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Review Article

Human Parasitic Vaccines - An Overview.

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Abstract

An effective vaccine against human parasitic infections remains elusive even after tremendous progress made in vaccine development for other microbial diseases. To date, the most promising parasite vaccine is that for malaria, which may go into commercial production in near future. The development of a parasite vaccine has been hampered not only by scientific and technological hurdles, but also due to economical considerations. The situation however, is slowly improving because of initiatives taken various non-profit organizations to boost up the research and development in this hitherto neglected field. Vaccines for leishmaniasis, hookworm, and schistosoma infections are now at an advanced stage of development, partly because of these efforts.

Key words: Parasites, vaccines, Developmental problems.

Introduction

Parasitic diseases affecting humans continue to be the leading cause of morbidity as well as mortality particularly in the tropical and subtropical countries. As per WHO estimates, 3.5 billion people worldwide are affected and 450 million have associated diseases due to infecting parasites. Drugs remain the mainstay for therapy for parasitic infections and also for disease control programmes. The existing anti-parasitic drugs have been in use for decades and drug resistance among the parasites is gradually expanding; the glaring example of which can be seen in *Plasmodium falciparum*. In spite of the tremendous strides in the fields of molecular medicine, genomics, and proteomics in the understanding of parasite biology; the discovery of a licensed human parasite vaccine continues to elude human efforts. This review intends to look into the current status of the promising human parasite vaccines which are in the late stages of development and may go into routine human use in not too distant future.

An effective vaccine stimulates the protective immune response of the host to fight invading pathogens. Even the simplest parasites have complex structure and life cycles. Moreover, there is a general lack of precise understanding of the host/parasite interaction[1]. Because of the complex nature of parasites, the immune system is confronted with a highly diverse and continuously changing spectrum of antigen. A number of biological characteristics of the parasite help in making the situation more difficult. First, many parasites go through a phase of sexual reproduction, with the associated exchange of genetic material. This results in new parasites with a different genetic and phenotypic make-up. There is a differential expression of genes during the successive life cycle stages. This results in a situation where the host seems to be harbouring parasites of different kinds, and so the host has to mount immune response for each variety. A number of species can express distinct variants of stage-specific molecules which are antigenically different. This ability allows them to avoid the host defences mechanisms.

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The site of infection may also affect the nature of the protective immune response and may constrain research on vaccine development.

The ability to produce recombinant parasite proteins in the early 1980's was considered a major breakthrough for vaccine development , but even after almost three decades, recombinant parasite vaccine for human use is non-existent. Producing protective recombinant parasite antigens has proven difficult. Efforts have been inhibited by the fact that recombinant proteins may be incorrectly folded and/or lack critical post-translational modifications, particularly the glycans that are attached to several of the native candidate antigens [2]. Other factors that add to the problem are that parasites avoid, deflect and confuse host immune system, the right parasite antigens has not been identified yet because of complicated life cycles, the protective host responses is not understood in most target species with multi-responses in majority of these parasites, candidate antigens may show efficacy in animal models but not in humans and moreover the animals may not be fully permissive, and finally absence of genome databases or bioinformatics algorithms for selecting candidate antigens of promise.

Apart from these scientific problems, the commercial viability of a vaccine depends on such factors as development and production costs, and specific characteristics, such as storage/transport conditions and shelf life. Perhaps the biggest barrier is the fact that current drugs have efficacies approaching 100%. It will not be easy to persuade users that a vaccine which is less than 100% effective can usefully control the disease. There is a market trend in favour of generic drug companies, which spend little on research and development and essentially do not invest in discovering new drugs or vaccines. Reasons for this are many and varied, with the demand for quick, high returns on investment reducing the opportunity for long-term discovery projects. As a result, very few pharmaceutical companies are currently committed to the discovery and development of antiparasitic vaccines [1,3].

