Parasitology

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Advances in vaccine development for human trichuriasis

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Review

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Abstract

Trichuriasis known as whipworm infection caused by *Trichuris trichiura*, is a highly prevalent soil-transmitted helminthiasis in low- and middle-income countries located in tropical and subtropical areas and affecting approximately 360 million people. Children typically harbour the largest burden of *T. trichiura* and they are usually co-infected with other soil-transmitted helminth (STH), including *Ascaris lumbricoides* and hookworm. The consequences of trichuriasis, such as malnutrition and physical and cognitive growth restriction, lead to a massive health burden in endemic regions. Despite the implementation of mass drug administration of anthelminthic treatment to school-age children, *T. trichiura* infection remains challenging to control due to the low efficacy of current drugs as well as high rates of post-treatment re-infection. Thus, the development of a vaccine that would induce protective immunity and reduce infection rate or community faecal egg output is essential. Hurdles for human whipworm vaccine development include the lack of suitable vaccine antigen targets and animal models for human *T. trichiura* infection. Instead, rodent whipworm *T. muris* infected mouse models serve as a major surrogate for testing immunogenicity and efficacy of vaccine candidates. In this review, we summarize recent advances in animal models for *T. trichiura* antigen discovery and testing of vaccine candidates, while providing an overall view of the current status of *T. trichiura* vaccine development.

Introduction

Epidemiology

Trichuris trichiura, known as whipworm, is one of the three major soil-transmitted gastrointestinal helminths (STH, *T. trichiura*, *Ascaris lumbricoides* and *Necator americanus*/*Ancylostoma duodenale*) and causes infection in over 360 million people annually worldwide (IHME, 2019). STHs are highly prevalent neglected tropical diseases (NTDs) remaining one of the most common infectious pathogens in humans globally (Hotez, 2018*b*; Hotez *et al*., 2008*a*, 2008*b*). Together, the three major STH infections rank high among the NTDs in terms of disability adjusted life years (DALYs). The most recent projections from the Institute of Health Metrics and Evaluation (IHME) estimate that the global burden of trichuriasis in 2019 alone was 236 000 DALYs (IHME, 2019). *Trichuris* is most commonly encountered in rural subtropical and tropical areas with overlapping extreme poverty in which sanitation facilities are inadequate (Pullan *et al*., 2014; Zawawi and Else, 2020). Trichuriasis is highly prevalent in Southeast Asian nations of Myanmar, Malaysia, Philippines, Laos, and Vietnam; Nepal and Bangladesh in South Asia; Somalia and Cameroon in Africa; and Venezuela, Ecuador, and Honduras in Latin America (IHME, 2019) (Fig. 1).

Children harbour the largest number of *T. trichiura* infection (Bundy *et al*., 1987). The basis for this age-dependent predilection is not well understood, and different theories have been proposed based on biological, environmental, or socioeconomic factors. Additionally, there is increasing evidence that in utero exposure may be linked to an increased risk of acquisition of worm infections (Weatherhead and Hotez, 2015). In a St. Lucia village, about 90% of infections were diagnosed in children aged 5–15 years old. Children also harbour the highest worm burden (Fig. 2) (Bundy *et al*., 1987; Stephenson *et al*., 2000), leading to a significant impact on childhood morbidity. However, more recent analyses suggest that high level of years lost from disability (YLDs) or related morbidity metrics can extend beyond early childhood and into adolescent and young adult age cohorts (IHME, 2019) (Fig. 3). For this illness, DALYs and YLDs are considered equivalent since no deaths are currently described directly.

Parasite–host interaction

T. trichiura is transmitted through a faecal–oral cycle. The life cycle of *T. trichiura* infection starts with the passage of un-embryonated eggs in the stool. Once in the soil, the eggs embryonate and become infective after 15–30 days. After oral ingestion of the eggs from

