Developing vaccines to combat hookworm infection and intestinal schistosomiasis

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Abstract | Hookworm infection and schistosomiasis rank among the most important health problems in developing countries. Both cause anaemia and malnutrition, and schistosomiasis also results in substantial intestinal, liver and genitourinary pathology. In sub-Saharan Africa and Brazil, co-infections with the hookworm, *Necator americanus*, and the intestinal schistosome, *Schistosoma mansoni*, are common. The development of vaccines for these infections could substantially reduce the global disability associated with these helminthiases. New genomic, proteomic, immunological and X-ray crystallographic data have led to the discovery of several promising candidate vaccine antigens. Here, we describe recent progress in this field and the rationale for vaccine development.

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In terms of their global health impact on children and pregnant women, as well as on adults engaged in subsistence farming, human hookworm infection (known as 'hookworm') and schistosomiasis are two of the most common and important human infections[1,2]. Together, their disease burdens exceed those of all other neglected tropical diseases[3–6]. They also trap the world's poorest people in poverty because of their deleterious effects on child development and economic productivity[7–9]. Until recently, the importance of these conditions as global health and economic problems had been underappreciated. Even the United Nations Millennium Development Goals for sustainable poverty reduction did not specifically mention these two conditions[10]. An important reason for this 'neglect' is that hookworm and schistosomiasis typically affect health without resulting in mortality, with infections such as HIV or malaria causing tenfold more deaths (TABLE 1). However, when the chronic morbidities of these two infections are fully considered according to disability-adjusted life years (DALYs; years of life lost owing to disability, ill health or death), hookworm and schistosomiasis combined rank among the most important diseases in developing countries, resulting in 4.5–92 million DALYs annually, the upper limit of which is greater than the DALYs due to malaria or HIV/AIDS[4–6]. Current efforts to control hookworm and schistosomiasis are inadequate and new tools are needed. Here we describe efforts to develop vaccines that combat hookworm and schistosomiasis, with an emphasis on disease caused by *Necator americanus*, the major hookworm of humans, and *Schistosoma mansoni*, the primary cause of intestinal schistosomiasis.

Global distribution and pathobiology

Hookworms are roundworm parasites that belong to the phylum Nematoda. They share phylogenetic similarities with the free-living nematode *Caenorhabditis elegans* and with the parasitic nematodes *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*, which are often used by immunologists to study T helper 2 (T_H2) cell and related responses in mice[11] (BOX 1). Schistosomes are platyhelminths (flatworms) that belong to the order Trematoda (commonly called the trematodes or flukes). Human infections with hookworms and schistosomes occur predominantly in areas of rural poverty in sub-Saharan Africa, Southeast Asia and tropical regions of the Americas. The epidemiology and pathobiology of both hookworm and schistosomiasis have been extensively reviewed recently[12–17] and are only briefly discussed here.

Hookworm. The global distribution and life cycle of hookworms are shown in FIG. 1. An estimated 600 million to 700 million people are infected worldwide, with the most infections occurring in the Asian countries of Indonesia, Bangladesh and India (60 million to 70 million people in each), followed by Nigeria and the Democratic

Table 1 | **Impact of hookworm, schistosomiasis, HIV/AIDS and malaria**

Disease	Main causative agents	Infections	DALYs*	Deaths (annual)	Refs
Hookworm	*Necator americanus* and *Ancylostoma duodenale*	576–740 million	1.5–22.1 million	65,000	15
Schistosomiasis	*Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum*	207 million	3–70 million	280,000	6,17
HIV/AIDS	HIV-1 and HIV-2	33.2 million	84.5 million	2.1 million	137,138
Malaria	*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium knowlesi*	515 million	46.5 million	1 million	138–141

*The wide range of disability-affected life year (DALY) estimates for hookworm infection and schistosomiasis reflects alternative disability weights assigned by different investigators. Such differences are expected to be resolved in the coming years through an initiative of the Institute of Health Metrics and Evaluation at the University of Washington, Seattle, USA.

Republic of the Congo in Africa, and Brazil (30 million to 40 million people in each)[15,18–20]. Approximately 85% of hookworm infections are caused by *N. americanus*, and the remainder are caused by *Ancylostoma duodenale*[21]. Microscopic infective larvae (third-stage larvae, or L3) live as non-feeding, non-replicating, developmentally arrested environmental stages in the soil, where they survive for a few days to weeks, depending on the temperature and the level of moisture. Hookworm L3 phenotypically resemble the developmentally arrested dauer larvae of *C. elegans*[22]. Human infection occurs when L3 come into contact with the skin. Larvae actively penetrate skin and migrate in the afferent vasculature to the lungs, where they ascend the pulmonary tree to the pharynx, are swallowed and moult to become adult male and female hookworms ~1 cm long. Adult hookworms burrow deep into the mucosa and submucosa of the small intestine, eventually rupturing capillaries and arterioles[12,13]. Blood ingestion ensues, followed by the lysis of erythrocytes, an ordered enzymatic digestion of host haemoglobin[23,24] and haeme detoxification[25,26]. Almost all of the pathology and morbidity owing to hookworm is the result of intestinal blood loss[13]. Female and male hookworms mate in the small intestine, and the females release microscopic eggs that exit the body in host faeces. The eggs hatch in the soil, resulting in a new generation of first-stage larvae, which feed on bacteria and other organic debris in the soil before they moult twice to the L3 stage and continue the life cycle.

Schistosomiasis. Approximately 90% of the world's 207 million cases of schistosomiasis occur in sub-Saharan Africa, with the most in Nigeria, Tanzania, the Democratic Republic of the Congo and Ghana[17]. In Africa, *Schistosoma haematobium* is the cause of urinary tract schistosomiasis (accounting for approximately two-thirds of the world's cases of schistosomiasis), whereas *S. mansoni* is the main cause of intestinal schistosomiasis (approximately one-third of total cases) (FIG. 1). *S. mansoni* also causes intestinal schistosomiasis in Latin America, with most of the cases occurring in Brazil, whereas *Schistosoma japonicum* and *Schistosoma mekongi* cause fewer than one million cases of intestinal schistosomiasis in Asia[17]. Schistosomiasis is a fresh-water-borne disease, and humans become infected when free-swimming microscopic cercariae penetrate the skin. These larvae shed their tails to become schistosomulae, which enter the vasculature and lungs before relocating to the venous system, where they become sexually mature adults that pair and mate[16]. Adult *S. haematobium* schistosomes migrate to the venous plexus, which drains the bladder and reproductive organs, whereas *S. mansoni* and *S. japonicum* go to the mesenteric veins draining the intestine[16]. Female schistosomes produce eggs that are equipped with a spine to facilitate penetration through blood vessels and into the urinary tract and genitals (*S. haematobium*) or into the intestine and liver (*S. mansoni* and *S. japonicum*). During chronic infection, which can last 5–7 years, much of the pathology from schistosome infection is a product of the immune response to parasite eggs in host tissues, and the resulting granulomatous lesions lead to fibrosis, which, in turn, can cause severe circulatory impairment of the affected organs[6,16,27].

Hookworms, schistosomes and anaemia

Both hookworm and schistosomiasis cause chronic anaemia that, over the long term, can manifest as impaired neurological and cognitive functioning in children, diminished work capacity in adults, and adverse outcomes of pregnancy in both mother and child[28]. The WHO defines anaemia as a blood haemoglobin concentration of below 11–13 g per 100 ml, depending on age, sex and pregnancy status; severe anaemia in pregnancy is defined as a haemoglobin concentration of below 7 g per 100 ml[29]. Approximately 50% of anaemia cases result from iron deficiency (iron deficiency anaemia (IDA)), with nearly three-quarters of the morbidity from IDA occurring in the poorest regions of Africa, Asia and the Americas[30]. IDA is associated with ~841,000 deaths and ~35 million DALYs annually, mostly by contributing to both maternal and perinatal mortality and through adverse effects on childhood development and cognition[30,31]. Young children are particularly susceptible to IDA, because of their increased iron requirements during growth periods, as are women of reproductive age, because of menstrual losses and the high iron demands of a growing fetus during pregnancy[28].

Cercaria
The free-swimming larval stage of a trematode helminth such as *Schistosoma mansoni*.

Fibrosis
The formation of fibrous connective tissue that replaces normal organ tissue, usually in response to an insult such as injury or infection.

Box 1 | **Immune evasion and regulation of helminth infections**

- Vaccination against hookworm and schistosomiasis requires more than the discovery of target antigens. An understanding of the immunology of the host–parasite relationship is necessary to avoid inducing ineffective immune mechanisms or amplifying a dangerous immunopathogenic response[132,133].
- Infection with these helminths occurs in childhood and (in the case of hookworms) sometimes plateaus or increases in intensity as adults age[134]; in addition, infected individuals can tolerate the presence of the parasite for years and even decades.
- Both infections are typically overdispersed in endemic areas, with a minority (5–10%) of infected individuals harbouring most (80%) of the parasites[15,134]. Severe immunopathological complications such as granulomatous disease and organ failure are also highly overdispersed, occurring in only 10% of people with these infections[135].
- Helminths have an elaborate life cycle in the human host, with a succession of developmental stages that occupy a range of exposed extracellular locations, such as the skin, vasculature, bloodstream and gastrointestinal tract[13,133].
- Helminth infection leads to a strong T helper 2 (T_H2)-type response, a phenomenon that is important for immunology as a whole[132,133]. The T_H2-type response is characterized by elevated levels of total and parasite-specific immunoglobulin E (IgE), as well as by increased levels of interleukin-4 (IL-4), IL-5 and IL-13, with concomitant expansion and mobilization of specific effector cells such as mast cells, eosinophils and basophils[132,133]. The high levels of total and specific IgE associated with hookworm are comparable to levels seen in allergic diseases in non-endemic countries[136].
- The T_H2 response is induced against a background of potent, parasite-induced immunoregulation, thought to be mediated by alternatively activated macrophages, $CD4^+CD25^+$ forkhead box $P3^+$ regulatory T cells and $CD4^+CD25^+$ IL-10-producing T regulatory 1 cells[132,133,136].
- This response creates an immune environment so extensively downregulated that it not only protects the host from the strong inflammatory effects of helminth infection (especially in *Schistosoma mansoni* infection), but may also partially reduce the effects of other IgE-mediated disorders such as atopy, asthma and anaphylaxis[132,136].
- Convincing proof-of-concept evidence that vaccines may be realistic goals comes from the successful immunization of laboratory animals (for *S. mansoni*) and canines (for *Ancylostoma caninum*) with radiation-attenuated, live larval parasites, or the vaccination of livestock with adult-stage antigens (linked with parasite blood feeding) to protect against blood-feeding nematode parasites that cause haemonchiasis.

