In June 1981, the Center for Disease Control (CDC) in the United States reported the first clinical evidence of a disease that would become known as Acquired Immunodeficiency Syndrome (AIDS). Twenty-three years later, the AIDS epidemic has spread all over the world. Since the beginning of the epidemic, 63 million people have been infected with HIV. Globally, over 38 million people are today living with HIV infection, with 5 million new infections acquired annually, and 14,000 daily. Over 95 per cent of new HIV infections occur in developing countries, mainly in Sub-Saharan Africa and South-East Asia. There is an urgent need to explore all possible approaches to control the epidemic, in particular, preventive measures such as health education, condom use, safe sex practices, and treatment of sexually transmitted diseases, preventive vaccines and topical microbicides. If considerable progress has been made in treatment with antiretroviral drugs and their large-scale implementation, the need for an AIDS vaccine has spurred an unprecedented effort of research and development worldwide, especially in South-East Asia. Preventive vaccines represent our only long-term hope to stop the epidemic.

Since the first report of HIV infection among sex workers in Chennai in 1986, it is estimated that over 5.1 million people were infected with HIV in India by the end of 2003, representing a high public health burden. HIV infection has spread to all the states in
India and high prevalence rates have been reported from Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Manipur, and Nagaland. A majority of HIV infections are due to sexual transmission followed by intravenous drug use and mother-to-child transmission. The HIV epidemic has been spreading from high risk to low risk populations and from urban to rural areas during the last 12-13 yr. The need to develop an HIV vaccine in India has become particularly pressing.

**Scientific and strategic challenges**

The main scientific obstacles to the development of an AIDS vaccine are summarized in Table I.

The high error rate of the viral reverse transcriptase leads to continuous mutations in the HIV genome. HIV-1 is characterised by its genetic diversity and hypervariability, especially in the envelope domain, to a lesser extent in core and regulatory genes. In addition, the genetic sequence of the full genome has allowed determining recombination between subtypes, now well established. In Asia, the HIV group M subtype A/E (E envelope, A gag/pol) is predominant (>75%) in Thailand and Myanmar, followed by subtype B (close to the North American and European B), essentially found in injecting drug users (IDUs), and subtype C in northern Thailand. In Indonesia, subtypes B (predominant) and E are both circulating. In China, the presence of subtypes B and C among injection drug using populations in southern Yunnan Province yielded CRF07_BC and CRF08_BC which subsequently became the predominant viruses as the HIV-1 epidemic spread to the north and the east. In India HIV-1 subtype C is predominant (91%) (subtype C accounts for 47.2% of all HIV infections worldwide, followed by subtypes B, A and E. Recombinants (A/C, B'/C) have also now been described in India. HIV-2 is also circulating. This may have tremendous implications for the design of HIV vaccines. A vaccine protecting against a subtype may not be protective or only partially protective against another subtype or recombinant.

The immune correlates of protection are still unknown. Both arms of the immune system (humoral and cell-mediated) are thought however to be important elements for protection. The best vaccine approach would prevent the establishment of HIV infection of the host upon exposure. A typical immune response to HIV infection involves the development of both neutralizing antibodies and cell-mediated immune responses. However, despite these immune responses, the persistence of HIV-1 reflects its ability to evade elimination by the immune system and to replicate predominantly in antigen-presenting and regulatory immune cells, enabling continuous virus replenishment. Efforts aim at developing vaccines that would induce both neutralizing antibodies and cytotoxic T lymphocytes (CTL) against CCR5 primary HIV isolates. The humoral arm produces virus-neutralizing antibodies that, when fully effective, completely prevent virions from infecting new host cells. Essential structural components of the glycoproteins required for host-cell interaction and entry are rendered inaccessible to antibody, and the remainder of the molecule is “hidden” from antibody assault. The complex interactions between the envelope and its receptors (CD4 and chemokines receptors), the partial protection from antibody by glycosylation of the envelope-chemokine receptor and stearic hindrance make the envelope a remarkable functional molecule that is appropriately exposed to the environment of the host cell surface but can also thwart effective antibody interaction. Several monoclonal antibodies have been described as “broadly” neutralizing against diverse HIV-1 isolates. However, the breadth and potency of their activity remains limited. Though the mechanisms that lead to neutralizing antibodies are relatively well understood, the development of an effective HIV vaccine remains a significant scientific and strategic challenge.
understood, scientists are still unable to make relevant immunogens capable of inducing such antibodies. An International Neutralizing Antibody Consortium has been set up and is co-ordinated and co-funded by International AIDS Vaccine Initiative.