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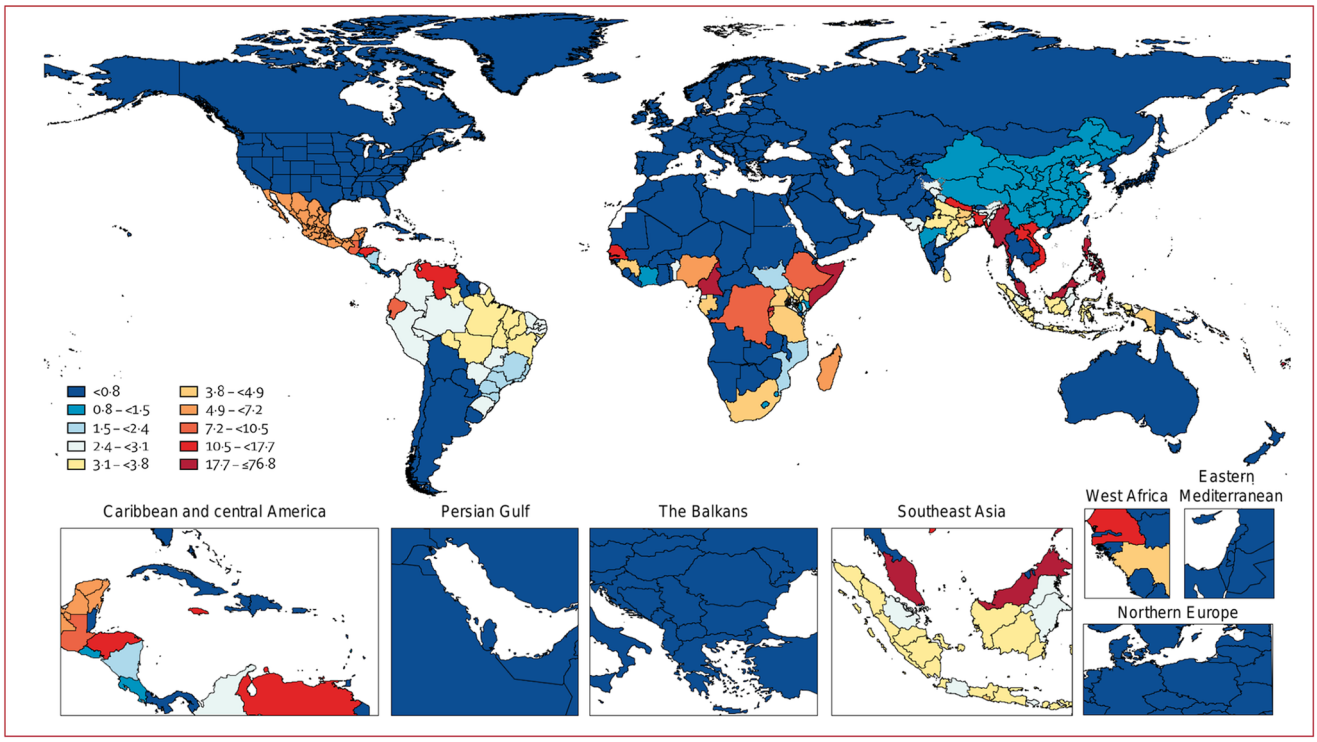

Fig. 1. Geographic distribution of trichuriasis, from the GBD 2019; Age-standardized DALY rates (per 100 000) by location, both sexes combined. 2019. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Results. Seattle, United States: Institute for Health Metrics and Evaluation (IHME), 2020. Available from http://ghdx.healthdata.org/gbd-results-tool. http://www.healthdata.org/results/gbd_summaries/2019/trichuriasis-level-4-cause.

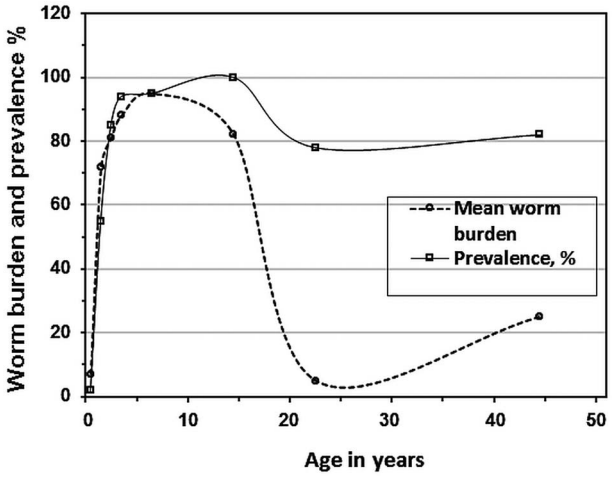

Fig. 2. Prevalence and intensity of *T. trichiura* infection by age in St. Lucia. From Stephenson *et al.* (2000), Used with permission.

