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Hookworm vaccines: current and future directions

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ABSTRACT

Introduction: Hookworms infect about half a billion people worldwide and are responsible for the loss of more than two billion disability-adjusted life years. Mass drug administration (MDA) is the most popular preventive approach, but it does not prevent reinfection. An effective vaccine would be a major public health tool in hookworm-endemic areas.

Areas covered: We highlight recent human studies where vaccination with irradiated larvae and repeated rounds of infection-treatment have induced partial protection. These studies have emphasized the importance of targeting the infective larvae to generate immunity to prevent adult worms from maturing in the gut. We summarize the current status of human and animal model vaccine trials.

Expert opinion: Hookworm infection is endemic in resource-poor developing regions where polyparasitism is common, and vaccine cold chain logistics are complex. Humans do not develop sterile immunity to hookworms, and the elderly are frequently overlooked in MDA campaigns. For all these reasons, a vaccine is essential to create long-lasting protection. The lack of a robust animal model to mimic human hookworm infections is a barrier to the discovery and development of a vaccine, however, there have been major recent advances in human challenge studies which will accelerate the process.

1. Introduction

Hookworms are one of the world's most common soil-transmitted helminth (STH) parasites and are prevalent in low-socio-economic settings. Although the prevalence of hookworm disease has significantly reduced in developed countries after World War II, it is still a major problem in economically disadvantaged rural and regional settings where people experience poor hygiene [1,2]. Currently, about 406–480 million people are living with hookworm infection [3], which accounts for approximately 2.1 million disability-adjusted life-years (DALYs) lost [4].

Hookworm infection of humans is caused by *Necator americanus*, *Ancylostoma duodenale* and *Ancylostoma ceylanicum*, each of which the adult stage resides in the small intestine. In endemic areas, hookworm infection is a potential threat to maternal and child health, and is responsible for 24 million cases of severe anemia each year, maternal morbidity and mortality, and the risk of fetal loss or premature birth due to high demand of iron [5–7]. The current therapeutic procedure involves individual or mass medication with albendazole or mebendazole for 1–3 days [3]. Unfortunately, no anthelmintic drugs account for 100% efficacy, and due to rapid re-infection and emerging benzimidazole resistance, anthelmintic drugs frequently fail to control hookworm infection, further contributing to the DALYs lost [7,8]. Alternative means of control, such as vaccines, can provide long-lasting immunity and protection against hookworm infection [5,9–11].

It should be noted from the outset that hookworm infections are rapidly cleared in many rodent models through a robust cellular and humoral immune response. In human hookworm infections however, a similar immune phenotype is observed, but rapid clearance of infection does not occur, and indeed adults often harbor heavy intensity hookworm infections through to old age. Hookworm infection induces a modified type 2 immune response, characterized by distinct cytokines, such as interleukins (IL) −4, −5, −13, −25, −33, and thymic stromal lymphopoietin (TSLP), leukocytes (e.g. eosinophils, mast cells, basophils, type 2 macrophages, type 2 dendritic cells (DC2) and type 2 innate lymphoid cells (ILC2)) and soluble factors (e.g. matrix metallo-proteinases and immunoglobulins G and E) (Figure 1) [14]. While features of a type 2 response are prominent in hookworm (and indeed most human helminth infections), type 1 cytokines are also evident in both experimental and naturally acquired hookworm infections, mediated by tumor necrosis factor TNF and type-1 helper T (Th1) cells producing interferon gamma (IFNγ) and IL-2 [15]. In terms of the type 2 response, IL-4 interacts with germinal center B cells, resulting in production of low-affinity IgE by plasma cells. IL-5 and IgE trigger histamine release by degranulating eosinophils and basophils/mast cells respectively to rapidly destroy and expel worms in experimental infections in animals. Interestingly, there is less evidence that this happens in humans, and instead only a very small

proportion of the serum IgE that is raised against *N. americanus* is highly antigen-specific [16]. IgE-armed basophils also contribute to trapping of larvae in the skin and limit larval migration through the lungs [17]. IL-13 accelerates worm expulsion by triggering a 'weep and sweep response' mediated by goblet cell hyperplasia and mucus secretion [18]. Furthermore, regulatory T cells (Tregs) inhibit the function of Th2 cells by secreting IL-10 and IL-6. Together these two cytokines along with IL-4, IL-5 and IL-13 contribute to ameliorate Th1- and Th17-mediated diseases [19].

While no effective vaccine against hookworm infection is available, a recent clinical study with UV-attenuated *N. americanus* larvae effectively protected human volunteers against a challenge infection with healthy unattenuated infective larvae [20]. Moreover, another recent clinical study assessed the vaccine efficacy of multiple rounds of short-term exposure to *N. americanus* third-stage infective larvae (L3) followed by successful chemotherapeutic intervention with anthelmintics,

and while significant protection against challenge infection was not observed, a trend toward reduced egg burdens in subjects who mounted a strong skin response to percutaneous infection was demonstrated [21]. These latest clinical studies prove that efficacious human responses to hookworm vaccines is possible and prove the utility of the human experimental challenge model in rapidly and efficiently assessing the efficacy of hookworm vaccines. However, additional trials with larger numbers of participants are required to confirm the efficacy of live/attenuated larval vaccines.