Malaria Vaccine: Modern malaria vaccine development stems from immunization studies of mice with irradiated sporozoites, conducted in the 1960s[4], and subsequent analyses of the mechanisms of immunity in this model. Challenge studies by Clyde in humans[5] demonstrated that a high level of protection could be induced in volunteers but required large numbers of bites by irradiated infectious mosquitoes. The emergence of a peptide-based candidate vaccine from Colombia, called SPf66, with apparent efficacy in new world monkeys and humans[6] generated enormous interest and controversy but eventually disappointment, successive, independent field efficacy trials in Africa and Asia failed to demonstrate protection. The difficulty of developing a highly effective malaria vaccine has led to the design and assessment of a very wide range of new approaches[7] as can be seen in Table 1. At present a large number of candidate antigens are in various stages of clinical trial as can be seen in Fig 1.

1. Sporozoite subunit vaccination, especially with the CS protein: e.g. RTS, S in adjuvant.
2. Irradiated sporozoite or genetically attenuated sporozoite immunization either by mosquito bite or using injected purified sporozoites.
3. Immunization with DNA and/or viral vectors to induce T cells against the liver-stage parasites, or to target other life cycle stages.
4. Use of whole blood-stage malaria parasites as immunogens.
5. Use of protein in adjuvant vaccines to reduce the growth rate of blood-stage parasites.
6. Use of protein (or long peptide) in adjuvant vaccines to induce antibody-dependent cellular inhibition (ADCI) of blood-stage parasites.
7. Use of peptide-based vaccines, mainly against blood-stage parasites—e.g. SPf66.
8. Development of anti-disease vaccines based on parasite toxins.
9. Immunization with parasite adhesion ligands.
10. Use of parasite antigens preferentially expressed in the placenta to prevent malaria in pregnancy.
11. Immunization with sexual stage parasite antigens as transmission-blocking vaccines.
12. Use of mosquito antigens as transmission-blocking vaccines.

Adapted from Hill AVS[7].

Table :1-Approaches to Malaria Vaccine Development

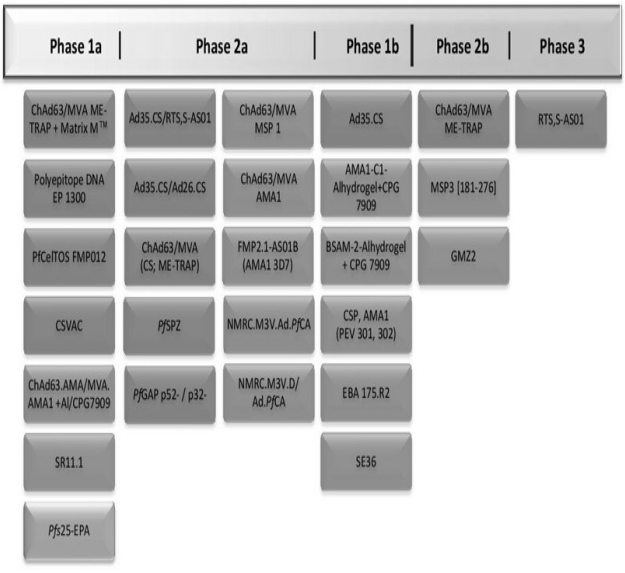

Fig: 1- Global Malaria Vaccine Pipeline: Candidate Vaccines in Various Phases of Trial.(Source: WHO).

The most effective malaria vaccine tested to date is RTS,S, a hybrid protein particle, formulated in a multi-component adjuvant named AS01. It is a pre-erythrocytic vaccine which consists of the central repeat ('R') of circumsporozoite protein fused to the C-terminal region known to contain T cell epitopes (hence 'T') fused in turn to the hepatitis B surface antigen ('S') which has yielded a yeast-expressed protein RTS[8]. To generate immunogenic particles, the RTS protein needed to be co-expressed with large amounts of the unfused S protein to yield RTS,S.

