

4. Parasitic diseases

Parasitic diseases caused by helminths and protozoa are major causes of human disease and misery in most countries of the tropics. They plague billions of people and kill millions annually, and inflict debilitating injuries such as blindness and disfigurement on additional millions. WHO estimates that one person in every four harbors parasitic worms. Attempts to develop vaccines against these pathogens have been hampered by the difficulty to cultivate them *in vitro*, the complexity of their multicellular organization and/or multistage development, added to their impressive antigenic variability. Although remarkable progress has been made in the last decade in the cloning and expression of protective antigens from a large number of parasites, the prospect of using these antigens for the development of preventive vaccines has been met with little enthusiasm from industrial vaccine manufacturers, due to general scepticism as to the capacity for defined antigens to elicit sterilizing immunity against complex organisms, especially metazoan organisms. Definite scientific and technical advances have nevertheless been made in recent years in the field, including the complete sequencing of the genome of *Plasmodium falciparum*, and quite a number of groups are now supporting research on vaccine development against parasitic diseases. Significant progress has been made over the past five years in the development of vaccines against malaria and leishmaniasis. Vaccine development efforts for Chagas' disease (American trypanosomiasis) have been curtailed because of successful efforts at vector control, whereas vaccine development for African sleeping sickness (African trypanosomiasis) still is hampered by the phenomenon of antigenic variability.

4.1. Amoebiasis

Amoebiasis is due to invasion of the intestinal wall by the protozoan parasite *Entamoeba histolytica*. Amoebic colitis results from ulcerating mucosal lesions caused by the release of parasite-derived hyaluronidases and proteases. Hepatic infection occurs as a consequence of entry of the parasite into the afferent bloodstream. The disease is prevalent throughout the developing nations of the tropics, at times reaching a prevalence of 50% of the general population and is estimated to cause more than 100 000 deaths per year.

Evidence from a cohort of Bangladeshi children suggests that mucosal IgA directed against the major amoebic adherence molecule, a 170 kD lectin, correlates with resistance to reinfection with *E. histolytica*. Gerbils immunized with this lectin antigen were reported to show significant decrease of liver abscesses following parasite challenge, suggesting that a subunit vaccine might elicit protective immunity.

4.2. Hookworm disease

4.2.1. Disease burden

Human hookworm infection is a soil-transmitted helminth infection caused by the nematode parasites *Necator americanus* and *Ancylostoma duodenale*. It is a leading cause of anaemia and protein malnutrition, afflicting an estimated 740 million people in the developing nations of the tropics. The largest numbers of cases occur in impoverished rural areas of sub-Saharan Africa, Latin America, South-East Asia and China. *N. americanus* is the most common hookworm worldwide, while *A. duodenale* is more geographically restricted.

Hookworm transmission occurs by skin contact with infective third-stage larvae (L3) that have the ability to penetrate through the skin, frequently entering the body through the hands, feet, arms, or legs. *A. duodenale* L3 also can be ingested. L3s migrate through the body and enter the lungs from which they are expelled by cough and swallowed into the intestine where they first moult twice to become adults. Adult hookworms are approximately one-centimeter-long parasites that cause host injury by attaching to the mucosa and submucosa of the small intestine and producing intestinal blood loss. The presence of between 40 and 160 adult hookworms in the human intestine results in blood loss sufficient to cause anaemia and malnutrition. The term “hookworm disease” refers primarily to the iron-deficiency anaemia with reduced host haemoglobin, serum ferritin, and protoporphyrin that results from moderate and heavy infections and is in direct correlation with the number of parasites (as measured by quantitative egg counts). In children, chronic hookworm infection has been shown to impair physical and intellectual development, reduce school performance and attendance, and adversely affect future productivity and wage-earning potential.

Unlike other soil-transmitted helminth infections, such as ascariasis and trichuriasis, in which the highest intensity infections occur primarily in school-aged children, high intensity hookworm infections also frequently occur in adult populations. This is an important health threat to adolescent girls, women of reproductive age, and to outcomes in pregnancy. Up to 44 million pregnant women are estimated to be infected with hookworm. In pregnant women, anaemia resulting from hookworm disease results in several adverse outcomes for both the mother and her infant, including low birth weight, impaired milk production, and increased risk of death for both the mother and the child.

Efforts to control hookworm infection include the sanitary disposal of faeces and educational campaigns about the proper use of latrines. At this time, the most cost-effective way to control hookworm infection has been through population-wide treatment with either albendazole or mebendazole. A resolution adopted at the 2001 World Health Assembly advocates the anthelmintic treatment of 75% of all at-risk school-aged children by 2010. In time this would become the largest health programme ever attempted. However, both children and adults usually become reinfected within a few months after deparasitation, which implies repeated and frequent use of the drugs, and there is concern that heavy and exclusive reliance on albendazole and mebendazole might lead to drug resistance. Therefore, a safe and cost-effective vaccine would provide an important new tool for the control of hookworm infection.