environmental exposure, *via* food or hands with contaminated soil, the eggs will hatch in the small intestine. First-stage larvae (L1) are released and penetrate the intestinal epithelial cells where they will create an intracellular niche and mature through the larval (L2, L3 and L4) and adult stages. The adult worms live in the caecum and ascending colon where the females will begin to oviposit 60–70 days after infection (Stephenson *et al.*, 2000; Else *et al.*, 2020).

The adult whipworm is slender with a tapered whip-like anterior end which embeds into the mucosa of the caecum. The engagement of the anterior whip with the host intestinal mucosa forms a syncytial environment in which whipworms take mucosal cells or blood as nutrition source (Stephenson *et al.*, 2000; Else *et al.*, 2020). Once inserted into the mucosa, whipworm secrets abundant proteins in the form of excretory–secretory (ES) products. ES products aid in immunomodulation of the host immune system to facilitate their parasitism in the human body (Else *et al.*, 2020). Secretome analysis of *T. muris* adult worm ES products by mass spectrometry identified 73 unique proteins, with 62 of them sharing homology to other nematode species, revealing high secretome conservation within nematodes (Tritten *et al.*, 2017). More than 14 high confidence miRNA were also identified in the *T. muris* adult ES products that are believed to be involved in host immunomodulation (Tritten *et al.*, 2017). Beyond proteins, more than 35 non-protein small polar metabolites were found within the ES products of *T. muris* adult worms, 17 of them exhibited various pharmacological activities (Tritten *et al.*, 2017; Wangchuk *et al.*, 2019). Another proteomic analysis on *T. muris* ES products using LC-MS/MS identified 147 proteins of which most were 'trypsin-like peptidase', 'thioredoxin-like' and 'tetratricopeptide repeat domains' proteins, but also hundreds of exosome-like extracellular vesicles (EVs) (Eichenberger *et al.*, 2018). The molecular components of ES and EV are important mediators in parasite–host communication and aid in immune evasion by parasitic organisms such as *Trichuris*.

Trchuris infection induced T helper 2 (Th2) or T helper 1 (Th1) immune response in the host determines the resistance or susceptibility to the infection. Th-2 cytokines are associated with resistance to infection and rapid parasite expulsion. Cytokines that have been found to play a major role in *Trichuris* control include IL-4, IL-5, IL-9 and IL-13. Blocking IL-4 receptor during *Trichuris* infection polarizes to a Th-1 response and promotes chronic infection. On the contrary, the administration of IL-4 to susceptible mouse strains results in a predominant Th-2 response and clearance of infection (Klementowicz *et al.*, 2012; McSorley and Maizels, 2012; Briggs *et al.*, 2018). Additionally, neutralization of IL-9 using IL-9-specific antibody prevents *Trichuris* worm expulsion from the caecum (Richard *et al.*, 2000). Type 2 cytokines induce gut hypercontractility, increase mucus production and promote epithelial cell turn over which leads to rapid expulsion of parasites (Khan *et al.*, 2003; Cliffe and Grencis, 2004).