Hookworm-associated anaemia. IDA is the hallmark of hookworm and results from intestinal blood loss caused by the feeding of adult worms at the site of parasite attachment in the intestine[13,32]. Infection with 25–30 adult *N. americanus* hookworms results in at least 1 ml of blood loss per day, a volume containing an amount of iron roughly equivalent to the daily requirement of an adolescent boy or girl and slightly more than the daily requirement of a younger child in order for them to grow[32,33]. As well as causing IDA, intestinal blood loss can result in protein malnutrition[12,13]. Numerous epidemiological studies confirm the substantial contribution of hookworm to the global burden of IDA[34]. Among school-aged children in Zanzibar, Tanzania, 41% of IDA and 57% of moderate to severe anaemia is attributable to hookworm[34]. Hookworm has been shown to be an important risk factor for anaemia in Brazilian schoolchildren[35], and it has been identified as a key determinant of IDA in preschool children in Kenya[36], Tanzania[37] and Malawi[38]. Similarly, studies have identified hookworm as an important cause of IDA in non-pregnant women in Zanzibar and Vietnam[34,39], whereas it accounts for 41–54% of the moderate to severe anaemia in pregnant women in Nepal[34,40]. The positive association between intensity of the hookworm infection and anaemia in children and during pregnancy was confirmed in recent meta-analyses[41,42]. For both children and women, anaemia is far more likely to be present in those with moderate to heavy hookworm infections[34,41], which are defined on the basis of quantitative faecal egg counts exceeding 1,999 eggs per gram (epg) of faeces compared with counts of individuals with no or light infection (<2,000 epg of faeces)[3].

Schistosomiasis-associated anaemia. All of the major forms of human schistosomiasis are associated with anaemia[4,6,43–50]. As found for hookworm, children and pregnant women infected with schistosomes are especially susceptible to anaemia[43–53], and reduced haemoglobin concentrations in both have been associated with moderate to high faecal or urine schistosome egg counts[28,44,45,47–49,51]. The anaemia associated with schistosomiasis has been attributed to several mechanisms, including iron deficiency due to blood loss in the intestine or urine, splenic sequestration and destruction of erythrocytes, autoimmune haemolysis, and the chronic inflammatory response to schistosome eggs deposited in host tissues[28,46].

Co-infections. In Africa and South America, co-infections with hookworm and schistosomes are common, and there are at least a dozen countries with more than five million cases of each helminth infection[2]. It has been proposed that co-infection with *N. americanus* and *S. mansoni* is synergistic with respect to worm burdens and the resulting pathologic sequelae, including anaemia[9,35,54–62]. A similar relationship has been suggested between hookworm and *S. japonicum* infection in East Asia[62]. In sub-Saharan Africa, there is also extensive geographical overlap among areas of hookworm, schistosomiasis, and malaria transmission resulting from infection with *Plasmodium falciparum*, another notable cause of anaemia[63,64]. In Kenya and Tanzania, the anaemias resulting from hookworm and *P. falciparum* co-infections have been shown to be additive[63]. There are conflicting data on whether hookworm or schistosomiasis increase host susceptibility to malaria or adversely affect the clinical course of the disease[5]. In Africa, female genital schistosomiasis caused by *S. haematobium* was shown to increase the odds ratio of acquiring HIV/AIDS threefold[65], and it has been suggested that *S. mansoni* may also affect susceptibility to HIV[66,67]. Hookworm and other intestinal nematode infections are immunomodulatory and may increase viral loads and the progression of HIV/AIDS[68], but larger studies are needed to confirm this relationship. Anaemia itself may adversely affect the course of HIV/AIDS[69], so hookworm and schistosomiasis may indirectly impact the progression of this disease. The global public health impact of anaemia and other pathological sequelae resulting from hookworm and schistosomiasis have stimulated efforts to develop more effective control strategies for these conditions. Such measures include the development of new control tools, foremost among these being anthelmintic vaccines. The current status of the development of vaccines is outlined below, first for hookworm and then for schistosomiasis.

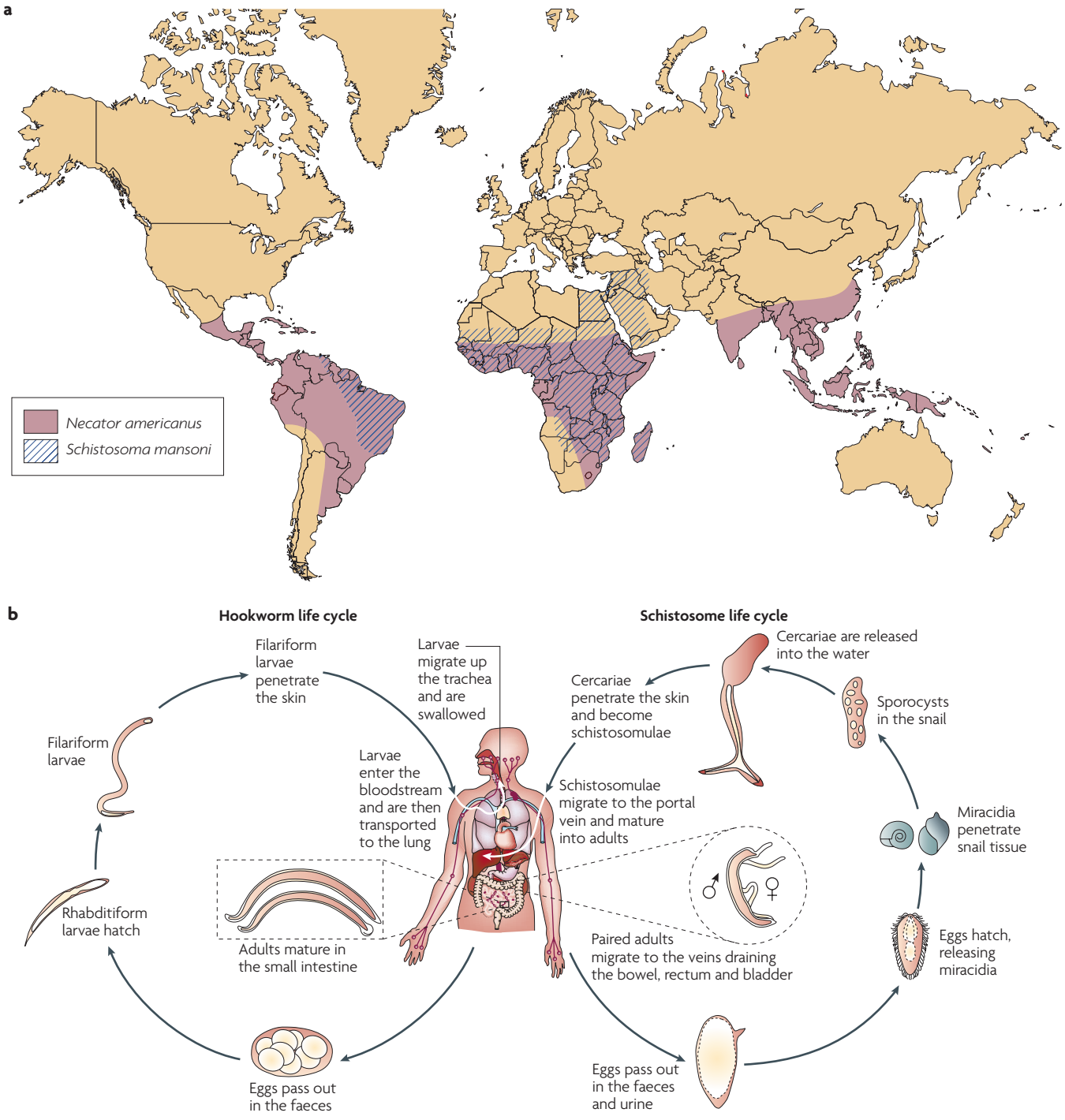

Figure 1 | **Global distributions and life cycles of hookworms and schistosomes. a** | The distribution of *Schistosoma mansoni*, which causes intestinal schistosomiasis, and *Necator americanus*, a hookworm, are shown[13,14,18]. **b** | The life cycles of hookworms and schistosomes.

Ascariasis
A clinical syndrome associated with infection by the intestinal, soil-transmitted nematode helminth *Ascaris lumbricoides*.

Trichuriasis
A clinical syndrome associated with infection by the intestinal, soil-transmitted nematode helminth *Trichuris trichiura*.

Rationale for a human hookworm vaccine

Hookworm and other common intestinal helminth infections such as ascariasis and trichuriasis are strongly associated with poverty and poor sanitation. However, improvements in sanitation or other environmental and personal protective control measures (such as footwear) frequently have a minimal impact on parasite prevalence or the intensity of infection, especially without accompanying programmes of health education and economic development[70,71]. The WHO has proposed annual mass treatment (that is, deworming of an entire population or age stratum in an endemic area) with a benzimidazole such as albendazole or mebendazole as the most cost-effective means of reducing the childhood morbidity related to chronic intestinal helminth infection (although the impact on improving

childhood cognition is variable)[42,72–75]. There is evidence that both albendazole and mebendazole interfere with invertebrate tubulin and microtubules and reduce the number of adult worms in the intestine[76]. In 2001, the 54th World Health Assembly committed to providing annual deworming for school-aged children wherever the prevalence in this age group exceeds 50%[42,72–75], although as of 2008 only 9% of school-aged children and 21% of preschool-aged children at risk of acquiring intestinal helminth infections have benefited from this intervention[73,75].

New information indicates that annual deworming may be less effective for hookworm than other intestinal helminth infections. For example, single-dose albendazole or mebendazole typically achieves either cure or substantial reductions in worm burdens and faecal egg counts for ascariasis[75,77]. However, for hookworm high rates of drug failure have been reported for mebendazole, with an average cure rate of only 15%[77]. Furthermore, after repeated administration in the same population, the efficacy of mebendazole has been reported to diminish over time[78], raising concerns about possible drug resistance. Indeed, drug failures have been shown to occur with benzimidazoles when used ubiquitously in livestock and have been associated with specific point mutations in the parasite gene encoding β-tubulin[79]. Although the same mutations have not yet been associated with drug failure in humans, efforts are underway to determine whether benzimidazole failures for hookworm and other helminth infections result from similar resistance mechanisms[79].