4.2.2. Vaccines

The feasibility of developing a human anti-hookworm vaccine is based on the previous success of using live, irradiated L3s as a vaccine for canine hookworm infection. The Human Hookworm Vaccine Initiative (HHVI), a programme of the Sabin Vaccine Institute, together with the George Washington University (USA), the Oswaldo Cruz Foundation (FIOCRUZ, Brazil), the Chinese Institute of Parasitic Diseases, the Queensland Institute of Medical Research (Australia), and the London School of Hygiene and Tropical Medicine (UK), has identified, isolated, cloned, and expressed the major L3 antigens, and then tested them as recombinant vaccines. The leading candidate, the *Ancylostoma*-secreted protein (ASP), was selected because it can be recognized in a subset of individuals who have low intensity hookworm infection, and is partially protective in laboratory hamsters and dogs against challenge with *A. ceylanicum* and *A. caninum*, respectively. With support from the Bill and Melinda Gates Foundation, as well as additional support from the NIAID, NIH, and the March of Dimes Birth Defects Foundation, the HHVI has completed manufacture of the Na-ASP-2 hookworm vaccine. A Phase I dose-escalating trial of the vaccine is tentatively planned to take place in the USA in early 2005. Further planning is in progress for a Phase

IIB trial to determine the vaccine's ability to protect against high intensity hookworm infection in Brazil. It is anticipated that industrial-scale manufacture of the vaccine will take place in Brazil.

Additional studies are in progress to develop a second antigen from adult hookworms. Candidates of choice are the haemoglobin-degrading proteases found to line the brush border membrane of the hookworm gastrointestinal tract. These have been expressed in eukaryotic expression systems such as yeasts or baculovirus, to keep their native conformation intact for better immunogenicity. Work is in progress to combine them with ASP in a multivalent vaccine.

4.3. Leishmaniasis

4.3.1. Disease burden

Leishmaniasis is caused by several species of flagellated protozoan parasites found in many areas of the world, particularly in Africa, Latin America, South and Central Asia, the Mediterranean basin and the Middle East. In its more severe forms, the disease can cause serious disfigurement as well as death. WHO estimates the worldwide prevalence to be approximately 12 million cases, with annual mortality of about 60 000. The size of the population at risk is about 350 million. Transmission is most often zoonotic: the parasites (*Leishmania*) are transmitted from a wild-animal reservoir (small rodents, dogs) by the bite of the female phlebotomine sandfly. It also can be anthroponotic, the parasite being transmitted by the sandfly from an infected human host. Several forms of the disease exist: cutaneous (CL), mucocutaneous (MCL) and visceral (VL, also called "kala-azar"), which, after treatment, is often followed by a dermal manifestation known as "post-kala-azar" dermal leishmaniasis (PKDL). CL and MCL in Central and South America are caused by members of the *L. mexicana* and *L. braziliensis* species, whereas CL in South and Central Asia and the Middle East is caused by *L. tropica* and *L. major*. The majority of MCL cases occur in Bolivia, Brazil and Peru, and 90% of CL cases occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria. VL ("kala-azar"), the most lethal form of the disease, is caused by *L. donovani* in Bangladesh, China, India, Nepal and Sudan by *L. infantum* in North Africa and southern Europe, and by *L. chagasi* in Latin America.

For many years, the public health impact of leishmaniasis has been grossly underestimated, as a substantial number of cases were never recorded. About 1.5–2 million new cases are estimated to occur annually, but only 600 000 are officially declared. In addition, deadly epidemics of VL periodically flare up but go mostly unnoticed in spite of case–fatality rates as high as 10% or more. In the 1990s Sudan suffered a crisis with an excess mortality of 100 000 deaths among people at risk. The expansion of leishmaniasis and the alarming rise in the number of cases is related to environmental changes such as deforestation, building of dams, new irrigation schemes and migration of non-immune people to endemic areas, and resulted in significant delay in the implementation of development programmes in the Amazon and the tropical regions of the Andean countries, Morocco and Saudi Arabia.

More recently, as a result of epidemiological changes, a sharp increase in the overlapping of HIV infection and visceral leishmaniasis has been observed, especially in intravenous drug users in South-Western Europe. The situation may soon worsen in Africa and Asia where the prevalence and detection of HIV and *Leishmania* co-infections still are probably largely underestimated.

The first line drugs for treatment of leishmaniasis are antimonials, which remain expensive, require repeated injections, and are associated with important side effects. Drug resistance also is becoming common in certain areas (i.e. Bihar, India). Miltefosin, a recently developed

drug which is active orally against VL, has not yet been widely used. Vector and reservoir controls may be useful under certain conditions but are not applicable in every epidemiological setting and require infrastructure and vigilance beyond the capability of many endemic countries. Vaccination, therefore, remains the best hope for control of all forms of the disease.

4.3.2. Vaccines

There is as yet no effective vaccine for prevention of any form of leishmaniasis. A first generation vaccine was prepared using whole killed parasites combined or not with BCG. The combination of autoclaved *L. major* promastigotes with BCG as adjuvant was tested in Iran against CL and in Sudan against VL. A limited efficacy was noted in converters to positive skin reaction to leishmania antigen (leishmanin) and unexpectedly in boys. Similar observations had been made earlier in Brazil using killed promastigotes without BCG. Alum precipitated autoclaved *L. major* promastigotes plus BCG have demonstrated safety and substantial immunogenicity in Phase I studies in Sudan. Additional trials are under way to test new formulations with IL-12. It is of note that treatments combining administration of antimonials and first generation leishmania vaccines in patients suffering from “post-kala-azar” dermal leishmaniasis (PKDL) have shown benefit to the patients, suggesting that even suboptimal leishmaniasis vaccines could have a role in the therapeutic setting.

