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The past, present and future of HIV-vaccine development: a critical view

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Despite the extensive efforts that have been made to combat AIDS, the global number of HIV-1 infections is still increasing. There is major consent among scientists worldwide, that the development of successful HIV vaccine strategies requires a profound understanding of the epidemiological principles of a viral pandemic, as well as deep insights into the molecular and immunological mechanisms of HIV pathogenesis. This review provides an overview of past and present developments, as well as future aspects of HIV vaccines, and also provides a summary of current clinical trials in man.

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▼ Since the discovery of HIV in 1983 [1], AIDS has become one of the greatest infectious disease threats to human health. A total of 13.9 million people have already died from AIDS since the beginning of the epidemic and the syndrome remains in the top five fatal diseases. Although effective antiretroviral therapy has limited the expansion of the epidemic in some industrialized countries, the numbers of new HIV infections, especially in the developing countries (including Africa, Asia and South-America) increase on a daily basis (http://www.unaids.org). It appears that the burden of AIDS weighs on the poorest societies, but the industrialized countries should take the responsibility to combat HIV and AIDS and control the epidemic as quickly as possible. Despite the efforts of local public relations to prevent viral transmission, the development of an effective vaccine is still one of the greatest hopes of controlling the HIV epidemic.

Indeed, HIV replication can be controlled successfully and one of the best examples is provided by several studies with so called long-term non-progressor patients (LTNPs) [2,3]. LTNPs comprise HIV seropositive individuals with stable $CD4^+$ T-cell counts (>500 cells μl^{-1}) and persistent low plasma viremia (<500 RNA ml^{-1}) over a period of at least 10 years post-infection, without developing any clinical symptoms of infection. When considering vaccine design, the immunological effectors that contribute to maintaining low viral loads are, as well as the virological [4] and host genetic determinants [5,6], the most interesting ones [7,8]. The possibility for natural immunity to efficiently control virus infection makes it possible to believe that, if the mechanisms of survival are known, we will be able to generate a successful HIV vaccine. Additionally, in contrast to Hepatitis B infection, for example, mucosal transmission of HIV-1 is relatively inefficient [9]. These observations further support the possibility of protecting against primary HIV-infection. Moreover, there is also clinical evidence that HIV-2 infection confers partial protection against HIV-1 [10], suggesting that a preventive vaccine could, in principle, be achievable.

HIV vaccines: effectors of immune responses

In general, vaccines work through the induction of adaptive immune responses, thus establishing immunological memory for antigenic structures presented by the infected cells or by the pathogen itself (Fig. 1). The basic components of adaptive immunity include B cells and T lymphocytes. There is still some debate about the components of the adaptive immune system that mediates protective immunity against HIV infection. For almost all currently licensed vaccines, antibodies are the major components that confer protection against virus infection [11]. Thus, initially it was suggested that neutralizing antibodies might also be a major effector of anti-HIV immunity. Antibody titres can be sustained at high levels in serum and – although in much lower concentrations – in mucosal secretions. Secretory antibodies are present at any time

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of infection and, in addition to strong mucosal humoral immune responses, could provide an effective initial barrier to HIV infection through sexual transmission. Although, a wide variety of antibodies is induced in HIV-infected patients, only a few of them neutralize heterologous strains or primary field isolates [12,13]. However, the generation of HIV vaccines that are capable of inducing strong cross-clade neutralizing mucosal and serum antibodies is complicated by the high variability of the HIV Env proteins. So far, nine genetic subtypes (A–K) within group M (main) as well as groups O (outlier) and N (new or non-M, non-O), have been characterized [14,15]. For most of these subtypes no prototype virus has yet been found. Moreover, an increasing number of intersubtype recombinants or mosaic viruses has been described recently, largely contributing to the antigenic diversity of HIV isolates [16,17]. Unfortunately, the lack of correlation between genetic subtypes and neutralization [18,19] makes vaccine development more complex [20].