The antiviral cellular immune response plays a crucial role in controlling virus replication, especially when effective during the phase of acute infection. The objective of a vaccine that elicits specific cellular immunity is to greatly enhance the pool of anti–HIV-1 CD4 memory and CD8 CTLs in the uninfected person. These established memory cells will then be capable of rapidly proliferating upon initial virus infection. The immediate proliferation of effector CTLs will, in theory, result in an interaction between the virus and the immune system whose dynamics favour the immune response, yielding a rapid clearance of infection and a lower persistent viral load. Such a vaccine will likely not prevent new HIV infection, but it should have both beneficial individual and epidemiological consequences. Encouragingly, the ability of the virus to fully “escape” from the cellular immune response through mutation may be limited by functional constraints on virus structure.

The vaccine strategies currently developed focus on the development of a cell-mediated immune response, essentially through HIV-specific CD8 cytotoxicity. However, the relevance CD8 T-cell measurements currently used in animal studies and clinical trials such as ELISpot INFγ have been questioned. If the ELISpot INFγ still represents the yardstick of measurement of CTL activity, such measurement is thought to give a partial image of the true immune responses susceptible to protect against HIV. There is a growing consensus that other parameters should be looked at, including the CD8 memory by measurement of IL2 production, the secretion of granzyme B and perforin as preferable markers of the CTL activity. However, such assays need to be standardized and validated.

The elicitation of cellular immunity by a vaccine requires that vaccine vectors be developed that can “deliver” genes encoding the target viral immunogens to immune system antigen-presenting cells. A number of such vectors are currently being developed and tested in preclinical and early phase clinical trials. The more promising technologies include various replication defective viruses and so-called “naked” DNA vectors used in multiple combinations. However, to date, individual vector systems have exhibited various deficiencies such as poor potency and negative effects of pre-existing antivector immunity.

Relevant animal models are lacking. In non-human primate models, these vaccines have elicited immune responses that range from highly to poorly effective in their ability to mitigate infection after challenge with the virus. The validity of animal models for protection will be resolved only when comparison of these animal results with the results of “proof of concept” (or phase IIb) and efficacy trials in humans are made possible.

Since most of HIV transmission occurs through sexual transmission, the development of an AIDS vaccine that would elicit protective immunity at the mucosal level has received special attention. The conduct of animal studies and clinical trials raised however methodological issues such as the need to harmonize and validate the methods of sample collection as well as of the measurement of immune responses at the mucosal level. The current cell-mediated immunity-oriented vaccine strategies may not be adapted to the situation since there seems to be a poor correlation between HIV shedding in semen and the systemic CD8 CTL response in humans.

**Table II. Programmatic difficulties to the development of AIDS vaccines**

<table>
<thead>
<tr>
<th>Difficulty</th>
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</thead>
<tbody>
<tr>
<td>Insufficient political leadership</td>
</tr>
<tr>
<td>Insufficient fundings allocated to AIDS vaccines</td>
</tr>
<tr>
<td>Lack or insufficient coordinated approach</td>
</tr>
<tr>
<td>Regulatory authorities in developing countries</td>
</tr>
<tr>
<td>Slow approval process</td>
</tr>
<tr>
<td>Standardization of assays and reagents</td>
</tr>
<tr>
<td>Length of clinical trials, especially of efficacy trials</td>
</tr>
</tbody>
</table>

