0021-7557/06/82-03-Suppl/S115
Jornal de Pediatria

doi:10.2223/JPED.1476

Vaccines under development: group B streptococcus, herpes-zoster, HIV, malaria and dengue

Luiz Jacintho da Silva,[1] Rosana Richtmann[2]

Abstract

Objectives: To review the current state of development of streptococcus B, herpes-zoster, HIV, malaria and dengue vaccines. These vaccines were selected both because of imminent commercial release and because of specific problems with their development.

Sources of data: A review of the literature was performed by means of a MEDLINE search, on the period 1996 to 2006, for the epidemiology and immunology of these diseases, analyzing both the greatest obstacles to creating a vaccine and the current state of research, with emphasis on studies in the most advanced stages.

Summary of the findings: Each of the five diseases chosen presents specific problems for vaccine development. Nevertheless, in the majority of cases these have been or are in sight of being resolved, allowing for the prediction that a safe and effective vaccine – or vaccines – will be available in the near future.

Conclusions: Despite the problems faced in developing these vaccines, advances in molecular biology and immunology have made it possible to overcome most obstacles, opening up the prospects for new vaccines.

J Pediatr (Rio J). 2006;82(3 Suppl):S115-24: Streptococcus B, herpes-zoster, malaria, dengue, AIDS, HIV, vaccine.

Introduction

Five of the innumerable vaccines in development were selected for this review, both because their commercial release is imminent, and because their development presented specific problems. The streptococcus B, herpes-zoster, human immunodeficiency virus (HIV), malaria and dengue vaccines were selected because they are all currently in phase III clinical trials. Each of these five diseases represents a significant burden on public health, and each of the vaccines presented development problems that were, and still are, difficult and complex to solve. Some, such as the malaria vaccine, have been in development for decades.

If the results of phase III studies are acceptable, without doubt these vaccines will be available in a short space of time, with the possible exception of the HIV vaccine, which still presents obstacles that will be difficult to overcome.

1. Professor titular, Disciplina de Infectologia, Departamento de Clínica Médica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil.
2. Médica infectologista, Instituto de Infectologia Emilio Ribas, São Paulo, SP, Brasil. Diretora, Departamento de Infectologia, Hospital e Maternidade Santa Joana / Pro Matre Paulista, São Paulo, SP, Brasil. Doutora, Universität Freiburg, Freiburg, Deutschland.

Suggested citation: da Silva LJ, Richtmann R. Vaccines under development: group B streptococcus, herpes-zoster, HIV, malaria and dengue. J Pediatr (Rio J). 2006;82(3 Suppl):S115-24.

Streptococcus B vaccine

Invasive disease caused by group B streptococcus (*Streptococcus agalactiae*, GBS) remains the main cause of death and morbidity among newborn infants (NB) and young children. Group B streptococcus is the main cause of early neonatal bacterial sepsis.[1,2] Around 80% of infections are acquired during passage through the birth canal. Studies undertaken in the USA identified anogenital colonization by GBS in 25 to 40% of healthy expectant mothers.[3] One of the most effective measures for the prevention of early neonatal bacterial sepsis by GBS was the institution of intrapartum antibiotics, as suggested and recommended by the Centers for Disease Control and Prevention (CDC) since 1996.[4] Despite this recommendation, there are still around 2,500 cases of GBS infection in the USA and approximately 100 deaths each year.[5] The use of intrapartum antibiotics led to a reduction of around 75% in cases of early neonatal bacterial sepsis from GBS, although it has had no influence on the incidence of late sepsis. More than half of the early GBS sepsis cases occur during the first week of life of the NB, with lethality of 25 to 50%. The major issue in the discussions on the use of intrapartum antibiotics for expectant mothers colonized by GBS is the rapid development of GBS strains resistant to penicillin, the drug used for this prophylaxis.[4]

It is believed that the development of a vaccine specifically for GBS is the only effective solution for preventing this infection that is so significant, prevalent and lethal among NB, in addition to minimizing impact on the bacterial resistance of the microorganism and possibly avoiding fetal death and prematurity related to this germ.

Development of the vaccine

The rationale behind the development of a vaccine against GBS is that the risk of neonatal GBS infection is inversely proportional to the quantity of maternal antibodies specific to the capsular polysaccharide antigen (CPA) that surrounds GBS. It is believed that the presence of IgG immunoglobulins in the NB is the result of their transplacental passage, conferring temporary protection to the NB.[6] The development of an effective vaccine could theoretically lead to a real reduction in the number of cases of both early and late GBS sepsis.

In 1970, Baker et al.[7] had already predicted the protective action of antibodies to type III CPA of GBS, finding that NB whose mothers had low levels of CPA type III specific antibodies presented increased incidence of early and late invasive GBS disease. Starting from this premise, it is believed that if there were sufficient levels of antibodies in human serum specific to the capsular polysaccharide antigen of GBS, individuals would be protected, since they exhibited adequate opsonization and phagocytosis. It is believed, based on the example of the polysaccharide pneumococcal vaccine, that by developing a vaccine with antibodies to the CPA of GBS for expectant mothers, we would be preventing the disease among NB, by transplacental transfer of protective antibodies. The best time to administer such a vaccine would be during the third trimester of pregnancy, in order to achieve serum antibody levels and transplacental transfer of protection to the NB. The first attempt was a vaccine specific to type III CPA, followed by other attempts with type I and II CPAs (these initial studies achieved immunogenic response in just 40 to 60% of those immunized).[8] Immunoresponse for serotype II was better (88%).