Evidence of widespread mebendazole drug failure indicates that the global control of human hookworm depends solely on the continued efficacy of albendazole. However, although treatment with albendazole cures existing infections, it does not confer protection from reinfection, which often occurs as early as 6 months after treatment in areas of high transmission[80]. Concerns about mebendazole drug failure, possible emerging resistance to the benzimidazoles and the rapid reinfection after treatment provided the impetus for establishing the Human Hookworm Vaccine Initiative, a non-profit product development partnership based at the Sabin Vaccine Institute (Washington DC, USA) that was established in 2000 to develop and test new vaccines for hookworm[1,2,12,81–86]. Prior efforts to develop human hookworm vaccines were limited to basic research conducted in university laboratories, although a live attenuated canine hookworm vaccine was developed by industry and briefly marketed for pet owners in the early 1970s[82,83,87]. The HHVI is the only partnership in the world that is currently working on vaccine development for hookworm and consists of investigators from the Sabin Vaccine Institute, the George Washington University (Washington DC, USA), The Fundação Oswaldo Cruz (FIOCRUZ; Brazil), the Instituto Butantan (São Paulo, Brazil), James Cook University (Cairns, Australia) and the London School of Hygiene and Tropical Medicine (UK). The ultimate aim of vaccine development efforts is to prevent moderate and heavy hookworm infections (that is, infections associated with faecal egg counts exceeding 1,999 epg of faeces), which are associated with substantial intestinal blood loss. Such a vaccine could be administered to very young preschool-aged children in a programme of 'vaccine-linked chemotherapy' (REF. 88) before their exposure to infective larvae in the environment, or to both preschool-aged and school-aged children who may have already been exposed and even infected.

Targeting hookworm blood feeding

The vaccines that are currently under development by the HHVI target the nutritional and metabolic requirements of the adult hookworm. The main approach has been to identify the essential components involved in parasite blood feeding, to genetically engineer these components as recombinant proteins and then to combine the recombinant components with one or more adjuvants to elicit protective antibodies on vaccination[82]. These protective antibodies would either directly neutralize the parasite macromolecules required for blood feeding and nutrition or indirectly damage important parasite structures. Protective immunity in vaccinated individuals would manifest as diminished hookworm-related blood loss and reduced numbers of hookworms in the intestine compared with levels in unvaccinated people. Based on prior experiences with live attenuated helminth vaccines for veterinary use, including those for canine hookworm and bovine lungworm, sterilizing immunity is not considered an attainable — or necessary — goal for anthelmintic vaccines[83,87,89] (TABLE 2). However, as the morbidity associated with hookworm is proportional to the number of worms harboured by individuals, a vaccine that prevents most moderate and heavy infections would be sufficient to have a major impact on the worldwide burden of hookworm and the associated anaemia.

Over the past decade, the molecular basis by which hookworms ingest and derive nutrition from host blood has been elucidated. As with all haematophagous parasites, *N. americanus* depends on host haemoglobin and serum proteins for survival. Adult hookworms ingest blood, lyse erythrocytes, degrade haemoglobin and serum proteins, and then absorb the digested peptides and amino acids[23,24] (FIG. 2). Following haemolysis, adult *N. americanus* hookworms use a hierarchical cascade of haemoglobinases (haemoglobin-degrading proteases), beginning with the cleavage of intact haemoglobin by an aspartic protease, APR1, followed by further proteolysis through the action of several cysteine proteases and metalloproteinases, all of which are expressed in the brush border membrane of the parasite's digestive tract[24]. The resultant small peptides and free amino acids are possibly absorbed through the parasite gut through a homologue of the membrane-spanning amino acid transporter of the free-living nematode *C. elegans*[90]. After their cleavage from digested globin, both iron-containing haeme and iron-containing haematin are potentially toxic to hookworms, because these compounds can generate oxygen radicals that may damage parasite structures[91]. Therefore, in addition

Benzimidazole
A member of a class of anthelmintic medications (including albendazole and mebendazole) that are active against nematode worms.

Adjuvant
A substance that enhances, accelerates or prolongs antigen-specific immune responses when used in combination with specific vaccine antigens.

Table 2 | **Successful* vaccines against helminth infections**

Parasite	Host		Treatment	Vaccine		Application	Status as of 2010
	Definitive	Intermediate		Target	Type		
Dictyocaulus spp.[142–144]	Cattle	NA	Benzimidazoles‡	L3	X-irradiated L3	Veterinary	Dictol and Bovilis (Intervet)
Ancylostoma caninum[87,145]	Canines	NA	Benzimidazoles‡	L3	X-irradiated L3	Veterinary	Discontinued (1974)
Haemonchus contortus[83,146]	Ruminants	NA	Benzimidazoles‡	H11, H-Gal-GP and TSBP	Excretory or secretory fractions	Veterinary	Experimental
Taenia ovis[147]	Canines	Sheep	Praziquantel	TO45	Recombinant	Veterinary	Experimental
Taenia saginata[147]	Humans	Cattle	Praziquantel	TSA9 and TSA18	Recombinant	Veterinary (transmission blocking)	Experimental
Taenia solium[147]	Humans	Swine	Praziquantel	TSOL18	Recombinant	Veterinary (transmission blocking)	Experimental
Echinococcus granulosus[147]	Canines	Sheep§	Benzimidazoles‡	EG95	Recombinant	Veterinary	Licensed‖

H11, membrane glycoprotein H11 (also known as aminopeptidase N); H-Gal-GP, gut membrane-associated protein complex of adult *H. contortus*; L3, third-stage larvae; NA, not applicable; TSBP, thiol sepharose-binding fraction of adult *H. contortus*. *Defined as an efficacy of greater than 90% in field trials. ‡Fenbendazole, oxfendazole or albendazole. §Humans are accidental hosts. ‖Licensed by the University of Melbourne, Australia, and AgResearch, New Zealand, with manufacture in China[147].

to haemoglobinases, all blood-feeding parasites such as hookworms and *P. falciparum* have evolved mechanisms to detoxify and transport haeme[25,92–94]. In the case of *N. americanus* and other hookworms, one putative mechanism for neutralizing these toxic moieties involves the pairing of glutathione *S*-transferase 1 (GST1) molecules as homodimers to create specific pockets capable of binding haeme and haematin[25,92,95] (FIG. 2).

Antigens of the human hookworm vaccine

From approximately two-dozen proteins that are putatively involved in the hookworm blood-feeding process[12,82], two lead candidate antigens have been selected for clinical development. The antigen selection programme of the HHVI is based on a ranking system that includes several key criteria, such as efficacy in animal trials, immuno-epidemiological observations in individuals resident in endemic areas, and the feasibility of protein expression and scaled-up manufacture using low-cost expression systems such as yeast, bacteria, or plants[82] (TABLE 3). In addition, antigens are prioritized if there is a plausible mechanism of protection associated with them, such as triggering the production of antibodies that inhibit crucial parasite enzymes or target important surface antigens. Both GST1 and APR1 are involved in parasite blood feeding, and it is thought that each antigen induces antibodies that interfere with the function of the respective protein and impair worm survival. Both antigens are therefore being considered for eventual combination in a human hookworm vaccine.

GST1 is a 24 kDa polypeptide that is expressed as a recombinant protein in the yeast *Pichia pastoris* to generate the antigen used for vaccines. Both GST1 from *N. americanus* and its orthologue from the canine hookworm *Ancylostoma caninum* have peroxidase activity that catalyses the conjugation of reduced glutathione to various electrophiles[25,92,95]. Both proteins belong to the Nu class of nematode GSTs, which is characterized by a reduced peroxidase activity relative to other classes of GSTs but an increased binding capacity for haeme and related products[25,92,95–97]. According to X-ray crystallography studies, *N. americanus* GST1 forms homodimers in solution to create atypically large binding cavities that are accessible to a diversity of ligands, including haeme[95], to which GST1 from both *A. caninum* and *N. americanus* binds with high affinity *in vitro*[25,92]. Both haeme and haematin contain oxidative iron that can result in the formation of oxygen radicals, which damage helminth structures. *In vivo*, GSTs may protect hookworms by binding and detoxifying haeme and the haematin by-products that are generated during the blood digestion process[25,92].

On the basis of their putative roles in hookworm blood feeding, GST1 from both *N. americanus* and *A. caninum* were tested in laboratory animal models of hookworm. In dogs, vaccination with recombinant *A. caninum* GST1 resulted in high levels of antibodies; following challenge with infective *A. caninum* larvae, the worm burdens and faecal egg counts of these vaccinated dogs were substantially lower than those observed in controls[25]. In hamsters, vaccination with recombinant *A. caninum* GST1 also resulted in cross-protection, with substantially lower worm burdens (less than half) following heterologous challenge with infective *N. americanus* larvae than those seen in controls[25,98], as did vaccination with *N. americanus* GST1 followed by homologous larval challenge[92]. A recombinant GST from the nematode parasite *Wuchereria bancrofti* is also showing promise in a jird model as a protective antigen against lymphatic filariasis[99]. Because of these encouraging results, recombinant *N. americanus* GST1 (formulated with Alhydrogel (aluminium hydroxide)) has been produced according to current good manufacturing practices in preparation for clinical trials.

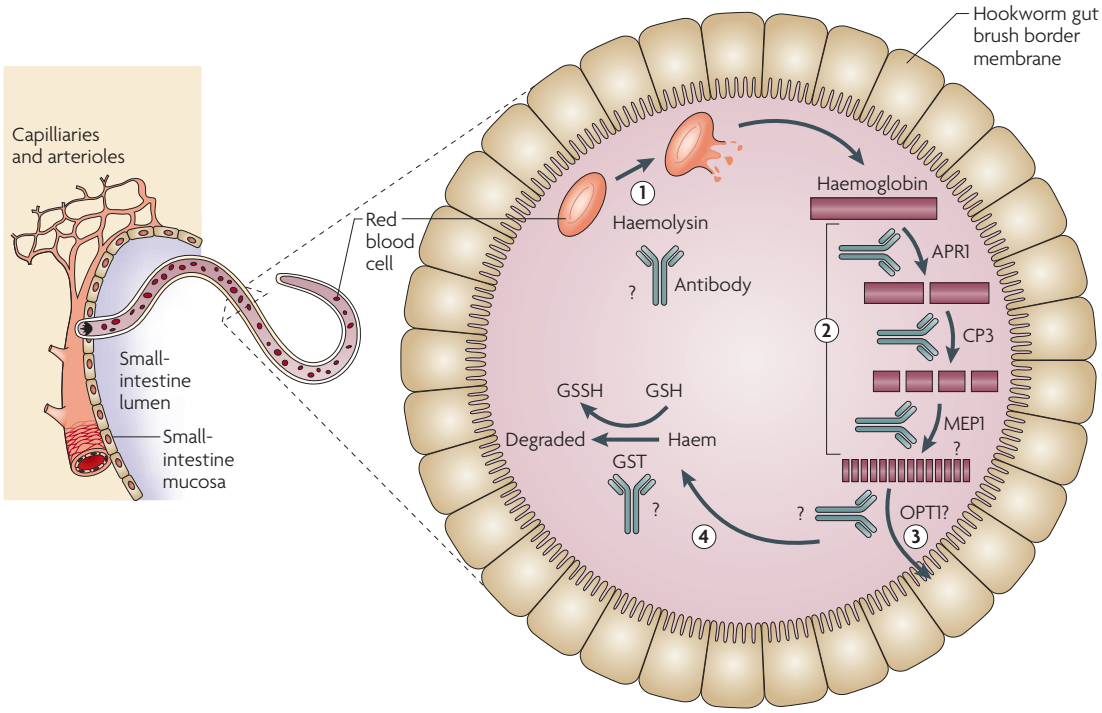

Figure 2 | ***Necator americanus* degradation of host blood components and potential vaccine targets.** Adult worms in the gut ingest blood, and parasite haemolysins drill pores into the erythrocytes, releasing haemoglobin into the parasite gut lumen (step 1). Haemoglobin is digested by the hierarchical and ordered cascade of haemoglobinases (APR1, an aspartic protease, CP3, a cysteine protease, and MEP1, a metalloproteinase) lining the brush border membrane of the parasite gut (step 2). The globin peptides and free amino acids that are released following haemoglobin digestion are absorbed into the gut cells, putatively being transported by OPT1 (step 3). Free haeme is detoxified by the action of glutathione *S*-transferase (GST) (step 4). Antibodies that could be induced by vaccination to neutralize the function of target proteins and interrupt blood feeding are shown. Question marks indicate steps that have not been experimentally confirmed. GSH, glutathione; GSSH, glutathione disulphide.