**Programmatic challenges**

The implementation of AIDS vaccine development programmes encounters several difficulties (Table II). The implementation and conduct of HIV vaccine clinical trials are difficult, long and costly. Recruitment
### Table III. Clinical trials in developing countries

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Phase</th>
<th>Product</th>
<th>No. of volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>China</td>
<td>II</td>
<td>V3 branched peptide</td>
<td>23</td>
</tr>
<tr>
<td>1994</td>
<td>Thailand</td>
<td>II</td>
<td>V3 branched peptide</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>II</td>
<td>V3 branched peptide</td>
<td>30</td>
</tr>
<tr>
<td>1995</td>
<td>Thailand</td>
<td>I/II</td>
<td>rgp 120 B (MN)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I/II</td>
<td>rgp 120 B (SF2)</td>
<td>54</td>
</tr>
<tr>
<td>1996</td>
<td>Cuba</td>
<td>I</td>
<td>V3-multi-epitope peptide</td>
<td>30</td>
</tr>
<tr>
<td>1997</td>
<td>Thailand</td>
<td>II</td>
<td>rgp 120 BE (SF2/CM235)</td>
<td>380</td>
</tr>
<tr>
<td>1998</td>
<td>Thailand</td>
<td>II</td>
<td>rgp 120 BE (MN/A244)</td>
<td>92</td>
</tr>
<tr>
<td>1999</td>
<td>Uganda</td>
<td>II</td>
<td>ALVAC vcp205</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Thailand</td>
<td>III</td>
<td>rgp 120 BE (MN/A244)</td>
<td>2,545</td>
</tr>
<tr>
<td>2000</td>
<td>Thailand</td>
<td>I/II</td>
<td>ALVAC vCP1521 + rgp 120 BE</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I/II</td>
<td>ALVAC vCP1521 + rgp 160 E</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I/II</td>
<td>ALVAC vCP1521 + rgp 120 BE</td>
<td>125</td>
</tr>
<tr>
<td>2001</td>
<td>Kenya</td>
<td>I</td>
<td>DNA-HIVA</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Haiti</td>
<td>II</td>
<td>ALVAC vCP1452 + rgp120 BB</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Trinidad</td>
<td>II</td>
<td>ALVAC vCP1452 + rgp 120 BB</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>II</td>
<td>ALVAC vCP1452 + rgp 120 BB</td>
<td>40</td>
</tr>
<tr>
<td>2002</td>
<td>Perú</td>
<td>II</td>
<td>ALVAC vCP1452 + rgp 120 BB</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Kenya</td>
<td>I</td>
<td>MVA-HIVA</td>
<td>18</td>
</tr>
<tr>
<td>2003</td>
<td>Uganda</td>
<td>II</td>
<td>DNA-HIVA + MVA-HIVA</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Botswana</td>
<td>I</td>
<td>DNA-multi-epitope</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Kenya</td>
<td>II</td>
<td>DNA-HIVA + MVA-HIVA</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>South Africa</td>
<td>II</td>
<td>MVA-HIVA</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>VEE-vectored C gag</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>South Africa &amp;</td>
<td>I/II</td>
<td>MRKAd-5 gag B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malawi</td>
<td></td>
<td>MRKAd-5 gag</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Brazil &amp; Peru</td>
<td>I/II</td>
<td>MRKAd-5 gag B</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Thailand</td>
<td>I/II</td>
<td>MRKAd-5 gag B</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Haiti &amp; Puerto Rico</td>
<td>I/II</td>
<td>MRKAd-5 gag B</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Thailand</td>
<td>III</td>
<td>ALVAC vCP1521 + rgp 120 BE</td>
<td>16,000</td>
</tr>
<tr>
<td>2004</td>
<td>USA, Later: Canada, Peru Dominican Republic, Haiti, Puerto Rico, Australia</td>
<td>I I b</td>
<td>MRKAd-5 gag/pol/nef B</td>
<td>1,500</td>
</tr>
</tbody>
</table>
of lower-risk volunteers for phases I trials, and of at-risk volunteers for phase II and III efficacy trials is rendered more difficult in less-educated populations exposed to stigma and discrimination, rumours and misunderstandings, and media opinion. Safeguarding the rights and welfare of individuals participating as research subjects in developing countries is a priority. In September 1997 the Joint United Nations Programme on HIV/AIDS (UNAIDS) embarked on a process of international consultation; its purpose was further to define the important ethical issues and to formulate guidance that might facilitate the ethical design and conduct of HIV vaccine trials in international contexts.