In 1988, Baker et al. developed a vaccination program with pregnant women. Humoral response was 90% for the type III CPA in pregnant women receiving the vaccine during the third trimester of pregnancy, at around week 31, i.e. there was *in vitro* observation of antibodies in serum from NBs with protective function (opsonization and bacterial death), detected up to 3 months after birth.[9] This was a landmark, demonstrating the real possibility of immunizing the expectant mother and protecting the unborn child from GBS.

Increases have recently been detected in the prevalence rates of invasive disease by GBS from other serotypes than I, II and III, with serotype V being of most concern. Unfortunately we have little data on the epidemiology and prevalence of the GBS serotypes that cause invasive diseases in Brazil. This epidemiological change raised awareness concerning the need for wider spectrum vaccines, covering many serotypes. It is estimated that a multivalent vaccine covering the serotypes Ia, Ib, II, III and V, could protect against virtually 100% of cases of the disease in infants and adults.[10]

The first conjugate vaccine with type III GBS CPA and tetanus toxoid as a carrier protein was developed based on this concept. The results with the new vaccine in expectant mothers demonstrated protection of up to 90% when compared with a placebo group.[11]

Since 1996, several new conjugate vaccines specific to the most important serotypes responsible for GBS disease have been tested. In general the vaccines are well tolerated, given to expectant mothers in one or two doses, via intramuscular injection. The most common adverse event is pain and sensitivity at the site of administration. Just 2% of 500 volunteers tested exhibited symptoms, such as low fever, headaches, shivering or myalgia, which resolved in 24 to 48 hours. The immune response to the different serotypes in the conjugate vaccine is dose-dependent, with the exception of serotype V. Doses such as 4 µg of type II CPA, 10 µg for type V and 15 µg for serotypes Ia, Ib, and III resulted in increases of four times in specific antibody titers in 80 to 93% of the volunteers tested 8 weeks after immunization. Peak antibody levels were detected between 4 and 8 weeks after vaccination, followed by a decrease in levels to the point where, after 1 year, GBS-specific antibody titers had dropped by 50%; however, in common with other conjugate vaccines, protection is maintained for prolonged periods.

The ideal vaccine would be a pentavalent conjugate vaccine. A bivalent combined (II-TT and III-TT) conjugate vaccine has already been developed and tested with good tolerance and immunogenicity.[12] Likewise, for the V serotype, vaccines have already been developed and tested, one conjugated with TT and another with mutant diphtheria toxin (CRM_{197}).[13] Both vaccines were well tolerated and there was no statistically significant difference between them in immunoresponse.

Anything that requires large-scale testing on pregnant women will come up against a debate on scientific ethics.[14] Many questions, however, remain to be answered with relation to the GBS vaccine: What is the ideal number of doses? Are boosters required? What is its true level of protection in clinical practice? When will we manage to develop a pentavalent vaccine? How should such a vaccine be approved and tested on a large scale? Would it be possible and effective to vaccinate non-pregnant women of fertile age?

Herpes-zoster vaccine

Recent studies have demonstrated the efficacy and safety of a herpes-zoster vaccine.[15] It is estimated that around 1 million cases of the disease occur annually in the USA. The importance of this new vaccine is directly related to the probable increase in the incidence of zoster as the decades pass, due to the increased longevity of the population and also to the increasingly frequent use of immunosuppressive drugs and treatments.

Zoster is linked with complications such as postherpetic neuralgia (PHN), herpes ophthalmitis, myocarditis, paresthesias, myopathies and others. The management and treatment of these complications is still far from acceptable.

Epidemiology

The clinical manifestations of latent varicella-zoster virus (VZV) reactivation can occur decades after the primary infection, having a profound effect on the quality of life of patients with the morbidity that is associated with the disease. Epidemiological studies report that the annual incidence of herpes-zoster is 2.9/1,000 in the USA,[16] 4.6/1,000 in Iceland,[17] 4.0/1,000 in Italy[18] and 4.8/1,000 in France.[19]

There are no Brazilian data since this is not a notifiable disease. In the Italian study, around 50% of cases occurred in individuals over 65 years old, and more than 75% of cases in people over 50 years old. There is a strong relationship between herpes-zoster incidence and advanced age, reaching figures of 10/1,000 per year in the 70 to 80-year-old population.[20] Theoretically, the tendency is for the number of cases to increase, since both longevity and the number of immunocompromised patients are increasing. Management of the disease and its complications still leaves much to be desired.[21]

Re-exposure to VZV appears to protect against zoster, whether by contact with infected children with the natural virus or through revaccination.[22] Young adults have CD4 and CD8 memory cells that recognize VZV, making the disease very rare in these immunocompetent individuals. In immunocompromised patients, loss of VZV-specific T lymphocytes can signify temporary susceptibility to VZV reactivation.

With increasing immunization of children against VZV, there will be reduced circulation of the wild virus in the population, which could lead to an increase in the number of cases of zoster in the elderly population who would no longer be given natural boosters at advanced ages. There are no concrete data on this impact, even for populations with high numbers of children vaccinated against varicella.

