DNA plus vector ($n = 22$), poxvirus vectors ($n = 101$), other vectors ($n = 93$), vector combinations ($n = 8$) and whole-inactivated SIV or proteins and/or peptides ($n = 21$). [c]Of the 256 monkeys immunized with protein or vectored vaccines, 200 (78%) had a plasma load reduction of less than 1 log.

We believe that understanding the basis for protection conferred by live attenuated SIV will facilitate the design of improved HIV vaccine strategies that will bring the field closer to the goal of a successful AIDS vaccine. Thus, this commentary addresses the scientific challenges associated with elucidating the mechanism of protection conferred by live attenuated SIV vaccines in monkeys. It also discusses the systematic approaches being undertaken by the Live Attenuated Consortium (LAC) of the International AIDS Vaccine Initiative (IAVI) to address this important vaccine research problem (**Fig. 1**).

Protection against pathogenic SIV challenge

There are at least three distinct strategies that have been used to attenuate SIV, which have resulted in robust protection from pathogenic SIV challenge in nonhuman primates. First, the administration of live attenuated SIV strains with deletion of *nef* (SIVΔ*nef*) or deletion of *nef* and *vpr* (SIVΔ*3*) has provided strong protection from pathogenic SIV challenge[7]. In some circumstances, vaccination has seemed to prevent infection. In other experiments, vaccination has led to a substantial reduction in plasma viral load during primary viremia (for example, a reduction of more than 5 logs, compared with the reduction of 1 log noted for other vaccine strategies) and complete control of viral replication in the post-acute phase (months to years) of infection.

Second, the administration of a highly attenuated, antibody neutralization–sensitive mutant of SIV with complete deletion of the V1–V2 region of the envelope protein (Env; SIVΔ*V1-V2*) has conferred potent protection from intravenous challenge by wild-type, pathogenic SIVmac239 (ref. 10). Protection has been achieved in some monkeys despite the fact that antiviral immune responses measured in peripheral blood are extremely weak or undetectable at the time of challenge (K. Mansfield *et al.*, unpublished observations). That result contrasts notably with the minimal protection reported with the leading candidate nucleic acid–based and viral vector–based vaccine candidates, which elicit robust cellular $CD8^+$ responses in the peripheral blood.

Finally, protection has also been demonstrated when rhesus monkeys have been inoculated intravenously with live, wild-type SIV and then have received a short course of antiretroviral drug treatment within days of inoculation. The drug treatment serves to limit viral replication during primary infection. In contrast to drug treatment begun in chronically infected macaques or humans, in which plasma viral load rebounds to pretreatment amounts after drug discontinuation[11], monkeys receiving early treatment effectively control pathogenic wild-type SIV replication after drug discontinuation. Furthermore, some of these monkeys are able to effectively control subsequent homologous and heterologous rechallenges with wild-type SIV, despite antiviral immune responses in some cases that are either extremely weak or not measurable in the peripheral blood[12].

Those results provide important lessons for AIDS vaccine development. They demonstrate that high antiviral immune responses, measured in the peripheral blood by standard assays, are not necessarily required for achieving vaccine-mediated protection from pathogenic SIV strains that are closely analogous to HIV. They collectively provide encouraging evidence that an effective HIV vaccine can be developed. However, they also notably emphasize that the mechanism for protection conferred by live attenuated SIV vaccines is not fully understood thus far and therefore critical information for the rational design of improved HIV vaccine candidates is lacking.

Potential mechanisms for protection

It is now well established that the primary site of SIV or HIV replication immediately after infection is in gut-associated lymphoid tissue (GALT), and this results in a rapid, profound depletion of primary lamina propria memory $CD4^+$ T cells[13]. Moreover, it has been shown that SIV infects resting memory $CD4^+$ T cells in GALT and in other lymphoid tissues, causing considerable depletion of the $CD4^+$ effector arm of the immune system[14,15]. Those data collectively suggest that successful immunization strategies against HIV and AIDS may need to blunt the tremendous amplification of HIV that occurs in GALT and the resulting acute substantial depletion of $CD4^+$ T lymphocytes. Those observations have now led to a series of testable hypotheses as to the mechanism(s) for protection conferred by live attenuated SIV vaccines.