Various subunit recombinant candidate vaccines also have been tested in mice and provided some degree of protection against infection. These vaccines were based on:

- recombinant surface antigen gp63, a glycoprotein with protease activity,
- lipophosphoglycan, a surface glycoconjugate;
- a 46 kD promastigote antigen derived from *L. amazonensis*;
- or the Leishmania-activated C kinase (LACK), among others.

Protection against *L. major* infection in mice was provided by DNA constructs encoding a number of *Leishmania* antigens, including gp63 and LACK.

It has been demonstrated in experimental animal models that a dominant Th1 lymphocyte response (IL-2, IFN- γ) is associated with self-limited disease, whereas a dominant Th2 response (IL-4, IL-5) is linked to progressive disease. Addition of Th1-driving adjuvants such as IL-12 or oligodeoxynucleotides (CpG) to leishmanial antigens (TSA, LeIF, LmSTI-1) resulted in complete protection of susceptible mice against progressive disease, whereas no protection was observed in the absence of adjuvant. The Bill and Melinda Gates Foundation has funded the development of a chimeric vaccine made of these three recombinant leishmanial antigens (LeIF, LmSTI-1 and TSA) in the form of a fusion protein combined with monophosphoryl lipid A in squalene oil as adjuvant. Phase I trials of this vaccine in healthy volunteers in the USA and initial efficacy testing as a therapeutic vaccine in patients in Latin America suggest the safety and immunogenicity of the vaccine.

Recent evidence indicates that a 15 kD protein antigen derived from the salivary glands of the sandfly vector also could be protective in mice when given as a vaccine.

Generally, recovery from CL leads to protection against future infections. For centuries, in some of the hyper-endemic areas of the Middle East, the pus of an active lesion was used to inoculate young children to protect them against future lesions on the exposed parts of the body, especially the face. *L. major* promastigotes grown in culture under good

manufacturing practice (GMP) guidelines, rather than the exsudates from active lesions, have been used for inoculation as a live vaccine. The practice is known as leishmanization. Genetically manipulated parasites with attenuated virulence or high sensitivity to chemotherapy might represent the ideal form of a live vaccine.

4.4. Malaria

4.4.1. Disease burden

Malaria is by far the world's most important tropical parasitic disease, killing more people than any other communicable disease except TB. Worldwide prevalence of the disease is in the order of 350–500 million clinical cases each year, with an estimated annual death toll of over 1.1 million deaths. The vast majority of deaths occur among children under five years of age, especially in remote rural areas with poor access to health services. One century ago, malaria was endemic across every continent except Antarctica. Control programmes based on the use of insecticides led to its elimination from Australia, Europe and the USA by the 1950s, but the disease still remains endemic in some 100 countries in Africa, the Americas, the Eastern Mediterranean Region, the South-East Asia Region, and the Western Pacific Region. These countries are inhabited by more than 2.4 billion people – 40% of the world's population.

Human malaria is caused by four species of the protozoan parasite *Plasmodium*. The disease thrives where the environment supports one of the 50 species of *Anopheles* mosquitoes that serve as the vector for transmission. Transmission of malaria is affected by climate and geography, and often coincides with the rainy season. Global warming and other climatic events such as "El Niño" also play their role in increasing the risk of disease, presumably because the associated weather disturbances influence vector-breeding sites. Quantitative leaps in malaria incidence coincident with ENSO (El Niño/southern Oscillation) events have been recorded in Africa, South America and Asia (in Pakistan and Sri Lanka). The disease has now spread to highland areas of Africa. A change in risk of malaria also can be the unintended result of economic activity or agricultural policy that changes the use of land, such as the creation of dams, new irrigation schemes, commercial tree cropping and deforestation. Urban malaria is increasing due to unplanned development around large cities, particularly in Africa and South Asia.

Symptoms associated with malaria include high fever, malaise, headache, myalgia, nausea and vomiting. Bouts recur every 48–72 hours. Severe disease cases occur more frequently with *P. falciparum* because of its ability to adhere to capillary walls. Acute renal failure, cerebral malaria and pulmonary oedema occur most commonly in populations that are immunonaïve, such as young children and travellers. Fatally afflicted children often die less than 72 hours after developing symptoms. In those children who survive, malaria also drains vital strength, impairing their physical and intellectual development. Malarial sickness is one of the principal reasons for poor school attendance. Other high-risk groups are women during pregnancy, non-immune travellers, refugees, displaced persons and labourers entering endemic areas. Malaria causes severe anaemia, a major factor contributing to maternal deaths in pregnant women. Pregnant mothers who have malaria and are HIV-positive also are more likely to transmit HIV to their newborn.

