Progress in HIV vaccine development
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Recent advances in HIV vaccine development include initiation of the first efficacy trials and substantial expansion of the preclinical pipeline. Several preclinical candidate vaccines have induced strong cellular immune responses and provided impressive protection against AIDS in non-human primate models; however, candidates that induce broadly neutralizing antibodies remain elusive.

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Abbreviations
CTL cytotoxic T lymphocyte
HIV human immunodeficiency virus
MHC major histocompatibility complex
SIV simian immunodeficiency virus
TCLA tissue-culture laboratory-adapted

Introduction
A safe and effective vaccine is the best hope for stopping the spread of HIV worldwide. As the 20th anniversary of the discovery of HIV approaches, considerable optimism is building that identification of an HIV vaccine is within reach. Advances in vaccine design, animal models and clinical research have recently converged to create a promising pipeline of candidate vaccines. However, overcoming remaining scientific, logistical and financial challenges will require the talents and resources of all stakeholders — academic researchers, pharmaceutical companies, philanthropic organizations, governments and communities. This review outlines the major scientific advances of the past two years and highlights important challenges in converting the current optimism into success.

Clinical trial results
The HIV envelope is the predominant target of neutralizing antibodies in HIV-infected individuals. Several adjuvanted recombinant monovalent HIV envelope proteins (e.g. gp160 or the mature exterior portion gp120), based on tissue-culture laboratory-adapted (TCLA) isolates of subtype B HIV, have been extensively studied in human trials. These candidates induced neutralizing antibodies in virtually all volunteers tested, but these antibodies exhibited little cross-reactivity against primary isolates of HIV [1]. Subsequently, bivalent candidates developed by VaxGen Inc. (AIDSVAX®, Brisbane, CA) have advanced to efficacy trials in the USA and Thailand (Table 1). The bivalent vaccine comprises two gp120s, one from a subtype B TCLA isolate of HIV and one from a subtype B or E primary isolate, and trial results are expected around the end of 2002.

Until recently, the frequency and strength of neutralizing antibodies and cytotoxic T lymphocytes (CTLs) induced by peptides based on the viral envelope or internal proteins have been disappointing. Peptide lipid-ation has shown some promise in improving immunogenicity — lipopeptides derived from env, gag and nef proteins induced CTLs to one or more peptides in up to two thirds of immunized volunteers [2]. Use of novel adjuvants, cytokines and co-stimulatory molecules are also under investigation. For example, a saponin adjuvant (QS21), although not well tolerated, decreased the dose of gp120 required to induce high-titer antibodies [3]. Peptides based on predictions of epitopes representing immunodominant, conserved, ‘supertype’

Table 1
HIV vaccine candidates in clinical trial.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>HIV subtype</th>
<th>Producer</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp120</td>
<td>B/B, B/E</td>
<td>VaxGen</td>
<td>Phase III trials ongoing in the US and Thailand</td>
</tr>
<tr>
<td>ALVAC-HIV</td>
<td>B, E</td>
<td>Aventis Pasteur</td>
<td>In phase II trials in the US, Haiti, Brazil and Trinidad (subtype B), and Thailand (subtype E); tested alone or in combination with gp120</td>
</tr>
<tr>
<td>ALVAC-HIV</td>
<td>A</td>
<td>Aventis Pasteur</td>
<td>Ready for phase I trial in Uganda</td>
</tr>
<tr>
<td>Lipopeptides LP5, LP6</td>
<td>B</td>
<td>ANRS</td>
<td>In phase I trials in France</td>
</tr>
<tr>
<td>Vaccinia TBC-3B</td>
<td>B</td>
<td>Therion</td>
<td>In phase I trials in the USA</td>
</tr>
<tr>
<td>DNA-HIV</td>
<td>B</td>
<td>Apollon</td>
<td>Phase I trials completed</td>
</tr>
<tr>
<td>DNA-HIV, MVA-HIV</td>
<td>A</td>
<td>University of Oxford</td>
<td>In phase I trials in the UK and Kenya</td>
</tr>
<tr>
<td>NYVAC-HIV</td>
<td>B</td>
<td>Aventis Pasteur</td>
<td>Ready for phase I trial in the USA</td>
</tr>
<tr>
<td>DNA-HIV, Adenovirus-HIV</td>
<td>B</td>
<td>Merck</td>
<td>In phase I trials in the USA</td>
</tr>
</tbody>
</table>