2. Current therapies and infection control

The best preventive measure to prevent hookworm infection is to avoid direct contact with contaminated soil, for example by wearing shoes and gloves when working with contaminated soil and sand. Furthermore, drinking safe water, sufficient water for hygiene, use of safe and clean sanitation facilities, such as toilets and sewage systems and handwashing (WASH) and school-based health education learning package (HELP) programs effectively reduced STH infections [22–24]. With an intensive WASH program, Japan is the first country to report a complete absence of positive stool samples for STH including *Ascaris lumbricoides*, *N. americanus*, *A. duodenale* and *Trichuris trichiura*, however, socioeconomically disadvantaged countries are still at high risk [25]. Moreover, routine veterinary management of dogs and cats, including proper disposal of feces, and regular deworming will reduce soil contamination and prevent zoonotic hookworm transmission with species including *A. ceylanicum* and *A. caninum*, both of which are transmitted from dogs (Figure 2) [26].
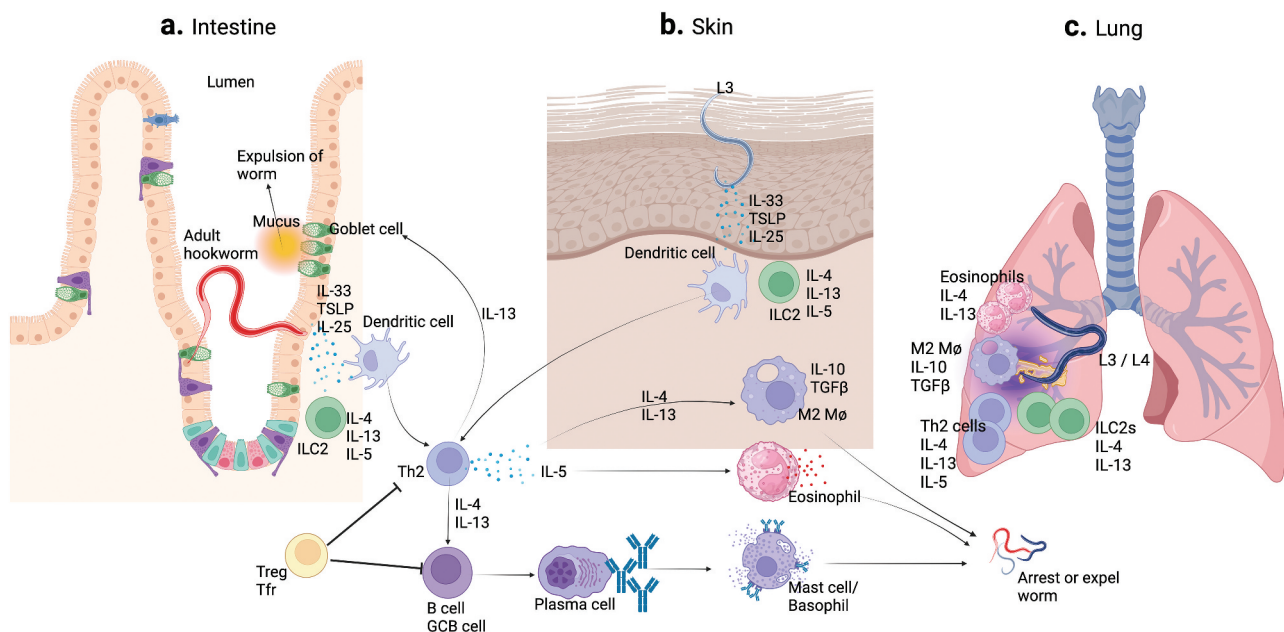
Figure 1. Organ specific immune response to hookworm infections. Hookworms damage a) intestinal and b) skin epithelia to secrete IL-33, TSLP and IL-25 alarmins that induce a local ILC2 response. Dendritic cells take up hookworm excretory/secretory products and induce differentiation of Th2 cells. IL-4 and IL-13 secreted by Th2 cells further induce activation and differentiation of B cells to IgE secreting plasma cells. IgE interacts with basophils or mast cells to stimulate degranulation. IL-13 also activates intestinal goblet cells to increase mucus secretion. Eosinophils are activated by the action of IL-5 to secrete histamine. IL-4 and IL-13 also polarize macrophages to the alternatively activated M2 type. c) The migratory L3 larval stage molts in the lung to L4, which can cause pulmonary damage. Distinct eosinophilia, M2 polarization, ILC2 and Th2 cell response is evident in the pulmonary stage of hookworm infection. Image adopted from [12,13] and created using Biorender.
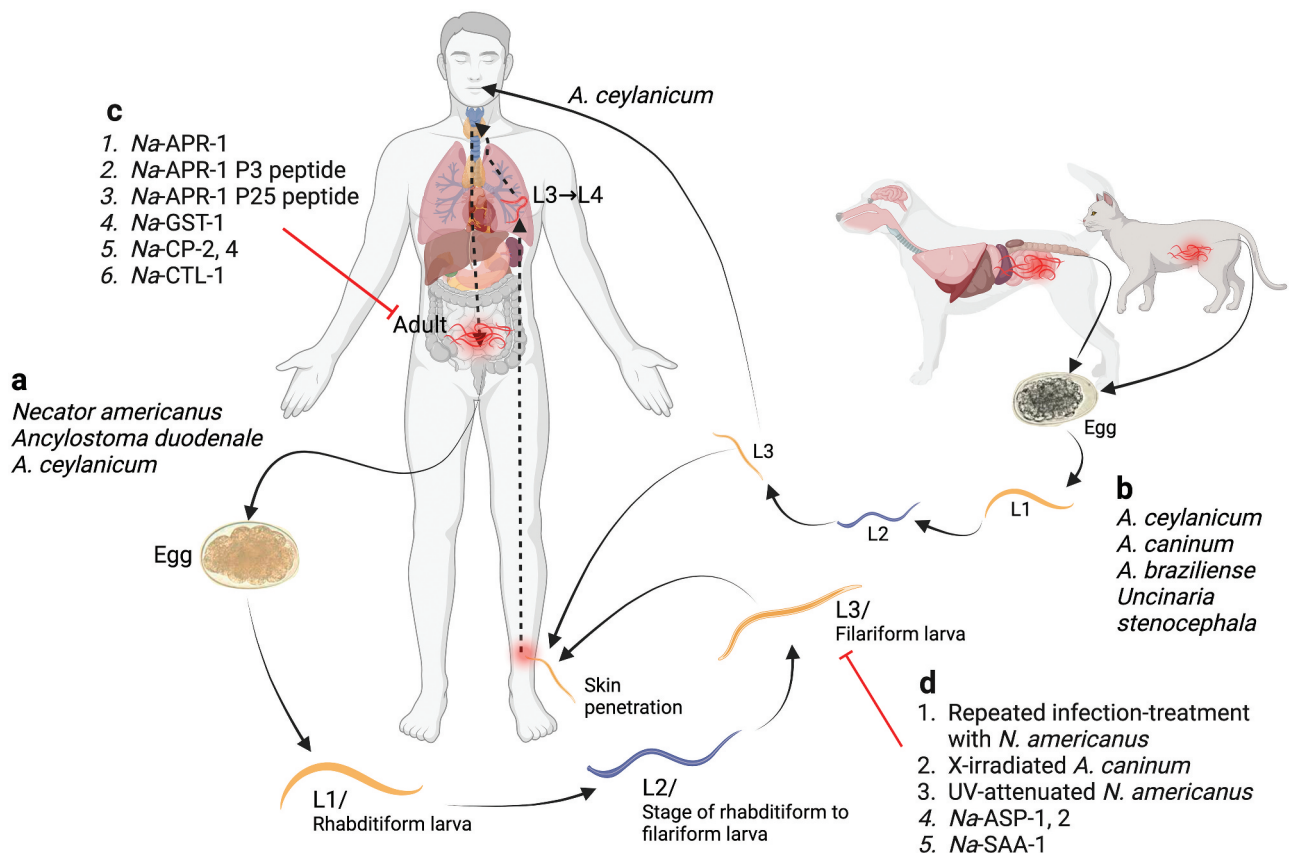
Figure 2. Lifecycle, mode of transmission and vaccine candidates of human hookworm infection. a) Human hookworms that complete lifecycle in human host; adults anchor to the small intestine and lay eggs that pass through the stool and hatch in the environment to L1 followed by L2 and L3. L3 penetrate skin, travel by blood to the lungs, then molt to L4. L4 crawl up the trachea whereupon they are coughed up and swallowed, thereby entering the gastrointestinal tract to finally take up residence as adult worms in the small intestine. *A. ceylanicum* L3 can infect by percutaneous or oral ingestion. b) Zoonotic hookworms usually undergo arrest in the skin following penetration. c) Candidates targeting the adult hookworm are mostly protein and peptide subunit vaccines. d) Infective L3 larvae are targeted using unattenuated/attenuated live helminths and protein subunit vaccines. Image created using Biorender.

2.1. Benzimidazoles

Major anthelmintics used for the treatment of hookworm infections are the benzimidazole group of compounds, such as mebendazole and albendazole. A single dose of 400 mg of albendazole or 500 mg of mebendazole is commonly used in Mass Drug Administration (MDA) campaigns. However, a 3-day treatment regimen with either drug, administered once daily with same dose, has been reported to offer better cure rates and reduced egg counts, although it is less practical for MDA programs. For uncomplicated cases, a 3-day regimen of mebendazole, with 100 mg taken twice daily 12 hours apart, is effective. Additionally, pyrantel pamoate, dosed at 11 mg/kg (up to a maximum of 1 g) daily for three days is also effective in treating hookworm infections [27].