Malaria therefore exacts an enormous toll in lives, in medical costs, and in days of labour lost. For the individual, costs include the price of treatment and prevention, and lost income. In rural areas, the rainy season is often a time of intense agricultural activity, when poor families earn most of their annual income. Malaria can make these families even poorer, hitting young adults especially hard: a single bout of the disease costs an estimated equivalent of 10 working days. The estimated costs of malaria, in terms of strains on the

health systems, are enormous: in endemic countries, as many as 3 out of 10 hospital beds are occupied by victims of the disease. The direct and indirect cost of malaria in sub-Saharan Africa is estimated to be 1–5% of the gross domestic product (GDP).

The battle to control malaria is being fought by efforts to implement improved diagnosis and chemotherapy, as well as integrated vector control through the use of insecticide-treated bednets and residual house spraying. International efforts to combat malaria are unprecedented, including among others a Global Malaria Control Strategy coordinated by the WHO, involving three UN agencies (UNDP, UNICEF and WHO) and, together with the World Bank and the Multilateral Initiative on Malaria (MIM), regroups a number of institutions wishing to promote malaria research in Africa. The UNDP/World Bank/WHO Special Programme on Tropical Diseases (WHO/TDR) has joined the initiative, establishing a task force to address the needs of endemic countries and to fund activities related to strengthening research capacities on malaria. However, emergence as well as resurgence of malaria continues to be evident worldwide, much of it due to drug-resistant parasites and insecticide-resistant vectors. Therefore, the development of a safe, effective and affordable malaria vaccine is a critical global public-health priority.

4.4.2. Parasitology

The agents of human malaria are four species of *Plasmodium* protozoa: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. All are transmitted by *Anopheles* mosquitoes. *P. falciparum* causes the greatest number of deaths, whereas *P. vivax* has the greatest geographic distribution. All strains of *Plasmodium* have a complex life cycle that begins when a female mosquito injects sporozoites into the human host when taking a blood meal. The sporozoites enter the bloodstream and within less than 30 minutes migrate to the liver and invade hepatocytes. Sporozoites mature in the liver where they give rise to tens of thousands of merozoites over a period of 6–16 days. Merozoites erupt into the bloodstream and invade erythrocytes where they multiply and mature over a period of 24–72 hours. Infected red blood cells (RBC) then lyse and liberate the merozoites which immediately proceed to invade new RBCs to repeat the cycle. The classical signs of malaria, acute febrile episodes and rigors that occur every 48 to 72 hours, coincide with the synchronized lysis of infected RBCs releasing the newly matured merozoites. Some of the merozoites eventually develop into sexual-stage gametocytes, which can sexually combine and develop into new sporozoites when taken up by an anopheline mosquito, thus reinitiating the cycle.

The complete nucleotide sequences of both the *Plasmodium* parasite and of the *Anopheles gambiae* vector have been determined.

4.4.3. Vaccines

The major malaria vaccine funding agencies are the NIH in the USA, the Wellcome Trust in the UK, the European Union – either directly through the European and Developing Countries Clinical Trials Partnership (EDCTP) or through the European Malaria Vaccine Initiative (EMVI) – USAID, the Malaria Vaccine Initiative (MVI) of PATH and the Bill and Melinda Gates Foundation. In addition, the African Malaria Network Trust (AMANET) was recently established to build African capacity to plan and coordinate malaria vaccine trials in Africa.

Several lines of evidence suggest that a prophylactic malaria vaccine for humans is feasible. Firstly, immunization with irradiated sporozoites was shown to confer 90% protection against experimental infection following laboratory-bred, sporozoite-infected mosquito bites in naïve human volunteers. Secondly, naturally acquired immunity progressively builds up during the first two decades of life in people living in malaria-endemic countries. This

immunity primarily impacts the severity of clinical disease, and appears to be linked to continuous antigenic stimulation, waning rapidly when exposure ceases. Thirdly, protection has been elicited by passive transfer of hyperimmune immunoglobulins from malaria-immune adults into malaria-naïve human volunteers.

However, key obstacles to the development of a vaccine include the lack of immune correlates of protection, the lack of reliable and predictive animal models, and the developmental and antigenic diversity and variability of the parasite. Much work has been done to determine which protective antigens or epitopes should be used in the construction of recombinant or synthetic malaria vaccines. The *Plasmodium* genome is A-T rich, unlike most of the microbial organisms or animal cells used to express recombinant antigens, and it also shows quite different codon usage. Enhanced expression of recombinant *Plasmodium* antigens therefore requires the use of synthetic genes reconstructed with optimized codons best suited for the expression system used for production.

The traditional approach to develop malaria vaccines has been the targeting of the different stages of parasite development (pre-erythrocytic, asexual and sexual stages).

Pre-erythrocytic vaccine strategies aim to generate an antibody response that will neutralize sporozoites and prevent them from invading the hepatocyte, and/or to elicit a cell-mediated immune response that will inhibit intra-hepatic parasites. This type of vaccine would be ideal for travellers because it would prevent the advent of clinical disease.

Asexual blood-stage (erythrocytic) vaccine strategies aim to elicit antibodies that will inactivate merozoites and/or target malarial antigens expressed on the RBC surface, thus inducing antibody-dependent cellular cytotoxicity and complement lysis; they also are meant to elicit T-cell responses that will inhibit the development of the parasite in RBCs. This type of vaccine would mostly serve as a disease-reduction vaccine in endemic countries by decreasing the exponential multiplication of merozoites.