N. americanus APR1 is a 45 kDa protein. For stability and safety reasons, including concerns about injecting humans with an active proteolytic enzyme, the version of this aspartic protease used for vaccination has been inactivated by substituting alanines for the catalytic aspartic acid residues[100]. The recombinant protein has been expressed in multiple systems, with *Escherichia coli*[100] and tobacco plants[101] producing the highest yields. The rationale for selecting *N. americanus* APR1 for development was based on successful laboratory animal trials. In dogs vaccinated with either *N. americanus* APR1 or *A. caninum* APR1, high levels of antibody were induced that inhibited protease activity *in vitro*; this was associated with substantially diminished blood loss (measured by haemoglobin levels) and worm burdens following challenge with *A. caninum* larvae compared with those seen in controls[100,102]. Vaccination with *A. caninum* APR1 also resulted in a substantial reduction in the worm burdens of hamsters challenged with *N. americanus* compared with burdens of controls[98]. These findings also suggest that cross-reactive immunity between *Necator* spp. and *Ancylostoma* spp. hookworms may occur. Following vaccination, APR1-specific antibodies are ingested by the parasite during blood feeding and localize to the parasite gut, where they can inhibit parasite feeding by neutralizing enzyme activity[98,102] (FIG. 2). Efforts to optimize both the yield and the solubility of *N. americanus* APR1 are in progress. A chimeric protein consisting of *N. americanus* GST1 fused to epitopes of *N. americanus* APR1, to generate neutralizing antibodies and inhibit parasite blood digestion, is already under development[103]. Additional key molecules involved in hookworm blood feeding have been identified from proteomic and transcriptomic analyses of the hookworm gut[104]. These molecules include a putative hookworm orthologue of a prolylcarboxypeptidase ('contortin') that protects sheep against *Haemonchus contortus* infection[105], and the extracellular domain of an intestinal peptide transporter that is essential for nutrient uptake and growth in *C. elegans*[90].

Previous vaccine antigens. ASP2 from *N. americanus* infective larvae was previously under consideration as a candidate vaccine antigen[106–109]. The canine hookworm orthologue of this molecule was determined to be a major immunogen associated with an effective vaccine consisting of live attenuated *A. caninum* L3 larvae[106,109]. In a Phase I trial conducted in healthy volunteers in the United States, ASP2 adjuvanted with Alhydrogel was shown to be safe and immunogenic[107]. However, in a second Phase I trial conducted at a hookworm endemic site

Table 3 | **Ranking of lead candidate *Necator americanus* vaccine antigens**

Necator americanus antigen	Target of IgE*	Measure of efficacy in animal model				Ease of manufacture#	Known function or structure**	Final Score‡‡
		Reduced adult worm counts (dog)‡	Reduced adult worm counts (hamster)§	Prevention of blood loss‖	Reduced faecal egg counts¶			
ASP2	Yes§§	2	3	1	3	3	2	14/23 (61%)
APR1	No	2	2	3	3	2	2	14/23 (61%)
GST1	No	2	3	1	1	3	2	12/23 (52%)
CP2	ND	1	2	ND	3	2	2	10/19 (53%)

GST1, glutathione S-transferase 1; IgE, immunoglobulin E; ND, not determined. *If individuals naturally infected with hookworm develop IgE to the protein, the antigen is immediately down-selected. ‡Reflects quintiles (represented by a score out of 5) of the reduced worm burdens of vaccinated dogs challenged with infective larvae compared with the burden of controls. §Reflects quintiles (represented by a score out of 5) of the reduced worm burdens of vaccinated hamsters challenged with infective larvae compared with the burden of controls. ‖Each level (from 1 to 3) represents an increment of 0.5 g per ml in the haemoglobin concentration of vaccinated and challenged animals (with 0 representing the concentration in a control group). ¶Reflects quartiles (represented by a score out of 4) of reduced faecal egg counts in vaccinated animals challenged with infective larvae, compared with controls. #A major impediment to the success of efficacious vaccine antigens is the ease and cost-effectiveness with which their production can be scaled up for synthesis according to good manufacturing practices: 0 means it is not feasible; 1 means it is difficult but potentially feasible; 2 means it will give modest yields and a relatively stable protein but substantial process development is required; 3 means it will give high yields of soluble, stable protein with a straightforward scale up for synthesis according to good manufacturing practices. **Known protein function and/or structure assists in determining the mechanism of protection and aids process development: 0 means that the function and/or structure are unknown; 1 means that the function or structure is known; 2 means that the function and structure are known. ‡‡Tally of scores for each category; if a category score was not obtained because the experiment was not carried out, the category is not counted for the final score. §§Recognition that naturally infected individuals develop *N. americanus* ASP2-specific IgE antibodies occurred late in the development process.

in Brazil, some of the adult volunteers experienced generalized urticaria immediately after vaccination (D.J.D., unpublished observations). The study was halted, and it was found that individuals who developed urticaria had high levels of immunoglobulin E (IgE) against ASP2. This finding has led to testing for the levels of IgE specific for candidate vaccine antigens using sera from individuals resident in hookworm-endemic areas. No detectable levels of IgE specific to *N. americanus* GST1 (J.M.B. and D.J.D., unpublished observations) or *N. americanus* APR1 (REF. 100) have been found in individuals living in hookworm-endemic areas of Brazil, thus permitting their continued development as candidate vaccine antigens. The reason neither recombinant adult hookworm protein induces IgE during natural infection is unknown, but it may be related to antigen structure or presentation to the immune system.

Vaccinating against schistosomiasis

In both Brazil and most of sub-Saharan Africa, *N. americanus* hookworm infections are co-endemic with intestinal schistosomiasis caused by *S. mansoni*[2]. Vaccines to combat each of these helminth infections are being developed because both diseases are associated with anaemia and malnutrition, especially in children. Ultimately, the two vaccines may be co-administered or combined in a multivalent anthelmintic vaccine (see below)[2]. The justification for developing vaccines against schistosomiasis has been reviewed recently[88,110] and includes the high disease burden[4,6], the high rates of post-treatment reinfection, the inability of chemotherapy-based morbidity control to interrupt transmission[111], the exclusive reliance on praziquantel for control and concerns about emerging drug resistance without new drugs in the development pipeline[88,110]. An important additional stimulus to develop new preventive approaches to schistosomiasis is the observation of so-called 'rebound morbidity': up to 80% of children living in high-transmission areas can suffer recurrent aggressive inflammation following interrupted annual chemotherapy because of reinfection. The feasibility of developing vaccines for schistosomiasis has been extensively reviewed[88,110,112]. Humans living in endemic areas can become resistant or partially immune to reinfection over time[113]. Furthermore, irradiated cercariae can elicit high levels of protective immunity in laboratory animals, and several recombinant-protein vaccines have been shown to elicit comparable levels of protective immunity in immunized animals that were subsequently challenged with cercariae[110].

Schistosome antigens under development

To date, one vaccine for urinary schistosomiasis has entered clinical trials. The Institut Pasteur and the French Institut National de la Santé et de la Recherche Médicale have taken a recombinant 28 kDa GST cloned from *S. haematobium* through both Phase I and Phase II clinical trials in Europe and West Africa[110,114] (see also Le Project Bilhvax 3). Known as Bilhvax, this GST formulated with an aluminium hydroxide adjuvant has been reported to be immunogenic and safe after testing in healthy adults[114]. However, further information regarding its efficacy, the duration of protection and the progress towards licensure are not available in the published literature. In addition, other vaccine candidates for intestinal schistosomiasis caused by *S. mansoni* will soon be ready for clinical testing[112]. One candidate is a 14 kDa fatty acid-binding protein known as Sm14 (REF. 115), which in experimental animals (mice and rabbits) elicits protection against *S. mansoni* as well as against *Fasciola hepatica*, another trematode fluke[116]. Recently, the group developing this vaccine reported success in stabilizing Sm14 by replacing a crucial cysteine residue in order to prevent dimerization[117]. Recombinant Sm14 is being developed as an anthelmintic vaccine by a partnership between private and government organizations in Brazil for use against both fascioliasis of livestock and human schistosomiasis caused by *S. mansoni*[116]. Another *S. mansoni* vaccine potentially moving into clinical development

Urticaria
A skin rash that is characterized by raised erythematous, pruritic lesions and is most often associated with intradermal mast cell degranulation due to an immediate-type hypersensitivity reaction (known colloquially as 'hives').

Praziquantel
An anthelmintic medication that is active against flatworms, including trematodes (flukes) and tapeworms (cestodes).

