AIDS VACCINES IN A GLOBAL PERSPECTIVE

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ABSTRACT

The undoubtable effectiveness achieved by the multidrug therapy, should not hamper the willingness and efforts of governments, the private sector and health organizations, nor diminish the interest of researchers, in the development of effective vaccines to control the HIV pandemic. The accessibility to retroviral polychemotherapy of large masses of infected and sick persons, as well as the long term persistence of the effectiveness of this approach, are far from being confirmed and even less, guaranteed. For these reasons many specialists are convinced that a safe, effective and accessible vaccine continues as a priority to bring the HIV/AIDS epidemics under control, specially in developing countries. Here a number of obstacles in the scientific field are analyzed which must be overcome or in some way circumvented to advance in the vaccine race. The multiple candidates and ingenious vaccine concepts explored at a laboratory, preclinical or clinical level are also reviewed, which demonstrate the motivation, imagination and creativeness of researchers facing the complex nature of this virus and the enormous challenge imposed by its global spreading. The state of the art regarding human trials at different phases is updated, including the efforts of developing countries involved in the trial and two of them in the development and trial of vaccine candidates, under strict ethical and methodological standards. The role of UNAIDS in the integration, coordination and support of these efforts is pointed out.

Keywords: AIDS vaccines, HIV/AIDS epidemics, HIV prevention, vaccine trials

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RESUMEN

La indudable efectividad alcanzada por la terapia multidroga, no puede disuadir la voluntad y esfuerzos de gobiernos, sector privado y organizaciones de salud, ni hacer desear el interés de los investigadores en el desarrollo de vacunas efectivas para el control de la pandemia de VIH. La accesibilidad de las grandes masas de infectados y enfermos a la poliquimioterapia retroviral, así como la perdurabilidad a largo plazo de la efectividad de este enfoque, están lejos de lograr confirmación y menos aun garantía. Por estas razones muchos especialistas están convencidos de que sigue siendo prioritario una vacuna segura, efectiva y accesible para poner bajo control la epidemia de VIH/SIDA, especialmente en países en desarrollo. Un número de obstáculos en el terreno científico analizados aquí, deben ser superados o evadidos de algún modo para avanzar en la carrera de la vacuna. Se revisan también los múltiples candidatos a ingeniosos conceptos vacunales explorados a nivel de laboratorio, preclínico y clínico, que demuestran la motivación, imaginación y creatividad de los investigadores que enfrentan la compleja naturaleza de este virus y el reto enorme que nos impone su diseminación a escala global. Se actualiza, además, el estado de avance de las pruebas en curso en distintas fases en humanos, incluidos los esfuerzos de países en desarrollo involucrados en la prueba y dos de ellos en el desarrollo y prueba de candidatos vacunales, bajo estricto control ético y metodológico. Se destaca la función de UNAIDS en la integración, coordinación y apoyo de estos esfuerzos.

Palabras claves: ensayos vacunales, epidemia de VIH/SIDA, prevención de VIH, vacunas de SIDA

Introduction

It is estimated that since the beginning of the HIV/AIDS pandemic, more than 30 million people have become infected with the human immunodeficiency virus (HIV), the etiologic agent of AIDS [1]. Of these, more than 6 million have already died of this disease, 77% of them in Sub-Saharan Africa.

Of the remaining 23 million people who today are living with HIV/AIDS, more than 90% are in developing countries. Sixty-three percent of all HIV infected people are in Africa, but the numbers in South- and South-East Asia are rapidly increasing, now representing 22% of all HIV-infected persons. It is estimated that more than 1.5 million people are infected with HIV in Latin America and the Caribbean, representing 9% of the total number of HIV infected persons in the world.

And the epidemics continues. Every day almost 9,000 people become infected with HIV, 90% of them in developing countries.