2.2. Emodepside

Due to low efficacy of current therapies, emodepside was recently shown to be efficacious for human STH infections, including for the treatment of hookworm disease in areas where resistance to benzimidazoles, levamisole and ivermectin were documented [28]. It is a widely used veterinary drug but has been used in humans for the treatment of onchocerciasis.

2.3. Limitations of anthelmintic treatment and emerging drug resistance

Although MDA campaigns are ongoing in many hookworm-endemic countries with particular focus on school children, reinfection within six months in communities with high worm burdens occurs. Furthermore, MDA with mebendazole does not reduce the prevalence of anemia caused by hookworm infections [29]. In fact, there are inconsistencies (single dose and multiple doses) of MDA with albendazole in children [30,31]. Additionally, the Global Burden of Disease (GBD) Study 2013 reported that MDA has an insignificant effect in reducing the prevalence of hookworm infection [7]. Most importantly, MDA programs are focused on pre-school and school-aged children [32,33], leaving other high-burden groups untreated [4]. Mathematical disease modeling also suggests that these programs will only be successful upon extensive and regular treatment over an extended duration [34,35]. Moreover, people do not tend to develop sterilizing immunologic resistance to hookworm infection, and adults are a major reservoir for transmission [36], highlighting a weak point in school-aged MDA programs.

Another major concern for MDA programs is emerging benzimidazole resistance [8]. Several lines of evidence suggest that the probable mechanism of resistance of hookworms

against benzimidazoles is due to a single nucleotide polymorphism in the β-tubulin gene at codon 198 [37]. In this regard, a systematic meta-analysis demonstrated that the efficacy of single-dose oral albendazole, mebendazole, and pyrantel pamoate against hookworm infections was 72%, 15%, and 31%, respectively [38]. A multi-drug resistant *A. caninum* (resistant to albendazole, moxidectin, or a combination of febantel-pyrantelmoxidectin) was reported in greyhound dogs in the USA and is due to the presence of the F167Y (TTC>TAC) resistance polymorphism in isotype 1 of the β-tubulin gene [39]. Advanced studies to explore horizontal or vertical transmission of resistance genes and newer drugs to inhibit expression of these genes are required to mitigate hookworm infections. Although resistance to emodepside in humans has not been reported, there remains a demand for new options to treat hookworm disease.

3. The need for a hookworm vaccine

An effective vaccine against hookworm is needed to prevent moderate to heavy infections, protect against re-infection and reduce the global burden of disease, especially with high-risk populations such as children and women of childbearing age. There are several vaccine candidates in clinical assessment and/or development, including irradiated parasites and subunit vaccines (Figure 2, Table 2) [5,20,40–42]. A challenge for human hookworm vaccine development is the absence of natural development of protective immunity over time [27]. Often the oldest individuals harbor the greatest worm burdens, and although a robust cellular and humoral immune response develops over time, it appears to have little impact on clearing the infection. The immune correlates of protection against human hookworm disease therefore remain poorly elucidated [10].

4. Antigen discovery and selection

Development of an effective hookworm vaccine is a complex process and requires selection of appropriate antigens so that an effective immune response against the parasite is generated. Moreover, the selected antigens should elicit a robust protective immune response that can be sustained for long periods without causing any harmful side effects. Hookworms possess important molecules on their external surfaces and within their excretory/secretory (ES) products. ES products are secreted from the parasite's buccal capsule primarily where they are exposed to the host's immune system. ES products of hookworms consist of structurally and functionally distinct and diversified molecules such as proteins, lipids, metabolites, and carbohydrates. In terms of parasite survival, development and the intricacies of host-parasite interactions, these molecules play critical roles, including host tissue penetration and migration, nutrient acquisition, reproduction, and evasion of host immune responses. The ES proteome is therefore of interest as a source of vaccine antigens [43–46].

To properly test subunit hookworm vaccines, a benchmark vaccine against which other vaccines can be compared is required. Inactivated or attenuated vaccines are often most effective in different bacterial and viral diseases [47,48].

Irradiated hookworm L3 retain skin-penetration capability but are non-viable in the host and cannot mature to adulthood [49]. Indeed, a canine hookworm vaccine was marketed in the 1970s consisting of irradiated *A. caninum* L3 [50]. While efficacious, irradiated hookworm vaccines are sub-optimal in terms of manufacturing difficulty, short shelf life, and high cost. As a result, the emphasis has been on the discovery of ES products as subunit vaccine antigens [43,51], and more recently, proteins on the surface of extracellular vesicles (EVs) found in ES products have been highlighted as a potential pathway to target for vaccine development [52,53].

5. Hookworm vaccine experimental models

5.1. Human hookworm challenge models

Comprehensive understanding of the mechanisms of hookworm-host interactions, especially how hookworms establish in their habitat and how the host's immune system responds is essential to develop new vaccination strategies. Host specificity of hookworms, for example, *A. caninum* in dogs and *N. americanus* in humans limits the use of animal models of human infections [54]. Moreover, mouse models (using the rodent hookworm, *Nippostrongylus brasiliensis*) and hamster models (*N. americanus* and *A. ceylanicum*) have been the primary choice to study human hookworm infections. However, each of these rodent models has its limitations (see below). Therefore, the human hookworm challenge model is becoming increasingly utilized [55] and is an important step in testing any new vaccine. Experimental infection of human volunteers with up to 150 *N. americanus* infective L3 is well tolerated in healthy uninfected subjects, and the safety profile is good (Tables 1 and 2). While obtaining adult worm burdens is a challenge in experimentally infected human subjects, worms in the intestine can be visualized using capsule endoscopy, and assessment of fecal egg counts is widely considered a suitable surrogate for adult worm burdens in the gut.

5.2. Marmoset model

Marmosets are tree-dwelling non-human primates. Infection of marmosets with laboratory-adapted strains of *N. americanus* was established successfully with characteristic immunological features of hookworm infection [62]. Major immune responses that mimic human infection were observed, including increased total IgE and IgG specific to adult worm ES products, IgE-induced basophil degranulation and histamine release. This model does however pose challenges, including cost and ethical considerations of using non-human primates for research purposes.

5.3. Hamster model

Infection of laboratory hamsters with *N. americanus* or *A. ceylanicum* represents a suitable rodent model of human hookworm infections [63,64]. Although *N. americanus* can infect hamsters, the percentage of challenge larvae that mature to adulthood in the gut is very low compared to that

Table 1. Irradiated hookworm vaccine studies, vaccine efficacy, and limitations.