The infection and the virus

Zoster, also known as "shingles," is characterized by radicular unilateral pain accompanied by vesicular exanthema, generally limited to a single dermatome. Zoster is the result of the re-emergence of latent VZV from the sensory ganglion. The disease is habitually diagnosed clinically. Treatment consists of antiviral drugs (anti-VZV) started a maximum of 72 hours after onset of symptoms, with the aim of reducing the extent of the disease, the length of its clinical course and, if possible, the principal complication: PHN.[23-25] Complications may be present in more than 50% of cases of the disease. The most common and most feared complication is neuralgia, which is a painful neuropathic syndrome, which may surface after the exanthema has resolved and persist for prolonged periods. The older the patient, the greater the risk and intensity of complications. Postherpetic neuralgia can last for years, even for the rest of the patient's life. Response to the treatments currently recommended is very limited. The use of antivirals in the treatment of acute zoster episodes reduces the duration and extent of the disease, but does not prevent neuralgia.

Development of a new vaccine

The immune status of the elderly, with progressive loss of cell-mediated immunity, predisposes towards VZV infections. The lower the level of cellular immunity, whether due to age or immunosuppressive diseases such as AIDS, the greater the incidence of zoster. In response to these facts, the Shingles Prevention Study ran a large and important project to establish the impact of a zoster vaccine. The objective of the study was to investigate the reduction of pain and discomfort caused by the disease and the impact on its overall incidence in addition to measuring the frequency of complications such as PHN in the elderly population.[26] Oxman et al. conducted the principal study published to date with the zoster vaccine.[26] They worked from the hypothesis that a vaccine against zoster would reduce the incidence and severity of the disease and of PHN in an adult population. This was a randomized, double-blind, placebo controlled study with 38,546 adult individuals over 60 years of age. The live attenuated vaccine Oka/Merck®, or placebo, was given in a single 0.5 mL subcutaneous injection. The estimated concentration of the vaccine employed varied from 18,700 to 60,000 plaque forming units (PFU) per dose, divided into 12 different batches. The mean concentration of the vaccine used was 24,600 PFU, at least 12 times (ranging from 10 to 30 times) the concentration of the vaccine used routinely since 1995 to immunize children against varicella (Oka/Merck® - Varivax® with a minimum of 1,350 PFU/dose). The primary objective of the study was to assess the impact of the disease (zoster), by means of plotting the incidence, severity and duration of pain associated

with zoster. The secondary objective was to evaluate the incidence of PHN. The mean follow-up period was 3.12 years, varying from one day to 4.90 years, with no difference between the vaccinated group and the placebo group. Immunodepressed patients were excluded. The mean age in both groups was 69 years, with 6.6% of the vaccinated group and 6.9% of the placebo group $\geq$ 80 years of age, respectively. During follow-up there were a total of 957 confirmed cases of zoster, 315 in the vaccinated group and 642 in the placebo group ($p < 0.001$). In 93% of all zoster cases, the disease was confirmed by PCR. The DNA of the vaccine virus was not detected in any of the cases. The use of adequate antiviral treatments during the zoster episodes was similar for both groups. There were 107 cases of PHN, 27 in the vaccinated group and 80 in the placebo group. The zoster vaccine reduced the impact of the disease in terms of pain and discomfort associated with zoster by 61.1% ($p < 0.001$), reduced incidence of the disease by 51% and reduced incidence of PHN by 66.5% ($p < 0.001$). When broken down by age group (60-69 years and > 70 years), reduction in disease impact was 65% for the 60-69 group and 55% for the over 70 years group.

There was a much larger number of adverse events in the vaccinated group when compared with the placebo group, with local reactions being most common, mostly mild ones.

Future expectations

With the progressive and universal use of the VZV vaccine in children, the circulation of the virus in the wild will probably diminish. The practical implication of this is that the adult population will be exposed to VZV less often and therefore have less opportunities for natural boosters, with resultant lowered cell-based immunity and antibodies specific to VZV.

Another aspect to be highlighted is that life expectancy is in constant expansion. It is estimated that the population over 85 years old in the USA increased by around 1 million between 1995 to 2005, and that the population of seniors from 60 to 85 years old increased even more. The Census Bureau in the USA estimates that in 2040 there will be from 8 to 13 million North-Americans aged over 85 years.[27]

Many important questions remain about the vaccine and the disease itself. Could the recently developed and tested vaccine be used with immunocompromised patients, with safety and efficacy? How long might protection last and what supplementary doses might be needed? Will populations given the varicella vaccine during childhood have less zoster in adulthood? Will the zoster vaccine be viable economically? What might the true number of PFU needed in the vaccine?[28]

This vaccine has already been submitted for approval by the US Food and Drug Administration (FDA) by the Merck® laboratory, and, if approved, it is estimated that it could prevent 250 thousand cases of zoster in the USA each year, in addition to reducing the severity and morbidity of the disease of another 250 thousand cases a year.

HIV vaccine

Any HIV vaccine, even if it was only partially effective, or if it just delayed the progression to AIDS, would be of enormous value.[29,30] The introduction of highly active antiretroviral therapy (HAART) during the second half of the 1990s represented a significant step forward in controlling the pandemic, but problems remain with tolerance, development of resistance and compliance, in addition to the elevated costs.[31,32]

Obstacles to developing a vaccine

There are currently around 30 AIDS vaccines in clinical trials with humans.[31,33] Despite these considerable efforts, the development of a vaccine to prevent AIDS faces serious difficulties, the principal of which is the lack of a model of immunity to HIV. The vast majority of people exposed to the virus acquire the infection and develop the disease. The mechanism through which this occurs is not yet sufficiently understood, resulting in traditional approaches to vaccine development failing to produce satisfactory results.[34,35]

Despite these problems there is a growing interest in achieving a safe and effective vaccine, with annual investment in HIV vaccine research estimated at more than US$ 680 million.[29,31,36]