Table 4 | **Ranking of lead candidate *Schistosoma mansoni* vaccine antigens**

Schistosoma mansoni antigen	Target of IgE*	Reduced adult worm counts (mice)‡	Reduced egg counts (mice)§	Preferential recognition by resistant humans‖	Intramammalian stage targeted¶	Ease of manufacture	Known function or structure#	RNAi phenotype**	Final Score‡‡
TSP2	No	4	4	3	3	3	1	2	20/23 (87%)
Sm29	ND	4	3	1	2	1	0	ND	11/21 (52%)
TSP1	ND	3	3	0	2	3	1	2	14/23 (56%)

Ig, immunoglobulin; ND, not determined. *If individuals naturally infected with *S. mansoni* develop IgE specific to the protein, the antigen is immediately down-selected. ‡Reflects quintiles (represented by a score out of 5) of the reduced worm burdens of vaccinated mice challenged with infective larvae compared with the burdens of controls. Vaccines used antigen formulated with an adjuvant that has been approved (or is under assessment) for human use. §Reflects quintiles (represented by a score out of 5) of the reduced liver egg and/or fecal egg burdens of vaccinated mice challenged with infective larvae compared with the burdens of controls. ‖Reflects tertiles (represented by a score out of 3) of the difference in the mean levels of IgG1 or IgG3 units between resistant (naturally resistant or drug-induced resistant) and chronically infected groups[110]. ¶Protein accessible to the immune system in intramammalian developmental stages: 1 means it can be detected on the surface of the fixed schistosomulum or adult; 2 means it can be detected on the surface of the live schistosomulum or adult; 3 means it can be detected on the surface of the live schistosomulum and adult. #Known protein function and/or structure assists in determining the mechanism of protection and aids process development: 0 means that the function and/or structure is unknown; 1 means that the function or structure is known; 2 means that the function and structure are known. **Reflects the essential nature of the target protein for parasite survival, based on RNA interference (RNAi) experiments. 0 means that RNAi results in no effect; 1 means that RNAi results in a deleterious *in vitro* phenotype; 2 means that RNAi results in a deleterious *in vitro* phenotype and affects survival *in vivo* (in animal models) after the transfer of parasites treated with double-stranded RNA into mice. ‡‡Tally of scores for each category; if a category score was not obtained because the experiment was not carried out, the category is not counted for the final score.

is Sm-p80, which is a DNA vaccine encoding the large subunit of a calcium-dependent neutral protease, providing levels of protection in baboons that are comparable to irradiated cercariae[118]. Finally, the schistosome molecule paramyosin is undergoing pilot-scale production in Asia for use against *S. japonicum* infection, possibly as a transmission-blocking vaccine, administered to water buffaloes[119].

The Sabin Vaccine Institute, in partnership with FIOCRUZ and the Instituto Butantan, is also working to transition a *S. mansoni* vaccine into clinical testing in Brazil (J.M.B., D.J.D. and P.J.H., unpublished observations). The primary targets of this vaccine development programme are schistosome membrane proteins identified by combined genomic, post-genomic and proteomic analyses of the adult *S. mansoni* outer surface, or tegument[120] (TABLE 4). The tegument of adult schistosomes is a single syncytium covering the body wall and is thought to be a dynamic layer involved in several key physiological processes, including parasite nutrition, osmoregulation and evasion of host immunity[120]. Hence, the schistosome tegument is a potentially vulnerable target for immunological attack by host antibodies. However, analysis of the schistosome proteome predicts that surprisingly few membrane-spanning proteins of the tegument are accessible to the host immune response[120,121]. They include a family of tetraspanin integral membrane proteins[122] and several outer-membrane proteins of unknown function, such as Sm29 (REFS 123,124). The tetraspanins are so named because they contain four transmembrane domains, with two extracellular loops that are predicted to interact with exogenous proteins or ligands (FIG. 3). The second extracellular domain fragment of a schistosome tetraspanin known as TSP2, from *S. mansoni*, has been selected for development as a human vaccine antigen. When this 9 kDa extracellular domain was expressed in either *P. pastoris* or *E. coli* and formulated with several adjuvants (including Freund's complete adjuvant[122], aluminium hydroxide, or aluminium hydroxide with CpGs) it provided high levels of protection in mice vaccinated with the antigen and then challenged with *S. mansoni* cercariae (A.L. and M.S.P., unpublished observations). In addition, evidence from human epidemiological studies indicates that putatively resistant individuals living in endemic areas of Brazil have elevated antibody responses to this protein compared with the responses of chronically infected individuals from the same endemic areas[122]. Recently, the *S. japonicum* orthologue of *S. mansoni* TSP2 was described and resulted in protection in mice that was similar to that described for *S. mansoni* TSP2, suggesting that this molecule may be effective against multiple human schistosome species[125].

S. mansoni TSP2 is thought to have a crucial role in tegument development and maturation[126]. The ultrastructural morphology of adult worms and schistosomula treated *in vitro* with *S. mansoni tsp2* double-stranded RNA (dsRNA) displays a distinctly vacuolated and thinner tegument compared with that of controls, suggestive of impaired closure of tegumentary invaginations[126]. Moreover, injection of mice with schistosomulae that had been pre-treated with *S. mansoni tsp2* dsRNA resulted in 83% fewer parasites being recovered from the mesenteric veins 4 weeks later when compared with recovery from mice injected with untreated schistosomulae[126]. These results suggest that tetraspanins have important structural roles in tegument development, maturation or stability. Other tegument tetraspanins are attractive vaccine candidates; for example, *S. mansoni tsp3* is the most highly upregulated mRNA in maturing schistosomula, a developmental stage that is widely accepted as being susceptible to damage by the human immune system[127,128]. In addition, Sj23 (23 kDa integral membrane protein of *S. japonicum*) is a tegument tetraspanin that is showing promise as a DNA vaccine for water buffaloes, which are an important reservoir host for *S. japonicum* in China[129].

Future directions

By targeting both hookworms and schistosomes, human helminth vaccines are being developed to reduce parasite-induced morbidity, the symptoms of which include intestinal blood loss and inflammation[2]. Administered in early childhood, such vaccines could prevent the major

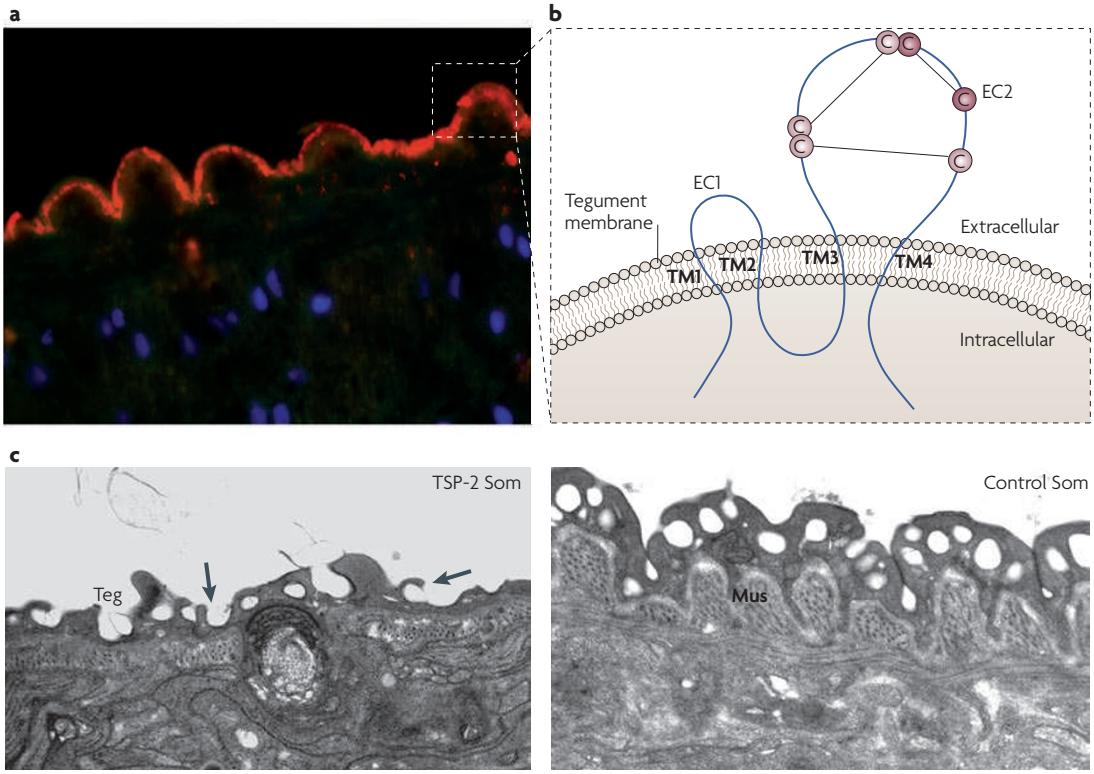

Figure 3 | ***Schistosoma mansoni* tegument. a** | A fluorescence micrograph of the tegument of an adult male *Schistosoma mansoni* probed with a mouse antibody raised to recombinant TSP2 (red), a tetraspanin. Nuclei, stained with 4′,6-diamidino-2-phenylindole (DAPI), are blue. **b** | A schematic representation of *S. mansoni* TSP2 in the tegument plasma membrane. Extracellular loops (ECs) are indicated, and cysteine residues are shown (the lines between them denote the disulphide bond pairing); transmembrane domains (TMs) are shown numbered from amino terminus to carboxyl terminus. **c** | The tegument of an *S. mansoni* schistosomula incubated for 7 days with *S. mansoni tsp2* (left) or luciferase (control; right) double-stranded RNAs. Digitate extensions (arrows) are more abundant on the surface of the tegument incubated with *tsp2* double-stranded RNA. Mus, muscle; Som, schistosomula; Teg, surface layer of the tegument. Part **a** image is reproduced, with permission, from REF. 120 © (2007) Elsevier. Part **c** image is reproduced from REF. 126.

paediatric sequelae of these infections, including anaemia, malnutrition, growth failure and impaired cognitive development.

The human hookworm vaccine is being developed as a bivalent product consisting of two co-formulated recombinant proteins (GST1 and APR1 from *N. americanus*) to prevent moderate and heavy hookworm infections caused by *N. americanus*. The vaccine is intended primarily for preschool- and school-aged children (<10 years of age) living in *N. americanus*-endemic regions. Similarly, TSP2 from *S. mansoni* is being developed as a recombinant-protein vaccine for the prevention of heavy-intensity infections of *S. mansoni*, the leading cause of intestinal schistosomiasis. A paediatric population will be targeted for both vaccines because this age group is at the greatest risk of developing the severe developmental, growth and cognitive impairments associated with these chronic infections[1,12–15]. Initially, vaccines containing the hookworm and schistosome antigens are being formulated with Alhydrogel; however, they will also be evaluated with an additional immunostimulant such as a lipid A derivative. The vaccines will be delivered by intramuscular injection, and the goal is to achieve the desired protection after one or two doses, depending on the number of doses required to achieve a protective response. The desired result is the prevention of moderate and heavy helminth infections, which would have a major impact on the anaemia, malnutrition and end-organ pathology associated with these parasitic infections in children[6,34]. The extension of protection into adulthood would also prevent the severe anaemia that is related to infection with these parasites during pregnancy, and reduce transmission. Such vaccines may also have an important impact on poverty reduction because of their anticipated effect on improving child and maternal health and development[81].

Both vaccines are being developed with the ultimate goal that even the most impoverished populations will have access to them as soon as they are available. As such, a strategic road map is being followed to ensure that low-cost manufacturing processes are used and that vaccine manufacturers in middle-income disease-endemic countries are involved from the start. In the Americas, Brazil is the furthest advanced, with two major vaccine manufacturers — FIOCRUZ/Bio-Manguinhos and the Instituto Butantan — actively engaged in development[130]. Accurate forecasting of the eventual demand for licensed vaccines is essential: for hookworm, it is estimated that

there are approximately one billion children at risk globally, so that covering a global birth cohort would require the vaccination of 100 million children annually[73]. Demand forecasting is underway for an intestinal schistosomiasis vaccine.