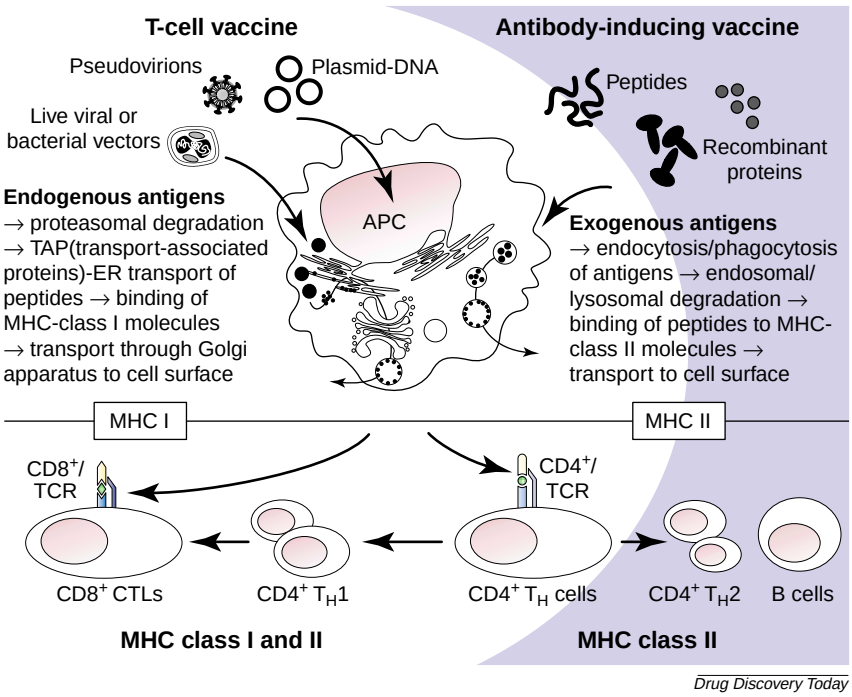

Figure 1. Principles of antigen-specific immune induction by vaccination. The mechanisms of T-cell vaccines (e.g. DNA vaccines, live viral vectors) to induce a predominantly MHC-class I, in addition to MHC-class II restricted immune responses, as well as the mechanisms of an antibody inducing vaccine, such as a subunit vaccine to induce MHC-class II immune responses, are shown. Abbreviations: CTL, cytolytic T-cells; MHC, major histocompatibility complex.

There are many examples underlining the importance of cytolytic $CD8^+$ T cells (CTL) in controlling HIV viral infection [21–23]. Most of these are derived from studies with LNTPs [7,24]. These observations suggest that, in addition to neutralizing antibodies, the induction of CTLs could significantly contribute to antiviral immunity. CTLs recognize an infected cell through a cognate interaction between the T-cell receptor and an antigenic peptide epitope presented in a groove of major histocompatibility complex (MHC)-class I molecules. Peptides are derived mainly from proteasomal degraded intracellular antigens expressed within virus-infected cells [25]. In general, the lysis of the infected cells is mediated by the secretion of perforin and granzymes, which penetrate the target cell membrane and induce apoptosis. Alternatively, FasL is upregulated on activated CTLs and its interaction with Fas on the target cell induces apoptosis through distinct pathways. An early cytolytic $CD8^+$ activity induced by an HIV vaccine could not only clear virus-infected cells rapidly but also diminish cytopathological effects associated with strong cellular immune responses to infection.

Furthermore, $CD4^+$ T cells (Th) are required for an efficient initiation of adaptive immune responses. In addition to some capacity to lyse HIV-infected cells [26], the $CD4^+$ T cells establish a microenvironment with a particular cytokine composition, shaping the immune system – dependent on the type of the infectious agent – to either Th1- or Th2-biased immunity [27]. Th1 cells are characterized by the secretion of, for example, interferon (IFN)-γ, interleukin (IL)-2 and IL-12 upon antigenic activation, thus playing an important role in the initiation of cell-mediated immune responses against intracellular pathogens. Controversially, Th2 cells are characterized by IL-4, IL-5, IL-6, IL-10 and IL-13 production. These cytokines control activation and differentiation of B cells into antibody-secreting cells, thus promoting humoral immunity against extracellular pathogens. Although HIV-specific $CD4^+$ cells provide help for both humoral, as well as cellular, immune responses, the need to initiate adaptive T-helper responses presents a dilemma because these cells are the major target for HIV-infection [28]. Again, this fact emphasizes the need for vaccine-induced immune responses pre-existing at the time of HIV exposure so that virus clearance can be accomplished before the burden of infected cells is sufficient to maintain persistent infection.

Recently, the innate immune response representing the first line of defence against pathogens started to merit

further attention in its role in combating HIV infection and disease progression [29]. Interferon producing cells (IPC), which are precursors of dendritic cells that enhance Th2-type responses, can contribute in a notable way to suppress HIV replication by engaging both innate (e.g. NK cells) as well as adaptive (e.g. $CD8^+$ cytotoxic cell) antiviral activities [30]. Another cell type involved in innate immune response is the $CD8^+$ cell showing noncytotoxic antiviral responses (CNAR). Although MHC-unrestricted and antigen non-specific, CNAR is mediated by an unidentified factor termed CAF ($CD8^+$ T-cell antiviral factor), which demonstrated suppression of HIV replication almost 15 years ago [31,32].