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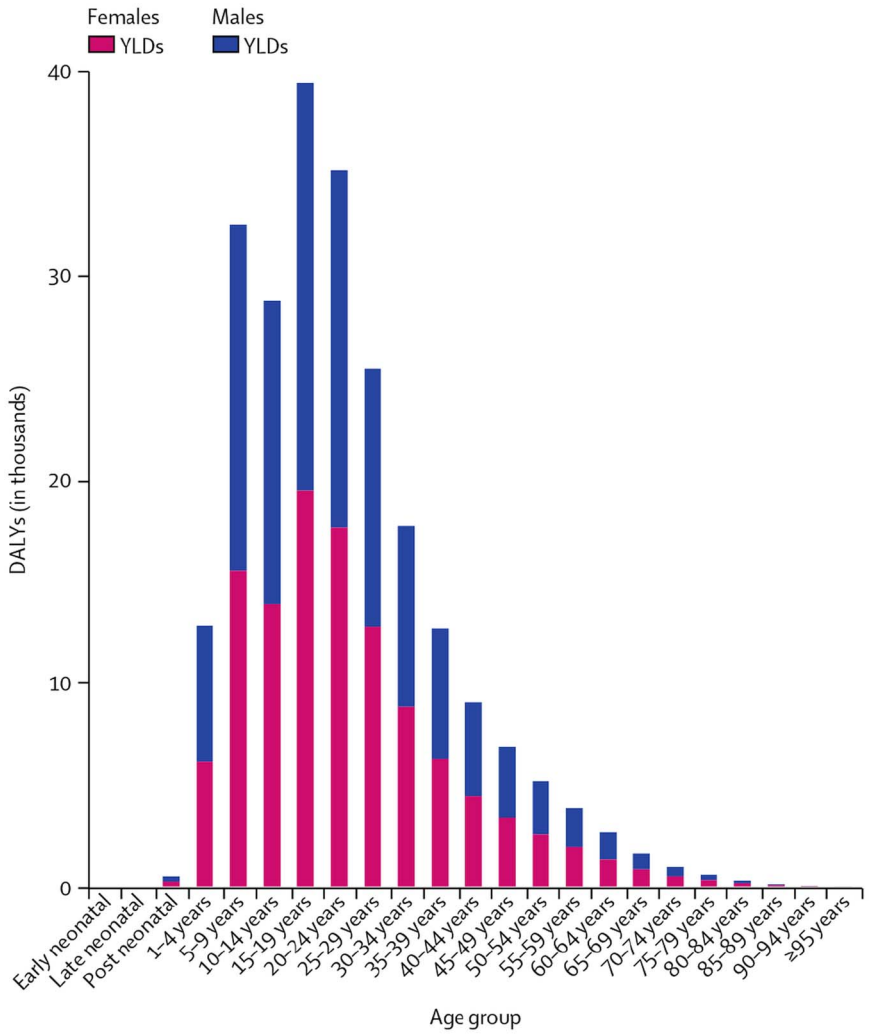

Fig. 3. Composition of years lost from disability (YLDs) by age group and sex, 2019. From Global Burden of Disease Study 2019 (GBD 2019) Results. Seattle, United States: Institute for Health Metrics and Evaluation (IHME), 2020. Available from http://ghdx.healthdata.org/gbd-results-tool; http://www.healthdata.org/results/gbd_summaries/2019/trichuriasis-level-4-cause.

However, during chronic trichuriasis, high concentrations of IFN-γ, IL-12 and IL-18, characteristic of Th1 response, have been identified. IL-18 drives suppression of IL-4 and IL-13 (Th2-related cytokines) reducing rapid parasite expulsion which makes the host more susceptible to persistent infection (Klementowicz *et al.*, 2012). But when IFN-γ is depleted there is a reduction in IL-18 which creates a more resistant immunologic profile in the host (Helmby *et al.*, 2001). Studies have shown that *T.* muris infected colonic tissues resembles mouse models of inflammatory bowel disease, with a defective epithelial barrier and a predominant Th1-related cytokines infiltrate. Mouse models of chronic trichuriasis have massive crypt hyperplasia driven by parasite-derived IFN-γ homologue (Grencis and Entwistle, 1997).

Clinical manifestations

Most infections with *T. trichiuria* are asymptomatic. The clinical symptoms usually develop with moderate to heavy infections. The most common manifestations are asthenia, abdominal pain and diarrhoea (Jourdan *et al.*, 2018). Trichuriasis has been associated with a form of inflammatory bowel disease which is linked to chronic diarrhoea and decreased nutrition intake, resulting in anaemia, physical and cognitive growth restriction in children. Heavy infections with *T. trichiura* can cause *Trichuris* dysentery syndrome (TDS) leading to severe malnutrition, bloody diarrhoea, tenesmus and rectal prolapse (Richard *et al.*, 2000; Stephenson *et al.*, 2000; Khuroo *et al.*, 2010; Weatherhead and Hotez, 2015; Zeehaida *et al.*, 2015). *T. trichiura* infection promotes poverty by impacting cognitive and physical growth, reducing educational performance and impairing economic productivity of the society further perpetuating the cycle of poverty. Trichuriasis has emerged as substantial public health problem in areas of poverty globally. Because of the significant individual and societal impact of trichuriasis, it is critical to work towards disease control and eradication.