In contrast to most AIDS vaccine strategies now being explored in clinical trials, administration of live attenuated SIV probably targets gut-associated memory $CD4^+$ T cells in a way similar to infection with wild-type SIV. Thus, it is important to determine if the protection provided by live attenuated mutants of SIV is due to the induction of GALT-specific immune responses. The LAC is addressing this problem directly by undertaking the first comprehensive, multicenter, large-scale, integrated, nonhuman primate study using more than 100 monkeys. This comprehensive study is meant to systematically assess the humoral and cellular immune responses mounted in all relevant compartments (including the peripheral blood, gut- and bronchus-associated lymphoid tissues, vaginal vault and lymph nodes) to SIVΔ*nef*, SIVΔ*V1-V2* and pharmacologically attenuated wild-type SIV. In addition, new viral vaccine vectors such as paramyxovirus-based candidates capable of specifically targeting GALT via mucosal administration will be compared for efficacy versus live attenuated SIV after challenge with pathogenic SIV.

Monkeys challenged with pathogenic SIV several months after being given a single administration of live attenuated SIV show much more protection than monkeys immunized by any other vaccine strategy. **Table 1** reviews vaccine-mediated protection against pathogenic SIV in Indian rhesus macaques. With suppression of viral load of more than 3 logs as a discriminating criteria for protection, 95% (74 of 78) of monkeys immunized with live attenuated SIV demonstrate protection, compared with only 7% (18 of 256) of monkeys immunized using other vaccine strategies. Moreover, as noted above, the amount of protec-

tion conferred by live attenuated SIV vaccines seems to improve as the interval between immunization and challenge increases from 8 to 79 weeks. Those observations suggest that protection may be due in part to maturation of the immune response resulting from sustained exposure to the 'protective' immunogen(s) expressed by the attenuated SIV. The LAC is addressing the importance of antigen persistence in conferring protection by systematically comparing the immune responses elicited by live attenuated SIV, to genetically engineered single-cycle SIV and to viral vectors known for their capacity to persist (such as herpes virus vectors)[16]. All the relevant systems to be compared will carry a set of functional genes similar to those found in live attenuated SIV.

Previous studies using small numbers of monkeys have shown that the administration of live attenuated SIV seems to confer greater protection against homologous virus challenge than against heterologous challenge[7]. For example, immunization with live attenuated SIVmac239-derived viruses does not protect immunized monkeys very well against challenge with SIVsmE660. This is a critical point, because the *env* sequence differences between SIVmac239 and SIVsmE660 represent an amount of sequence heterogeneity comparable to that commonly noted among different HIV isolates circulating in the human population. Those observations raise the possibility that the protection noted with live attenuated SIV vaccines could be dependent on the presence of strain-specific neutralizing antibodies. Although the administration of broadly neutralizing monoclonal antibodies to monkeys protects them from SHIV challenge, passive transfer of concentrated sera from monkeys immunized with live attenuated SIV has failed to protect naive monkeys from pathogenic SIV challenge thus far[17]. Therefore, it is imperative to expand heterotypic vaccination-challenge studies with a much larger pool of monkeys of different major histocompatibility complex type to determine unequivocally the amount of protection conferred by the live attenuated SIV against infection with mismatched wild-type virus strains and to determine if the protection is due to the induction of neutralizing antibodies or other immune mechanisms. The LAC also plans to undertake a series of comprehensive studies with chimeric live attenuated SIVs differing in *env* genes with the goal of further delineating the function of *env* in the protection noted.

The set of HIV antigens and, by analogy, SIV antigens that must be included in an effective vaccine to confer protection against HIV and SIV, respectively, remains unclear. Several antigens are potential targets for cell-mediated immune responses, and the envelope glycoproteins gp41 and gp120 are the principal targets for neutralizing antibodies. Designing an immunogen to elicit broadly neutralizing antibodies to HIV remains a chief scientific challenge; human efficacy trials of subunit gp120 vaccines have failed to show protection from HIV infection or suppression of viral load in subjects subsequently exposed and infected with HIV. Moreover, the various HIV vaccine candidates now in human clinical trials focusing on the induction of cell-mediated immune responses express incomplete subsets of the full complement of HIV genes. Furthermore, none of the candidates matches the set of analogous antigens incorporated in live attenuated SIV and none confers the level of protection obtained with live attenuated SIV. Thus, it is important to systematically determine which antigens need to be included in the vaccine to confer maximum protection.