The difficulties of implementing HIV vaccine efficacy trials in developing countries have been reviewed elsewhere. HIV incidence rates although still high in some high-risk groups such as discordant couples or intravenous drug users and commercial sex worker groups, show a general trend to decreasing. This is mainly due to the implementation of aggressive intervention programmes of prevention and treatment in these groups over the past 10 yr. As a consequence of lower incidence rates, vaccine efficacy trials will need to be multi-centric and/or multi-country in order to reach the number of participants needed at analysis for a given mode of transmission.

Over the past 10 yr, several developing countries have joined the international effort of AIDS vaccine development (Table III). The implementation of clinical trials in developing countries raised new challenges regarding the approval process and regulatory issues. In several instances, the approval process was poorly defined, ethics committees and regulatory authorities unprepared and inadequately staffed for reviewing complex dossiers of “high tech” genetically engineered products and dealing with “last minute” changes that characterizes research and development. This situation leads most of the time to lengthy approval process and delays in trial initiation. Although considerable efforts have been made in strengthening the capacity of ethics committees and regulatory agencies in developing countries, more remains to be done to shorten delays to trial initiation.

AIDS vaccine approaches

Since 1987, when the first preventive HIV vaccine candidate entered clinical trials, more than 40 vaccine candidates have been evaluated in safety and immunogenicity trials, and one candidate has progressed to efficacy trials. More than 10,000 HIV-uninfected volunteers have participated in clinical trials of HIV vaccines between 1987-2003. Databases of AIDS vaccines in human trials have now been established by the International AIDS Vaccine Initiative (IAVI) (www.iavi.org) and US National Institutes of Health Vaccine Research Center (www.vrc.nih.gov), and provide details and references for the individual trials. Several vaccine concepts, schedules of immunization, routes of administration, and adjuvants have been tested. Classical vaccine strategies based on live-attenuated or whole-inactivated HIV have severe limitations. Most efforts to develop an AIDS vaccine have therefore focused on newer vaccine approaches.

Recombinant envelope subunits: Recombinant soluble HIV and Simian immunodeficiency virus (SIV) glycoproteins have been used as subunit vaccines. HIV-1 gp160, gp140, or gp120 induced only a transient response in primates even with strong adjuvants or special delivery formulations. They did not protect macaques from challenge with the virulent SIVmac. These studies have underlined the importance of the conformational integrity of the envelope glycoprotein for the induction of neutralizing antibodies: current envelope vaccine candidates elicit high gp120-binding antibody titres with neutralizing activity against matched tissue culture laboratory-adapted virus strains but not primary HIV-1 isolates. The antibodies induced by gp120 are usually incapable of neutralizing primary CCR5 isolates, even though they can neutralize the homologous CXCR4 virus strain. They can therefore prevent infection if animals are challenged with a homologous CXCR4 virus but not with a CCR5 virus strain. Efficacy trials with monomeric gp120 derived from circulating primary isolates have demonstrated their absence of efficacy against HIV infection in humans.

Several approaches have been undertaken to overcome these obstacles including mixing gp120
from primary isolates, and soluble gp140 glycoprotein trimers. The resulting molecules showed enhanced immunogenic potency, but the improvement was far from optimal. The removal of glycosyl moieties to unmask neutralization epitopes, the making of gp120-CD4 receptor complexes or the deletion of the 1st and 2nd variable loops from gp120 (currently tested in human volunteers) have been used as immunogens and shown to induce broadly neutralizing antibodies \[^{50}\].