Current management approach

Benzimidazoles (albendazole and mebendazole) are the most commonly used anthelmintic therapy for the treatment of *T. trichiura* (Keiser and Utzinger, 2008) and have been recommended by the World Health Organization (WHO) as part of mass drug administration (MDA) and preventive chemotherapy approach to control STH infections (Keiser and Utzinger, 2008). Despite highly commendable efforts led by WHO to make therapeutic drugs available to everyone at risk, in 2019 the WHO estimated that of the 613 million children who required regular deworming, only two-thirds actually received treatment. In addition, there is evidence that pregnant women do not consistently receive anthelminthic treatments even though the WHO recommends deworming after the first trimester of pregnancy in high prevalence areas (Brooker *et al.*, 2008; Hotez *et al.*, 2014).

However, preventive chemotherapy and targeted treatment strategies alone are not sufficient to achieve elimination of trichuriasis due to the following reasons:

(i) Single-dose albendazole and mebendazole is highly efficacious against *A. lumbricoides* (95.7% and 96.2%,

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respectively), however, both have poor efficacy against *T. trichiura* (30.7% and 42.1% respectively) (Keiser and Utzinger, 2008; Soukhathammavong *et al.*, 2012; McCarty *et al.*, 2014; Clarke *et al.*, 2019).

(ii) High rates of post-treatment re-infection have been observed, especially in areas of intense transmission, requiring a higher frequency of deworming (WHO, 2006; Yap *et al.*, 2013).

(iii) There are concerns about the potential development of *Trichuris* resistance to benzimidazoles as control programmes continue to be scaled up worldwide. This resistance threat has also been seen in areas of intense transmission with post-treatment infection and higher frequency of deworming (Vercruysse *et al.*, 2011; Geary, 2012; Clarke *et al.*, 2019).

(iv) Some studies have shown that drug combination are superior to single-dose albendazole, however, there are multiple operational and financial barriers that would need to be considered for large-scale deworming programme (Clarke *et al.*, 2019; Keller *et al.*, 2020).

On the basis of these limitations, the current approach to deworming using treatment alone will not lead to the elimination of STH infections. Therefore, new technologies are required (Keenan *et al.*, 2013). There is increasing concerns about the suitability of preventive chemotherapy programmes for trichuriasis that rely exclusively on monotherapy with benzimidazole anthelminthic drugs (Keller *et al.*, 2020; Patel *et al.*, 2020). In areas of Asia and Africa where ivermectin is being deployed for treatment of lymphatic filariasis or onchocerciasis, the combination of albendazole and ivermectin may offer superior results against trichuriasis, although there is recognition that we will require additional agents for the elimination of trichuriasis and other STH infections (Palmeirim *et al.*, 2018). The development of a preventive vaccine given to children before exposure to the helminths or during programmes linked to deworming (vaccine-linked chemotherapy to prevent helminth reinfection) would lead to acquisition of immunity at an earlier age and reduce community infection, representing a key technology for shaping global trichuriasis control and elimination strategies (Keenan *et al.*, 2013).

However, compared to other infectious pathogens, the vaccine development for trichuriasis has made modest progress and no human vaccine candidates have so far entered the critical path towards the clinic. A significant hurdle is the absence of an adequate animal model due to *T. trichiura* tropism for humans. Another challenge for trichuriasis vaccine development is the lack of effective antigens identified to induce protective immunity against trichuriasis. Lastly, consensus on what would be a suitable product development strategy and the minimal or preferred target product profile for such a vaccine has not been reached.

Animal model

Establishing an appropriate animal model is key for vaccine development against human whipworm. Trichuridae family are host-specific nematodes. Human whipworm *T. trichiura* predominately infects human, although have been found in the intestine of some non-human primates (NHP) (Ghai *et al.*, 2014). The *T. trichiura* collected from infected macaques, baboons and humans are morphologically indistinguishable and some studies have suggested that primate and human *T. trichiura* share the same evolutionary line. Knowing that NHP can sustain infection provides a potential animal model for human whipworm research, however, genetic analysis of these pathogens suggests the phylogenetic structure of *T. trichiura* is complicated (Foth *et al.*, 2014; Ghai *et al.*, 2014; Xie *et al.*, 2018). Nevertheless, there is no NHP model established for laboratory infection of *T. trichiura* up until now. Despite the recent advancements of NHP trichuriasis models, they largely remain impractical due to cost, availability, ethical reasons and large space required for breeding colonies (VandeBerg and Williams-Blangero, 1997).