ALVAC-HIV, recombinant canarypox expressing multiple HIV genes; ANRS, National Agency for AIDS Research, France; MVA-HIV, modified vaccinia Ankara, an attenuated vaccinia vector, expressing multiple HIV genes; NYVAC-HIV, an attenuated vaccinia vector expressing multiple HIV genes; TBC-3B, attenuated vaccinia vector expressing multiple HIV genes.
epitopes (e.g., recognized by multiple alleles) are also under development [4∗].

DNA candidates, thus far, have not fulfilled the expectations arising from early studies in mice. DNA vaccines encoding *env* and *gag-pol* genes were safe in doses of up to 3 mg, but failed to induce strong immune responses (Goepfert *et al.*, *Int Conf AIDS* 1998,12:635) [5∗]. Codon-optimized, adjuvanted and particle-formulated candidates are expected to perform better.

Live recombinant vectors expressing one or more HIV genes are among the most promising candidate vaccines. The first HIV recombinant viral vector evaluated in humans was an attenuated vaccinia that expressed the HIV gag and env genes required for complete replication and/or particle formation. The most extensively studied vector in human trials is ALVAC®, a recombinant canarypox developed by Aventis Pasteur. Five canarypox-HIV recombinants, alone or in combination with gp120 subunit vaccines, have been evaluated in humans. Although HIV-specific CTL responses were detected in only about one-third to one-half of volunteers, the concomitant induction of neutralizing antibodies and T-helper responses in volunteers boosted with gp120 has made this ‘prime-boost’ a promising approach [6∗∗,7∗∗]. A phase II study of a canarypox HIV candidate (ALVAC®vCP1452) in combination with gp120 (AIDSVAX®/B/B, VaxGen) is underway in the USA. This study will lead to an efficacy trial in late 2002 if immunogenicity criteria are met. Another recombinant pox vector, modified vaccinia Ankara (MVA, IDT Germany, under contract to the University of Oxford, UK) expressing HIV gag and a number of CTL epitopes, has recently entered clinical trial in the UK and Kenya.

**Preclinical studies**

Preclinical studies have truly fueled the current optimism. First, several candidate vaccines have produced promising results in rather stringent non-human primate models of AIDS. Second, the number of candidates advancing toward phase I human trials has increased dramatically in the past three years (Table 2).

Advancements in the field of HIV and SIV (simian immunodeficiency virus) immunology have permitted more thorough and sensitive evaluation of cellular responses to HIV and SIV candidate vaccines (Table 3). Until a few years ago, cellular immune assays were limited to measuring proliferation of T cells exposed to antigen
Transmissibility of virus from these animals has also not yet been determined, although viral load in the plasma of HIV-infected persons strongly correlates with transmission to sexual partners and to newborns [24**,25]. Whether vaccine-induced long-term control of HIV replication will prevent HIV transmission remains to be determined.

Most candidate vaccines that controlled infection through strong cellular immune responses did not induce high-titer neutralizing antibodies. However, cocktails of antibodies passively transferred have protected macaques against pathogenic challenge — protection correlated with in vitro neutralization results [26,27**]. Studies with strains of HIV that have been genetically modified have provided additional evidence that antibody can contribute to the control of viremia [28]. Thus, a candidate vaccine that induces broadly neutralizing antibodies as well as strong cellular responses could provide improved protection against infection or disease.

Additional optimism has also come from the substantial increase in the number of vaccine candidates that are scheduled to enter clinical trial in the coming 1–3 years. In view of the high risk and relatively poor global market forces that dissuade aggressive private sector investment in product development, particularly for candidate vaccines based on HIV subtypes that predominate worldwide, government and philanthropic sources have supported the preclinical development of many of these (Table 2).