Vaccine antigen and formulation	Immunization schedule	Target species	Current stage of development	Major findings	Limitation	References
Irradiated Ac L3-1000	SC at day 0, 21, 42, challenge at day 56 with live Ac L3-500 by footpad infection, egg counted 3-times/week up to day 70	A. caninum	Animal trial in beagle dogs	87% reduction of eggs in feces. High anti-Ac-ASP-2-IgG1, -IgG2, -IgA, and low -IgE. Strong PBMC proliferation to crude L3 antigens, elevated IL-4. Sera from vaccinated dogs inhibited L3 skin penetration in vitro by 60%	Not an acceptable proposition for human vaccination	[56]
Live Na-L3-150	SC at day 0, challenge with live L3-150 SC at day 7, 14, 21, 42 and 70, sacrificed at day 95 or, live Na-L3-150 SC at day 0, single dose oral albendazole 100 mg/kg treatment at day 42, and challenge at day 70 and 98	N. americanus	Animal trial in golden hamsters	Vaccinated hamsters were 99-100% protected upon challenge. Anti-Na-L3-IgG developed in 7 days post L3 infection and lasted up to 10 weeks. Histology showed trapped dead larvae in skin and surrounded by inflammatory cells	Hamster model has limitations; low recovery of adult worms from high larval challenge doses	[57]
Na-L3-25/50	Skin inoculation, single dose, monitored at day 3, 7 and weekly until day 84 or day 126	N. americanus	Phase 1 human trial	Na-L3-50 induced tolerable and patent Na infection in 90% individuals until day 126 post infection, mediated by persistent eosinophilia	Efficacy of the vaccine was not assessed by challenge	[58]
Live ultra-violet C (UVC)-attenuated N. americanus L3-50	Skin inoculation at day 1, 42, challenge at day 84 with unattenuated L3-30	N. americanus	Phase 1 clinical trial	Reduced larvae in feces, mediated by eosinophilia, increased IgG response to larval extract, and greater IFNγ, TNFα, IL-2, IL-4, and IL-5 production by PBMCs	Impractical for widespread use	[21]
Na-L3-50	Skin inoculation at week 0, 3 & 6, albendazole treatment after each inoculation, challenge at weeks 13 & 15	N. americanus	Phase 1 clinical trial	Non-significant reduction of egg load, higher IgG1 titers, blood eosinophilia, and greater IFNγ concentration in serum; correlation between skin reactivity and reduced EPG	Severe skin rash in 57% infected volunteers	[22]

Abbreviations: Na: Necator americanus, Ac: Ancylostoma caninum, SC: subcutaneous, PBMC: peripheral blood mononuclear cell
Abbreviations: Na: Necator americanus, Ac: Ancylostoma caninum, Ay: A. ceylanicum, GIT: gastro-intestinal tract

Table 2. Hookworm protein vaccine candidates and mechanisms of action.

Candidate	Full name	Hookworm species	Life cycle interrupted	Mechanism of action/function	Remarks	References
irL3	Irradiated and attenuated live L3	N. americanus, A. caninum	L3	Kill L3 or arrest migration or further development by host immune response	Safe, immunogenic and protective in humans	[21,39,41]
APR-1	Aspartic protease-1	N. americanus	Adult	Digestion and detoxification of hemoglobin in GIT	Safe and immunogenic in humans, completed two phase I clinical trials	[11,58,59]
GST-1	Glutathione S-transferase-1	N. americanus	Adult	Digestion and detoxification of hemoglobin in GIT	Safe and immunogenic in humans, completed two phase I clinical trials	[9,11,60]
ASP-1, 2	Ancylostoma-secreted protein-2	N. americanus, A. caninum, A. ceylanicum	L3	Precise function not known	Immunogenic, tested in two different phase I clinical trials involving unexposed healthy volunteers and hookworm-infected subjects. ASP-2 vaccine development has been terminated due to adverse reactions.	[43,61–65]
rAceyCP1	Recombinant Ay cysteine protease	A. ceylanicum	Adult	Digestion of blood in GIT and parasite survival	Immunogenic and efficacious in hamsters	[66]
CP-2	Cysteine protease-2	A. caninum	Adult	Involved in parasite nutrition by proteolysis	Immunogenic and efficacious in dogs	[67]

seen in humans [27], and knowledge about the adaptation of *N. americanus* to this specific immune environment is not well understood [65]. Hamsters infected with *A. ceylanicum* develop anemia and weight loss, as in heavy human infections [66]. Hamsters effectively accelerate mucosal mastocytosis and generate high systemic IgG1 and IgE titers [67]. Infection also induces a strong modified Th2 response, represented by elevated IL-10, IL-4, IL-5, and IL-13 production [68]. These findings confirm that the *A. ceylanicum* hamster infection model could be useful in investigating host-parasite interactions during human hookworm infections. In fact, different hookworm vaccine candidates have been investigated in the hamster model, with some showing significant efficacy [64,69]. Nonetheless, development of natural resistance and the lack of hamster specific reagents limits the utility of this model of hookworm infection.

5.4. Rat and mouse model

N. brasiliensis is a rodent strongyle nematode that establishes natural infections in rats and displays a permissive infection in laboratory rodents with a life cycle and pathophysiology similar to that of the human hookworms – *N. americanus* and *A. duodenale* [70]. Although more prevalent in rats, *N. brasiliensis* can also infect mice [71]. Like human hookworms, *N. brasiliensis* infects rodents by the percutaneous route, travels through the circulation and lungs, then exits the circulation to creep up the trachea and is finally swallowed into the GI tract [72]. Systemic transit of the worm has enabled researchers to conduct both mucosal and systemic immunological studies [73]. More importantly, the robust modified Th2 immune response induced by *N. brasiliensis* has been extensively studied and provided insights about the cellular and effector cell mechanisms that underpin this immune response [74].

Although mice and rats can be infected with the human hookworms, they do not reach maturity and usually become arrested in the tissues following skin penetration in mice [45]. Apart from this, *N. brasiliensis* infection in rodents differs from human infections in numerous ways. After skin penetration, *N. brasiliensis* reaches the gut much more quickly (usually 7 days) than human hookworms (4–6 weeks) [70]. Moreover, Th2 cell-mediated protective immunity develops in mice after a self-cure process, and *N. brasiliensis* adult worms are expelled from the gut after 24 days [70]. Rodents develop robust and sterilizing resistance against reinfection over time, and this is not typically seen in human hookworm infections.

6. Current status and limitations of hookworm vaccines

6.1. Live hookworm vaccines

Initial studies on hookworm vaccine production revealed that live attenuated larvae provide more robust protection against infection than dead larvae [75,76]. Moreover, our research group recently performed a human phase 1 clinical trial with ultra-violet-C (UVC) attenuated *N. americanus* larvae and showed that vaccinated individuals were partially protected against challenge with non-attenuated infective stage larvae (Tables 1 and 2) [20]. A more recent study with live unattenuated *N. americanus* larvae showed a non-significant reduction of fecal egg output with repeated exposure, as well as a correlation between severe skin rash at the site of larval inoculation and reduced EPG after challenge infection [21]. Despite the promise they hold, the major limitations of live attenuated or unattenuated hookworm vaccines are their impracticalities for widespread use, limited shelf-life of the vaccines, and high cost of manufacture. Where such studies are of greatest value (in our opinion) is in the provision of antigen discovery reagents. With the advent of immunomics, recombinant proteome arrays have been produced for hookworm antigens [77] and these arrays should be screened with sera from human subjects vaccinated with irradiated larvae or repeated rounds of infection-treatment. Therefore, most studies now are focused on development of an effective protein or peptide subunit vaccine that can prevent establishment of different developmental stages within the host.

6.2. Protein subunit vaccines

Instead of live parasites, the ultimate hookworm vaccine would consist of a specific immunogenic recombinant antigen, or a combination of multiple antigens, that induces an effective immune response to prevent infection [78,79]. Recombinant vaccine antigens can be produced in the lab by expressing them in a heterologous expression system such as Chinese Hamster Ovary (CHO) cells, *Escherichia coli*, yeast, insect cells, and even transgenic plants. Although, recombinant protein subunit vaccines can be challenging to produce and to generate the desired immune response, they are an ideal choice and have been investigated by many groups [10,80–82]. Major challenges of recombinant vaccines are the requirement for an adjuvant, multiple immunizations, and consideration of post-translational modifications (phosphorylation and glycosylation), proteolytic activity, production yield, etc [79]. Of the most successful *N. americanus* recombinant vaccines, aspartic protease-1 (*Na*-APR-1) and glutathione-S-transferase-1 (*Na*-GST-1) have been most extensively studied as single antigens [9,83,84], and most recently as a co-administered vaccine [11]. The two most recent clinical trials with *Na*-APR-1 and *Na*-GST-1 co-administered in adult and school-aged children reported high levels of anti-*Na*-APR-1 IgG in adults and anti-*Na*-GST-1 IgG in children [10,11]. Co-administration of these two vaccines might therefore ensure robust antibody responses against at least one antigen through both childhood and adulthood. Despite being tested in multiple clinical trials and shown to be immunogenic, none of these hookworm subunit vaccines have yet been tested for efficacy via a challenge infection or natural exposure in a hookworm-endemic area. Table 2 and **Supplementary Table 1** describe the current vaccine candidates tested in human clinical trials and in animal models.