The HIV-1 Tat protein is more conserved than envelope proteins, is essential in the virus life cycle and is expressed very early upon virus entry. In addition, both humoral and cellular responses to Tat have been reported to correlate with a delayed progression to disease in both humans and monkeys. This suggested that Tat is an optimal target for vaccine development aimed at controlling virus replication and blocking disease onset. Subunit vaccines based on the accessory protein Tat have been developed and have demonstrated at least partial protective efficacy in HIV envelope and SIV core genes chimeric construct or SHIV macaque models and are currently tested in humans \[^{51}\]. Gp120 nef/tat fusion adjuvanted subunit protein vaccine induces mediocre immune responses in humans measured by ELISpot INF\(\gamma\) \[^{52}\].

**Synthetic peptides:** Synthetic peptide immunogens either linear or branched initially concentrated on the V3 loop of gp120. Well-tolerated, they were unable to induce neutralizing antibodies against primary isolates \[^{53,54}\]. Although peptides and proteins usually do not induce a class I–restricted CD8 response *in vivo*, lipopeptides have such a capacity and may represent an interesting formulation for inducing or boosting the CTL immune response to HIV. Indeed, long lipopeptides with a fatty acid tail can induce broad cellular immune responses in humans as well as in animals. Lipopeptides tested in France gave impressive immune responses measured by ELISpots INF\(\gamma\) with up to 83 per cent of HIV-specific CD8 responders. The lipopeptides with a monolipid tail and a tetanus toxoid peptide appeared to be superior to double lipid tail peptides \[^{55-57}\].

**Live recombinant vectors:** Live recombinant vector vaccines are either a live attenuated viral or bacterial strain used as a vector to carry HIV genes encoding the antigens of interest (Table IV). They are able to stimulate both humoral and cell-mediated immunity.
Pox vectors such as vaccinia virus vectors were the first to be tested in animals and humans. They induce HIV-specific CTL responses but weak and transient anti-HIV antibody responses and can confer protection in macaque models. The lack of safety of vaccinia virus recombinants in immunocompromised people has led to the prefer the use of attenuated vaccinia virus strains such as NYVAC or the Modified Vaccinia virus Ankara (MVA), a highly attenuated, host range–restricted strain of vaccinia virus. NYVAC HIV-1 subtype C (an attenuated slow replicative genetically engineered vaccinia) was recently tested in humans. A maximum of 50 per cent ELISpot INFγ response was observed58.

MVA, Modified vaccinia Ankara, AAV, Adeno-associated virus, SFV, semliki forest virus; NYVAC, Attenuated vaccinia by genetic engineering

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Defective alphavirus “replicons” such as Venezuelan Equine Encephalitis (VEE) virus, Sindbis virus and Semliki Forest virus (SFV) replicons offer the advantages of an important amplification of the viral message after infection and are able to target DNA glycosylase (udg-) gene. Macaques immunized with this MVA udg- exhibited significantly higher levels of CD8 and CD4 T-cell proliferation one week post-immunization compared to normal MVA vector. In prime-boost experiments, measles vector prime and MVA udg- boost induced stronger responses than measles or MVA udg- alone60.

Other pox vectors, non-replicative in mammalian cells, have been developed including canarypox (ALVAC) or fowlpox viruses. Although very safe in humans, since non-replicative, they are less immunogenic than vaccinia. Results from several Phase I and II trials with recombinant canarypox vectors have demonstrated the induction of CD8 CTL in a limited proportion of vaccinees (15-30%) at any given time point post-immunization. Interestingly, some CTL responses generated by recombinant canarypox vectors were cross-clade61.