In addition to the complexity and inadequacy of the natural immunoresponse to HIV, the virus itself exhibits major genetic variations, an elevated rate of reproduction and a high proportion of mutations, all making development of a vaccine more difficult.[37-39]

HIV and the immunoresponse

The different strains and variants of HIV-1 are classed into three primary groups: M (main), O (outlier) and N (new). Each group is further divided into subtypes or clades. The main group (M), is subdivided into clades A to J. A clade (from the Greek *klados*, branch) is made up of phenotypical and genotypical variants of the virus. Genetic homology across clades is approximately 60%. Different parts of the world exhibit predominance of different clades. In addition to this diversity, HIV exhibits an elevated rate of mutation, caused by the absence of mechanisms for correcting reproductive errors in its RNA. This results in a large number of variants, even within a single individual.[37-39]

The human immunodeficiency virus is composed of an external envelope that surrounds a capsid containing RNA, which determines the formation of structural proteins and glycoproteins and its three enzymes, protease, reverse transcriptase and integrase. The structural glycoproteins and proteins, particularly the external ones, such as gp120, gp41 and p24, are examples of primary targets of the initial immunoresponse. In order to fuse with the cell there must be interaction between gp120 and CXCR4 and CCR5 and CD4 receptors. This bonding, together with the bonding of co-receptors, causes alterations that allow gp41 to fuse with the cell membrane, allowing the virus to enter. Once inside the cell, the viral RNA produces DNA that integrates with the cell's DNA, allowing HIV to be produced for the rest of the life of the cell. The first cells to be infected are local immune cells, such as dendritic cells and monocytes. Once infected, these cells migrate to the lymph nodes, where HIV will infect $CD4^+$ T lymphocytes. Despite an intense immunoresponse, HIV is capable of resisting eradication and goes on to destroy the $CD4^+$ T lymphocytes, with consequent immunosuppression and progression to clinically manifest AIDS.[34,39]

Despite the similarity with infection of monkeys by SIV, this has not proven a practical animal model for studying vaccines.[40-42]

Another subject of investigation are individuals who, despite being exposed to HIV, do not become infected, and also those individuals who, once infected, do not progress to the immunosuppression phase.

Strategies for the development of a vaccine

Traditional strategies

the traditional strategies used to develop vaccines have not proven either effective or viable for HIV. The inadequacy of immunoresponse and the severity of the disease rule out the use of attenuated live viruses, and inactivated viruses have not been capable of inducing adequate immunoresponse.

The use of part or parts of the virus, obtained by recombination or by inactivation and splitting has been widely adopted, however, without success to date. Clinical trials with subunit vaccines have not been successful. Several different approaches are being employed to develop vaccines against HIV and AIDS.[33,35,40]

Subunit vaccines

Subunit HIV vaccines are developed using recombinant proteins derived from surface (envelope) proteins of laboratory HIV strains, designed to stimulate specific humoral immunity.

Many different HIV proteins and genes have already been evaluated, including structural genes and proteins (gag, env, gp120, gp41 and gp160), viral enzymes (pol) and regulating proteins (*nef*, *tat*, *rev* and *vpr*). The capacity of these proteins to induce humoral or cell-based immunity varies immensely, but results have been discouraging even though the formation of neutralizing antibodies has been achieved. Currently, work is directed towards vaccines that have the three-dimensional structure of the virus, following the example of the HPV vaccine.

The recombinant vaccine that has achieved the greatest response, despite the results of phase II clinical trials being discouraging, is AIDSVAX®, which uses the gp120 protein, with different versions for different clades.[33,35,40]

Synthetic peptides

Synthetic peptides are laboratory-prepared immunogenic fragments of viral proteins. Efforts are concentrating on a portion of the gp120 protein, but, while well tolerated and with encouraging results in laboratory tests, immunogenicity in clinical trials has not been achieved.[33,35,40]

Recombinant vector vaccines

Usually the virus or bacteria used as vector for HIV antigen coding genes will be attenuated or non-pathogenic. Recombinant vector vaccines stimulate both cell-based and humoral immunity and appear to be one of the most promising strategies for developing vaccines for HIV and AIDS.

A vaccine using vaccinia as the vector has shown itself capable of inducing both cell-based and humoral immunity, which, although transitory, was enough to protect monkeys.

Vaccines currently in evaluation use canarypox, a more attenuated strain of the vaccinia virus (MVA – modified vaccinia Ankara), adenovirus, alphavirus (Venezuelan equine encephalitis virus, Sindbis and Semliki Forest viruses), in addition to bacteria such as Bacille Calmette-Guérin (BCG), *Salmonella spp*, *Listeria monocytogenes* and *Shigella spp*.[33,35,40]

DNA vaccines

This is a promising vaccine development strategy. It does, however, present some yet-to-be-resolved problems of a biological nature. These are fragments of the virus DNA that contain just the genes that code for some of the antigenic proteins and are, therefore, incapable of coding the complete virus. When injected, they integrate with the cell DNA and code for the desired antigens. Both cell-based and humoral immunity is induced. These vaccines are still in the initial phases of clinical trials.[33,35,40]

Prime-boost strategies

In order to circumvent the difficulties involved in

inducing protective cell-based and humoral immunity from HIV with the vaccines evaluated so far, this alternative strategy combines vaccines: the first is used to induce cell-based immunity, for memory, and the second to induce the formation of antibodies. The strategy aims to potentialize the positive features of different vaccines. The combination of DNA vaccines with subunit vaccines or recombinant vector vaccines is one strategy being evaluated.[33,35,40]

Clinical trials in progress

Despite the problems with obtaining a vaccine that effectively controls HIV or even retards progression to AIDS, there are several vaccines in different stages of the evaluation process, from laboratory studies with animal models to phase III clinical trials.