**Vaccine design**

As noted above, recombinant monomeric gp120 envelope candidate vaccines elicit antibodies that are generally subtype specific and neutralize TCLA isolates but few if any primary isolates of HIV. For this reason, there is little confidence that the candidate recombinant envelope vaccines now in clinical trials will induce neutralizing antibodies with the breadth necessary for worldwide use. At a minimum, cocktails of gp120s would be necessary. Antibodies induced in human volunteers by ALVAC and gp120 have been reported to neutralize five out of 14 primary isolates of HIV, including HIV with different co-receptor usage [29*].

Table 3

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Assessment</th>
<th>Specific assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humoral immune responses</td>
<td>Antibody binding assays</td>
<td>ELISA, Western blots</td>
</tr>
<tr>
<td></td>
<td>Antibody neutralization assays</td>
<td></td>
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<tr>
<td></td>
<td>Antibody-mediated fusion inhibition assay</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antibody-dependent cytotoxicity</td>
<td></td>
</tr>
<tr>
<td>Cellular immune responses</td>
<td>Proliferation to soluble antigens (mostly CD4+ cells)</td>
<td>Chromium-release assay</td>
</tr>
<tr>
<td></td>
<td>Cytotoxicity</td>
<td>Tetramer binding</td>
</tr>
<tr>
<td></td>
<td>Enumeration of antigen-specific T cells</td>
<td>ELISPOT, intracellular staining</td>
</tr>
<tr>
<td></td>
<td>Enumeration of cytokine-producing cells (IFN-γ, TNF-α, etc.)</td>
<td>(flow cytometry)</td>
</tr>
</tbody>
</table>

ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor α.
Unfortunately, although new viral vectors that enter human trial this year and next may prove to induce more consistent or higher levels of CTLs, candidates likely to induce broadly neutralizing antibodies have not yet been identified.

Efforts to design a vaccine that induces broadly reactive antibodies against primary isolates were given a boost with the report that a fused cell preparation (comprising cells expressing HIV envelope and cells expressing HIV receptors) induced antibodies in transgenic mice that neutralized 23 of 24 primary isolates from different HIV subtypes [30]. Although this result has not been reproduced, several groups have constructed modified envelopes that might reveal conserved conformational epitopes critical to HIV entry with somewhat encouraging results. The V2 loop is one of three highly variable sequences of the HIV envelope. Removal of this from a DNA vaccine resulted in a candidate vaccine that induced antibodies that were somewhat more broadly reactive than the parent molecule [31]. A stabilized envelope trimer, designed to resemble the functional envelope glycoprotein on the surface of the virion, induced neutralizing antibodies against select primary isolates and TCLA HIV, whereas trimers derived from TCLA HIV induced antibody that neutralized only the homologous virus [32]. Other approaches — including stable oligomerization, removal of carbohydrate molecules, modification of envelope to be independent of CD4, and gp120–CD4 fusion proteins or complexes — are also under investigation [28,33–35,37]. No outstanding envelope candidate has yet emerged.

Other remaining challenges

One achievement that would advance the field of HIV vaccine development more than any other would be identifying a candidate vaccine that shows some protection in human trials and determining the immune correlate(s) — the type, magnitude, breadth and/or location of immune responses — that are associated with protection. Sensitive and quantitative antibody assays have been in existence for decades. The new cellular assays described above are now being employed in vaccine clinical trials, increasing our ability to detect and quantify vaccine-induced cellular responses. This has improved hope that an immune correlate can be identified in the context of large efficacy trials.

Table 4

Possible outcomes of immunization against HIV.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Specific effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilizing immunity</td>
<td>No cells contain integrated provirus (no virus detected at any time in blood, lymph nodes, or at the site of exposure using the most sensitive PCR assay) No seroconversion to HIV proteins not in the vaccine No CTLs to HIV proteins not in the vaccine</td>
</tr>
<tr>
<td>Transient infection</td>
<td>Low level of virus detected only very early following exposure (no virus detected in blood, lymph nodes, or at the site of exposure using the most sensitive PCR assay at 6 months and all later times) No or transient seroconversion to HIV proteins not in the vaccine No or transient CTLs to HIV proteins not in the vaccine</td>
</tr>
<tr>
<td>Controlled infection</td>
<td>Virus levels fall to and remain at low to undetectable levels (&lt;1000 RNA copies/ml) following the acute stage of infection Seroconversion to HIV proteins not in the vaccine occurs CTLs to HIV proteins not in the vaccine are present</td>
</tr>
<tr>
<td>Lack of transmission to others</td>
<td>Virus levels in blood and secretions remain insufficient to infect others</td>
</tr>
</tbody>
</table>