The evolution of divergent viral variants and escape mutants as a consequence of extensive HIV replication undoubtedly renders it more difficult for the immune system to control virus load and to further prevent the infection of latently infected cellular reservoirs, such as stem cells or resting lymphoid cells [33,34]. Therefore, an effective vaccine should block events that occur immediately after HIV-1 exposure. One of the most frequently discussed issues is whether or not a successful HIV vaccine has to induce a sterile immunity. By definition, sterilizing immunity provides complete protection from infection. Ideally, no seroconversion to non-vaccine antigens should be notified and no HIV replication should be detectable at any time following exposure, so that transmission to other individuals is excluded. Of note, none of the currently licensed vaccines for other viral pathogens is known to fully prevent infection. Moreover, most of these vaccines are effective because they limit viral replication and spread of the pathogen by keeping the virus titre below the threshold for clinical expression of disease, without completely blocking viral replication. Alternatively, a more realistic HIV vaccine should allow transient infection, characterized by a temporary detection of HIV at mucosal sites or in the blood at an early stage. At later time points, viral replication should be controlled so that HIV infection is rapidly cleared before the establishment of latently infected cells or widespread dissemination. Nevertheless, in recent years there has been an increasing demand for an altruistic vaccine, which just decreases viral load in infected individuals to protect the individual from disease and prevent or diminish transmission raises, especially in developing countries. Although there is still no protective HIV vaccine, there is at least the chance of controlling an HIV epidemic in countries with the highest HIV prevalence by an altruistic HIV vaccine.

The ideal HIV vaccine: what should it look like?

As discussed above, the concerted interaction of innate immune responses, neutralizing antibodies and strong cellular immune responses could correlate with the protection of HIV infection and control of viral replication. Accordingly, which viral antigen should be included in an ideal HIV vaccine composition and how should this antigen be presented to the immune system in the most favourable manner to induce efficient responses? During the early days of HIV-vaccine development it seemed clear that antibodies directed against the envelope proteins gp120 and/or gp41 of HIV could neutralize the virus and, therefore, could be an effective component of a successful HIV vaccine. To date, we still have to deal with the problem of inducing broad cross-neutralizing antibodies against Env and yet none of these approaches alone seems to be very promising. The group-specific antigen Gag, which encodes for structural components of HIV virions is well conserved among diverse HIV subtypes [35]. In addition, strong and broad cross-clade CTL recognition against Gag specific targets, as well as multiple T-helper proliferate responses, have been well documented in both vaccinated animal and HIV-infected individuals [36,37]. Furthermore, non-structural regulatory proteins of HIV, such as Tat, Rev and Nef, which are the first to be expressed in the viral lifecycle, could also be important components of a candidate vaccine to prevent initial HIV replication. Strong cellular immune responses against Rev and Nef could contribute to efficiently eliminating virus-infected cells, whereas neutralizing antibodies against Tat might block transactivation by exogenous Tat and could, therefore, suppress rapid viral replication [38–41]. In conclusion, an ideal HIV vaccine should induce a wide variety of immune responses against multiple viral antigens to combat infectious viral particles, as well as HIV-infected cells at any time of HIV replication (Fig. 2).

In the past 10 years there has been an enormous boost of new vaccine approaches to improve the immunogenicity of vaccine antigens and their delivery into appropriate immunological compartments (Table 1). In addition to conventional recombinant protein immunization strategies, the use of synthetic peptides, recombinant proteins, whole killed HIV, non-replicating HIV-like particles (pseudovirions) or live-attenuated HIV are under investigation. Genetic immunization strategies using naked or liposomal entrapped DNA, live bacterial or viral vectors, as well as replication-defective replicons that carry selected viral genes, represent second- and third-generation delivery systems with promising concepts of innovative vaccination strategies. Eventually, a combination of different vaccine approaches could possibly result in an additive or synergistic effect capable of inducing stronger, broader or more prolonged immune responses.

Moreover, an HIV vaccine should induce immune responses that are broadly reactive in order to confer protection

against almost all genetic HIV subtypes. Although such a candidate vaccine does not exist so far, it remains a realistic and achievable goal. CTLs directed against highly conserved epitopes have been shown to be broadly cross-reactive. However, no one has yet devised an approach that is capable of inducing broadly neutralizing antibodies against variants of the Env antigen. Thus, for most of the currently performed or planned clinical trials, candidate vaccines are originating from representative viral strains that predominant within predefined cohorts in that country. Alternatively, vaccines should include genetic information or subunit proteins from selected HIV subtypes, covering the relevant clades or 'neutralizing groups' and ensuring an overall broad cross-clade immunogenicity.

In conclusion, the ideal HIV vaccine should be a complex, multi-component vaccine containing multiple, precisely selected antigen-encoding genes, as well as proteins or peptides of representative and diverse subtypes, delivered by several approaches of a well-reasoned prime–boost regimen.