The situation of these studies is extremely dynamic and it is recommended that Internet websites that provide up-to-date information be consulted, for example the HIV Vaccines Trials Network (http://www.hvtn.org/), the International AIDS Vaccine Initiative (http://www.iavi.org/) and Clinical Trials (http://www.clinicaltrials.gov/) which is run by the National Institutes of Health in the USA. This website lists no less than 27,383 studies in progress or already approved.[8]

Future prospects

Research to create an effective vaccine has already entered its third decade and major advances have been achieved, in particular with relation to understanding natural immunity to HIV. The absence of natural immunity is still a major obstacle, but lessons learnt from experiments conducted so far, while themselves often very frustrating, point to promising alternatives, such as the prime-boost strategy.

A vaccine that was just partially effective, even if it raised ethical issues, would be of great utility in public health. Thanks to the progress achieved in the area of treatment, a vaccine that could reduce the initial viral load or retard development of the disease allowing antiretroviral therapy to be delayed would be a step forward.[31,41]

Dengue vaccines

Dengue, together with malaria, is one of the two most important vector-borne diseases today. Dengue cases are counted in millions every year. It is estimated that around 2/5 of the world's population is exposed to the risk of contracting dengue. In 2001 almost 400 thousand cases of dengue were notified in Brazil.[43,44]

The dengue vector, *Aedes aegypti*, is extremely well adapted to the urban environment, and, together with the extremely poor urban infrastructure of most large cities and metropolitan areas in the third world, this virtually rules out any chance of controlling dengue, meaning that a vaccine is the only sure solution for the disease.[45,46]

Obstacles to developing a vaccine

The use of traditional vaccine development techniques to create an effective dengue vaccine does not create major problems. There are already effective vaccines for other types of flavivirus, such as the live attenuated yellow fever virus vaccine and the Japanese encephalitis vaccine with inactivated whole viruses.[47] Many attenuated strains of the four serotypes of the dengue virus have already been produced and evaluated, proving themselves to be immunogenic and capable of providing protection.[48-50] The major problem is the particular nature of the pathogenesis of the more severe forms of dengue, with the occurrence of a phenomenon known as antibody disease enhancement (ADE).[51-53]

Any vaccine, attenuated or inactivated, must be a combined vaccine that induces immunity against all four serotypes. This aspect in particular makes more careful pre-clinical and clinical trials obligatory, or, alternatively, the development of new strategies for vaccine development.

The dengue virus and immunoresponse

The dengue virus is a single-strand, positive-sense, RNA virus of the *Flaviviridae* family. Its genome is contained in a single strand of RNA with a single long open reading frame (ORF). The ORF is translated into a single polyprotein which is cleaved by proteases from the virus and the infected cell into 10 proteins: three structural (C, M and E) and seven non-structural (NS 1, NS 2a, NS 2b, NS 3, NS 4a, NS 4b and NS 5).[44,54]

There are four different serotypes, DEN-1, DEN-2, DEN-3 and DEN-4. The virion is approximately 50 nm in diameter, and its genome has an 11 kb extension. All four serotypes have been sequenced.[52]

Each serotype confers permanent specific immunity and short-term cross-immunity, and all four serotypes are capable of causing severe and fatal diseases. There is genetic variation within each serotype, with at least five DEN-1, five DEN-2, four DEN-3 and two DEN-4 genotypes. Certain genetic variants of each serotype appear to be more virulent or to have greater epidemic potential.[44,52] Immunity conferred by natural infection is long lasting, but type-specific.

The three-dimensional structure of the E protein consists of a complex dimer with two identical subunits and is subdivided into three distinct domains:

I – the central domain, containing the radical amino terminal;

II – contains the majority of the dimer contacts;

III – includes the C terminal and is related to the virulence of different viral strains.

Antibodies against the E protein are directed to epitopes that are present all over the external surface of the molecule. The neutralization mechanism is related to dissociation of the E dimer due to the presence of the antibody, preventing the alterations that lead to the formation of the trimer form of the molecule.

Humoral response is generally vigorous, specific IgM antibodies are detectable from the fourth day after onset of symptoms, reaching their highest levels by around the seventh or eighth day before slowly declining until they are undetectable after some months. Specific IgG antibodies are observed in low concentrations from the fourth day after onset of symptoms and climb to high levels in 2 weeks, remaining detectable for many years and conferring immunity to the type with which the individual was infected, probably lifelong. Antibodies that appear during infection by a given serotype of the dengue virus protect against infection by other serotypes, but this protection is short lived.

The antibodies provoke the lysis of the envelope or block its receptors with consequent viral neutralization. The E protein, located in the outer leaflets of the dengue virus envelope, is fundamental to viral bonding with the membrane receptors. The E protein epitopes define the production of antibodies specific to the viral serotype and to the whole genus and can be detected by many serological tests.[44,46]

Cellular cytotoxic immunoresponse by T lymphocytes occurs in response to stimulation by the NS1, NS3 and E proteins. T helper lymphocytes act in the presence of cells infected with dengue that express type II HLA receptors, producing IFN-γ, IL-2 and granulocyte-macrophage colony stimulating factor. The cytotoxic lymphocytes directly attack cells infected with dengue that express type I HLA receptors.[52]

Strategies for the development of a vaccine

There are currently two main approaches to developing a vaccine with efficacy against all four serotypes of the dengue virus, attenuation and viral chimeras with characteristics from all four serotypes.[47,50,54]

Live attenuated virus

There are two vaccines made with attenuated viruses in advanced stages of development. One of these was developed in Thailand with three strains of the virus attenuated by successive passages through primary dog kidney (PDK) cells and a strain of DEN-3 attenuated by successive passages through African green monkey cells. This vaccine is being developed by Sanofi-Pasteur®.