6.3. Peptide vaccines

With the great success of recombinant protein vaccines, several groups including ours are now investigating the development of a peptide subunit vaccine against hookworm infections [85,86]. The peptide vaccines are preferred over protein vaccines as the precise molecular composition of the peptide vaccine antigen is known, there is less risk of reversion or contamination with genomic material or infectious agents during purification, more stable and easily fit into delivery vehicles to delay antigen release in the host. However, a major risk of peptide vaccines is insufficient immunogenicity due to the small size of the immunogen. The peptide vaccine candidates studied to date are sourced from the *Na*-APR-1 protein. Two major epitope vaccine candidates studied are the B-cell epitope A291Y-GCN4 and its extended version called p3 fused to the Th-cell epitope P25. A B-cell peptide epitope A291Y-GCN4 vaccine in association with an in-built LCP (lipid core peptide)-2 adjuvant was able to induce IgG in mice that could completely inhibit the enzymatic activity of *Na*-APR-1, however, the efficacy of the vaccine was not assessed using the challenge model [87]. Other recent studies with combined p3 and P25 peptide vaccine candidates was able to reduce up to 98% of the intestinal worm burden and 99% of egg burdens following challenge with *N. brasiliensis* [85,86]. The major peptide vaccine candidates with their immunization schedules, major findings and limitations are summarized in Tables 2, 3.

Abbreviations: *Na: Necator americanus, Nb: Nippostrongylus brasiliensis*, IP: intra-peritoneal, SC: subcutaneous, APR: aspartic protease, LCP: lipid core peptide, BL10: branched polyleucine, LL10: linear polyleuc

7. Challenges of hookworm vaccines

Developing an efficacious vaccine against STH infections faces more challenges than crafting vaccines against bacteria or viruses because of the size, complexity, heterogeneity and sophistication of these eukaryotic, multicellular pathogens. Major hurdles involved in hookworm vaccine development include safety concerns (because of potential IgE responses), defining an effective immunization schedule, lack of a suitable animal model for testing immunogenicity, and route of vaccine administration to shape the immune response [90,91].

The complex lifecycles of hookworms limit our current understanding of immunological interactions between the host and parasite, as well as immune mechanisms involved in protection [90,91]. A good example is the *Na*-ASP-2 vaccine, which was considered as a promising subunit vaccine after efficacious animal studies and a successful (safe and immunogenic) phase 1 trial in a non-endemic site. Development of this vaccine, however, was terminated due to urticarial hypersensitivity reactions in vaccinated hookworm endemic participants with a preexisting IgE response from natural exposure to hookworms prior to vaccination [83]. Therefore, there is a risk of high-affinity IgE production to other hookworm vaccine candidates.

Other barriers to developing a hookworm vaccine include commercialization challenges, geopolitical barriers, and low industry interest. Moreover, the anti-vaccine movement in the US and around the world has had a significant effect on STH vaccine development [92]. Because hookworm infection is mostly found in low-income countries, there is little commercial potential for a hookworm vaccine as it would be used

Table 3. Peptide subunit vaccine studies with their efficacy and limitations.

Vaccine antigen and formulation	Immunization schedule	Target species	Current stage of development	Major findings	Limitation	References
30/60 μg *Na*-APR-1 B-cell peptide epitope A291Y-GCN4-LCP-2	IP at day 0, 21, 33, 43, culled on day 70	*N. americanus*	Animal trial in BALB/c mice	High anti-*Na*-APR-1 IgG, completely inhibited the enzymatic activity of *Na*-APR-1	Efficacy of the vaccine was not assessed by challenge	[87]
0.5 mg *Na*-APR-1 peptide p3-LCP and P25-LCP	Oral on day 0, 7, 14, 21, 28, 35, challenge at day 49 with *Nb*-L3-500 SC, culled at day 56	*N. brasiliensis*	Animal trial in BALB/c mice	Up to 98% and 99% reduction of intestinal worm and egg burdens, high serum anti-*Na*-APR-1 IgG and salivary anti-*Na*-APR-1 IgA titers	Multiple dose schedule is less practical	[85]
100 μg *Na*-APR-1 peptide p3 and P25	Oral on day 0, 7, 14, 21, 28, 35, challenge at day 49 with *Nb*-L3-500 SC, culled at day 56	*N. brasiliensis*	Animal trial in BALB/c mice	Significantly reduced worm and egg burdens, high anti-p3 and anti-*Na*-APR-1 IgG responses	Multiple dose schedule is impractical	[88]
100 μg *Na*-APR-1 p3 peptide-based vaccines: LCP, polymethylacrylate (PMA), branched polyleucine (BL10) and linear polyleucine (LL10) in PBS	Oral at day 0, 14, 28, 42, challenge at day 56 with *Nb*-L3-750 SC, culled at day 63	*N. brasiliensis*	Animal trial in BALB/c mice	BL10 and LL10 induced the highest serum anti-p3 and anti-APR-1 IgG titters and no anti-APR-IgE titers. Reduced intestinal worm burdens by 55-80% and fecal egg count by 55-85% with the highest reduction of worm burden by LL10	IgG titers were assessed from gut-associated lymphoid tissues and not in the blood	[89]
25 μg *Na*-APR-1 peptide p3-P25 in LCP, PMA, BL10, LL10 or alum/CpG	IP at day 0, 14, 28, challenge at day 42 with *Nb*-L3-750 SC, culled at day 49	*N. brasiliensis*	Animal trial in BALB/c mice	Alum/CpG adjuvanted -p3-P25, BL10 and LCP reduced worm burden by 75%, 77% and 59%. BL10 and LCP generated the highest serum anti-*Na*-APR-1 IgG and fecal anti-*Na*-APR-1 IgA titers	Duration of gut mucosal immune response not assessed; antibodies failed to neutralize activity of *Na*-APR-1	[86]

exclusively for the benefit of residents of these areas [93]. Considering the above facts, the development of a new hookworm vaccine becomes a complex and multidisciplinary task that requires an understanding of host-pathogen interactions, epidemiology, and meeting the manufacturing parameters [94].

8. Conclusion

Despite many complexities and challenges, progress has been made in developing vaccines against hookworm infections. Notably, pre-clinical vaccine studies have progressed to identifying vaccine candidates from either the dog hookworm *A. caninum* (tested in dogs), *A. ceylanicum* (tested in golden hamsters), or a laboratory strain of *N. americanus* adapted to golden hamsters. Current lead hookworm protein subunit vaccine candidates target the adult developmental stage of the hookworm, but recent clinical trials with irradiated and non-irradiated L3 have emphasized the importance of targeting larvae in the skin, and discovery of the target L3 antigens that drive these protective responses in human subjects should be a priority. That said, such screens also need to exclude IgE-inducing antigens to ensure safety in hookworm endemic populations.