Vaccines targeting the sexual stage of the parasite do not aim to prevent illness or infection in the vaccinated individual, but to prevent or decrease transmission of the parasite to new hosts.

Other novel approaches being currently taken include the development of combination multicomponent vaccines, a commercial irradiated sporozoite vaccine, and an anti-parasite toxin vaccine. This type of potential anti-disease vaccine would target parasite toxins contributing to the disease, such as the glycosylphosphatidyl inositol (GPI) anchor.

4.4.3.1. Pre-erythrocytic vaccines

These vaccines aim to protect against malaria infection and ideally should provide sterilizing immunity for non-immune individuals.

- The most advanced and well-documented pre-erythrocytic (liver-stage) vaccine candidate is derived from the circumsporozoite protein (CSP) that is found at the surface of the sporozoite and of the infected hepatocyte. This candidate vaccine, RTS,S/AS02, developed by GSK and the WRAIR, comprises the antigenic C-terminus (amino acids 207–395) of the CSP from *P. falciparum* fused to the hepatitis B surface antigen and expressed in the form of VLPs in *Saccharomyces cerevisiae*. Initial Phase I clinical trials of RTS,S formulated with GSK AS02 adjuvant (containing MPL, QS21 and an oil-in-water emulsion) showed protection against malaria challenge in six out of seven volunteers. A dose-range Phase I/II study showed levels of efficacy from 30% (single dose) to 55% (3 doses), with an overall protective efficacy of 41% among 41 vaccinees.

However, protective efficacy was not long-lived. Further trials in the Gambia demonstrated a 70% protection efficacy against infection for the first 9 weeks, with efficacy waning rapidly thereafter. Volunteers who received a fourth dose of the vaccine the following year, prior to the onset of the malaria season, again exhibited a 47% protection over a 9-week follow-up period. Recent field testing on 2022 children in Mozambique showed that efficacy of three doses of RTS,S/AS02 at preventing a first malaria attack in 1–4 year-old children was about 30%, with a 37% decrease in blood parasitemia frequency at six months, and a 58% overall decrease of severe disease incidence (the figure was 77% in children between ages 1 and 2). Other clinical studies are under way, including one aimed at combining RTS,S with the blood-stage antigen MSP-1 (*see below*).

- Another CSP-based candidate vaccine includes a 102-amino acid synthetic peptide representing the C-terminus of the CSP antigen formulated with Montanide ISA 720. The formulation, which is developed by Dictagen Inc., in collaboration with the University of Lausanne (Switzerland), was shown to be safe in human volunteers and to elicit both an antibody and a cellular immune response including secretion of IFN- γ . The vaccine has now undergone further Phase I studies comparing doses and adjuvants, and is currently undergoing Phase IIa clinical trial at the University of Nijmegen (Netherlands).
- An alternative approach to synthetic vaccines was to incorporate several copies of a protective CSP epitope into a multiple antigenic peptide (MAP). As expected, the resulting vaccine was found to elicit an immune response only in the volunteers with cognate HLA haplotypes. In an effort to bypass MHC restriction, the peptide was linked to a “universal” T-cell epitope. This construct was shown to elicit robust immune responses in humans with diverse genetic background. The B-cell and T-cell epitopes from the MAP trials have been incorporated into a recombinant VLP based on hepatitis B core particles. This CSP-HBc particle vaccine, known as ICC-1132, is being developed by Apovia in the USA with the help of the MVI, but results of a Phase II study have been disappointing.
- Other vaccines based on the CSP antigen include live recombinant vaccines that use MVA, Adenovirus, Sindbis virus, or a cold-adapted attenuated influenza virus strain as a vector. Prime-boost combinations of some of these vaccines with the RTS,S/AS02 vaccine are in progress.
- The United States Department of Defense, in collaboration with Vical Inc., has developed candidate DNA vaccines for malaria (the Multi-Stage DNA Operation, MuStDO), including a liver-stage DNA vaccine that encodes the CSP of *P. falciparum*. This DNA vaccine was tested as a “proof-of-concept” in a Phase I study carried out by the United States Navy Malaria Program. The vaccine elicited cell-mediated immune responses but only modest antibody responses. No serious adverse events were recorded, but the vaccine did not elicit protection against experimental challenge.
- A multiple-antigen DNA vaccine, MuStDO-5, has been designed to encode five different liver-stage antigens: CSP, liver stage antigens 1 and 3 (LSA-1 and -3), exported protein 1 (EXP1), and the sporozoite surface protein 2 (SSP2, also known as thrombospondin-related adhesive protein, TRAP). Various studies conducted in endemic areas have linked LSA-1 and LSA-3 with protective immunity. MuStDo-5 is manufactured as a combination of five separate plasmids. The vaccine, administered with GM-CSF DNA as an adjuvant, was safe and well tolerated in mice and rabbits, but showed only weak immunogenicity in primates. In addition, competition between the plasmids was observed, due to immunodominance of one of the antigens.