The adjuvant contains the immunostimulants, mono-phosphoryl lipid A (MPL, a toll-like receptor 4 agonist) and QS21 (a derivative of Quill A). A related adjuvant AS01, which contains liposomes is used in this vaccine. This vaccine induces a very high concentration of antibodies, often of hundreds of micrograms per millilitre, that target the conserved central repeat region of the circumsporozoite protein. The level of these antibodies correlates with protection against infection or disease[9]. It has demonstrated 51% efficacy in reducing the rate of all episodes of clinical malaria over fifteen months of follow-up in a Phase 2 trial in children aged 5-17 months in Kenya[10]. The ongoing Pivotal Phase 3 trial started in May 2009 and has enrolled 15,460 children. The trial, conducted at 11 sites in seven countries across sub-Saharan Africa, reported that RTS,S reduced the incidence of all episodes of clinical malaria by 55%[11]. The second set of results has become available in 2012, and the salient findings were as follows[12]:

a) The incidence of a first or only episode of clinical malaria meeting the primary case definition during 12 months of follow-up was 0.37 per person-year in the RTS,S/AS01 group and 0.48 per person-year in the control group, for a vaccine efficacy of 31.3% .
b) Efficacy was higher at the beginning than at the end of the follow-up period.
c) Before vaccination, 34.3% of infants were positive for anti-circumsporozoite antibodies but at low titers. After vaccination, 99.7% were positive for anti-circumsporozoite antibodies, with a geometric mean titer of 209 EU per milliliter.

The full trial results are expected in 2014 with the aim of licensure and deployment in 2015. This vaccine will be evaluated as a potential addition to, not a replacement for, integrated approaches of existing preventive, diagnostic and treatment measures tailored to a given endemic setting.

Leishmania Vaccine: The evidence that most individuals who were once infected with Leishmania are resistant to clinical infections when later exposed to it provided the justification for vaccine development [13]. The leishmaniases are unique among parasitic diseases because a single vaccine could successfully prevent and treat disease and has the potential to protect against more than one *Leishmania* parasite species[14]. The development of a Leishmania vaccine can be divided into the following stages:

1. Live Leishmania vaccine (Leishmanization, LZ) : It is not licensed, but used in Uzbekistan, former USSR, Iran, and Israel. Live virulent *L. major* promastigotes are harvested from cultures and used as vaccine. At present, there is only one prophylactic live vaccine in use. This is a mixture of live virulent *L. major* mixed with killed parasite registered in Uzbekistan. Adverse side effects include development of large persistent lesions, psoriasis and immunosuppression[15].
2. First generation vaccines consisting of whole killed Leishmania or fractions of the parasite: Whole killed vaccines have been experimented with for both old- and new- world Leishmania. Mayrink and his colleagues developed a killed vaccine composed of five isolates of *Leishmania* containing four different species in 1970s[16]. Convit and his group in Venezuela introduced their autoclaved *Leishmania mexicana* (*L. mexicana*) + BCG for immunotherapy and/or immunochemotherapy[17]. Several prophylactic studies were done with inconclusive results or low protection induced by the vaccine (killed *Leishmania* injected 3 times without any adjuvant) when given to leishmanin-negative (Montenegro skin test, MST) individuals. However, a highly significant finding of this group, which has been confirmed repeatedly by others, is that the incidence rate amongst the MST converted individuals in the vaccine group was significantly lower than those in the control (unvaccinated) group or vaccinated but MST non-converted individuals[18]. For old world leishmaniasis, autoclaved *L.major* + BCG (ALM+BCG) has been extensively studied. Two doses of the vaccine reduced the incidence by 43% in Leishmanin Skin Test converted volunteers in Sudan against Visceral Leishmaniasis involving 2306 volunteers[19]. To enhance the immunogenicity of the ALM+BCG vaccine, ALM was adsorbed to alum and the resulting alum-ALM was mixed with BCG just prior to injection. It appears to constitute a safe vaccine and an appropriate candidate for further development. A subunit vaccine utilising the fucose mannose ligand (FML) antigen has been shown to be a potent immunogen in mice and rabbits and a sensitive, predictive and specific antigen in serodiagnosis of human and canine kala-azar[20].