9. Expert opinion

The current preventive chemotherapeutics for hookworm infection are limited to adolescents mostly. Development of an effective hookworm vaccine will potentially reduce DALYs, especially in tropical developing regions where this infection is prevalent. Combined vaccination and MDA programs will be the best strategy to prevent hookworm infection. Live-attenuated hookworm vaccines as well as repeated rounds of infection/treatment show promise, but are impractical for widespread use, therefore, an effective protein or peptide subunit vaccine will be a viable and economic solution.

The major limitations of developing a hookworm vaccine are lack of appropriate animal model, complex disease course and immune response, and lack of interest by the pharmaceutical industry in neglected tropical disease vaccines. Multiomics technologies such as proteomics and immunomics should be applied to identify protective proteins using sera from protected human subjects, such as those vaccinated with irradiated hookworm larvae. Although *Na*-APR-1 and *Na*-GST-1 and their epitopes show great promise for inducing protective immunity, additional proteins, particularly those that are important for larval penetration, migration and immune evasion are required.

While there are clinical candidate vaccines in development, these all focus on adult stage antigens, and it is unclear whether they will prove to be efficacious in humans given that challenge studies in human volunteers have yet to be conducted. Further research on identifying the best protective proteins from the two key developmental stages – adults and infective larvae – is imperative. We emphasize two key biological process that are instrumental in host-parasite interactions: (i) proteins on the external surface of hookworm extracellular vesicles are viable targets of serum antibodies and can interrupt host-parasite molecular communication; (ii) proteins secreted by infective larvae that allow them to traverse the skin and migrate to the lungs are viable targets of serum antibodies that interrupt parasite migration to the gut. A protein or peptide vaccine (ideally for oral delivery) in freeze-dried form for easy transport and storage will be a cheaper option than one which requires cold chain processes.

With the tremendous success of COVID mRNA vaccines the question now begs, would mRNA vaccines for large eukaryotic pathogens, such as hookworms be sufficiently efficacious? Indeed, a combination of a hookworm mRNA vaccine with monovalent or polyvalent vaccines targeting other pathogens is worthy of consideration to reduce cost and human intervention.

The future of hookworm vaccine development lies within sustainable protection into adulthood. The meticulous development of proteomics and immunomics technologies have paved the way to identify immunogenic and protective proteins in a high-throughput fashion. Advanced molecular tools such as single cell RNA sequencing and bioinformatics will be of tremendous help to identify T cell and B cell epitopes of hookworm protein and peptide vaccine candidates. Perhaps most importantly, integration of the excellent human challenge models that exist for hookworm infection will accelerate the up- and down-selection of vaccine candidates in a time- and co-effective manner.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Author contribution statement

E Sarkar drafted the manuscript, S Sikder and A Loukas significantly improved the writing, P Giacomin, and A Loukas contributed through rigorous review.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Brooker S, Bethony J, Hotez PJ. Human hookworm infection in the 21st century. Adv Parasitol. 2004;58:197–288.
2. Thompson RC, Reynoldson JA, Garrow SC, et al. Towards the eradication of hookworm in an isolated Australian community. Lancet. 2001;357(9258):9258:770–1.
3. CDC. Soil-Transmitted Helminths 2024 [cited 2024 Jun 13]. Available from 2024 Jun 13: https://www.cdc.gov/sth/about/hookworm.html?CDC_AAref_Val=https://www.cdc.gov/parasites/hookworm/index.html
4. Bartsch SM, Hotez PJ, Asti L, et al. The Global economic and health burden of human hookworm infection. PLoS Negl Trop Dis. 2016;10(9):e0004922.
5. Hotez PJ, Diemert D, Bacon KM, et al. The human hookworm vaccine. Vaccine. 2013;31(Suppl 2):B227–32.
6. Brooker S, Hotez PJ, Bundy DAP. Hookworm-related anaemia among pregnant women: a systematic review. PLoS Negl Trop Dis. 2008;2(9):e291.
7. Vos T. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386(9995):743–800.
8. Siwińska AM, Bąska P, Daniłowicz-Luebert E, et al. Cloning and molecular characterization of cDNAs encoding three Ancylostoma ceylanicum secreted proteins. Acta Parasitol. 2013;58(1):112–118.
9. Diemert DJ, Freire J, Valente V, et al. Safety and immunogenicity of the Na-GST-1 hookworm vaccine in Brazilian and American adults. PLoS Negl Trop Dis. 2017;11(5):e0005574.
10. Adegnika AA, de Vries SG, Zinsou FJ, et al., Safety and immunogenicity of co-administered hookworm vaccine candidates Na-GST-1 and Na-APR-1 in Gabonese adults: a randomised, controlled, double-blind, phase 1 dose-escalation trial. Lancet Infect Dis. 2021;21(2):275–285
    • **First clinical study with combined *N. americanus* APR-1 and GST-1 to assess safety and immunogenicity and observed high anti-GST-1 and anti-APR-1 IgG in serum of Gabonese adults.**
11. Zinsou JF, Diemert DJ, Dejon-Agobé JC, et al., Safety and immunogenicity of the co-administered Na-APR-1 and Na-GST-1 hookworm vaccines in school-aged children in Gabon: a randomised, controlled, observer-blind, phase 1, dose-escalation trial. Lancet Infect Dis. 2024;24(7):760–774.
    • **First clinical study with combined *N. americanus* APR-1 and GST-1 to assess safety and immunogenicity and observed in Gabonese school-aged children.**
12. Ramalingam TR, Pesce JT, Sheikh F, et al. Unique functions of the type II interleukin 4 receptor identified in mice lacking the interleukin 13 receptor α1 chain. Nat Immunol. 2008;9(1):25–33.
13. Gaze S, McSorley HJ, Daveson J, et al. Characterising the mucosal and systemic immune responses to experimental human hookworm infection. PLoS Pathog. 2012;8(2):e1002520.
14. Pritchard DI, Walsh EA. The specificity of the human IgE response to Necator americanus. Parasite Immunol. 1995;17(11):605–607.
15. Obata-Ninomiya K, Ishiwata K, Tsutsui H, et al. The skin is an important bulwark of acquired immunity against intestinal helminths. J Exp Med. 2013;210(12):2583–2595.
16. Bąska P, Norbury LJ. The role of the intestinal epithelium in the "Weep and Sweep" response during Gastro—Intestinal Helminth infections. Animals (Basel). 2022;12(2):175.
17. Imai S, Tezuka H, Fujita K. A factor of inducing IgE from a filarial parasite prevents insulin-dependent diabetes mellitus in nonobese diabetic mice. Biochem Biophys Res Commun. 2001;286(5):1051–1058.
18. Nair MG, Herbert DBR. Immune polarization by hookworms: taking cues from T helper type 2, type 2 innate lymphoid cells and alternatively activated macrophages. Immunology. 2016;148(2):115–124.
19. Chapman PR, Webster R, Giacomin P, et al., Vaccination of human participants with attenuated Necator americanus hookworm larvae and human challenge in Australia: a dose-finding study and randomised, placebo-controlled, phase 1 trial. Lancet Infect Dis. 2021;21(12):1725–1736.
    •• **First clinical study using ultra-violet C irradiated *N. americanus* L3 with challenge with unattenuated L3 reported reduced larvae in faeces and mediated by serum IgG response to L3 extracts.**
20. Hoogerwerf M-A, Janse JJ, Kuiper VP, et al., Protective efficacy of short-term infection with Necator americanus hookworm larvae in healthy volunteers in the Netherlands: a single-centre, placebo-controlled, randomised, controlled, phase 1 trial. Lancet Microbe. 2023;4(12):e1024–34.
    •• **Last clinical study with live *N. americanus* L3 with challenge with live L3 found non-significant reduction of faecal egg load with skin rush as an adverse effect.**
21. Baker JM, Trinies V, Bronzan RN, et al. The associations between water and sanitation and hookworm infection using cross-sectional data from Togo's national deworming program. PLoS Negl Trop Dis. 2018;12(3):e0006374.
22. Al-Delaimy AK, Al-Mekhlafi HM, Lim YAL, et al. Developing and evaluating health education learning package (HELP) to control soil-transmitted helminth infections among Orang Asli children in Malaysia. Parasit Vectors. 2014;7(1):416.
23. Phillips AE, Ower AK, Mekete K, et al. Association between water, sanitation, and hygiene access and the prevalence of soil-transmitted helminth and schistosome infections in Wolayita, Ethiopia. Parasit Vectors. 2022;15(1):410.
24. Hasegawa M, Pilotte N, Kikuchi M, et al. What does soil-transmitted helminth elimination look like? Results from a targeted molecular detection survey in Japan. Parasit Vectors. 2020 ;13(1):6.
25. CDC. Parasites - Zoonotic Hookworm 2020 [cited 2024 Feb 15]. Available from: https://www.cdc.gov/zoonotic-hookworm/hcp/clinical-features/?CDC_AAref_Val=https://www.cdc.gov/parasites/zoonotichookworm/gen_info/faqs.html
26. Loukas A, Hotez PJ, Diemert D, et al. Hookworm infection. Nat Rev Dis Primers. 2016;2:16088.
27. Mrimi EC, Welsche S, Ali SM, et al. Emodepside for Trichuris trichiura and hookworm infection. N Engl J Med. 2023;388(20):1863–1875.
28. Smith JL, Brooker S. Impact of hookworm infection and deworming on anaemia in non-pregnant populations: a systematic review. Trop Med Int Health. 2010;15(7):776–795.
29. Soukhathammavong PA, Sayasone S, Phongluxa K, et al. Low efficacy of single-dose albendazole and mebendazole against hookworm and effect on concomitant helminth infection in Lao PDR. PLoS Negl Trop Dis. 2012;6(1):e1417.
30. Adegnika AA, Zinsou JF, Issifou S, et al. Randomized, controlled, assessor-blind clinical trial to assess the efficacy of single- versus repeated-dose albendazole to treat Ascaris lumbricoides, Trichuris trichiura, and hookworm infection. Antimicrob Agents Chemother. 2014;58(5):2535–2540.
31. Hotez PJ, Brindley PJ, Bethony JM, et al. Helminth infections: the great neglected tropical diseases. J Clin Invest. 2008;118(4):1311–1321.
32. Knopp S, Mohammed KA, Rollinson D, et al. Changing patterns of soil-transmitted helminthiases in Zanzibar in the context of national helminth control programs. Am J Trop Med Hyg. 2009;81(6):1071–1078.
33. Dunn JC, Bettis AA, Wyine NY, et al. Soil-transmitted helminth reinfection four and six months after mass drug administration: results from the delta region of Myanmar. PLoS Negl Trop Dis. 2019;13(2):e0006591.
34. Farrell SH, Coffeng LE, Truscott JE, et al. Investigating the effectiveness of current and modified World Health Organization Guidelines for the control of soil-transmitted helminth infections. Clin Infect Dis. 2018;66(suppl_4):S253–9.
35. Zacharia F, Silvestri V, Mushi V, et al. Burden and factors associated with ongoing transmission of soil-transmitted helminths infections among the adult population: a community-based