- The Oxford University Malaria Vaccine Clinical Trials Group conducted studies of a DNA, a fowlpoxvirus (FPV), and an MVA-based vaccine expressing TRAP fused to a polyepitopic construct, demonstrating strong correlation between the induction of IFN- γ -secreting CD4+ and CD8+ T-cell responses and protection against malaria in a mouse model. The DNA and MVA candidate vaccines were combined in a prime-boost immunization trial in human volunteers in the Gambia. No protection was observed against occurrence of disease, but malaria-related mortality was reduced. A similar recombinant FPV/MVA prime-boost immunization regimen is currently being tested in Kenya.
- Additional antigens that have been targeted for pre-erythrocytic vaccine development include the liver stage antigens 1 and 3 (LSA-1 and -3), the sporozoite and liver stage antigen (SALSA) and the sporozoite threonine and asparagine rich protein (STARP). LSA-3, a highly conserved pre-erythrocytic antigen, has been demonstrated to induce protective immunity against *P. falciparum* sporozoite challenge in chimpanzees and Aotus monkeys. LSA-3 formulated with AS02 and a LSA-3 lipopeptide candidate are about to enter Phase I trials.
- A glutamate-rich protein (GLURP) long synthetic peptide vaccine developed by the Statens Serum Institut in Denmark in collaboration with EMVI is in early clinical studies in European adult volunteers.
- The SPf66 vaccine candidate that had been developed in Colombia was a synthetic multiepitope, multistage peptide vaccine mixed with alum as an adjuvant. The vaccine was tested in several Phase III field trials involving thousands of volunteers, but its reported efficacy was too low to warrant further development, although one may suspect that the vaccine might have fared better with more potent adjuvants.

4.4.3.2. Asexual blood-stage vaccines

These vaccines are aimed to primarily protect against severe malaria disease, and not against infection.

- The most advanced asexual blood stage vaccines are based on the use of merozoite surface protein 1 (MSP-1), which is part of a complex involved in red blood cell invasion, MSP-2, MSP-3, the apical membrane antigen 1 (AMA-1), a type 1 integral membrane protein and the glutamate-rich protein (GLURP). Antibodies to MSP-1 have been shown to block parasite invasion of red blood cells in vitro. AMA-1 is a natural target of protective responses in vivo. Both AMA-1 and MSP-1 have their 3-D conformation stabilized by intramolecular disulphide bonds which are critical for optimal immunogenicity of the molecule. MSP-1 contains two cysteine-rich epidermal growth factor (EGF)-like domains that generate protective antibodies and are conserved across all species of *Plasmodium*.

Current work has concentrated on either the entire MSP-1 molecule, its 42 kD C-terminal moiety, or a further-processed 19 kD fragment. These were expressed either as such or as part of fusion molecules using baculovirus, *E. coli*, or yeast (*Saccharomyces* or *Pichia*). Recombinant MSP-1 42 kD and 19 kD fragments have been shown to protect both mice and Aotus monkeys against lethal parasite challenge, but a Phase I trial of the 19 kD fragment carried out at Baylor University (USA) demonstrated that the vaccine was poorly immunogenic and had unacceptable side effects.

- The MSP-1 42 kD fragment formulated in AS02 adjuvant by GSK in collaboration with WRAIR and the MVI was found to be safe and very immunogenic in human volunteers in Kenya, Mali and the USA. The vaccine is presently entering Phase II trial in Kenya.
- Another vaccine studied in collaboration between WRAIR and GSK is based on the AMA-1 protein formulated in AS02. The vaccine was studied in a Phase I study in the USA and should now proceed to Phase I studies in Kenya and Mali.
- A MSP-1/AMA-1 fusion antigen was produced in Shanghai using *Pichia pastoris* and Montanide ISA 720 adjuvant and showed good immunogenicity in rabbits and non-human primates. WHO sponsored and coordinated the first clinical testing of this vaccine (PfCP 2.9) in collaboration with the Second Military Medical University in Shanghai. A Phase I trial occurred in China and showed the vaccine to be safe and immunogenic. Plans are under way for further clinical development.
- MSP-3 is being developed both as a long synthetic peptide by the Pasteur Institute and EMVI and as a recombinant protein by the Pasteur Institute. The vaccine construct contains B and T-cell epitopes that were selected based on their targeting by cytophilic antibodies that interact with monocytes in the antibody-dependent cellular inhibition (ADCI) assay. AMANET, in collaboration with EMVI, sponsored and coordinated a recently completed Phase I study of the vaccine in Burkina Faso, where the vaccine was shown to be safe. Vaccine-induced antibodies demonstrated ADCI activity in vitro and in vivo in a new mouse model of *P. falciparum* malaria.
- Another long synthetic peptide vaccine, developed by the Staten Serum Institute in Denmark in collaboration with EMVI, is based on the glutamate-rich protein (GLURP). GLURP formulated in alum and Montanide ISA 720 has been tested in a Phase Ia clinical trial and is planned for further clinical development.
- Furthest along the vaccine development pathway of blood-stage malaria vaccine candidates is the “Combination B” vaccine, which results from a collaborative effort by the Papua New Guinea Institute for Medical Research along with the Australian Cooperative Research Center for Vaccine Technology in Queensland, The Walter and Eliza Hall Research Institute and the Swiss Tropical Institute. This vaccine combines MSP-1 and MSP-2 with *P. falciparum* ring-stage infected erythrocyte surface antigen (RESA) in a Montanide adjuvant formulation. Recent Phase I/IIb trials in 120 5–9 year-old children in Papua New Guinea showed a 62% reduction in parasite density in vaccinees. Analysis of the genotype of breakthrough parasites showed a significant increase in the opposite dimorphic form of MSP-2. A new version of the vaccine is being developed using both variants of MSP-2 in order to target both genotypes.
- Additional merozoite surface antigens under development as vaccine candidates include MSP-4, -5, -8 and -9. These molecules contain one or more of the hallmark EGF-like domains present in MSP-1. MSP-5 is of particular interest because it lacks the sequence variation between different isolates of *P. falciparum* from different geographical locations that is typically seen with most of the MSPs.
- The erythrocyte-binding antigen (EBA-175) and its paralog in *P. vivax*, the Duffy binding antigen (DBA), are two other antigens currently developed as recombinant vaccine candidates expressed either in *E. coli* or in *Pichia pastoris* or as a DNA prime-boost vaccine.