The other, developed in the USA, employs strains from all four serotypes of the dengue virus attenuated by successive passages through PDK cells and a final passage through fetal Rhesus monkey lung cells. This vaccine is being developed by GlaxoSmithKline Biologicals®.

Both vaccines exhibit elevated efficacy, tolerance and safety in phase I and II clinical trials. Phase III clinical trials are ongoing.[48,50,55]

Chimeras

Chimeras are viable vaccines obtained by inserting genes that code for certain desirable antigens from one virus or viruses into another virus, this last termed the backbone.

There are at least four chimeric vaccines in advanced stages of development, all promising:[50,55]

- prM and E genes from all four serotypes of the dengue virus inserted into a non-structural portion of the 17D yellow fever vaccine virus (Acambis & Sanofi-Pasteur®).
- prM and E genes from all four serotypes of the dengue virus inserted into a non-structural portion of an attenuated DEN-2 (16681, PDK 53) virus (CDC).
- prM and E genes from the DEN-1, DEN-2 and DEN-3 dengue viruses inserted into a DEN-4 virus attenuated by deletion of specific nucleotides (NIH).
- prM and E genes from the DEN-2, DEN-3 and DEN-4 dengue viruses inserted into a DEN-1 virus attenuated by deletion of specific nucleotides (FDA).

Future prospects

The two vaccines using attenuated live viruses appear to be the most promising and have already proven their efficacy in clinical trials. One aspect yet to be confirmed is whether this efficacy is uniform and consistent for all four serotypes, under the theoretical threat of creating an increased risk of severe forms if not.

Chimeric vaccines should be an interesting alternative for the future, but they present the same basic problem as the attenuated vaccines, which is of increasing the risk of development of severe forms if the immunity provided is not homogeneous for all four serotypes.[54,56]

Malaria vaccines

It is estimated that around 2.7 million people, the majority of them children, die each year from malaria and that more than 2 billion people are exposed to the risk of acquiring the disease worldwide. In Brazil, while the number of deaths may not be excessive, for decades the number of cases has been counted in hundreds of thousands.

Malaria, in common with AIDS, has proven an enormous challenge to vaccine development, not just due to the complexity of the immunoresponse to the infection, but also because of a lack of political will.[57-59]

Almost all of the vaccines under development are directed at *Plasmodium falciparum*, which is responsible for severe forms of malaria and for the vast majority of deaths.[60]

Obstacles to developing a vaccine

Plasmodium parasites have a complex lifecycle, with many different stages, both within the definitive host and the vector. Different antigens are expressed at different stages in the lifecycle, although the malaria genome project has demonstrated that the same antigen can be expressed at different stages.[61,62]

The major problem, however, is the complexity of the immunoresponse to the parasite. To date, there is no safe and effective vaccine against protozoa, organisms that are infinitely more complex than viruses and bacteria. Plasmodia have more than 5 thousand genes, as against 5 or 10 in the majority of viruses.[61-63]

Plasmodium spp. *and the immunoresponse*

The main stages of the lifecycle of *P. falciparum* can be summed up thus:

Pre-erythrocytic phase: the parasite is injected by the anopheles vector, which introduces around 15 sporozoites into the bloodstream. These sporozoites migrate rapidly to the liver and lodge in hepatocytes, where they go through a process of asexual reproduction that lasts for an average of 6 to 7 days, releasing around 20 to 40 thousand merozoites into the bloodstream.

Erythrocytic or bloodstream phase: the merozoites go through a cycle of infection of red blood cells, asexual reproduction and bursting of the blood cells, liberating even more merozoites. It is during this phase that the clinical manifestations of malaria surface, with duration and intensity depending on the host's immunoresponse.

Sexual phase: some of the merozoites are transformed into male and female gametocytes. These forms may be aspirated once more by the anopheles vector, where they will reproduce sexually, completing the lifecycle by producing sporozoites to be inoculated into another host.[57]

The immunoresponse to malaria is partial and both species and strain specific, but there is experimental evidence to demonstrate that a protective vaccine is possible:[64,65]

- The immunization of humans and animals with irradiated sporozoites results in partial or complete protection from an experimental infection with viable sporozoites.
- Repeated infection leads to natural immunity.
- Passive transfer of the immunoglobulin of an immune person confers immunity on children.
- Several phase I and II clinical trials of a variety of vaccines have demonstrated protection, although these vaccines have had low efficacy.

Strategies for developing a vaccine

More than three decades have passed since it was first demonstrated that irradiated sporozoites were capable of inducing immunity. Due to the complexity of the parasite's lifecycle and its equally complex antigenic structure, traditional attenuation and inactivation techniques have not met with success. The identification of different proteins capable of inducing immunity, although only partial, in animals and humans has made subunit vaccines the principal strategy currently in employment.