cross-sectional survey in Muleba district, Tanzania. PLoS One. 2023;18(7):e0288936.

36. Furtado LFV, Dos Santos TR, de Oliveira V, et al. Genotypic profile of benzimidazole resistance associated with SNP F167Y in the beta-tubulin gene of *Necator americanus* helminths obtained from Brazilian populations. Infect Genet Evol. 2020;86:104594.

37. Keiser J, Utzinger J. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. Jama. 2008;299(16):1937–1948.

38. Jimenez Castro PD, Venkatesan A, Redman E, et al. Multiple drug resistance in hookworms infecting greyhound dogs in the USA. Int J Parasitol Drugs Drug Resist. 2021;17:107–117.

39. Bottazzi ME. The human hookworm vaccine: recent updates and prospects for success. J Helminthol. 2015;89(5):540–544.

40. Bethony J, Loukas A, Smout M, et al. Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. FASEB J. 2005;19(12):1743–1745.

41. Noon JB, Aroian RV. Recombinant subunit vaccines for soil-transmitted helminths. Parasitology. 2017;144(14):1845–1870.

42. Hewitson JP, Grainger JR, Maizels RM. Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. Mol Biochem Parasitol. 2009;167(1):1–11.

43. Pearson MS, Tribolet L, Cantacessi C, et al. Molecular mechanisms of hookworm disease: stealth, virulence, and vaccines. J Allergy Clin Immunol. 2012;130(1):13–21.

44. Loukas A, Prociv P. Immune responses in hookworm infections. Clin Microbiol Rev. 2001;14(4):689–703.

45. Chen CC, Louie S, McCormick BA, et al. Helminth-primed dendritic cells alter the host response to enteric bacterial infection. J Immunol. 2006;176(1):472–483.

46. Tlaxca JL, Ellis S, Remmele RL Jr. Live attenuated and inactivated viral vaccine formulation and nasal delivery: potential and challenges. Adv Drug Deliv Rev. 2015;93:56–78.

47. Minor PD. Live attenuated vaccines: historical successes and current challenges. Virology. 2015;479-480:379–392.

48. Bain RK. Irradiated vaccines for helminth control in livestock. Int J Parasitol. 1999;29(1):185–191.

49. Miller TA. Industrial development and field use of the canine hookworm vaccine. Adv Parasitol. 1978;16:333–342.

50. Harnett W. Secretory products of helminth parasites as immunomodulators. Mol Biochem Parasitol. 2014;195(2):130–136.

51. Coakley G, McCaskill JL, Borger JG, et al. Extracellular vesicles from a helminth parasite suppress macrophage activation and constitute an effective vaccine for protective immunity. Cell Rep. 2017;19(8):1545–1557.

52. Mekonnen GG, Pearson M, Loukas A, et al. Extracellular vesicles from parasitic helminths and their potential utility as vaccines. Expert Rev Vaccines. 2018;17(3):197–205.

53. Fujiwara RT, Geiger SM, Bethony J, et al. Comparative immunology of human and animal models of hookworm infection. Parasite Immunol. 2006;28(7):285–293.

54. Chapman PR, Giacomin P, Loukas A, et al. Experimental human hookworm infection: a narrative historical review. PLoS Negl Trop Dis. 2021;15(12):e0009908.

55. Diemert D, Campbell D, Brelsford J, et al. Controlled human hookworm infection: accelerating human hookworm vaccine development. Open Forum Infect Dis. 2018;5(5):ofy083.

56. Pearson MS, Bethony JM, Pickering DA, 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. 2009;23(9):3007–3019.

57. Zhan B, Perally S, Brophy PM, 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. 2010;78(4):1552–1563.

58. Goud GN, Zhan B, Ghosh K, et al. Cloning, yeast expression, isolation, and vaccine testing of recombinant *Ancylostoma*-secreted

protein (ASP)-1 and ASP-2 from *Ancylostoma ceylanicum*. J Infect Dis. 2004;189(5):919–929.