Candidate HIV vaccines for clinical trials

Candidate HIV subunit vaccines

The first Phase I HIV vaccine trial was conducted with the full-length *env* gene product (rgp160) produced by MicroGeneSys (Meriden, CT, USA) in 1987 [42–44]. Since that time, additional Phase I trials have been performed using at least 13 different gp160 or gp120 candidate vaccines produced either in insect cells, yeast or mammalian cells. These conventional Env-based subunit vaccines administered with commercial adjuvants, such as $Al(OH)_3$ and MF59, were among the earliest attempts of HIV vaccine development, based on the premise that the HIV Env protein is the prominent target for neutralizing antibodies. Highest

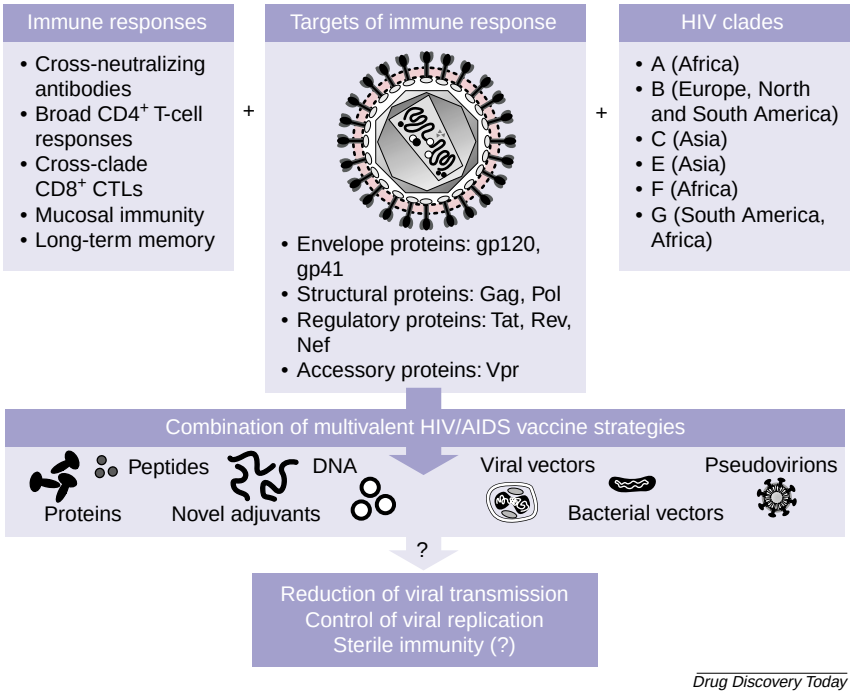

Figure 2. Concepts of the development of a successful HIV vaccine. The knowledge of the effectors for immune protection, genetic clades and viral targets for immune responses could contribute to the development of novel vaccine strategies to reduce viral transmission, control HIV replication and perhaps induce sterile immunity.

Table 1. HIV vaccine strategies

Vaccine strategies	Description
Subunit vaccines	Recombinant viral proteins (e.g. Env, p24, Tat)
Live attenuated vaccines	Live attenuated HIV viruses containing deletions of *nef* and/or *vpr* genes
Whole killed	Chemically inactivated HIV viruses
Peptide-based vaccines	Chemically synthesized HIV protein fragments and/or defined immunogenic epitopes
Pseudovirions	Non-replicating and non-infectious virus-like particles (VLPs) consisting of, for example, Gag, GagPol and Env
Replicons	Non-HIV viruses engineered to carry genes encoding HIV proteins
Live bacterial vectors	Harmless bacteria engineered to carry genes encoding HIV proteins [e.g. *Salmonella typhimurium*, Bacille Calmette-Buerin (BCG)]
Live viral vectors	Non-HIV viruses engineered to carry genes encoding HIV proteins (e.g. Vaccinia, MVA, NYVAC, Canarypox, VEE)
DNA vaccines	Naked plasmid-DNA containing one or more HIV genes
Combined vaccines	Combination of different vaccines in a mixed modality immunization schedule [e.g. DNA vaccine(s) plus live viral vector(s) plus recombinant protein(s)]

titres and most broadly reactive neutralizing antibodies were induced by an rgp120 vaccine produced in mammalian CHO cells [42,45–47]. However, these vaccines were based on laboratory-adapted clade B strains of HIV (e.g. LAI, IIIB, MN or SF-2). There is no surprise that neutralization was observed mainly towards T-cell line adapted strains (TCLA) and some primary syncytia inducing (SI) isolates, but not to primary non syncytia inducing (NSI) isolates [48]. Nevertheless, all recombinant proteins tested to date appear to be safe and to induce $CD4^+$ T-cell proliferate responses [49]. Unfortunately, no specific CTL responses were observed in most vaccinated volunteers [46,49]. Three gp120 vaccines produced in mammalian cells have been evaluated in Phase II clinical trials. Currently, VaxGen (Brisbane, CA, USA) is testing two bivalent preparations of rgp120 [AIDSVAX gp120 (B/B) and AIDSVAX gp120 (B/E)] based on laboratory clade B strains and one primary clade E isolate of HIV in a Phase III clinical trial in the USA and Thailand [50]. Interim results from the first Phase III clinical trials, started in October 1998, are likely to be available by the end of 2001 and in 2002 [51]. In addition to recombinant Env variants, the efficacy of several other recombinant viral proteins, such as p24 and Tat, is currently evaluated in Phase I clinical trials [52,53].