Thus, the intense national and international efforts to control the HIV/AIDS pandemic, based on education and behavioural change, have not been sufficient to control the spread of HIV. To complement these interventions, new HIV preventive technologies are being explored, including: (a) early diagnosis and treatment of other sexually transmitted diseases, (b) female controlled methods, such as vaginal viricides and female condoms, (c) anti-retroviral drugs to prevent mother-to-child transmission; and (d) preventive vaccines.

However, many people believe that a safe, effective and affordable vaccine is required to bring the HIV/AIDS epidemic under control, especially in developing countries.

Scientific Obstacles

The development of HIV vaccines, however, has encountered a number of scientific obstacles [2-4].

Lack of information on potential immunological correlates of protection [5-7] AIDS may be different from other vaccine preventable diseases in that the infection persists, and AIDS develops, despite a broad range of immune responses
by the host. Thus, at the present time it is not clear what type of immune response a vaccine should induce to confer protective immunity in the recipient.

There are rare situations, however, in which host responses seem to confer some degree of protection against HIV infection or disease. There are documented cases of individuals who despite continuous exposure to HIV fail to seroconvert. Different explanations have been proposed: presence of a mutant gene for the “second” HIV receptor (CCR5); alloimmunization phenomena; generation of protective HIV-specific cytotoxic T cells (CTL) in the absence of detectable humoral response. Likewise, there are individuals who after many years of being HIV-infected, show little or no indication of immunological deterioration (the so called “long term non-progressors”). Again, more than one “protective” mechanism could be operating in these individuals, including: strong CTL responses; high levels of broadly neutralizing antibodies; or infection with naturally attenuated HIV strains.

Different candidate vaccines which aim at stimulating different arms of the immune system (humoral or cell mediated immunity) are being developed. However, at the present time it is not clear which vaccine approach, or approaches, will work.

Genetic variability of HIV [8-12]

HIV strains isolated from different parts of the world exhibit divergent nucleotide sequences, especially in the gene coding for the envelope glycoproteins (gp120 and gp41). These sequence differences have been used to classify HIV into a major group M (subtypes A through I), and the genetically more distant (“outlier”) group O.

HIV genetic subtypes are unevenly distributed in different parts of the world. The prevalent subtype in the Americas and Europe is subtype B, representing approximately 20% of all HIV infections worldwide. The most widespread HIV subtype is probably subtype C, with 36% of all infections in the world. Subtype C is the most prevalent subtype in southern Africa and India, and it has also been found in South America and, more recently, in China. Every subtype is present in Africa, where subtype A is also prevalent (representing 22% of the world total).

The more or less artificial definition of genetic subtypes has resulted in the false belief that these genetic subtypes may correspond to immunological subtypes, with all the consequences which this could have for vaccine development. Actually, genetic subtypes do not seem to correspond to immunological subtypes. It has been reported that sera from HIV-infected persons can cross-neutralize, in vitro, clinical isolates from different subtypes. Likewise, persons vaccinated with candidate vaccines based on B strains, develop CTLs which cross-react with target cells expressing env or gag antigens from different subtypes. These experiments suggest, however, that the E subtype strains (prevalent in South East Asia) may be immunologically more distant from prototypic B strains, than strains from other group M subtypes. These humoral and cell-mediated immunological cross-reactivities may indicate the possibility of cross-protection between different subtypes.

On the other hand, more than one immunotype may be represented within a single genotype. How can we be sure, for instance, that genetic subtype C viruses prevalent in South Africa, Ethiopia, China, India and Brazil, all represent the same vaccine-relevant immunological subtype? This general question is of capital importance to developing countries, where multiple genetic subtypes are circulating. We need to know if vaccines against each subtype are needed, or if an appropriate combination of carefully selected HIV strains could be sufficient to provide protective immunity against a broad range of viruses.

Protection experiments in animal models [13, 14]

The two animal models most extensively used in HIV vaccine research (HIV infection of chimpanzees and Simian Immunodeficiency Virus (SIV) infection of macaque monkeys) have resulted in conflicting, and some times paradoxical results.