Recombinant vector vaccines and DNA vaccines are also being assessed, but it is the subunit vaccines that have advanced furthest. This group can be subdivided according to the phase of the cycle aimed at.[65,66]

Pre-erythrocytic

The best known antigen is the circumsporozoite protein (CSP) which is expressed in extracellular sporozoites and in the intrahepatocytic forms of the parasite. This protein, whether recombinant or synthesized, has been demonstrated as safe and antigenic, but offers only partial protection.

The CSP-based vaccine with the best and most promising results, already in phase III trials, is RTS,S, the result of a partnership between the Walter Reed Army Research Institute in the USA and GlaxoSmithKline Biologicals®.

This vaccine consist of a hybrid in which CSP is fused to the surface antigen of the hepatitis B strain used for the hepatitis B vaccine, in association with a powerful adjuvant, AS02 (adjuvant system 02). This adjuvant is a combination of monophosphoryl lipid A (MPL), a purified bacterial wall component, a saponin (QS-21) and a water-oil emulsion.[67-69]

Erythrocytic

The majority of the vaccines aimed at the erythrocytic stage are based on the plasmodium protein responsible for the entrance of the merozoites into the blood cells. The best studied so far is merozoite surface protein 1 (MSP1). Antibodies to MSP1 offer resistance to clinically manifest malaria, suggesting that a vaccine based on the protein would offer protection, which has indeed been demonstrated in animal models. several similar vaccines have already been or are currently being submitted to phase I clinical trials. Other antigens, generally variants of MSP1, are also being studied, still in the initial stages.[60,65,66]

Transmission blockers

These are vaccines that are capable of blocking the transmission of plasmodia to mosquitoes, but do not protect the person being vaccinated. By blocking the gametocytes, the lifecycle of plasmodium is halted making sexual reproduction impossible.

Many vaccines are being evaluated, none of them in an advanced stage of development. Since they do not protect the person being vaccinated, they would possibly be administered in conjunction with other vaccines.[60,65]

Future prospects

Despite a series of frustrations over more than 30 years of research into a malaria vaccine, major advances have been achieved in recent years and, for the first time, there is now a vaccine that is truly promising in phase III clinical trials: RTS,S.

The complete sequencing of the *P. falciparum* genome has allowed for the identification of potential antigens for investigation.[70] In common with AIDS and dengue, even a partially effective vaccine for malaria would be an advance, since it would reduce transmission, prevent epidemics, reduce the risk of resistance to anti-malarial drugs and, together with mosquito nets impregnated with permetrine and the use of prophylactic medication, could make more effective control possible.[59]

Final comments

This review has demonstrated that the development of a safe and effective vaccine is a process that is long, difficult and, very often, of uncertain success. Of the vaccines described here, it is the one to combat HIV that presents the greatest problems, but the current progress of research permits, perhaps with a little optimism, the prediction that these problems will be overcome in the near future.

Conflict of interest

Luiz Jacintho da Silva declares that he is an HPV vaccine consultant for GlaxoSmithKline Biologicals and that he has been a member of the speaker's bureau and participated in assistant committees for GlaxoSmithKline Biologicals, Sanofi-Pasteur, Wyeth, and Chiron. Rosana Richtmann declares that she is a member of the speaker's bureau for Laboratório MSD and Laboratório Wyeth.