However, human trichuriasis can be modelled using naturally occurring whipworm species in other animals, such as *T. suis* in pig (Beer, 1976) and *T. muris* in mice (Else *et al.*, 1989). Porcine *T. suis* and human *T. trichiura* are morphologically and genetically closely related (Beer, 1976) potentially leading to cross-infections between humans and pigs (Beer, 1976). However, *T. suis* fecundity in humans remains uncertain as it typically does not develop into mature adult worms in the human host (Beer, 1976; Nejsum *et al.*, 2012). However, it is possible that *T. suis* may develop to stages capable of secreting ES products that influence the host immune response which could provide insights into potential vaccine targets as well as reveal important pathogen–host interactions at the mucosal barrier (Leroux *et al.*, 2018). This concept forms the basis of several human clinical trials which used *T. suis* egg infection to treat inflammatory bowel diseases (IBD), allergic airway disease, allergic rhinitis, asthma, and multiple myeloma due to the known immunomodulatory properties of the ES products (Summers *et al.*, 2005*a*, 2005*b*; Bager *et al.*, 2010; Bourke *et al.*, 2012; Jouvin and Kinet, 2012; Bager, 2013; Voldsgaard *et al.*, 2015; Huang *et al.*, 2018). However, despite the immunomodulatory properties of *T. suis* ES products, the meta-analysis on randomized, double-blind, placebo-controlled trials of *T. suis* ova therapy (TSO) showed no statistical benefit for IBD patients (Huang *et al.*, 2018). *T. muris* is the rodent whipworm and shares a similar oral–faecal life cycle as well as extensive homology at genomic and transcriptomic levels as human *T. trichiura* (Klementowicz *et al.*, 2012). As a result of these similarities, the *T. muris* mouse model has been largely used as a surrogate for immunogenicity and efficacy models for vaccine development against human trichuriasis (Dixon *et al.*, 2008; Klementowicz *et al.*, 2012). The model was specifically used to better characterize the natural history of infection with and without inhibiting key mediators in protective immunity (Else *et al.*, 1989). Genetically altered mice have a range of *T. muris* susceptibility (Else *et al.*, 1989). Genes within the H-2 allele and some non-H-2 genes cause resistance to *T. muris* infection (Klementowicz *et al.*, 2012). Mice with H-2^q, H-2^b H-2 alleles have been found to expel parasites faster than mice having H-2^k and H-2^d alleles (Else and Wakelin, 1988). Furthermore, targeted deletion of genes related to the immunological response significantly affect parasite expulsion kinetics (Else *et al.*, 1992). As a result different genetic knockout strains implications on *T. muris* susceptibility, experimental mouse models of trichuriasis can be classified as high-responder (HR), low-responder (LR), or non-responder (NR) strains to *T. muris* infection (Else and Wakelin, 1988). HR mice such as BALB/c mainly elicit a type-2 immune response, associated with elevated IL-4 and IgG1, enhancing early expulsion of worms from the intestines prior to maturation (Patel *et al.*, 2009; Dixon *et al.*, 2010). HR strains are ideal for examining the natural protective immune response to *T. muris* infection as well as the immunological response to vaccination and post-vaccine challenge models. LR mice (such as C57BL/10 and B10.B) or NR (AKR) induce a type-1 immune response (Else *et al.*, 1994; Patel *et al.*, 2009) which allows survival of worms in the large intestine and leads to chronic patent infections. Susceptible NR strains, such as AKR, are useful in testing the efficacy of potential vaccines by measuring reduction in worm burden but are less effective in understanding vaccine-induced protective immunity (type-2 immunity) due to the polarized type-1 immune response (Else *et al.*, 1994; Robinson *et al.*, 1995; Wangchuk *et al.*, 2019). Therefore, selection of mouse strains for vaccine development studies needs to balance

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host susceptibility to infection with the development of vaccine-induced protective immunity (Robinson *et al.*, 1995).