Human adenovirus types 4, 5, and 7 can be administered orally or intranasally and can induce both systemic and mucosal immunity. Recently, an Ad5 recombinant expressing HIV-1 Gag was found to successfully induce cellular immune responses in rhesus macaques and to attenuate infection and mitigate disease progression after challenge with a pathogenic SHIV. Recently DNA priming and recombinant Ad5 boosting was able to induce strong cell-mediated immune responses in human volunteers. The priming effect of DNA was however limited. Adeno 5 subtype B was tested in human volunteers62. DNA prime does not bring substantial advantage over Ad5 alone, especially in Ad5 naïve subjects. Volunteers without pre-existing immunity to Ad5 develop strong ELISpot responses in up to 82 per cent of them. In contrast, the percentage of responders is inversely proportional to the titres of pre-existing Ad5 antibody titer (down to 28%). In developed countries the percentage of Ad5 immune individuals is about 30 per cent with low titers increasing to 70 per cent with high titers in developing countries63.

In non-human primates, MVA recombinants were found to induce potent CTL responses able to partially control virus loads after challenge with pathogenic SHIV or SIV59. A new MVA vector was engineered that is genetically blocked for progression through the poxvirus replication cycle via the deletion of the uracil-
dendritic cells, resulting in efficacious antigen presentation 64.

AAV is a naturally occurring virus that depends on a helper virus for replication. Although pre-existing immunity to AAV is common in the US and Europe, extensive epidemiological studies have found this virus to be non-pathogenic. The AAV-based vaccine is not capable of replication as it lacks the requisite AAV viral elements that respond to helper adenovirus co-infection and the vaccine is thus incapable of replication, even in the presence of helper viruses. Persistence of vaccine DNA sequences in cells occurs by a mechanism involving an unintegrated episomal concatamer 65. Although 25-60 per cent of humans in the US and Europe have binding antibodies against AAV, only a small percentage of the population exhibit neutralizing antibody, and the low titres observed likely will not prevent a ‘vaccine take’. No data on pre-existing immunity to AAV is available for Southeast Asia and Africa. Preclinical data show that AAV-based vaccines induce both antibody and T-cell responses against the inserted genes when delivered as a single intramuscular injection. Immune responses in mice and macaques persist at least six months after a single intramuscular injection. AAV-based AIDS vaccine clinical trials have been initiated in Europe.

Numerous other virus vector systems like the attenuated vaccine strains of measles or yellow fever viruses, poliovirus replicons, rabies virus, vesicular stomatis virus, or Sendai virus are being developed for the preparation of live recombinant HIV vaccines (Table IV). Live recombinant bacterial vaccines have also been developed including bacillus Calmette-Guerin (BCG), Salmonella, Listeria monocytogenes and Shigella 66.

However, in individuals previously exposed to the vector and who developed a residual immunity to the vector, most live recombinant viral or bacterial vectors show a decreased immunogenicity compared to naïve hosts. Volunteers with low pre-existing Ad5 antibody titres developed strong cell-mediated immune responses but not those with high pre-existing immunity 67. This serious limitation is not observed with canarypox or fowlpox vectors but applies to BCG, poliovirus, or human adenovirus. It also applies to MVA and NYVAC in the populations vaccinated against smallpox. This might also be an issue for booster injections, as successive immunizations with the same vector will induce immunity to the vector.

**Naked DNA**: Injection into the muscle or the epidermis of a purified plasmid DNA that carries a gene encoding an antigen under the control of an appropriate mammalian transcription promoter leads to expression of the antigen in situ and triggers an immune response, mostly of the Th1 type. DNA vaccines alone even when engineered as synthetic HIV-1 genes with optimized codons or formulated with cytokines or various delivery systems have however been disappointing, inducing weak immune responses in primates and humans 68.

**Prime-boost concept**: In animals, priming with a viral vector or nucleic acid vaccine, followed by boosting with either another vector or subunit/peptide vaccine, induced stronger immune responses compared to vaccination with either vaccine alone 69. Several prime-boost strategies have been tested over the past decade. They are summarized in Table V.