Chimpanzees vaccinated with recombinant gp120 or gp160 candidate vaccines have been reproducibly protected from challenges with homologous, or with closely related, HIV strains. The observed protection has correlated with the level of neutralizing antibodies. More recently, successful chimpanzee protection experiments have been reported using multigenic (especially gag and env carrying) poxvirus vectors, adenovirus vectors and naked DNA vaccines, although boosts with recombinant envelope antigens were often used.

Contrary to the situation in chimpanzees, macaque monkeys are notoriously difficult to protect with experimental SIV vaccines. Only live-attenuated vaccines (especially nef deleted mutants) seem to induce solid protection against pathogenic SIV challenges.

The more recent development of chimeric viruses generally containing the envelope gene of HIV and the genomic backbone of SIV (SHIV). Pathogenic strains capable of infecting and producing disease in macaque monkeys, may offer the opportunity to better explore the possible role of envelope antigens in protection. The SHIV/macaque model may be the most practical approach to rapidly obtain information on the possible significance of the different HIV genetic subtypes in relation to vaccine induced protection.

Of course, the key question here is: Which animal model is more relevant to predict vaccine-induced protection in humans? The truth is that we will not have that answer until these animal models are “validated” through vaccine efficacy trials in humans. However, one thing is clear: animals can be protected with experimental vaccines, even if we do not know the mechanism of protection. Thus, these animal protection experiments might be one of the most compelling reasons to test the efficacy of HIV candidate vaccines in human trials.

Candidate Vaccines

A great deal has been learned about the basic structure of the HIV itself, and that information has guided the design of different types of “candidate” HIV vaccines (sometimes referred to as “vaccine concepts”):
Subunit vaccines [15]
These are based on parts of the virus, especially the envelope glycoproteins (gp120, or its precursor, gp160), generally produced by genetic engineering in mammalian cells. The "envelope concept" was the first to be developed and tested in human trials, and it is primarily aimed at inducing humoral immunity (neutralizing antibodies).

Most recombinant gp120 or gp160 candidate vaccines represent native processed proteins, although they have a monomeric rather than an oligomeric configuration. This could explain their inability to induce antibodies capable of neutralizing the infectivity of clinical isolates of HIV (those isolates which have not been adapted to grow in transformed cell lines). That observation has led some people to propose that monomeric gp120 candidate vaccines may not be effective in protecting humans against clinical (wild) isolates of HIV. However, chimpanzees vaccinated with monomeric gp120 have been protected against a challenge with a clinical isolate of HIV.

Peptide vaccines [16, 17]
The identification of the third hypervariable region of gp120 (the V3 loop) as the dominant neutralization determinant (PND) of HIV, has stimulated the development of different candidate vaccines based on synthetic peptides representing that important group of epitopes. Some of these candidate vaccines include V3 loop sequences representative of different strains and/or genetic subtypes. Other synthetic peptide vaccines include gag-derived sequences, including p17, the matrix protein, and p24, the core protein.

Peptide vaccines could be designed to stimulate humoral or cell-mediated immunity, depending on their presentation and on the adjuvant strategy used. Primate protection data using peptide vaccines are very limited, and it seems likely that these vaccines could possibly be used as boosters, to broaden immune responses initially induced by other more potent immunogens.

Live-vectorated vaccines [18, 19]
Genes coding for immunologically relevant HIV proteins (especially gag and env, but also other genes) have been inserted in a variety of viral and bacterial expression vectors, including poxviruses (vaccinia and canarypox), adenovirus, picornaviruses, BCG, salmonella, and shigella, among others. Live-vectorated vaccines could be engineered to induce both humoral and cell-mediated immunity. Oral administration of certain live-vectorated vaccines could also result in the induction of mucosal immunity. The development of live-vectorated HIV vaccines could be of great importance for developing countries in view of their potentially lower cost.