59. Loukas A, Bethony JM, Mendez S, et al. Vaccination with recombinant aspartic hemoglobinase reduces parasite load and blood loss after hookworm infection in dogs. PLoS Med. 2005;2(10):e295.

60. Mendez S, Zhan B, Goud G, et al. Effect of combining the larval antigens *Ancylostoma* secreted protein 2 (ASP-2) and metalloprotease 1 (MTP-1) in protecting hamsters against hookworm infection and disease caused by *Ancylostoma ceylanicum*. Vaccine. 2005;23(24):3123–3130.

61. Noon JB, Schwarz EM, Ostroff GR, et al. A highly expressed intestinal cysteine protease of *Ancylostoma ceylanicum* protects vaccinated hamsters from hookworm infection. PLoS Negl Trop Dis. 2019;13(4):e0007345.

62. Griffiths GD, Brown AP, Hooi DS, et al. Development of a model of hookworm infection exhibiting salient characteristics of human infection. Am J Trop Med Hyg. 2008;78(6):936–945.

63. Garside P, Behnke JM. *Ancylostoma ceylanicum* in the hamster: observations on the host-parasite relationship during primary infection. Parasitology. 1989;98(Pt 2):283–289.

64. Xue J, Zhan B, Guo J, et al. Acquired hookworm immunity in the golden hamster (*Mesocricetus auratus*) elicited by living *Necator americanus* third-stage infective larvae. Exp Parasitol. 2012;130(1):6–12.

65. Shepherd C, Wangchuk P, Loukas A. Of dogs and hookworms: man's best friend and his parasites as a model for translational biomedical research. Parasit Vectors. 2018;11(1):59.

66. Garside P, Behnke JM, Rose RA. Acquired immunity to *Ancylostoma ceylanicum* in hamsters. Parasite Immunol. 1990;12(3):247–258.

67. Behnke IM, Guest J, Rose R. Expression of acquired immunity to the hookworm *Ancylostoma ceylanicum* in hamsters. Parasite Immunol. 1997;19(7):309–318.

68. Geiger SM, Massara CL, Bethony J, et al. Cellular responses and cytokine production in post-treatment hookworm patients from an endemic area in Brazil. Clin Exp Immunol. 2004;136(2):334–340.

69. Xiao S, Zhan B, Xue J, et al. The evaluation of recombinant hookworm antigens as vaccines in hamsters (*Mesocricetus auratus*) challenged with human hookworm, *Necator americanus*. Exp Parasitol. 2008;118(1):32–40.

70. Camberis M, Le Gros G, Urban J Jr. Animal model of *Nippostrongylus brasiliensis* and H*eligmosomoides polygyrus*. Curr Protoc Immunol. 2003;19:1–27.

71. Traub RJ. *Ancylostoma ceylanicum*, a re-emerging but neglected parasitic zoonosis. Int J Parasitol. 2013;43(12–13):1009–1015.

72. Sotillo J, Sanchez-Flores A, Cantacessi C, et al. Secreted proteomes of different developmental stages of the gastrointestinal nematode *Nippostrongylus brasiliensis*. Mol Cell Proteomics. 2014;13(10):2736–2751.

73. Nair MG, Herbert DR. Immune polarization by hookworms: taking cues from T helper type 2, type 2 innate lymphoid cells and alternatively activated macrophages. Immunology. 2016;148(2):115–124.

74. Vacca F, Le Gros G. Tissue-specific immunity in helminth infections. Mucosal Immunol. 2022;15(6):1212–1223.

75. Fujiwara RT, Loukas A, Mendez S, et al. Vaccination with irradiated *Ancylostoma caninum* third stage larvae induces a Th2 protective response in dogs. Vaccine. 2006;24(4):501–509.

76. Vinayak VK, Gupta NK, Chopra AK, et al. Efficacies of vaccines against canine hookworm disease. Parasitology. 1981;82(Pt 3):375–382.

77. Tang YT, Gao X, Rosa BA, et al. Genome of the human hookworm *Necator americanus*. Nat Genet. 2014;46(3):261–269.

78. Baxter D. Active and passive immunity, vaccine types, excipients and licensing. Occup Med (Lond). 2007;57(8):552–556.

79. Nascimento IP, Leite LC. Recombinant vaccines and the development of new vaccine strategies. Braz J Med Biol Res. 2012;45(12):1102–1111.

80. Ghosh K, Hawdon J, Hotez P. Vaccination with alum-precipitated recombinant *Ancylostoma*-secreted protein 1 protects mice against

challenge infections with infective hookworm (*Ancylostoma caninum*) larvae. J Infect Dis. 1996;174(6):1380–1383.

81. Loukas A, Bethony JM, Williamson AL, et al. Vaccination of dogs with a recombinant cysteine protease from the intestine of canine hookworms diminishes the fecundity and growth of worms. J Infect Dis. 2004;189(10):1952–1961.

82. Pérez O, Batista-Duharte A, González E, et al. Human prophylactic vaccine adjuvants and their determinant role in new vaccine formulations. Braz J Med Biol Res. 2012;45(8):681–692.

83. Diemert DJ, Pinto AG, Freire J, et al. Generalized urticaria induced by the *Na*-ASP-2 hookworm vaccine: implications for the development of vaccines against helminths. J Allergy Clin Immunol. 2012;130(1):169–76.e6.

84. Diemert DJ, Zumer M, Campbell D, et al. Safety and immunogenicity of the *Na*-APR-1 hookworm vaccine in infection-naïve adults. Vaccine. 2022;40(42):6084–6092.

85. Bartlett S, Eichenberger RM, Nevagi RJ, et al. Lipopeptide-based oral vaccine against hookworm infection. J Infect Dis. 2020;221 (6):934–942.

86. Shalash AO, Becker L, Yang J, et al. Development of a peptide vaccine against hookworm infection: immunogenicity, efficacy, and immune correlates of protection. J Allergy Clin Immunol. 2022;150(1):157–69.e10.

87. Skwarczynski M, Dougall AM, Khoshnejad M, et al. Peptide-based subunit vaccine against hookworm infection. PLoS One. 2012;7(10): e46870.

88. Bartlett S, Skwarczynski M, Xie X, et al. Development of natural and unnatural amino acid delivery systems against hookworm infection. PRNANO. 2020;3:471–482.

89. Shalash AO, Becker L, Yang J, et al. Oral peptide vaccine against hookworm infection: correlation of antibody titers with protective efficacy. Vaccines (Basel). 2021 Sep 17;9:(9).

90. Knox DP, Redmond DL, Skuce PJ, et al. The contribution of molecular biology to the development of vaccines against nematode and trematode parasites of domestic ruminants. Vet Parasitol. 2001;101(3–4):311–335.

91. Brune KD, Howarth M. New routes and opportunities for modular construction of particulate vaccines: stick, click, and glue. Front Immunol. 2018;9:1432.

92. Hotez PJ. The global fight to develop antipoverty vaccines in the anti-vaccine era. Hum Vaccin Immunother. 2018;14(9):2128–2131.

93. Hotez PJ, Molyneux DH, Fenwick A, et al. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. PLoS Med. 2006;3(5):e102.

94. Cunningham AL, Garçon N, Leo O, et al. Vaccine development: from concept to early clinical testing. Vaccine. 2016;34(52):6655–6664.