Substantial hurdles must be overcome during clinical development of hookworm and schistosomiasis vaccines, not least of which is securing adequate funding to conduct the clinical trials required for licensure. Additional obstacles include obtaining access to the novel adjuvants that may be required to induce an adequate immune response and the difficulty of conducting large-scale efficacy studies in endemic areas. As hookworm and schistosomiasis are prevalent in resource-limited, rural areas of the tropics and subtropics, this is where Phase III clinical trials must be conducted, which can be logistically challenging. Furthermore, because the clinical effects of these parasitic infections are chronic, with sequelae such as IDA often appearing only after months or years of infection, efficacy trials will be necessarily long.

Combining hookworm and schistosomiasis vaccines. In the future, vaccines for hookworm and intestinal schistosomiasis could be combined in a multivalent anthelmintic vaccine[2], which may increase vaccine efficiency and reduce the timeframe for widespread distribution in affected areas of Africa and Latin America. The Sabin Vaccine Institute is currently working with the WHO to build consensus on the use of hookworm and schistosomiasis vaccines in resource-poor settings[131]. Health systems established through the integrated control of mass drug administration for neglected tropical diseases and expanded global deworming efforts for preschool- and school-aged children[8,10] could ultimately provide an infrastructure for linking newly developed vaccines with anthelmintic chemotherapy.

1. Hotez, P. J. *et al.* Helminth infections: the great neglected tropical diseases. *J. Clin. Invest.* **118**, 1311–1321 (2008).
2. Hotez, P. J., Bethony, J. M., Oliveira, S. C., Brindley, P. J. & Loukas, A. Multivalent anthelminthic vaccine to prevent hookworm and schistosomiasis. *Expert Rev. Vaccines* **7**, 745–752 (2008).
3. WHO Expert Committee. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. *World Health Organ. Tech. Rep. Ser.* **912**, 1–57 (2002).
4. King, C. H., Dickman, K. & Tisch, D. J. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* **365**, 1561–1569 (2005).
5. Hotez, P. J. *et al.* Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. *PLoS Med.* **3**, e102 (2006).
6. King, C. H. & Dangerfield-Cha, M. The unacknowledged impact of chronic schistosomiasis. *Chronic Illn.* **4**, 65–79 (2008).
7. Bleakley, H. Disease and development: evidence from hookworm eradication in the American South. *Q. J. Econ.* **122**, 73–117 (2007).
8. Hotez, P. J., Fenwick, A., Savioli, L. & Molyneux, D. H. Rescuing the bottom billion through control of neglected tropical diseases. *Lancet* **373**, 1570–1575 (2009).
9. King, C. H. Parasites and poverty: the case of schistosomiasis. *Acta Trop.* **113**, 95–104 (2010).
10. Hotez, P. J. *et al.* Control of neglected tropical diseases. *N. Engl. J. Med.* **357**, 1018–1027 (2007).
11. Finkelman, F. D. *et al.* Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunol. Rev.* **201**, 139–155 (2004).
12. Brooker, S., Bethony, J. & Hotez, P. J. Human hookworm infection in the 21st century. *Adv. Parasitol.* **58**, 197–288 (2004).
13. Hotez, P. J. *et al.* Hookworm infection. *N. Engl. J. Med.* **351**, 799–807 (2004).
14. Hotez, P. J., Bethony, J., Bottazzi, M. E., Brooker, S. & Buss, P. Hookworm: "the great infection of mankind". *PLoS Med.* **2**, e67 (2005).
15. Bethony, J. *et al.* Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* **367**, 1521–1532 (2006).
This paper provides a comprehensive overview of the life cycles, epidemiology, global disease burden, clinical manifestations and immunology of soil-transmitted helminth infections.
16. Gryseels, B., Polman, K., Clerinx, J. & Kestens, L. Human schistosomiasis. *Lancet* **368**, 1106–1118 (2006).
17. Steinmann, P., Keiser, J., Bos, R., Tanner, M. & Utzinger, J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect. Dis.* **6**, 411–425 (2006).
18. de Silva, N. R. *et al.* Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol.* **19**, 547–551 (2003).
19. Hotez, P. J., Bottazzi, M. E., Franco-Paredes, C., Ault, S. K. & Periago, M. R. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. *PLoS Negl. Trop. Dis.* **2**, e300 (2008).
20. Hotez, P. J. & Kamath, A. Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Negl. Trop. Dis.* **3**, e412 (2009).
21. Stoll, N. R. This wormy world. *J. Parasitol.* **33**, 1–18 (1947).
22. Tissenbaum, H. A. *et al.* A common muscarinic pathway for diapause recovery in the distantly related nematode species *Caenorhabditis elegans* and *Ancylostoma caninum*. *Proc. Natl Acad. Sci. USA* **97**, 460–465 (2000).
23. Williamson, A. L. *et al.* A multi-enzyme cascade of hemoglobin proteolysis in the intestine of blood-feeding hookworms. *J. Biol. Chem.* **279**, 35950–35957 (2004).
24. Ranjit, N. *et al.* Proteolytic degradation of hemoglobin in the intestine of the human hookworm *Necator americanus*. *J. Infect. Dis.* **199**, 904–912 (2009).
25. Zhan, B. *et al.* Biochemical characterization and vaccine potential of a heme-binding glutathione transferase from the adult hookworm *Ancylostoma caninum*. *Infect. Immun.* **73**, 6903–6911 (2005).
26. Zhan, B. *et al.* Molecular cloning, biochemical characterization, and partial protective immunity of the heme-binding glutathione *S*-transferases from the human hookworm *Necator americanus*. *Infect. Immun.* **78**, 1552–1563 (2010).
This work documents the expression of recombinant GST proteins of *N. americanus* in *P. pastoris*, the characterization of these proteins and their effectiveness in preventing infection in a hamster vaccination–challenge model.
27. Wilson, M. S. *et al.* Immunopathology of schistosomiasis. *Immunol. Cell Biol.* **85**, 148–154 (2007).
This article discusses how the granulomatous response to *S. mansoni* and *S. japonicum* eggs develops in the liver, as well as how regulatory cells and cytokine decoy receptors limit the extent of the immunopathology associated with these infections.
28. Tolentino, K. & Friedman, J. F. An update on anemia in less developed countries. *Am. J. Trop. Med. Hyg.* **77**, 44–51 (2007).
29. WHO. Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. (WHO, Geneva, 2001).
30. Stoltzfus, R. J. Iron deficiency: global prevalence and consequences. *Food Nutr. Bull.* **24**, S99–S103 (2003).
31. Larocque, R., Casapia, M., Gotuzzo, E. & Gyorkos, T. W. Relationship between intensity of soil-transmitted helminth infections and anemia during pregnancy. *Am. J. Trop. Med. Hyg.* **73**, 783–789 (2005).
32. Crompton, D. W. The public health importance of hookworm disease. *Parasitology* **121**, S39–S50 (2000).
33. Food and Agriculture Organization (FAO) & WHO. *Human Vitamin and Mineral Requirements*: *Report of a Joint FAO/WHO Expert Consultation* 113–221 (FAO/WHO, Rome, 2002).
34. Stoltzfus, R. J., Dreyfuss, M. L., Chwaya, H. M. & Albonico, M. Hookworm control as a strategy to prevent iron deficiency. *Nutr. Rev.* **55**, 223–232 (1997).
35. Brooker, S. *et al.* Age-related changes in hookworm infection, anaemia and iron deficiency in an area of high *Necator americanus* hookworm transmission in south-eastern Brazil. *Trans. R. Soc. Trop. Med. Hyg.* **101**, 146–154 (2007).
36. Brooker, S. *et al.* The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. *Trans. R. Soc. Trop. Med. Hyg.* **93**, 240–246 (1999).
37. Sousa-Figueiredo, J. C. *et al.* A parasitological survey, in rural Zanzibar, of pre-school children and their mothers for urinary schistosomiasis, soil-transmitted helminthiases and malaria, with observations on the prevalence of anaemia. *Ann. Trop. Med. Parasitol.* **102**, 679–692 (2008).
38. Calis, J. C. *et al.* Severe anemia in Malawian children. *N. Engl. J. Med.* **358**, 888–899 (2008).
39. Nguyen, P. H. *et al.* Risk factors for anemia in Vietnam. *Southeast Asian J. Trop. Med. Public Health* **37**, 1213–1223 (2006).
40. Dreyfuss, M. L. *et al.* Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. *J. Nutr.* **130**, 2527–2536 (2000).
41. Brooker, S., Hotez, P. J. & Bundy, D. A. Hookworm-related anaemia among pregnant women: a systematic review. *PLoS Negl. Trop. Dis.* **2**, e291 (2008).
42. Smith, J. L. & Brooker, S. Impact of hookworm infection and deworming on anaemia in non-pregnant populations: a systematic review. *Trop. Med. Int. Health* **15**, 776–795 (2010).
43. Stephenson, L. The impact of schistosomiasis on human nutrition. *Parasitology* **107**, S107–S123 (1993).
44. Sturrock, R. F. *et al. Schistosomiasis mansoni* in Kenya: relationship between infection and anaemia in schoolchildren at the community level. *Trans. R. Soc. Trop. Med. Hyg.* **90**, 48–54 (1996).
45. Friedman, J. F. *et al.* Relationship between *Schistosoma japonicum* and nutritional status among children and young adults in Leyte, the Philippines. *Am. J. Trop. Med. Hyg.* **72**, 527–533 (2005).
46. Friedman, J. F., Kanzaria, H. K. & McGarvey, S. T. Human schistosomiasis and anemia: the relationship