Plasmodium species do not carry out N- or O-linked glycosylation. It has been observed that glycosylation occurring in some expression systems may mask the immunogenicity of

protective epitopes. Thus, a recombinant version of AMA-1 in which the glycosylation sites had been mutagenized was shown to elicit protective immunity in Aotus monkeys whereas the glycosylated version did not.

Despite encouraging progress, the lack of immune correlates of protection and that of predictive animal models, together with the polymorphism and strain variability of many asexual stage antigens constitute major challenges to the development effort of asexual stage vaccines. In contrast with pre-erythrocytic vaccine candidates, asexual stage vaccine candidates also lack a human artificial challenge model and have to rely on natural challenge in field trials to provide proof-of-concept. Their development is therefore slower and necessitates major commitment, intensive collaboration as well as high-level coordination supported by adequate funding.

4.4.3.3. Transmission-blocking vaccines

These vaccines are aimed to induce antibodies against the sexual stage antigens in order to prevent the development of infectious sporozoites in the salivary glands of the *Anopheles* mosquitoes. The leading candidate vaccines contain the *P. falciparum* surface protein antigens Pfs25 and Pfs28 or their *P. vivax* homologues Pvs25 and Pvs28. These vaccines are currently being developed at the NIH as recombinant yeast-secreted proteins (*S. cerevisiae*). Initial human Phase I trials have been conducted for Pfs25 and should follow soon for Pvs25. Other sexual stage-specific antigens that are being developed as transmission-blocking vaccines are Pfs48/45 and Pfs230.

4.4.3.4. Other approaches

Various groups such as the US CDC, NMRC and WRAIR are developing multi-antigens, multi-stage vaccine concepts. Advantages of a combination vaccine include the potential to address the problem of antigenic variation, that of inducing immunity in genetically heterogeneous populations and that of possible immune escape of the parasite. However, the risk of interference between components of the vaccine and increased reactivity of the formulation must be taken into account.

The concept of attenuation and parasite challenge to elicit immunity also is explored. An attempt to develop a commercial attenuated sporozoite vaccine has been undertaken by Sanaria Inc., with the support from the Bill and Melinda Gates Foundation and the NIH.

Finally, the glycosylphosphatidylinositol (GPI) anchor, which tethers several of the *Plasmodium* antigens to the membrane, has been shown to be highly toxic in mouse models. An anti-toxic vaccine is currently being developed as a carbohydrate anti-GPI vaccine. A proof-of-principle study testing synthetic GPI as a vaccine in rodent models of malaria showed that the candidate vaccine was immunogenic and protected the animals from significant malaria pathology and mortality. Whether further development of malaria toxin neutralization as a vaccine strategy will continue is not however certain.

4.5. Schistosomiasis

4.5.1. Disease burden

Schistosomiasis, also known as bilharziasis, is second only to malaria in public health importance. It is estimated that 200 million people worldwide are infected with the snail-transmitted, water-borne parasitic helminth, and that 20 000 deaths are associated with the severe consequences of infection, including bladder cancer or renal failure (*Schistosoma haematobium*) and liver fibrosis and portal hypertension (*S. mansoni*). In sub-Saharan Africa

where schistosomiasis constitutes an important public health problem, a survey in 2000 of disease-specific mortality reported that 70 million individuals out of 682 million had experienced haematuria and 32 million dysuria associated with *S. haematobium* infection. It was estimated that 18 million suffered bladder wall pathology and 10 million hydronephrosis. Infection with *S. mansoni* was estimated to cause diarrhoea in 0.78 million individuals, blood in stool in 4.4 million and hepatomegaly in 8.5 million. Using the very limited data available, mortality rates due to non-functioning kidney (from *S. haematobium*) and haematemesis (from *S. mansoni*) have been estimated at 150 000 and 130 000 per year, respectively. Although these are global estimates of the schistosomiasis disease burden, the public health impact of schistosomiasis in the field has been poorly evaluated and is still subject to controversy. Apart from a few situations where schistosomiasis is or was recognized as an obvious public health problem, as in Brazil, China, Egypt, the Philippines, northern Senegal and Uganda, the disease is often not a priority for health authorities. Moreover, the lack of a simple clinical case definition does not enable rapid identification of the disease by health personnel.