Peptide-based vaccine approaches

Peptide vaccines, based on vaccination with defined immunogenic B-cell and T-cell epitopes, were thought to induce stronger and more specific immune responses. Clinical trials for an octamer $V3_{MN}$-based peptide have been conducted by United Biomedical (Hauppauge, NY, USA), in seronegative volunteers in the USA, Australia, Thailand, Brazil and China. Although the vaccine was well tolerated and no clinical abnormalities were observed, only antibodies that neutralized the homologous, laboratory adapted isolate, but not primary isolates, were induced in 60–90% of the volunteers [54,55]. Other clinical trials using multivalent chimeric peptides derived from diverse HIV proteins, as well as several studies in primates, further confirmed that a peptide-based approach might not be suitable for inducing broadly cross-neutralizing antibodies [56]. Nevertheless, further improvements of peptide vaccines could provide an efficient tool for inducing selective cellular immunity against multiple targets.

DNA vaccines

Genetic immunization with naked or formulated DNA plasmids, by intramuscular injection or intradermal gene gun-delivery, elicits both humoral and cellular responses by imitating natural viral infection in vaccinated individuals [57]. In general, after DNA uptake by somatic and/or immune cells, the encoded antigens are expressed, and either released from cells to initiate immune responses by cross-priming events or degraded by the proteasome. The processed antigenic peptides are then presented to CTLs via the classical MHC-class I pathway. Additionally, directly transfected professional antigen-presenting cells enable the presentation of intracellular processed peptides on MHC-class II molecules, so DNA vaccination efficiently primes both arms of the immune system. Clinical trials to test the potency of candidate DNA vaccines have now been started. Recently, an initial Phase I clinical trial of a therapeutic DNA vaccine encoding for Env and/or Rev proteins in 15 asymptomatic HIV-infected patients, has proven the safety and potential immunogenicity of an HIV-directed DNA-based vaccine [58–60]. Additional Phase I clinical trials of several DNA candidate vaccines encoding for Env and GagPol proteins of HIV clade B and C are in the pipeline for 2001 and 2002, and more trials are being planned [51]. In these future studies, the use of codon-optimized genes, which have been shown to improve expression levels [61,62], as well as multicomponent DNA immunization, could hold promise for increased cellular and humoral immune responses. Furthermore, DNA vaccination could gain special importance in the context of heterologous prime–boost regimens, showing that antigen-specific immune responses are drastically increased when boosting a DNA-primed response with antigenic proteins or a live-viral-vector vaccine [63,64]. Nevertheless, so far there is little clinical experience in DNA vaccination. Therefore, safety concerns, such as plasmid integration and induction of anti-DNA autoimmunity, should be addressed carefully in the near future.

Combined vaccine strategies

In recent years, vaccination strategies have been getting increasingly complex, with the aim of inducing more potent cellular immune responses, for example, by applying combined prime–boost regimens using different antigen-delivery systems. Examples of currently used delivery systems that carry one or more HIV genes are non-pathogenic live-viruses and bacteria, such as vaccinia virus (VV) [65] and Bacille Calmette-Buerin (BCG) [66]; these enable the expression of selected HIV antigens following infection of cells in vaccinated individuals. Although antigens are made efficient to the immune system by using a similar method as an HIV-infected cell, vector immunity could limit the effectiveness of subsequent immunizations using the same agent. In several Phase I clinical trials, human volunteers were vaccinated with recombinant VV expressing HIV gp160 and received subsequent boosts with protein antigens, such as rgp160, or rgp120. The majority

of these individuals developed both Env-specific CTLs and neutralizing antibodies against at least some laboratory strains [67–69]. Further clinical trials with recombinant VV expressing Gag–Pol, in addition to Env, are in progress [51].

Recently, the replication competent VV-based vectors were almost exclusively taken over by attenuated forms that are replication incompetent in most human cells. The most prominent representatives of these highly attenuated VV-vectors include the New York Vaccinia (NYVAC), a genetically engineered variant of Copenhagen VV vaccine strain that lacks immune evasion genes [70], and the Modified Vaccinia Ankara (MVA) derived from the VV strain Ankara by serial passages in chicken embryo fibroblasts. MVA viruses are highly avirulent, even in immunosuppressive individuals, because of six major deletions in the genome [71,72]. Several studies have already been conducted using other recombinant apathogenic viruses, such as canarypox virus (ALVAC), which is the most prominent representative of avian poxviruses [73]. Canarypox viruses are characterized by a replication defect on mammalian cells. Initial Phase I clinical trials [73–79] demonstrated that vaccination with recombinant canarypox containing several HIV genes induced CTL responses in 50% of human volunteers. In another clinical trial, neutralizing antibodies against laboratory strains were induced in >95% of volunteers primed with recombinant canarypox and boosted with rgp120 or gp160. Further developments of more complex recombinant canarypox viruses, that contain a variety of HIV genes or coding sequences for defined immunogenic epitopes, are currently in progress to proceed to Phase I and II clinical trials. Alternative vector-based approaches are currently being developed for HIV vaccines, including recombinant poliovirus, influenza virus, mengovirus, herpesvirus, Semliki forest virus (SFV), Venezuelan equine encephalitis virus (VEE), salmonella, BCG, shigella and lactococcus (http//www.niaid.nih.gov/daids/vaccine/concepts.htm). Some of these live vectors have already been tested successfully in the SIV animal model and further studies are under way to test their immunogenicity and safety in more detail. In the near future, a Phase I clinical trial will be conducted by VaxGen in collaboration with the Center for Vaccine Development (University of Maryland, Baltimore, MD, USA), using live attenuated recombinant *Salmonella typhimurium* expressing rgp120 (LAI) in combination with a booster immunization with rgp120 (MN) [80].