and potential mechanisms. *Trends Parasitol.* **21**, 386–392 (2005).
This summarizes the animal and human data about the impact of schistosomiasis on anaemia and discusses four possible mechanisms: extracorporeal blood loss, autoimmune haemolysis and anaemia of inflammation, and splenomegaly with erythrocyte sequestration.
47. Koukounari, A. *et al.* Morbidity indicators of *Schistosoma mansoni*: relationship between infection and anemia in Ugandan schoolchildren before and after praziquantel and albendazole chemotherapy. *Am. J. Trop. Med. Hyg.* **75**, 278–286 (2006).
48. Koukounari, A. *et al. Schistosoma haematobium* infection and morbidity before and after large-scale administration of praziquantel in Burkina Faso. *J. Infect. Dis.* **196**, 659–669 (2007).
49. Koukounari, A. *et al.* Relationships between anaemia and parasitic infections in Kenyan schoolchildren: a Bayesian hierarchical modelling approach. *Int. J. Parasitol.* **38**, 1663–1671 (2008).
50. Tohon, Z. B. *et al.* Controlling schistosomiasis: significant decrease of anaemia prevalence one year after a single dose of praziquantel in Nigerian schoolchildren. *PLoS Negl. Trop. Dis.* **2**, e241 (2008).
51. Prual, A. *et al.* Consequences of *Schistosoma haematobium* infection on the iron status of schoolchildren in Niger. *Am. J. Trop. Med. Hyg.* **47**, 291–297 (1992).
52. Friedman, J. F., Mital, P., Kanzaria, H. K., Olds, G. R. & Kurtis, J. D. Schistosomiasis and pregnancy. *Trends Parasitol.* **23**, 159–164 (2007).
53. Ajanga, A. *et al. Schistosoma mansoni* in pregnancy and associations with anaemia in northwest Tanzania. *Trans. R. Soc. Trop. Med. Hyg.* **100**, 59–63 (2006).
54. Stephenson, L. S. Helminth parasites, a major factor in malnutrition. *World Health Forum* **15**, 169–172 (1994).
55. Stephenson, L. S. *et al.* Relationships of *Schistosoma haematobium*, hookworm and malarial infections and metrifonate treatment to growth of Kenyan school children. *Am. J. Trop. Med. Hyg.* **34**, 1109–1118 (1985).
56. Lwambo, N. J., Siza, J. E., Brooker, S., Bundy, D. A. & Guyatt, H. Patterns of concurrent hookworm infection and schistosomiasis in schoolchildren in Tanzania. *Trans. R. Soc. Trop. Med. Hyg.* **93**, 497–502 (1999).
57. Guyatt, H. L., Brooker, S., Kihamia, C. M., Hall, A. & Bundy, D. A. Evaluation of efficacy of school-based anthelmintic treatments against anaemia in children in the United Republic of Tanzania. *Bull. World Health Organ.* **79**, 695–703 (2001).
58. Friis, H. *et al.* Effects on haemoglobin of multi-micronutrient supplementation and multi-helminth chemotherapy: a randomized, controlled trial in Kenyan school children. *Eur. J. Clin. Nutr.* **57**, 573–579 (2003).
59. Raso, G. *et al.* An integrated approach for risk profiling and spatial prediction of *Schistosoma mansoni*–hookworm coinfection. *Proc. Natl Acad. Sci. USA* **103**, 6934–6939 (2006).
60. Brito, L. L. *et al.* Moderate- and low-intensity co-infections by intestinal helminths and *Schistosoma mansoni*, dietary iron intake, and anemia in Brazilian children. *Am. J. Trop. Med. Hyg.* **75**, 939–944 (2006).
61. Fleming, F. M. *et al.* Synergistic associations between hookworm and other helminth species in a rural community in Brazil. *Trop. Med. Int. Health* **11**, 56–64 (2006).
62. Ezeamama, A. E. *et al.* The synergistic effect of concomitant schistosomiasis, hookworm, and trichuris infections on children's anemia burden. *PLoS Negl. Trop. Dis.* **2**, e245 (2008).
63. Brooker, S. *et al.* Epidemiology of plasmodium-helminth co-infection in Africa: populations at risk, potential impact on anemia, and prospects for combining control. *Am. J. Trop. Med. Hyg.* **77**, 88–98 (2007).
64. Demissie, F., Kebede, A., Shimels, T. & Beyene, P. Assessment of public health implication of malaria-geohelminth co-infection with an emphasis on hookworm-malaria anemia among suspected malaria patients in Asendabo, southwest Ethiopia. *Ethiop. Med. J.* **47**, 153–158 (2009).
65. Kjetland, E. F. *et al.* Association between genital schistosomiasis and HIV in rural Zimbabwean women. *AIDS* **20**, 593–600 (2006).
66. Chenine, A. L. *et al.* Acute *Schistosoma mansoni* infection increases susceptibility to systemic SHIV clade C infection in rhesus macaques after mucosal virus exposure. *PLoS Negl. Trop. Dis.* **2**, e265 (2008).
67. Da'dara, A. A. & Harn, D. A. Elimination of helminth infection restores HIV-1C vaccine-specific T cell responses independent of helminth-induced IL-10. *Vaccine* **28**, 1310–1317 (2010).
68. Bentwich, Z., Teicher, C. L. & Borkow, G. The helminth HIV connection: time to act. *AIDS* **22**, 1611–1614 (2008).
69. Moore, R. D. Anemia and human immunodeficiency virus disease in the era of highly active antiretroviral therapy. *Semin. Hematol.* **37**, 18–23 (2000).
70. Moraes, L. R., Cancio, J. A. & Cairncross, S. Impact of drainage and sewerage on intestinal nematode infections in poor urban areas in Salvador, Brazil. *Trans. R. Soc. Trop. Med. Hyg.* **98**, 197–204 (2004).
71. Asaolu, S. O. & Ofoezie, I. E. The role of health education and sanitation in the control of helminth infections. *Acta Trop.* **86**, 283–294 (2003).
72. WHO. Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers. (WHO, Geneva, 2006).
73. WHO. Soil-transmitted helminthiasis. Progress report on number of children treated with anthelmintic drugs: an update towards the 2010 global target. *Wkly Epidemiol. Rec.* **82**, 237–252 (2008).
74. Olsen, A. Efficacy and safety of drug combinations in the treatment of schistosomiasis, soil-transmitted helminthiasis, lymphatic filariasis and onchocerciasis. *Trans. R. Soc. Trop. Med. Hyg.* **101**, 747–758 (2007).
75. Hotez, P. J. Mass drug administration and integrated control for the world's high-prevalence neglected tropical diseases. *Clin. Pharmacol. Ther.* **85**, 659–664 (2009).
76. Geary, T. G. *et al.* Unresolved issues in anthelmintic pharmacology for helminthiases of humans. *Int. J. Parasitol.* **40**, 1–13 (2010).
77. Keiser, J. & Utzinger, J. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA* **299**, 1937–1948 (2008).
78. Albonico, M. *et al.* Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. *Bull. World Health Organ.* **81**, 343–352 (2003).
79. Geerts, S. & Gryseels, B. Drug resistance in human helminths: current situation and lessons from livestock. *Clin. Microbiol. Rev.* **13**, 207–222 (2000).
80. Albonico, M. *et al.* Rate of reinfection with intestinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area. *Trans. R. Soc. Trop. Med. Hyg.* **89**, 538–541 (1995).
81. Hotez, P. J. & Ferris, M. T. The antipoverty vaccines. *Vaccine* **24**, 5787–5799 (2006).
82. Loukas, A., Bethony, J., Brooker, S. & Hotez, P. Hookworm vaccines: past, present, and future. *Lancet Infect. Dis.* **6**, 733–741 (2006).
83. Bethony, J. M., Loukas, A., Hotez, P. J. & Knox, D. P. Vaccines against blood-feeding nematodes of humans and livestock. *Parasitology* **133**, S63–S79 (2006).
A summary of the progress that has been made towards vaccine development using defined larval-stage and adult-stage antigens against hookworms in humans and against *H. contortus* in livestock, as well as the anticipated impact of the host immune response on vaccine design.
84. Diemert, D. J., Bethony, J. M. & Hotez, P. J. Hookworm vaccines. *Clin. Infect. Dis.* **46**, 282–288 (2008).
This article discusses the feasibility of developing a hookworm vaccine on the basis of the previous success of an attenuated larval vaccine against the canine hookworm *A. caninum*. It also summarizes potential protective mechanisms, the stability of potential candidate antigens and the results of clinical testing of the first human hookworm candidate vaccine antigen, ASP2.
85. Bottazzi, M. E. & Brown, A. S. Model for product development of vaccines against neglected tropical diseases: a vaccine against human hookworm. *Expert Rev. Vaccines* **7**, 1481–1492 (2008).
86. Hotez, P. J. & Brown, A. S. Neglected tropical disease vaccines. *Biologicals* **37**, 160–164 (2009).
87. Miller, T. A. Industrial development and field use of the canine hookworm vaccine. *Adv. Parasitol.* **16**, 333–342 (1978).
88. Bergquist, R., Utzinger, J. & McManus, D. P. Trick or treat: the role of vaccines in integrated schistosomiasis control. *PLoS Negl. Trop. Dis.* **2**, e244 (2008).
89. Urquhart, G. M. Field experience with the bovine lungworm vaccine. *Dev. Biol. Stand.* **62**, 109–112 (1985).
90. Meissner, B., Boll., M., Daniel, H. & Baumeister, R. Deletion of the intestinal peptide transporter affects insulin and TOR signaling in *Caenorhabditis elegans*. *J. Biol. Chem.* **279**, 36739–36745 (2004).
91. Brophy, P. M. & Pritchard, D. I. Metabolism of lipid peroxidation products by the gastro-intestinal nematodes *Necator americanus*, *Ancylostoma ceylanicum* and *Heligmosomoides polygyrus*. *Int. J. Parasitol.* **22**, 1009–1012 (1992).
92. Zhan, B. *et al.* Molecular cloning and purification of *Ac*-TMP, a developmentally regulated putative tissue inhibitor of metalloprotease released in relative abundance by adult *Ancylostoma* hookworms. *Am. J. Trop. Med. Hyg.* **66**, 238–244 (2002).
93. Deponte, M. & Becker, K. Glutathione *S*-transferase from malarial parasites: structural and functional aspects. *Methods Enzymol.* **401**, 241–253 (2005).
94. Jani, D. *et al.* HDP—a novel heme detoxification protein from the malaria parasite. *PLoS Pathog.* **4**, e1000053 (2008).
95. Asojo, O. A. *et al.* X-ray structures of *Na*-GST-1 and *Na*-GST-2 two glutathione s-transferase from the human hookworm *Necator americanus*. *BMC Struct. Biol.* **7**, 42 (2007).
This work describes the crystal structure of the leading hookworm candidate vaccine antigen GST1, revealing it to exist as a homodimer with a cavity in which ligands such as haeme can potentially be bound and thereby detoxified.
96. van Rossum, A. J. *et al.* Binding of hematin by a new class of glutathione transferase from the blood-feeding parasitic nematode *Haemonchus contortus*. *Infect. Immun.* **72**, 2780–2790 (2004).
97. Schuller, D. J. *et al.* Crystal structure of a new class of glutathione transferase from the model human hookworm nematode *Heligmosomoides polygyrus*. *Proteins* **61**, 1024–1031 (2005).
98. Xiao, S. *et al.* The evaluation of recombinant hookworm antigens as vaccines in hamsters (*Mesocricetus auratus*) challenged with human hookworm, *Necator americanus*. *Exp. Parasitol.* **118**, 32–40 (2008).
This article describes the protective effects of several candidate hookworm vaccine antigens, including *N. americanus* ASP2, *A. caninum* APR1 and *A. caninum* GST1, in hamsters challenged with infective *N. americanus* larvae following vaccination.
99. Veerapathran, A., Dakshinamoorthy, G., Gnanasekar, M., Reddy, M. V. & Kalyanasundaram, R. Evaluation of *Wuchereria bancrofti* GST as a vaccine candidate for lymphatic filariasis. *PLoS Negl. Trop. Dis.* **3**, e457 (2009).
100. Pearson, M. S. *et al.* An enzymatically inactivated hemoglobinase from *Necator americanus* induces neutralizing antibodies against multiple hookworm species and protects dogs against heterologous hookworm infection. *FASEB J.* **23**, 3007–3019 (2009).
This study shows that vaccination of dogs with an *N. americanus* APR1 in which the catalytic aspartic acid residues are mutagenized results in substantially reduced parasite egg burdens after challenge with infective larvae of *A. caninum*, and induces antibodies that bind the native enzyme in the parasite gut, neutralizing its enzymatic activity.
101. Yusibov, V. & Rabindran, S. Recent progress in the development of plant derived vaccines. *Expert Rev. Vaccines* **7**, 1173–1183 (2008).
102. Loukas, A. *et al.* Vaccination with recombinant aspartic hemoglobinase reduces parasite load and blood loss after hookworm infection in dogs. *PLoS Med.* **2**, e295 (2005).
This investigation finds that vaccination of dogs with recombinant *A. caninum* APR1 results in protection from anaemia, the major clinical manifestation of hookworm, after challenge with infective *A. caninum* larvae.
103. Pearson, M. S. *et al.* Neutralizing antibodies to the hookworm hemoglobinase *Na*-APR-1: implications for a multivalent vaccine against hookworm infection and schistosomiasis. *J. Infect. Dis.* **201**, 1561–1569 (2010).
104. Ranjit, N., Jones, M. K., Stenzel, D. J., Gasser, R. B. & Loukas, A. A survey of the intestinal transcriptomes of the hookworms, *Necator americanus* and *Ancylostoma caninum*, using tissues isolated by laser microdissection microscopy. *Int. J. Parasitol.* **36**, 701–710 (2006).