Prime-boost combinations using a DNA vaccine for priming and recombinant MVA or fowlpox virus vaccines for boosting elicited the best CTL responses in macaques 70 that showed lower virus loads and prolonged survival following pathogenic SHIV challenge 71. Recombinant vaccinia, MVA, and fowlpox constructs were comparable in their immunogenicity. Moreover, whereas the magnitude of the peak vaccine-elicited T lymphocyte responses in the recombinant pox virus-boosted monkeys was substantially greater than that seen in the monkeys immunized with plasmid DNA alone, the magnitude of the CTL responses decayed rapidly and was comparable to those of the DNA-alone-vaccinated monkeys by the time of viral challenge. Consistent with these comparable memory T-cell responses, the clinical protection seen in all groups of experimentally vaccinated monkeys was similar. This study, therefore, indicates that the steady-state memory, rather than the peak effector vaccine-elicited T lymphocyte responses, may be the critical immune correlate of protection for a CTL-based HIV vaccine 72. Recently, a prime-boost regimen with DNA + MVA expressing strings of CTL epitopes from HIV-1 subtype A was
tested in human volunteers in United Kingdom, Kenya and Uganda and showed disappointing results. Less than 20 per cent of vaccinees developed ELISpot INFγ responses\textsuperscript{73,74}. Whether different MVA constructs will add significant benefit remain to be demonstrated.

A prime-boost regimen using DNA prime and fowlpox (FPV) boost was tested in macaques subsequently SHIV-challenged. Interestingly, priming with two DNA injections followed by one FPV booster injection was equivalent to three DNA injections only as measured by decrease in viral load after challenge although the first regimen induces stronger ELISpot INFγ responses. This may suggest that ELISpot IFNγ may not be a good immune correlate of protection. In Australian volunteers, the low (1.5 mg) and high (4.5 mg) doses of DNA vaccine induced similar immune responses\textsuperscript{75}.

Prime-boost with lipopeptides and canarypox did not show improvement in immune responses over lipopeptides alone. Lymphoproliferation following peptides was detected in all vaccinees and in only 38 per cent with canarypox alone\textsuperscript{76}.

Ad5 prime followed by ALVAC (canarypox) boost induces a synergistic response in animals\textsuperscript{77}, which was unfortunately not observed in humans.

In order to circumvent the pre-existing immunity issue to the vaccine vector, efforts now focus on developing vaccines derived from adenovirus subtypes rarely infecting humans such as Ad11 and Ad35. Ad11 prime boosted either by Ad5, Ad35 or Ad11 shows that heterologous is superior to homologous boost (Ad11+Ad35)\textsuperscript{78}.

In animals, AAV subtype 1 seems to induce stronger immune response measured by ELISpot INFγ as compared to AAV subtype 2. Several prime-boost regimens using homologous or heterologous AAV constructs and MVA were compared in macaques: AAV2+AAV1>AAV2+AAV2>AAV2+MVA>MVA+MVA. When using recombinant vectors, heterologous prime-boost strategies seem to induce stronger cell-mediated immune responses than homologous ones\textsuperscript{79}.

**Pediatric trials:** The rapidly increasing prevalence of mother-to-child transmission (MTCT) of HIV-1 worldwide has resulted in an urgent need for effective preventive strategies\textsuperscript{80}. Although antiretroviral regimens have shown considerable efficacy in reducing vertical transmission of HIV, it is still not known whether such regimens would prevent the transmission of HIV-1 through breastfeeding beyond the neonatal period\textsuperscript{81}. In addition, children who escape MTCT are again at risk for infection when they become sexually active as adolescents. An infant vaccine regimen, begun at birth, represents an attractive immunization strategy and might also provide the basis for lifetime protection against HIV-1 infection. However, the development of protective immunity may take time and passive coverage until immunization is complete would be important in preventing breast milk transmission of HIV-1. Since the majority of infants acquire infection at or within 6 months of birth, efficacy data could be available as early as 6 months - 1 yr following the initiation of the Phase III trial.