Finally, adoptive transfer studies using retrovirus infection in mice have shown that live attenuated Friend leukemia virus vaccine protects against pathogenic challenge through a mechanism that includes a combination of neutralizing antibody, $CD4^+$ memory and $CD8^+$ cytotoxic immune responses. The basic premise of that model relies on virus-specific antibodies that provide the first line of defense by neutralizing a substantial proportion of the virus challenge, thereby providing time for the cell-mediated memory responses to activate, expand and 'mop up' any breakthrough infection. However, it is unclear whether live attenuated SIV requires a combination of neutralizing antibody, $CD4^+$ memory T cells and $CD8^+$ cytotoxic immune responses; systematic studies are needed to clarify the involvement of various immunological components. Studies evaluating the efficacy of live attenuated SIV vaccines generally have used small numbers of monkeys in the context of investigator-initiated grants, with limited capacity for comprehensive immunologic analyses. Prospective studies using larger numbers of monkeys, together with state-of-the-art assays for assessing cellular, humoral and innate immune responses, offer for the first time the means to determine the relative contribution made by the different immune responses to conferring protection. Systematic depletion studies, such as with monoclonal antibody to CD8, provide a complementary approach for delineating the protective mechanism(s) involved.

State of the global HIV vaccine field

Approximately US$650 million is now being spent annually on HIV vaccine research and development, yet it is now generally accepted that the goal of a safe, effective widely accessible AIDS vaccine is at best years away and that solutions to the key scientific challenges remain the principal rate-limiting factor[18]. The main focus of vaccine candidates in the clinical pipeline now is to elicit robust cellular immune responses capable of suppressing viral load and thus slowing the progression to AIDS and further blunting the rate of transmission due to lowered viral loads. At present, the most advanced candidate in clinical trials for testing the cell-mediated immunity-based hypothesis is the Merck replication-defective adeno-5 vector, which has entered a proof-of-concept phase IIB trial in 1,500 subjects at risk for HIV infection. Data from this clinical trial should be available in 3–4 years. Mathematical models have demonstrated that suppression of viral load by more than 1 log could produce a substantial public health benefit, so these trials represent the first real-life test for the cell-mediated immunity-based vaccine strategy. However, the long-term goal for an effective HIV vaccine is to prevent the establishment of HIV infection, which so far has been achieved only by the live attenuated SIV vaccines in monkeys. Therefore, it is crucial to elucidate the mechanism of protection conferred by live attenuated SIV and to make full use of this new information in the design of better HIV vaccine candidates.

The LAC initiative

A common theme to the approaches described above is that considerable protection against pathogenic SIV challenge is apparently conferred by genetically attenuated or pharmacologically attenuated SIV in the absence of robust systemic immune responses, as measured by standard screening assays such as interferon-γ enzyme-linked immunospot assay. For systematically addressing that scientific problem, a highly collaborative, interactive and problem-solving approach is needed. To meet that need, the IAVI has established the LAC, composed of a flexible and expanding number of leading investigators with diverse backgrounds. This initiative, patterned after a similar consortium assembled to address the HIV-neutralizing antibody problem[19], is predicated on the belief that progress toward solving critical vaccine design problems will be accelerated by building on basic research advances through a coordinated and greatly expanded applied research agenda.

The LAC is placing great emphasis on the systematic assessment of the scientific hypotheses outlined above that are most relevant to understanding the mechanism of protection conferred by administration of live attenuated SIV. We view the resolution of these questions as a chief starting point from which to apply rational HIV vaccine design, to create safe but equally effective analogous vaccine alternatives to

live attenuated SIV. We also realize that that these studies will need to be undertaken on a large scale and with rigorous systems in place to achieve standardized results with statistically significant outcomes. Traditionally, academic laboratories working with nonhuman primates tend to be very effective at the innovative phase of discovery but less effective at large-scale systematic studies, because of limitations of resources and industrial project-management systems. The LAC is placing considerable emphasis on the standardization of assays, protocols and reagents across the consortium and on quality control across the participating laboratories (**Fig. 1**). Finally, we are also seeking to accelerate progress by sharing results, 'know-how' and experience and by generating in bulk the critical reagents needed for HIV vaccine research and making these available for communal use. A series of 'enabling' projects is being implemented for the LAC, including a diverse array of activities from reagent design to the development and validation of methods for immunological assessment. These enabling projects provide the critical support for the large-scale multicenter studies that will be necessary to successfully address this scientific problem.

Concluding remarks

Elucidating the mechanism of protection conferred by live attenuated SIV vaccines is now one of the priorities for the collective AIDS vaccine effort. The solution to this problem and the subsequent design of HIV vaccines that mimic and improve on the efficacy of live attenuated SIV approaches will probably require both systematic testing of scientific hypotheses and innovation in immunogen design. We believe that the LAC represents a logical approach for addressing this important scientific challenge and offers a model for rapidly translating advances from vaccine research to the development of improved vaccine candidates, which collectively advance the field and bring the goal of a safe and effective AIDS vaccine nearer.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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