High rates of schistosomiasis occur near bodies of fresh water. Environmental changes linked to water resource development, population movements and population growth have led to the spread of the disease to previously low or non-endemic areas, particularly in sub-Saharan Africa. The building of the Diama dam on the Senegal River for example introduced intestinal schistosomiasis into both Mauritania and Senegal. Refugee movements and population displacements in the Horn of Africa introduced intestinal schistosomiasis to Somalia and to Djibouti. In contrast, successful schistosomiasis control has been achieved in several countries in Asia, the Americas, North Africa and the Middle East. Schistosomiasis has been eradicated from Japan and some of the islands in the Lesser Antilles. Four national control programmes (Brazil, China, Egypt, and the Philippines) have demonstrated that concerted control efforts together with economic development can decrease morbidity to low levels. Chemotherapy was central to these successes. The current drug of choice, praziquantel, reverses pathology in as little as six months after treatment in *S. haematobium* infections. The cost of praziquantel has decreased significantly over the past 20 years. Nevertheless, large scale use of praziquantel can impose a heavy burden on health systems. In addition, concerns remain over the potential threat of the emergence of praziquantel-resistant parasites.

4.5.2. Parasitology

The three major species of schistosomes, *S. mansoni*, *S. haematobium*, and the *S. japonicum* complex (including *S. japonicum* and *S. mekongi*) are distinguished by their snail vectors, location within the host vasculature, and egg morphology. *S. haematobium* is found in the Middle East and Africa, including the islands of Madagascar and Mauritius. Intestinal schistosomiasis due to *S. mansoni*, is now found in the Arabian peninsula, most African countries north of the equator (Egypt, Libya, Sudan, Somalia, Mali, Senegal, Mauritania), as well as in Brazil, some Caribbean islands, Suriname and Venezuela. *S. japonicum* is endemic in China where bovines are the main reservoir, as well as in Indonesia and the Philippines (with dogs and pigs as reservoir). *S. mekongi* is mostly found in Cambodia and Laos, along the Mekong River.

Asexual reproduction of the parasites occurs in fresh-water snails that release in the water large numbers of free-swimming larval schistosomes known as *cercariae*. The cercariae are attracted to the human skin through which they penetrate then lose their tail upon entry to become *schistosomulae* which migrate through the blood stream and the lung of the host until they reach the liver. Schistosomules differentiate in the liver into male and female schistosomes that migrate through the portal vasculature to settle in the mesenteric or bladder venules where they lay eggs. The latter exit from the body in faeces or urine and hatch in

fresh water, giving birth to *miracidia* that swim via the action of their cilia until they find a suitable snail host in which they will give rise to thousands of progeny.

Most of the morbidity associated with Schistosomiasis occurs when eggs remain trapped in the intestinal or bladder wall or in the liver, eliciting the formation of granulomas and fibrosis. In the liver, fibrosis leads to portal hypertension and splenomegaly. The most severe forms of the disease are due to *S. japonicum*.

4.5.3. Vaccines

The administration of radiation-attenuated cercariae to laboratory animals provided protection against experimental *S. mansoni* infection by blocking the migration of the parasite out of the lung. IFN- γ and Th1 cellular immune responses appear to play a key role in this process.

Great attention has been paid to the use of antigens from schistosomules, with disappointing protection results so far. Somewhat better results have been obtained with antigens that are shared between schistosomules and schistosomes, such as the 63 kD parasite myosin, the 97 kD paramyosin, the 28 kD triose phosphate isomerase (TPI), a 23 kD integral membrane protein (Sm23), and the 26 and 28 kD glutathione-S-transferases (GSTs). In recent Phase I and II clinical trials, the 28 kD *S. haematobium* GST (Sh28GST) developed by Institut Pasteur de Lille (France), was safe and showed good immunogenicity in human volunteers in France, Niger and Senegal.

The Schistosomiasis Vaccine Development Programme (SVDP), based in Egypt and supported by USAID, has focused on two *S. mansoni* antigens: paramyosin and a synthetic peptide construct containing multiple antigen epitopes (MAP) from the triose phosphate isomerase (Bachem Company, Los Angeles, USA).

Another candidate vaccine, which is developed by FIOCRUZ (Rio de Janeiro, Brazil), is based on the use of Sm14, a 14 kD fatty acid-binding *S. mansoni* protein with cross-reactivity with *Fasciola hepatica*. In mice, Sm14 provided a 67% protection against challenge with *S. mansoni* cercariae and full protection against *F. hepatica* metacercariae.

None of the above candidate vaccines has, however, been able so far to provide more than a partial reduction in challenge-derived worm burdens relative to non-immunized controls. It is hoped that better success can be achieved using cocktails of recombinant antigens.

Another approach to vaccination against schistosomiasis has been to target the fecundity of the female schistosome in order to diminish egg excretion. Success with this approach has been reported in mice and large animal reservoir hosts, including pigs and water buffaloes, using *S. japonicum* 26 kD GST and paramyosin. The suggestion was made, and the hope entertained, that vaccination of the reservoir host might be sufficient to reduce *S. japonicum* transmission to humans.