105. Geldhof, P. & Knox, D. The intestinal contortin structure in *Haemonchus contortus*: An immobilised anticoagulant? *Int. J. Parasitol.* **38**, 1579–1588 (2008).
106. Bethony, J. *et al.* Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. *FASEB J.* **19**, 1743–1745 (2005).
107. Goud, G. N. *et al.* Expression of the *Necator americanus* hookworm larval antigen *Na*-ASP-2 in *Pichia pastoris* and purification of the recombinant protein for use in human clinical trials. *Vaccine* **23**, 4754–4764 (2005).
108. Fujiwara, R. T. *et al.* Immunogenicity of the hookworm *Na*-ASP-2 vaccine candidate: characterization of humoral and cellular responses after vaccination in the Sprague Dawley rat. *Hum. Vaccin.* **1**, 123–128 (2005).
109. Fujiwara, R. T. *et al.* Vaccination with irradiated *Ancylostoma caninum* third stage larvae induces a T_H2 protective response in dogs. *Vaccine* **24**, 501–509 (2006).
110. McManus, D. P. & Loukas, A. Current status of vaccines for schistosomiasis. *Clin. Microbiol. Rev.* **21**, 225–242 (2008).
111. King, C. H., Sturrock, R. F., Kariuki, H. C. & Hamburger, J. Transmission control for schistosomiasis -– why it matters now. *Trends Parasitol.* **22**, 575–582 (2006).
112. Oliveira, S. C., Fonseca, C. T., Cardoso, F. C., Farias, L. P. & Leite, L. C. Recent advances in vaccine research against schistosomiasis in Brazil. *Acta Trop.* **108**, 256–262 (2008).
113. Correa-Oliveira, R., Caldas, I. R. & Gazzinelli, G. Natural versus drug-induced resistance in *Schistosoma mansoni* infection. *Parasitol. Today* **16**, 397–399 (2000).
114. Capron, A., Riveau, G., Capron, M. & Trottein, F. Schistosomes: the road from host–parasite interactions to vaccines in clinical trials. *Trends Parasitol.* **21**, 143–149 (2005).
115. Moser, D., Tendler, M., Griffiths, G. & Klinkert, M. Q. A 14-kDa *Schistosoma mansoni* polypeptide is homologous to a gene family of fatty acid binding proteins. *J. Biol. Chem.* **266**, 8447–8454 (1991).
116. Tendler, M. & Simpson, A. J. The biotechnology-value chain: development of Sm14 as a schistosomiasis vaccine. *Acta Trop.* **108**, 263–266 (2008).
117. Ramos, C. R. *et al.* Stability improvement of the fatty acid binding protein Sm14 from *S. mansoni* by Cys replacement: structural and functional characterization of a vaccine candidate. *Biochim. Biophys. Acta* **1794**, 655–662 (2009).
118. Zhang, W. *et al.* Sm-p80-based DNA vaccine provides baboons with levels of protection against *Schistosoma mansoni* infection comparable to those achieved by the irradiated cercarial vaccine. *J. Infect. Dis.* **201**, 1105–1112 (2010).
119. Jiz, M. *et al.* Pilot-scale production and characterization of paramyosin, a vaccine candidate for schistosomiasis japonica. *Infect. Immun.* **76**, 3164–3169 (2008).
120. Loukas, A., Tran, M. & Pearson, M. S. Schistosome membrane proteins as vaccines. *Int. J. Parasitol.* **37**, 257–263 (2007).
This article describes the tetraspanin family of integral membrane proteins that are abundantly represented in the outermost surface of *Schistosoma* spp., and discusses the evidence from a mouse vaccine model and from human immunoepidemiology studies that indicate the potential of tetraspanins as vaccine candidates.
121. Braschi, S. & Wilson, R. A. Proteins exposed at the adult schistosome surface revealed by biotinylation. *Mol. Cell. Proteomics* **5**, 347–356 (2006).
122. Tran, M. H. *et al.* Tetraspanins on the surface of *Schistosoma mansoni* are protective antigens against schistosomiasis. *Nature Med.* **12**, 835–840 (2006).
123. Cardoso, F. C., Pacifico, R. N., Mortara, R. A. & Oliveira, S. C. Human antibody responses of patients living in endemic areas for schistosomiasis to the tegumental protein Sm29 identified through genomic studies. *Clin. Exp. Immunol.* **144**, 382–391 (2006).
124. Cardoso, F. C. *et al.* Schistosoma mansoni tegument protein Sm29 is able to induce a Th1-type of immune response and protection against parasite infection. *PLoS Negl. Trop. Dis.* **2**, e308 (2008).
125. Yuan, C. *et al. Schistosoma japonicum*: Efficient and rapid purification of the tetraspanin extracellular loop 2, a potential protective antigen against schistosomiasis in mammalian. *Exp. Parasitol.* **126**, 456–461 (2010).
126. Tran, M. H. *et al.* Suppression of mRNAs encoding tegument tetraspanins from *Schistosoma mansoni* results in impaired tegument turnover. *PLoS Pathog.* **6**, e1000840 (2010).
127. Gobert, G. N. *et al.* Transcriptional changes in *Schistosoma mansoni* during early schistosomula development and in the presence of erythrocytes. *PLoS Negl. Trop. Dis.* **4**, e600 (2010).
128. Fitzpatrick, J. M. *et al.* Anti-schistosomal intervention targets identified by lifecycle transcriptomic analyses. *PLoS Negl. Trop. Dis.* **3**, e543 (2009).
129. Da'dara, A. A. *et al.* DNA-based vaccines protect against zoonotic schistosomiasis in water buffalo. *Vaccine* **26**, 3617–3625 (2008).
130. Morel, C. M. *et al.* Health innovation networks to help developing countries address neglected diseases. *Science* **309**, 401–404 (2005).
131. WHO. *Deworming for Health and Development* 27 (WHO, Geneva, 2005).
132. Maizels, R. M. *et al.* Helminth parasites – masters of regulation. *Immunol. Rev.* **201**, 89–116 (2004).
This paper reviews the immunoregulation induced by helminth infections, which is dominated by the T_H2 phenotype and the selective loss of effector activity against a background of regulatory T cells, T_H2-inducing dendritic cells and alternatively activated macrophages.
133. Maizels, R. M., Holland, M. J., Falcone, F. H., Zang, X. X. & Yazdanbakhsh, M. Vaccination against helminth parasites - the ultimate challenge for vaccinologists? *Immunol. Rev.* **171**, 125–147 (1999).
134. Bethony, J. *et al.* Emerging patterns of hookworm infection: influence of aging on the intensity of *Necator* infection in Hainan Province, People's Republic of China. *Clin. Infect. Dis.* **35**, 1336–1344 (2002).
135. Andrade, Z. A. Schistosomiasis and liver fibrosis. *Parasite Immunol.* **31**, 656–663 (2009).
136. Erb, K. J. Helminths, allergic disorders and IgE-mediated immune responses: where do we stand? *Eur. J. Immunol.* **37**, 1170–1173 (2007).
137. Cohen, M. S., Hellmann, N., Levy, J. A., DeCock, K. & Lange, J. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *J. Clin. Invest.* **118**, 1244–1254 (2008).
138. WHO. World health report 2004: changing history (WHO, Geneva, 2004).
139. Greenwood, B. M. *et al.* Malaria: progress, perils, and prospects for eradication. *J. Clin. Invest.* **118**, 1266–1276 (2008).
140. Rowe, A. K. *et al.* The burden of malaria mortality among African children in the year 2000. *Int. J. Epidemiol.* **35**, 691–704 (2006).
141. Snow, R. W., Guerra, C. A., Noor, A. M., Myint, H. Y. & Hay, S. I. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* **434**, 214–217 (2005).
142. Vercruysse, J., Knox, D. P., Schetters, T. P. & Willadsen, P. Veterinary parasitic vaccines: pitfalls and future directions. *Trends Parasitol.* **20**, 488–492 (2004).
143. Ploeger, H. W. *Dictyocaulus viviparus*: re-emerging or never been away? *Trends Parasitol.* **18**, 329–332 (2002).
144. McKeand, J. B. Vaccine development and diagnostics of *Dictyocaulus viviparus*. *Parasitology* **120**, S17–S23 (2000).
145. Steves, F. E., Baker, J. D., Hein, V. D. & Miller, T. A. Efficacy of a hookworm (*Ancylostoma caninum*) vaccine for dogs. *J. Am. Vet. Med. Assoc.* **163**, 231–235 (1973).
146. Knox, D. P. *et al.* The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichostrongyloids. *Int. J. Parasitol.* **33**, 1129–1137 (2003).
147. Lightowlers, M. W. Cestode vaccines: origins, current status and future prospects. *Parasitology* **133**, S27–S42 (2006).

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Competing interests statement
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FURTHER INFORMATION
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