Beside the genetic background, the sex of mouse also affects worm expulsion dynamics in the intestines, with female mice being more resistant to infection while males are more susceptible (Robinson *et al.*, 1995; Bancroft *et al.*, 2000; Klementowicz *et al.*, 2012). This sex difference susceptibility to *T. muris* infection is likely related to discrepancies in production of the type-2 cytokine IL-13 (Bancroft *et al.*, 1998, 2000) and type-1 cytokines IFN-γ or TNF-α (Hayes *et al.*, 2007; Hepworth and Grencis, 2009). Studies have revealed that male-associated dihydrotestosterone hormones inhibit dendritic cell (DC) activation of T cells and skew T cell differentiation towards Th1 response *via* IL-18-dependent mechanisms. Female-related hormones increase the generation of Th2 response leading to enhanced *Trichuris* resistance (Hepworth *et al.*, 2010). This sex-specific immune polarization to *T. muris* infection has been linked to variation in sex-specific genes. A significant quantitative trait locus (QTL) gene on chromosome 5 associated with IFN-γ production was found only in male mice. This QTL was in the same location as a QTL for TNF-α and IL-6 production in male mice suggesting a locus of pro-inflammatory cytokines in male mice compared to female mice (Hayes *et al.*, 2014).

Infectious dose can also influence the relative resistance *vs* susceptibility of mice to *T. muris* (Klementowicz *et al.*, 2012). High dose infection with 200–300 eggs triggers a type-2 immune response and early expulsion of worms from the caecum during acute infection. Acute trichuriasis is further associated with the production of IL-13, which enhances mucus production and epithelial turnover in the caecum (Bancroft *et al.*, 1998; Hasnain *et al.*, 2011; Klementowicz *et al.*, 2012) and IL-9 which induces intestinal hypercontractility (Khan *et al.*, 2003). Thus, during high burden of infection, IL-13 and IL-9 are both critical for worm eradication from the gastrointestinal track. However, a low-dose infection with 10–25 eggs stimulates a type-1 immune response associated with IFN-γ-dominated $CD4^+$ cells and subsequent chronic infection. As a result the low-dose inoculum is more reflective of natural infection (Bancroft *et al.*, 2001). Despite these differences, both high- and low-dose infection induce immunity mediated by IRF8- and IRF4-dependent dendritic cells and protect against re-infection (Bancroft *et al.*, 2001; Demiri *et al.*, 2017).

Lastly, different *T. muris* strains or isolates can affect the success of a mouse model for trichuriasis. The Edinburgh, Japan and Sobreda *T. muris* isolates consist of different molecular components in their ES products (Wakelin *et al.*, 2002) and thus induce different host immune responses affecting their susceptibility (Koyama and Ito, 1996). B10.BR, CBA and C57BL/10 mice are usually resistant to both the Edinburgh and Japan isolates, but can develop chronic infection with the Sobreda isolate. The increased infectivity of the Sobreda isolate is related to its ability to increase type-1 associated high levels of IFN-γ and Th1-associated IgG2a production while inhibiting type-2 immune responses in HR mouse strains (Bellaby *et al.*, 1996). The Sobreda isolate is also capable of inducing high concentrations of Tregs in the mouse gut potentially inhibiting the Th2-related protective immunity and promoting chronic infection (D'Elia *et al.*, 2009).

Identification of *Trichuris vaccine* candidates

Development of *Trichuris* vaccines requires the identification of antigens that induce protective immunity. However, vaccine development against *Trichuris* infection remains in the early stages without many vaccine candidate antigens identified. Most of the successes with *T. muris* have been associated with adult-stage worm extracts and stichosome-derived proteins (Table 1).

Adult worm extracts

Vaccination with whole worm extracts of *T. muris* induces a high degree of protective immunity in mice as assessed by reduction in larval worm burden (92%) (Wakelin and Selby, 1973). Extract antigens from the anterior region of the adult worms that contain a parasite organ known as the stichosome induce higher protection than antigens prepared from the posterior region, indicating antigens released by stichocytes in the secretory glands in the anterior head elicit higher protective immunity (Wakelin and Selby, 1973). Another study for mice vaccinated with whole worm extracts and stichosome extracts of adult *T. muris* also induce a high degree of protective immunity as assessed by reduction in larval worm burden further suggesting that the proteins originating from the stichosome may be strong immunogens (Jenkins and Wakelin, 1977).