Since the first clinical trials conducted in man in 1987, ~30 different HIV vaccine candidates have been tested in Phase I trials, but only two vaccine approaches were further submitted to Phase III clinical trials (http//hiv-web.lanl.gov/reviews). Those are both based on the Env protein of HIV, although it is well accepted that cross-clade neutralizing antibodies are hardly induced by existing recombinant Env antigens and that, moreover, antibodies in the absence of strong cellular immune responses are unlikely to protect against HIV infection.

Clinical trials under investigation: which are the promising candidates?

As discussed previously, the only antigen that has so far been tested extensively, at least in Phase I clinical trials, is the full length gp160 or gp120, either as a subunit vaccine, peptide vaccine, DNA-vaccine or using live attenuated vectors (such as VV or canarypox virus) for antigen delivery. Other HIV antigens, such as Gag, or GagPol, which have been known for years to be highly immunogenic, are currently being investigated for clinical Phase I and II trials. Although the immunogenicity of several candidate vaccines against viral regulatory proteins, like Tat, Rev or Nef, has already been proven extensively in several animal models, no clinical trials have thus far been initiated.

To date, >60 Phase I and II trials of 30 candidate vaccines have been conducted worldwide (Tables 2 and 3). Future plans for Phase III clinical trials, scheduled to start in 2002–2003 in Kenya, South Africa, Thailand and the United States, using DNA, recombinant VEE or canarypox virus and rgp120 from HIV-clades, predominating in the testing area, already exist [51]. Meanwhile, basic research is proceeding rapidly and several sets of data (e.g. on the evaluation of multiple vaccine concepts) will be available before any of the currently considered Phase III clinical trials are completed. The usual time span of 10+ years between a vaccine concept and its Phase III clinical trial is the worst dilemma of HIV vaccine research. Other limitations, such as the lack of knowledge on the correlates of immune protection or the relevance of more than nine genetic subtypes of HIV, in addition to inadequate animal models for testing safety and immunogenicity before clinical trials, renders testing of vaccine candidates in man extremely difficult.

However, if we are not ultimately going to test vaccine candidates in man, we will never get all the required crucial information. If we do not conduct further Phase III clinical trials as soon as possible, we definitively will not have an HIV vaccine within the next 15–20 years. Unfortunately, efficacy trials in developing countries, which indeed provide the best prerequisite for testing candidate HIV vaccines because of their high HIV and AIDS incidence, are extremely difficult to establish. Only a few of them meet the regulatory requirements of developed countries for production (Good Manufacturing Practice; GMP) and clinical evaluation (Good Clinical

Table 2. HIV/AIDS candidate vaccines of clinical trials

Designation	Vaccine antigen (HIV strain)	Production method	Vaccine developer	Clinical trial
Envelope-based vaccines				
rgp160 (LAI)	(LAI)	Baculovirus/insect cells	MicroGeneSys[a]	Phase I
rgp160 (IIIB)	(IIIB)	Vaccinia/Vero cells	Immuno-AG[b]	Phase I
Env 2–3 (SF2)*	(SF-2)	Yeast	Chiron[c]/BIOCINE	Phase I
rgp120 (IIIB)	(IIIB)	CHO cells	Genentech[d]	Phase I
rgp120 (MN)	(MN)	CHO cells	Genentech	Phase I/II
rgp120 (SF2)	(SF2)	CHO cells	Chiron/BIOCINE	Phase I/II
rgp160 (MN)	(MN)	Vaccinia/Vero cells	Immuno-AG	Phase I
rgp160(MN/LAI)	(MN/LAI)	Vaccinia/BHK-21 cells	Pasteur Merieux[e]	Phase I/II
AIDSVAX gp120 (B/B)	(MN/GNE8)	CHO cells	VaxGen[f]	Phase III
AIDSVAX gp120 (B/E)	(MN/A244)	CHO cells	VaxGen	Phase III
rgp120 (B/E)	(SF2/CM235)	CHO cells	Chiron	Phase II
rgp140 (E)	92TH023/LAI	Vaccinia/Vero cells	Aventis Pasteur[e]	Phase I/II
Non-envelope-based vaccines				
Ty p17/p14 VLPs	(LAI)	Yeast	British Biotech[g]	Phase I
p24		CHO cells	Chiron	Phase I
Tat	(LAI)	*Escherichia coli*	B. Ensoli, Italy	Phase I
Peptide-based vaccines				
P3C541b	Gag lipopeptide	Synthetic linear peptide	United Biomedical[h]	Phase I
HGP-30	p17 (LAI)	Synthetic linear peptide	Viral Technologies[i]	Phase I/II
V3 loop of gp120	(MN)	Synthetic linear peptide	United Biomedical	Phase I
V3 loop of gp120	(15 strains)	Synthetic linear peptide	United Biomedical	Phase I
gp120 C4-V3	(MN/EV91/RF/CANO)	Polyvalent peptide	Wyeth-Lederle[j]	Phase I
Nef/Gag/V3	Nef/Gag (LAI)/V3 (BX08)	Synthetic linear peptides	Bachem[k]	Phase I