3. Second generation vaccines include all defined vaccines, i.e., recombinant proteins, DNA vaccines and combinations. A variety of Leishmania vaccine candidates consist of recombinant proteins. More recent efforts have aimed at increasing the immunogenicity of DNA cloned vaccines, including the use of genetic adjuvants and plasmid-based expression of viral replicons. Some of the important recombinant protein candidate vaccines include: surface expressed glycoprotein leishmaniolysin (gp63), Leishmania activated C kinase (LACK), parasite surface antigen (PSA), Leishmania derived recombinant polyprotein (Leish-111f) and serine proteases[21]. Leish-111f is a single polyprotein composed of three molecules fused in tandem: the *L. major* homologue of eukaryotic thiol-specific antioxidant, TSA; the *L. major* stress-inducible protein-1, LmSTI1; and the *L. braziliensis* elongation and initiation factor, LeIF. The Leish-111f product is the first defined vaccine for leishmaniasis to go in to human clinical trials and has completed phase 1 and 2 safety and immunogenicity testing in normal, healthy human subjects[22].

4.Live-attenuated Leishmania vaccines: The live-attenuated anti-leishmanial vaccine is still at its early stages of development. The use of dihydrofolate reductase thymidylate synthase (dhfr-ts) knockout parasites led to the protection in a mouse model but not in monkeys[18]. Recently, use of *L. donovani* centrin null mutants (LdCEN-/-) in mice showed clearance of virulent challenge parasites in 10 weeks after challenge, with significantly reduced parasite burden in the spleen and no parasites in the liver[23].

Given the rapid progress in the fields of parasite immunology and genetic engineering, a successful anti-*Leishmania* vaccine should be achievable in the near future. Based on the past and present experience on *Leishmania* vaccine studies, it appears that future experiments should include appropriate adjuvants as components in order to achieve effective vaccines against human leishmaniasis[18].

Amebiasis Vaccanie: The serine-rich *E.histolytica* protein mediates the binding of trophozoites of *E. histolytica* to the mammalian cells. In animal studies, 85% of the vaccinated gerbils in a total of 3 trials were completely protected from developing amebic liver abscess. The safety and immunogenicity has also been well-documented in African green monkeys. The N-Acetylgalactosamine –inhibitable *E. histolytica* lectin (GAL / GALNAC) mediates the adherence of trophozoites . Although the vaccination has been protective in 66% of the animals using this antigen, in the remaining there has been evidence of a significant increase in liver abscess size. Other candidates in experimental stages are 29-kDa cysteine-rich protein (peroxiredoxin) , lipophosphoglycan, and oral/ intranasal administration of lectins[24].

Human Hookworm Vaccine: A number of hurdles complicate the development of an effective vaccine for hookworm, and for that matter other helminths. Some of these include

1. The difficulty of maintaining human hookworms in animal models and the cost of maintaining the hookworm in laboratory-canine model.
2. The absence of a laboratory animal that is permissive to human hookworms and can accurately reproduce human disease (anemia).
3. Paucity of *in vitro* functional tests to determine the efficacy of the immune response induced by an experimental hookworm vaccine.
4. The lack of a protective immune response in humans and the consequent absence of correlates of protection that can guide the discovery of vaccine antigens and be used to assess their effectiveness in preclinical and clinical trials.
5. No model of an effective immune response in humans to determine the biological consequences of the vaccine in humans.

In 1964, Miller [25]showed that *Ancylostoma caninum* larvae could be attenuated using 40,000 röntgens of X-ray. Industrial manufacture and US licensing of the 1st hookworm vaccine commenced in 1970s, which consisted of gamma-irradiated infective *A. caninum* L3 vaccine for canine[26]. The vaccine was discontinued in 1975 due to commercial failure. Although this vaccine failed commercially, it provided compelling evidence that human hookworm vaccine is a possibility.