**Adolescents:** It is acknowledged that adolescents should benefit in priority of a safe and efficacious AIDS vaccine once available before they enter into their sexual life. The participation of adolescents in efficacy trials raises several ethical and legal concerns and is thought to be unnecessary for licensure at least in the general population. The debate however remains open for some high-risk groups such as sex workers and men having sex with men that de facto include some adolescents. The growing consensus is that once efficacy is demonstrated in adults, bridging studies should be conducted in adolescents\textsuperscript{82}.

**Role of India:** The last two years have been for the Government of India through leading institutions including the Indian Council of Medical Research (ICMR), the National AIDS Control Organisation (NACO) and their partner International AIDS Vaccine Initiative (IAVI), a period of intense activities and great achievements in the domain of AIDS vaccine development. Other institutions under the Department of Biotechnology (DBT) leadership are also actively involved in several vaccine approaches.

In 2002, a multiple AIDS vaccine candidate strategy approach was adopted. This strategy would allow to speed up the testing of several vaccines in
parallel rather than sequentially to ensure that an effective vaccine is available at the earliest. As a consequence, it was decided to set up centres of excellence for AIDS vaccine clinical and laboratory evaluation at two ICMR Institutes in India. This strategy would contribute significantly to the research and development capacity building in India.

One centre is located at the National AIDS Research Institute (NARI), Pune, working towards conducting the first phase I clinical trial with the AAV-based AIDS vaccine in 2005, assuming all approvals are granted. This centre is fully operational including a clinical centre and of the state-of-the art immunology laboratory entirely dedicated to clinical trials. The second AIDS vaccine clinical evaluation centre is being set up at the Tuberculosis Research Centre, Chennai. A Phase I trial with the MVA vaccine is expected to begin in 2005.

The implementation of clinical trials to test the vaccine efficacy is the bottleneck of clinical development for any vaccine, in particular for AIDS vaccines. Such implementation represents a tremendous scientific and organisational challenge that must be anticipated years in advance. An important step is therefore to define the feasibility of such trials in India. In this regard, assessments of various infrastructures and organisations working with different communities and their epidemiological characteristics were conducted by a team comprising of several national and international experts. The team visited select scientific institutions and non-governmental organisations in view of providing recommendations for the conduct of site and community preparedness studies.

The AIDS vaccine development programme in India is based on transparency and accountability. To ensure that the trials are ethical and safe, broad-based community support is required. Several mechanisms and bodies have been put in place including the: (i) National AIDS Vaccine Advisory Board providing guidance to the Government of India. (ii) Informed Consent Group to develop a template for the Informed Consent documents used in the Phase I trials; (iii) Gender Advisory Board and Training to help oversee and guide the Indian vaccine programme’s efforts to incorporate gender concerns in AIDS vaccine trials. Training modules and a framework for implementing gender training for trial staff was developed with their assistance; (iv) Non-Governmental Organisation Working Group to ensure that communities are better informed of the clinical trial processes and that the trials are conducted in a safe and ethical manner with community support and representation; and (v) National Consultation on HIV Care and Treatment in AIDS Vaccine Trials. The consultation helped define the policy and technical guidelines for the care and treatment of trial participants, including those who become HIV infected, during the course of AIDS vaccine clinical trials, within the larger framework of the NACO HIV/AIDS care and treatment policy guidelines.

Conclusion

The quest for an AIDS vaccine presents an unprecedented scientific and human challenge for this 21st century. AIDS vaccines must be seen as the ultimate prevention tool that will complement the existing prevention strategies. The acceleration of vaccine development through the parallel exploration of several scientific approaches and implementation of clinical trials are the best and probably only way to reach this goal. This long-term commitment endeavour requires strong and renewed political leadership and commitment, flexibility of processes, medical and scientific dedication and collaboration on a mission mode along with community participation for immediate action. The recent achievements in India highlight clearly the commitment of the Government of India and the scientific community to a long-term global effort to develop an AIDS vaccine.

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