Various potential outcomes might result from immunization (Table 4). Because HIV integrates into the host cell’s DNA, once infection occurs, it may not be possible to completely eliminate the virus. Long-term control may be the only feasible outcome. In any case, for a vaccine to have substantial public health value it should prevent the vaccine recipient from passing the virus on to others. Evaluating outcomes other than sterilizing immunity, defined as the absence of detectable infection, will require long-term follow-up and will present enormous challenges.

Another challenge is to decipher the relevance of different HIV subtypes to vaccine development. Several studies have demonstrated that antibody recognition does not correlate completely with genetic subtype [38,39]. Further, CTLs directed against one HIV subtype can kill cells

Table 5

Challenges to conducting preventive HIV vaccine efficacy trials.

<table>
<thead>
<tr>
<th>Industrialized countries</th>
<th>Developing countries</th>
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<tbody>
<tr>
<td>Relatively low incidence of HIV infection even in higher risk groups requires large trials of thousands per arm</td>
<td>Concerns regarding exploitation and unequal partnerships</td>
</tr>
<tr>
<td>At-risk populations present recruitment and retention challenges particularly women at sexual risk, men at heterosexual risk and intravenous drug users</td>
<td>Concerns that the country will not have affordable access to the vaccine if proven efficacious</td>
</tr>
<tr>
<td>Distrust of researchers and government</td>
<td>Infrastructure needs: clinics, labs, equipment, supplies</td>
</tr>
<tr>
<td>Growing misunderstandings and distrust of vaccines in general</td>
<td>Training needs: science, good clinical practice, ethics, lab assays, data management</td>
</tr>
<tr>
<td></td>
<td>National authorities and institutional review boards poorly supported or nonexistent</td>
</tr>
</tbody>
</table>
infected with HIV from other subtypes, due largely to the more highly conserved nature of the internal HIV proteins. The pattern of such cross-killing varies and is less efficient relative to homologous targeting against cells infected by HIV from the same genetic clade; however, the magnitude needed to provide protection remains unknown [24**,40,41]. In addition, individuals with different human leukocyte antigen (HLA) backgrounds are likely to focus CTL responses on different epitopes, which could theoretically impact immune responses to vaccines and efficacy of vaccines found to be effective in other populations [42]. Until a correlate of immune protection is validated, clinical trials must be carried out in multiple countries, where different HIV subtypes circulate, to determine whether any vaccine will be broadly efficacious. Some of the problems associated with conducting such trials are shown in Table 5.

Conclusions

With the advent of improved cellular immune assays, there is a strong desire to move candidate vaccines that could prove at least partially effective into efficacy trials to attempt to define immune correlates. However, as the properties required in a successful HIV vaccine remain unknown, academic creativity in the design of vaccines, animal models and clinical trials is needed. This should ensure that improvements would continue if the candidate vaccines in trials or in the pipeline prove lacking in the degree, breadth or durability of efficacy. Fortunately, in recent years a number of promising new candidate vaccines that induce strong cellular immune responses have yielded improved results in preventing AIDS in animal models. Several of these candidates have recently or will soon enter clinical trials, fueling the current optimism that identifying a safe and at least partially effective HIV vaccine in this decade is an achievable goal.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest •• of outstanding interest


In comparison to alum, QS-21 adjuvant significantly reduced the maximal effective dose of gp120 envelope subunit vaccine with respect to antibody induction, T-cell proliferation in response to antigen, and delayed hypersensitivity skin responses in volunteers.