Practice; GCP) of candidate vaccines. Several years of intense collaboration between industrialized countries and developing countries, as well as considerable funding by external national grants [such as the European Vaccine Efforts or the IAVI (International AIDS Vaccine Initiative)] are required, and intensive time will be spent to define appropriate cohorts of individuals with a high risk of HIV infection containing the basic infrastructure for clinical Phase II and III trials of upcoming HIV-candidate vaccines.

To proceed in a more rapid and co-ordinated way, national organizations, as well as international co-operative foundations, have to define common goals and find pragmatic solutions for financing intellectual property right protection and, finally, for the clinical testing of candidate vaccines. Experimental protocols for the evaluation of safety and immunogenicity profiles in both primates and man must standardize to better compare results from different working groups and to reduce overall experimental costs. Furthermore, with regards to the dramatic AIDS epidemic in developing countries, we have to put more emphasis on clinical trials in Africa, Asia and South America, to ultimately combat the AIDS/HIV epidemic.

European efforts in HIV vaccine development

During the past decade, the national governments of distinct European countries, and also the European Union (EU), have spent considerable amounts of money on basic research for HIV and the development of powerful candidate vaccines. In addition to national task forces to coordinate programmes for developing and testing candidate vaccines in Phase I clinical trials, the EU has followed a straightforward policy to gain deeper insights into the epidemiological situation in developing countries, to bring concepts of innovative antigen-delivery systems from

Table 2. HIV/AIDS candidate vaccines of clinical trials: continued

Designation	Vaccine antigen (HIV strain)	Production method	Vaccine developer	Clinical trial
DNA vaccines				
APL 400-003	env/rev (LAI)	GeneVax® vector	Wyeth-Lederle	Phase I
APL 400-047	gag/pol (LAI)	GeneVax® vector	Wyeth-Lederle	Phase I
gag			Merck[l]	Phase I
HIVA	Multiple epitopes (A)	pTHr vector	MRC[m]	Phase I
Recombinant pox viruses				
HIVAc-1e	gp160 (LAI)	Vaccinia	Bristol-Myers[n]	Phase I
TCB-3B	Env, Gag, Pol (IIIB)	Vaccinia	Therion[o]	Phase I
vCP125	gp160 (MN)	Canarypox (ALVAC)	Aventis Pasteur	Phase I
vCP205	gp120 (MN) and TM gp41 gag and protease p15 (LAI)	Canarypox (ALVAC)	Aventis Pasteur	Phase I/II
vCP300	gp120 (MN), TM gp41, gag protease p15 (LAI), nef and pol (LAI)[†]	Canarypox (ALVAC)	Aventis Pasteur	Phase I
vCP1433	gp120 (MN), TM gp41, gag, protease p15 (LAI), pol and nef (LAI)[#]	Canarypox (ALVAC)	Aventis Pasteur	Phase I
vCP1452	gp120 (MN), TM gp41, gag, pol (protease) (LAI), pol and nef (LAI)[•]	Canarypox (ALVAC)	Aventis Pasteur	Phase I/II
vCP1521 (E/B)	gp120 (92TH023), TM gp41 (LAI), gag/protease p15 (LAI)	Canarypox (ALVAC)	Aventis Pasteur	Phase I/II
MVA-(A)	polyepitope insert	MVA	A. McMichael[m]	Phase I

[*]Env 2–3 is a non-glycosylated form of gp120.
[†]Contains a part of the *nef* gene (LAI) encoding two peptides (9.1kDa and 3.3 kDa) and a part of the *pol* gene (LAI) encoding three peptides (5.5, 7.2 and 7.0 kDa) all of them containing multiple CTL epitopes.
[#]Contains an epitope string consisting of all known human CTL epitopes of Nef and Pol proteins.
[•]Contains an epitope string consisting of all known human CTL epitopes of Nef and Pol proteins as well as the VV genes *E3L* and *K3L*, which enable high and sustained production of HIV antigens.
[a]Meridan, CT, USA; [b]Deerfield, IL, USA; [c]Emeryville, CA, USA; [d]Vacaville, CA, USA; [e]Lyon, France; [f]Brisbane, CA, USA; [g]Oxford, UK; [h]Hauppauge, NY, USA; [i]Vienna, VA, USA; [j]St. Davids, PA, USA; [k]Bubendorf, Switzerland; [l]Whitehouse Station, NJ, USA; [m]Oxford, UK; [n]New York, NY, USA; [o]Cambridge, MA, USA.