References

1. Gibbs RS, Schrag S, Schuchat A. Perinatal infections due to group B streptococci. Obstet Gynecol. 2004;104:1062-76.
2. Vaciloto E, Richtmann R, Costa HPF, Kusano EJU, Almeida MFB, Amaro ER. A survey of the incidence of neonatal sepsis by group B streptococcus during a decade in a Brazilian maternity hospital. Braz J Infect Dis. 2002;6:55-62.
3. Schuchat A. Group B streptococcus. Lancet. 1999;353:51-6.
4. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep. 2002;51:1-22.
5. Centers for Disease Control and Prevention. Active Bacterial Core Surveillance (ABCs) Report, Emerging Infections Program Network, group B streptococcus, 2002. http://www.cdc.gov/ncidod/dbmd/abcs/survreports/gbs02.pdf. Access: 04/03/2006.
6. Lin FY, Weisman LE, Azimi PH, Philips JB 3rd, Clark P, Regan J, et al. Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen. J Infect Dis. 2004;190:928-34.
7. Baker CJ, Kasper DL. Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. N Engl J Med. 1976;294:753-6.
8. Baker CJ, Kasper DL. Group B streptococcal vaccines. Rev Infect Dis. 1985;7:458-67.
9. Baker CJ, Rench MA, Edwards MS, Carpenter RJ, Hays BM, Kasper DL. Immunization of pregnant women with a polysaccharide vaccine of group B streptococcus. N Engl J Med. 1988;319:1180-5.
10. Zaleznik DF, Rench MA, Hillier S, Krohn MA, Platt R, Lee ML, et al. Invasive disease due to group B streptococcus in pregnant women and neonates from diverse population groups. Clin Infect Dis. 2000;30:276-81.
11. Kasper DL, Paoletti LC, Wessels MR, Guttormsen HK, Carey VJ, Jennings HJ, et al. Immune response to type III group B streptococcal polysaccharide-tetanus toxoid conjugate vaccine. J Clin Invest. 1996;98:2308-14.
12. Baker CJ, Paoletti LC, Wessels MR, Guttormsen HK, Rench MA, Hickman ME, et al. Safety and immunogenicity of capsular polysaccharide-tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib. J Infect Dis.1999;179:142-50.
13. Baker CJ, Paoletti LC, Rench MA, Guttormsen HK, Edwards MS, Kasper DL. Immune response of healthy women to 2 different group B streptococcal type V capsular polysaccharide-protein conjugate vaccines. J Infect Dis. 2004;189:1103-12.
14. Heath PT, Feldman RG. Vaccination against group B streptococcus. Expert Rev Vaccines. 2005;4:207-18.
15. Poland GA. The growing paradigm of preventing disease: vaccines to prevent herpes zoster and pertussis in adults. Ann Intern Med. 2005;143:539-41.
16. Donahue JG, Choo PW, Manson JE, Platt R. The incidence of herpes zoster. Arch Intern Med. 1995;155:1605-9.
17. Helgason S, Petursson G, Gudmundsson S, Sigurdsson JA. Prevalence of postherpetic neuralgia after a first episode of herpes zoster: prospective study with long term follow up. BMJ. 2000;321:794-6.
18. di Luzio Paparatti U, Arpinelli F, Visona G. Herpes zoster and its complication in Italy: an observational survey. J Infect. 1999;38:116-20.
19. Chidiac C, Bruxelle J, Daures JP, Hoang-Xuan T, Morel P, Leplege A, et al. Characteristics of patients with herpes zoster on presentation to practitioners in France. Clin Infect Dis. 2001;33:62-9.
20. Hodson AH. Epidemiology of shingles. J R Soc Med. 1991;84:184.
21. Mounsey AL, Matthew LG, Slawson DC. Herpes zoster and postherpetic neuralgia: prevention and management. Am Fam Physician. 2005;72:1075-80.
22. Thomas SL, Wheeler JG, Hall AJ. Contacts with varicella or with children and protection against herpes zoster in adults: a case-control study. Lancet. 2002;360:678-82.
23. Wood MJ, Kay R, Dworkin RH, Soong SJ, Whitley RJ. Oral acyclovir therapy accelerates pain resolution in patients with herpes zoster: a meta-analysis of placebo-controlled trials. Clin Infect Dis. 1996;22:341-7.
24. Tyring SK, Beutner KR, Tucker BA, Anderson WC, Crooks RJ. Antiviral therapy for herpes zoster: randomized, controlled clinical trial of valacyclovir and famciclovir therapy in immunocompetent patients 50 years and older. Arch Fam Med. 2000;9:863-9.
25. Jackson JL, Gibbons R, Meyer G, Inouye L. The effect of treating herpes zoster with oral acyclovir in preventing postherpetic neuralgia. A meta-analysis. Arch Intern Med. 1997;157:909-12.
26. Oxman MN, Levin MJ, Johnson GR, Schmader KE, Straus SE, Gelb LD, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med. 2005;352:2271-84.
27. Campion EW. The oldest old. N Engl J Med. 1994;330:1819-20.

55. Chaturvedi UC, Shrivastava R, Nagar R. Dengue vaccines: problems and prospects. Indian J Med Res. 2005;121:639-52.
56. Damonte EB, Pujol CA, Coto CE. Prospects for the therapy and prevention of dengue virus infections. Adv Virus Res. 2004;63:239-85.
57. Schlagenhauf P. Malaria: from prehistory to present. Infect Dis Clin North Am. 2004;18:189-205.
58. Greenwood B. Malaria vaccines. Evaluation and implementation. Acta Trop. 2005;95:298-304.
59. Breman JG, Alilio MS, Mills A. Conquering the intolerable burden of malaria: what's new, what's needed: a summary. Am J Trop Med Hyg. 2004;71:1-15.
60. Moorthy VS, Good MF, Hill AV. Malaria vaccine developments. Lancet. 2004;363:150-6.
61. Good MF, Stanisic D, Xu H, Elliott S, Wykes M. The immunological challenge to developing a vaccine to the blood stages of malaria parasites. Immunol Rev. 2004;201:254-67.
62. Engwerda CR, Good MF. Interactions between malaria parasites and the host immune system. Curr Opin Immunol. 2005;17: 381-7.
63. Good MF, Xu H, Wykes M, Engwerda CR. Development and regulation of cell-mediated immune responses to the blood stages of malaria: implications for vaccine research. Annu Rev Immunol. 2005;23:69-99.
64. Good MF. Vaccine-induced immunity to malaria parasites and the need for novel strategies. Trends Parasitol. 2005;21:29-34.
65. Webster D, Hill AV. Progress with new malaria vaccines. Bull World Health Organ. 2003;81:902-9.
66. Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. Lancet. 2005;365:1487-98.
67. Heppner DG Jr, Kester KE, Ockenhouse CF, Tornieporth N, Ofori O, Lyon JA, et al. Towards an RTS, S-based, multi-stage, multi-antigen vaccine against falciparum malaria: progress at the Walter Reed Army Institute of Research. Vaccine. 2005;23: 2243-50.
68. Ballou WR, Arevalo-Herrera M, Carucci D, Richie TL, Corradin G, Diggs C, et al. Update on the clinical development of candidate malaria vaccines. Am J Trop Med Hyg. 2004;71:239-47.
69. Hill AV. Pre-erythrocytic malaria vaccines: towards greater efficacy. Nat Rev Immunol. 2006;6:21-32.
70. Vernick KD, Waters AP. Genomics and malaria control. N Engl J Med. 2004;351:1901-4.

Correspondence:
Luiz Jacintho da Silva
Rua Nanuque, 432, ap. 164
CEP 05302-030 – São Paulo, SP – Brazil
E-mail: ljsilva@unicamp.br