Eleven highly conserved peptide sequences predicted to represent strong binding supermotifs for MHC class II DR alleles and thus capable of inducing T-cell helper responses, were selected after confirming such binding and tested for antigen-specific proliferation on cells from 31 HIV-1 infected individuals. Each peptide was recognized by several patients and many patients recognized multiple peptides. Recognition was mediated by human T cells.


The immunogenicity of a DNA vaccine encoding env and rev genes was tested in healthy volunteers. Although proliferative responses were observed at various times in volunteers who received a 300 μg dose, no CTLs were detected by either ELISPOT or lytic chromium release CTL assays.


A recombinant HIV-1-canarypox (ALVAC vCP-205) administered with or without gp120 subunit was evaluated in a phase II trial that enrolled 435 volunteers at low or high risk for HIV infection. Vaccine safety was demonstrated. One-third of the volunteers developed CTL responses and 94% and 56% of those receiving the combination or ALVAC alone, respectively, developed homologous neutralizing antibody responses.


In a phase I trial 62% of volunteers receiving vCP-205 – a recombinant canarypox vector expressing the HIV gp120, gag and protease – as well as a recombinant gp120 envelope subunit developed HIV-specific CTL responses. Over 90% of volunteers developed neutralizing antibodies to the vaccine parental strain.


An ELISPOT assay was developed that identifies cells producing interferon-γ (IFNγ) after antigen-specific stimulation. Peptide pools representing the antigen sequences were incubated with peripheral blood mononuclear cells (PBMCs) in ELISA plates coated with antibody to IFNγ and then the number of spots of cell proliferation were read visually. Background spots ranged from 1–50 per million PBMCs, whereas responses to malaria antigens tested were 100–450 spots per million PBMCs. Within assay and between assays variation coefficients were 21.9% and 24.7%, respectively, making this assay acceptable for evaluation of vaccine-induced T-cell responses.


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A codon-optimized SIV gag DNA vaccine elicited high-frequency SIV-specific CTLs in peripheral blood and lymph node lymphocytes. When challenged intravenously with pathogenic SIVmac60 the immunized monkeys developed a secondary CTL response and contained viral replication for a 50-day follow-up, although peak viral RNA levels post-challenge were comparable to controls. These results demonstrate the applicability of soluble MHC Class I tetramer technology to monitor vaccine-induced CTL responses and to detect tetramer-positive cells in unstimulated whole blood.


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Macaques immunized with DNA vaccine encoding SIV gag and HIV-1 env admixed with interleukin 2 (IL-2)/IFN-gamma and challenged with SHIV-89.6P demonstrated potent secondary CTL responses, stable CD4+ T-cell counts, low to undetectable setpoint viral loads and no evidence of clinical disease by 140 days after the challenge. Improved outcome correlated with augmented vaccine-elicited CTL responses. Data suggested that gag-specific CTLs above approximately 0.3–0.5% of CD3+CD8+ T cells provided little additional discernible benefit. The role of neutralizing antibodies in controlling viral replication was less clear as no neutralizing antibody responses were detected in vaccinated monkeys prior to challenge. These results demonstrate the importance of vaccine-elicited CTL responses in protecting monkeys from AIDS-like disease.


The authors introduced disulfide bonds between the C-terminal region of gp120 and the immunodominant segment of the gp41 ectodomain of a primary R5 isolate with up to 50% efficiency. This protein, when cleaved by furin, was more efficiently recognized by potent neutralizing antibodies, not detectably recognized by non-neutralizing anti-gp120 or anti-gp41 monoclonal antibodies, and exposed the epitope for Mab 17b upon binding sCD4. Whether this or similar proteins prove to have immunogenic properties different than unmodified gp120 or gp140 remains to be determined. Gp140 is an engineered truncated form of the full-length gp160 molecule, which is cleaved into the mature gp120 external envelope protein and the gp41 transmembrane envelope protein during viral replication.


Single-chain chimeric molecules, comprising gp120 and CD4 and deleted in one or more portions of the gp120 molecule, were characterized with respect to their ability to bind the CCR5 second receptor and to block HIV infection in cultured cells. These constructs were shown to present complex-dependent epitopes and thus could be interesting immunogens if they are able to induce broadly neutralizing antibodies.