the laboratory to preclinical studies and to allow research networks, which enable a highly systematic testing of candidate vaccines matching the GMP regularities in multicentre clinical trials. Accordingly, our own lab has been co-ordinating an INCO-DC programme, which finally succeeded with the help of the Biomedical Primate Research Centre (BPRC; Rijswijk, The Netherlands) and the Chinese Academy of Preventive Medicine (CAPM; Beijing, China) to define the prevalent clade B and clade C virus strains circulating throughout China. A follow-up programme, EUROVAC I and II, comprises >20 European HIV working groups, including a prominent industrial partner. All of them are specialists in different fields of antigen delivery systems, GMP production, set-up of multicentre clinical trial platforms, as well as the analysis of various aspects of humoral, cell mediated and mucosal immune responses. This programme aims to compare the safety and immunogenicity of multiple viral antigens in HIV-1 negative volunteers in a European Phase I clinical trial. Various delivery systems will be combined in carefully selected prime–boost regimes. This study includes various DNA candidate vaccines, which are based on synthetic, codon-optimized genes and have been developed in our laboratory [81,82], purified recombinant proteins in combination with potent adjuvants, recombinant attenuated vaccinia viruses, such as MVA and NYVAC, as well as genetically modified recombinant alphaviruses. Antigens to be presented include genetically modified and functionally

Table 3. Clinical trials (Phase II and III) of candidate HIV vaccines

Designation (HIV strain)	Production method	Adjuvant	Vaccine developer
Phase II			
Envelope-based vaccines			
rgp120 (SF2)	CHO cells	MF59	Chiron[a]/BIOCINE
rgp120 (MN)	CHO cells	$Al(OH)_3$	Genentech[b]
rgp120 (SF2/CM235 clade E)	CHO cells	MF59	Chiron
Recombinant proteins in combination with Pox virus recombinants			
vCP205 (MN/LAI)	Recombinant canarypox	MF59	Pasteur Merieux[c]
+ rgp120 (SF2)	CHO cells		Chiron/BIOCINE
vCP1452 (MN/LAI)	Recombinant canarypox	$Al(OH)_3$	Aventis Pasteur[c]
+ rgp120 (MN and/or GNE8)	CHO cells		VaxGen[d]
vCP1521 (E/B)	Recombinant canarypox	$Al(OH)_3$	Aventis Pasteur
+ rgp120 (SF2/CM235)	CHO cells		VaxGen
+ rgp140 (92TH023/LAI)			
Phase III			
Envelope-based vaccines			
AIDSVAX rgp120 (B/B)	CHO cells	$Al(OH)_3$	VaxGen (USA)
AIDSVAX rgp120 (B/E)	CHO cells	$Al(OH)_3$	VaxGen (Thailand)

[a]Emeryville, CA, USA; [b]Vacaville, CA, USA; [c]Lyon, France; [d]Brisbane, CA, USA.

inactivated variants of Gag, Pol, Nef and Env proteins, which are derived from the prototype B, as well as a primary, representative clade C isolate from China [83], respectively. The primary goal of this European HIV-vaccine study is to determine the safety and immunogenicity of the above named candidate vaccine approaches. Furthermore, based on the outstanding expertise of contributing organizations, we aim to develop standard operating procedures enabling highly reproducible analysis and quantification of the immune responses. Another equally important objective of this study is to gain additional information on the induction of cross-clade specific B- and T-cell mediated immune responses, respectively.

Conclusions

Despite the complexity of HIV epidemiology and pathogenesis, and the difficulties associated with the development and evaluation of HIV/AIDS vaccines, there is still reason for optimism. Major progress has been made to better understand the correlates of protective immunity and to develop and improve vaccine approaches to enhance overall immunogenicity *in vivo*. Therefore, the combination of multicomponent DNA vaccines, live viral vectors (such as ALVAC or MVA) and recombinant proteins is only one example of a promising approach for a successful candidate vaccine. Recently, a multiprotein DNA–MVA vaccine has been demonstrated to efficiently control a highly pathogenic virus challenge via the mucosal route in a rhesus macaque model [84]. These observations lead us to believe that an effective, broad-reactive HIV vaccine would prevent and/or control initial viral replication when transmitted via the natural route of infection. As the best marker of vaccine efficacy is its protection in man, results from additional Phase III clinical trials would provide the best proof. However, in addition to intensive costs and the ethical, political and geographical issues associated with implementations, Phase III clinical trials take at least three years to conduct. In conclusion, it could take at least another 5–10 years until we obtain the first results on the efficacy of the most promising, recently developed and novel HIV/AIDS candidate vaccines in man.

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