The most extensively studied viral vector for HIV vaccines has been the avian poxvirus known as canarypox. The canarypox virus undergoes an abortive cycle of replication when infecting mammalian cells, but it is capable of expressing foreign gene sequences placed under the control of early promoters. Although these characteristics make the canarypox a safe vector for human use, it also results in relatively low immunogenicity, requiring high doses of the inoculum, and/or boosting with recombinant envelope proteins for an efficient response. Canarypox vectored HIV vaccines have been shown to confer some degree of protection against an HIV challenge.

Likewise, recent results with a BCG vectored HIV vaccine have shown some protection in monkeys challenged with a nonpathogenic SHIV clone.

Naked DNA vaccines [20-22]
Naked DNA vaccines represent a novel and promising approach for the development of HIV vaccines. These vaccines are theoretically capable of inducing both CTLs and neutralizing antibodies. However, in practice, available naked DNA vaccines may need boosting with other constructs (such as rgp120), to elicit reasonable levels of antibodies. Early immunization protocols using SIV in monkeys failed to show protection in vaccinated animals. However, more recently, chimpanzees have been protected with HIV naked DNA vaccines.

The production of naked DNA vaccines is technologically less complex than the production of recombinant vaccines. This may be relevant for developing countries, since it would facilitate the production of candidate vaccines based on different genetic subtypes.

Whole-inactivated virus
Although vaccination of macaque monkeys against SIV using whole inactivated virus showed some success initially, subsequent research has demonstrated that such protection was due, not to a specific anti-SIV immune response but to immune responses to human host cell proteins incorporated into the SIV virions. Since then, research on whole inactivated vaccines has decreased, although not completely abandoned.

Inactivated HIV vaccines are being evaluated in HIV-positive individuals as immunotherapeutic agents. However, safety concerns related to faulty inactivation and/or the possibility of integration of viral genetic material into host cell DNA, have slowed further development of this time-honoured vaccine approach.

Live-attenuated virus [23]
The development of live-attenuated HIV vaccines is based on the initial observation that nef deleted mutants of SIV were not pathogenic in macaque monkeys, and protected the animals against infection and disease when superinfected with pathogenic virus. Additional deletions can also be introduced in other non-essential regulatory genes, to increase the safety of the candidate vaccine. Very interestingly, some "long-term non progressors" have been found to be infected with nef deleted HIV viruses, and it has been proposed that those naturally attenuated strains should be seriously considered as potential HIV vaccines.

As mentioned before, live-attenuated vaccines are the only ones that have been capable of inducing solid protection against challenge with pathogenic SIV. For this reason, some investigators believe that this approach should be energetically followed for the development of HIV vaccines in humans. Of course, this approach raises important concern about the
safety of the product which should be addressed before proceeding with human trials.

**Human Trials**

**Phase I Trials**

Despite the scientific uncertainties described above, several HIV candidate vaccines have proceeded to Phase I and Phase II clinical trials in humans [3, 4, 24, 25].

Some 15 different HIV candidate vaccines have been tested in more than 20 Phase I trials since 1987, enrolling a total of approximately 3,000 HIV-negative volunteers, mostly in the United States, but also in several European countries.

Most of the candidate vaccines tested in Phase I trials have been based on the recombinant envelope concept, either gp160 (from MDA5GeneSys, Immuno AG, Pasteur-Merieux-Connaut, and the Free University of Brussels) or gp120 (from Biocine/Chiron, SmithKline Beecham, and Genentech/VaxGen). Several peptide-based vaccines have also been tested (from United Biomedical Inc, Serum and Vaccine Institute, Viral technologies, Pasteur-Merieux-Connaut, Free University of Brussels, Chiron, and the Cuban Center for Genetic Engineering and Biotechnology). In addition, a “particle” vaccine, based on a yeast retrotransposon, has been tested (from British Biotechnology).