The Human Hookworm Vaccine Initiative (HHVI) is the only group currently working on vaccines targeting this parasite. Ancylostoma Secreted Protein-2 of *N. americanus* (Na-ASP-2) is a 21 kDa protein that is secreted by infective hookworm larvae upon entry into the host and Na-ASP-2 was chosen as a lead hookworm vaccine candidate[27]. In a phase 1 study in hookworm-naïve adults living in the USA, *Na*-ASP-2 adjuvanted with Alhydrogel® was well-tolerated and immunogenic[28]. However, a phase 1 safety and immunogenicity trial of this vaccine in healthy adults from a hookworm endemic area in rural Brazil had to be halted when 3 participants developed immediate, generalized urticarial reactions. The urticarial reactions were associated with elevated levels of IgE antibodies specific for Na-ASP-2, which were present before immunization most likely due to previous hookworm infection[29]. In November, 2012 Sabin Vaccine Institute an-

start of Part II of its Phase I clinical trial of the *Necator americanus*-glutathione S-transferase 1 (*Na*-GST-1) vaccine candidate. Part II of the trial commenced in Americaninhas, Brazil, following successful vaccinations in Part I of the study, which began in Belo Horizonte, Brazil in late 2011. Ultimately, Na -GST-1 and *Necator americanus* Aspartic-Protease 1 (Na -APR-1) would be used together a bivalent vaccine and the aim of the vaccine will be to reduce moderate to heavy infections in the host[30].

Schistosoma Vaccine: The Institut Pasteur has taken a recombinant 28 kDa Glutathione S-transferase (GST) cloned from *S. haematobium* through both phase 1 and 2 clinical testing in Europe and West Africa (Senegal and Niger). Sh28-GST (Bilhvax) is a recombinant protein formulated with an aluminum hydroxide adjuvant . Bilhvax appears to be immunogenic and well-tolerated in healthy adults from non-endemic (France) *and S. haematobium* endemic areas in Africa. The most important vaccine target of the schistosome is the tegument. The tegument is thought to be involved in several key physiologic processes: parasite nutrition, osmoregulation, and the evasion of host immunity. Tetraspanins found in outer tegument play an important role in maintaining the integrity of the tegument. *Schistosoma mansoni* -TSP-2 has been selected by the HHVI for development as a human vaccine antigen[31,32]. The Sm -TSP-2 recombinant schistosomiasis vaccine would be intended primarily for school-aged children living in the *S. mansoni* endemic regions of sub-Saharan Africa and Brazil. The vaccine ideally would prevent the reacquisition of schistosomes in the blood stream following initial treatment with Praziquantel (vaccine-linked chemotherapy)[33].

Taenia Solium Vaccine: (Transmission Blocking Veterinary Vaccine): Independent vaccine trials for *Taenia solium* carried out in pigs with the TSOL18 antigen in Mexico, Peru, Honduras, and Cameroon have all achieved 99–100% protection. Results were published of the first field trial of the TSOL18 vaccine, which was carried out in north Cameroon. The vaccine completely eliminated the transmission of *T. solium* by the pigs involved in the trial[34].

Conclusion

In addition to the technological hurdles, the economic challenges have until very recently discouraged the multinational pharmaceutical companies from embarking on parasite vaccine research and development. In recent years, Product development-Public Private Partnerships (PD-PPP); which are non-profit organizations that use industry practices or partner with industry for purposes of developing, manufacturing, and clinically testing vaccines have been taking the initiative in this field. Because of their non-profit status, they attract private and public donor support. The availability of funds has speeded up the process of vaccine development for many neglected diseases. At present, the aim is to pursue vaccine manufacture through partnerships with innovative developing countries (IDC). IDCs are middle-income countries, such as Brazil, Cuba, China, and India, with modest economic productivity but which have achieved a high level of innovation in biotechnology. Therefore PDP-IDC liaisons represent a promising avenue for the development and testing of the parasite vaccines.

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