Live-vectorized vaccines have also been tested in human trials, including vaccinia carrying env or env/gag/pol (from Bristol-Meyers-Squibb, and Therion Biologics), or different constructs of canarypox carrying env/gag/pol (from Pasteur-Merieux-Connaut).

Finally, a naked DNA vaccine based on gag/env (from Apollon) is in Phase I trial in the US.

Some of these Phase I trials have combined priming with one candidate vaccine, followed by boosting with a second candidate. The best studied prime-boost combinations are vaccinia-HIV recombinants, followed by gp160, or more recently, canarypox-HIV recombinants, followed by gp120.

Ten years of experience with Phase I trials has shown that the candidate vaccines are safe, causing only minor discomfort at the site of injection. In addition, these Phase I trials have provided important information on the immunogenicity of these candidate vaccines. All the vaccines induced antibody formation, but only gp120 induced high levels of neutralizing antibodies. However, as explained before, these antibodies are capable of neutralizing the infectivity of laboratory-adapted strains of HIV, but not of field isolates (although, some investigators have reported that using a “resting cell assay”, neutralization of field isolates can be detected in vaccines).

Although gp120 vaccines can induce neutralizing antibodies, these candidate vaccines are incapable of eliciting CTLs. This type of cellular immune response has been detected in approximately 50% of volunteers receiving canarypox-HIV live-vectorized vaccines. Very interestingly, CTLs induced by a canarypox based on a B subtype strain, are capable of inducing lysis of target cells expressing antigens from other subtypes, suggesting the presence of cross-reactive T-cell epitopes.

**Phase II trials**

Two vaccine concepts have entered Phase II trials in the United States: gp120 (from Biocine/Chiron and Genentech) and, more recently, canarypox-HIV recombinant boosted with gp120 (Pasteur-Merieux-Connaut and Biocine/Chiron).

These trials are being conducted to further test the safety and immunogenicity of these vaccine concepts in persons at higher risk of HIV infection.

Despite receiving careful counseling against high-risk behavior, some 26 volunteers participating in Phase II trials have become infected with HIV due to natural exposure. These cases are being extensively studied virologically and immunologically, to try to identify potential correlates of protection (or lack of protection). A temptation which should be avoided is to overinterpret these results in terms of the potential efficacy of the different vaccines; which is not possible in view of the small number of volunteers enrolled, and insufficient to draw any statistically significant conclusion.

It is generally accepted that the only scientific way to assess the efficacy of HIV candidate vaccines is through the conduct of well designed, large scale, efficacy trials.

**Progressing to Efficacy Trials**

To date (September 1997), no efficacy trials of an HIV vaccine have been conducted. However, plans are being discussed to initiate efficacy testing of two vaccine concepts (gp120 and canarypox-HIV followed by gp120) in the US and in Thailand.

Efficacy trials are very complex studies [26-31]. They require the enrollment of hundreds or thousands of volunteers in double-blind, placebo-controlled studies, half receiving the experimental vaccine, and the other half receiving a control injection. The populations to be enrolled in these trials should have a measurable incidence of HIV infection, so that the effect of the vaccine in preventing infection is detectable and quantitated with statistical certainty. The higher the HIV-incidence in the study population, the lower the number of volunteers required in the study. Typical HIV-vaccine efficacy trials would be conducted in populations with an HIV incidence between 2 and 5% per year, which would require the enrollment of 2 to 4 thousand volunteers, to be followed for a period of up to three years.

For ethical reasons, study populations enrolled in vaccine trials should receive sufficient education on avoidance of risk behavior, which hopefully would decrease the incidence of HIV infection, a situation which should be considered when estimating the sample size for the trial.

HIV vaccine efficacy trials are being designed to detect “primary end-points” (protection against HIV infection) as well as “secondary end-points” (modification of the infection). If the vaccine is able to confer “sterilizing immunity”, the trial may show that participants enrolled in the active arm of the trial have significantly less infections than volunteers in the control arm. However, it is possible that effective vaccines may not be able to entirely prevent initial HIV infection in vaccinees, but that the preexist-
ing vaccine-induced immunity could modify the infection, allowing for a recovery from the infection, with the elimination of the virus, or for the establishment of a low-level infection, which might correlate with better long-term prognosis.

**HIV Vaccine Trials in Developing Countries**

There are several reasons to conduct HIV vaccine trials in developing countries: (a) most new HIV infections occur in these countries and they need a vaccine to control the epidemic; (b) for statistical reasons, efficacy trials must be conducted in populations with high HIV incidence, and many of these are in developing countries; (c) vaccines should be tested against infection by different HIV subtypes in different geographical areas, and also in different populations that may differ in health and genetic backgrounds [32-35].

With the assistance of the World Health Organization and of UNAIDS, several developing countries are preparing for the conduct of HIV vaccine trials which must be implemented with the highest scientific and ethical standards.

Three countries (Brazil, Thailand and Uganda) have developed WHO/UNAIDS-endorsed National AIDS Vaccine Plans. These plans describe the national policies in relation to HIV/AIDS vaccine research, development and evaluation, the process of review and approval of research proposals, and suggestions for preparatory research. That research includes virus subtype monitoring, epidemiological studies (including the establishment of cohorts of HIV-negative volunteers), vaccine-related social and behavioural research, and repeat Phase II/III trials of selected candidate vaccines.

Within the framework of its National AIDS Vaccine Plan, Thailand has already conducted three Phase I HIV preventive vaccine trials (V3 synthetic peptides from UBI, gp120B from Genentech/VaxGen and from Bioence/Chiron) and is presently discussing plans for Phase II/III evaluation of gp120BE candidate vaccines. Brazil has conducted a Phase I HIV vaccine trial (V3 synthetic peptides from UBI), and Uganda is ready to initiate its first trial (canarypox-HIV live vector, from Pasteur-Merieux-Connaught).

As mentioned before, the other two developing countries which have been involved in Phase I HIV vaccine trials are China (which tested a V3 synthetic peptide vaccine from UBI) and Cuba (which is testing a locally developed V3 based recombinant protein).

**Role of UNAIDS**

The overall objective of UNAIDS' vaccine strategy is to promote the development, evaluation and future availability of safe, effective and affordable HIV preventive vaccines for worldwide use, especially in developing countries.

To achieve this objective, UNAIDS is implementing the following actions, which were recommended by its Vaccine Advisory Committee:

- Collect, exchange, analyse and disseminate information necessary to make decisions about HIV/AIDS vaccine research and clinical trials of candidate vaccines, especially in developing countries.
- Promote the creation of collaborative networks of scientists and institutions working on HIV/AIDS vaccine research in industrialized and developing countries, to foster a better understanding of the challenges arising and their possible solutions.
- Assist in building capacity in developing countries to support HIV/AIDS vaccine research, including clinical trials.
- Provide independent and authoritative scientific and ethical advice to developing countries on the conduct of HIV/AIDS vaccine trials.
- Identify and address ethical, regulatory and legal barriers to international HIV/AIDS vaccine development and future availability.
- Advocate for worldwide commitment to accelerate the development and future availability of HIV/AIDS vaccines, especially in developing countries.
Los recientes avances en el estudio de la biología molecular han tenido un importante impacto en el conocimiento de los virus de hepatitis y las infecciones causadas por ellos. Este impacto se hace especialmente manifiesto en los nuevos métodos disponibles para el diagnóstico y estudio de estos virus, así como de las enfermedades por ellos causadas y el desarrollo de vacunas. El propósito de este libro es brindar a los lectores los conocimientos básicos de biología molecular, además de una información actualizada de los más recientes avances relacionados con el conocimiento de los virus de hepatitis. Está dirigido a profesionales (médicos, biólogos, investigadores y otros) vinculados al diagnóstico y al manejo de las hepatitis virales.