Oral vaccinations with *T. muris* adult worm extracts formulated with cholera toxin adjuvant induced significant protection in both HR BALB/c and LR C57BL/10. This response was associated with *T. muris*-specific intestinal IgA expression in these mice, but was not effective in the LR B10.BR mice (Robinson *et al.*, 1995). Likewise, subcutaneous immunization with *T. muris* worm extracts formulated with Freund's adjuvant induced high level of circulating IgG1 and significant protection against subsequent *T. muris* egg challenge (Robinson *et al.*, 1995). Further proteomic and immunological analysis of *T. trichiura* adult worm extract fractions identified that a homologue of macrophage migration inhibitory factor and heat-shock protein 70 could contribute to the immunomodulatory effects on host immune responses and may be related to the protective immunity (Santos *et al.*, 2013).

Interestingly, *Trichinella spiralis* and *T. muris* that are genetically related nematodes, share cross-reactive antigen. Mice infected with each nematode or immunized with each soluble crude worm extracts elicited protective immunity against heterologous challenge infections with accelerated worm expulsion. This cross protection could also be achieved by adoptive transfer of mesenteric lymph node cells taken from mice infected with the heterologous parasite, indicating that there is a specific cross-immunity between *T. spiralis* and *T. muris* due to shared antigens (Lee *et al.*, 1982). Exploring this cross-reactivity in vaccine development may shed light on the development of a pan-helminthic vaccine strategy.

Stichosome and excretory-secretory products

Trichuroidea superfamily nematodes, including *Trichuris* sp and *Trichinella* sp, possess a unique structure of stichosome at the anterior portion of the worms, which is a longitudinally arranged cell layer called stichocytes around the oesophagus. The stichosomes or stichocytes contain secretory granules that can be secreted or released as ES products through the anterior ends of the adult worms into the colonic mucosa (*Trichuris* sp) to facilitate their parasitism in the host (Despommier and Muller, 1976; Lee *et al.*, 1982). Mice immunized subcutaneously with secretory exo-antigen extracted from stichosome of *T. muris* formulated with Freunds' adjuvant induced high levels of immunity to *T. muris* challenge (Dixon *et al.*, 2010; Briggs *et al.*, 2018). The protective immunity was dose-dependent with 1 μg of stichosome extracts reducing worm burden by 50% and 10 μg reducing by 80–90%. The major protective antigen was a protein of 30 kDa (Jenkins and Wakelin, 1983).

Trichuris adult worm secreted ES product contains a range of proteins with prominent components at 52–54, 35–45 and 17 kDa, with the most abundant protein at 43 kDa in *T. muris* or 47 kDa in *T. trichiura* (Lillywhite *et al.*, 1995). Immunological

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Table 1. Major *Trichuris* vaccine candidates discovered to date

Antigen	Stage	Function(s)	Vaccine type	Adjuvant	Imm route	Worm reduction%	Imm effector	References
Worm extracts	Adult	Various	Crude extracts	Freund's	SC	92%	Unknown	He *et al.* (2016), Wakelin and Selby (1973)
				Cholera toxin	Oral	97.8% (C57BL/10)	IgG1, IgA(mucosa), IL-5	Jenkins and Wakelin (1977)
				Freund's	SC in humanized mice	Not measured	Human IgG1, IgA, IL-5	Robinson *et al.* (1995)
ES products	Adult	Immunomodulation	Crude ES	Freund's ISA720	SC	80–97%	Th2 (IgG1, IL-5, IL-9, IL-13), Goblet/M2	Briggs *et al.* (2018), Taylor and Else (2002)
Extracellular vehicles (EVs)	Adult	Immunomodulation	Crude EVs	Freund's	SC	~50%	IgG1, IgG2a/c	Dixon *et al.* (2010)
TM43/TT47	Adult	Pore forming, IL-13 agonist	Recombinant protein	Unknown	Unknown	Similar to crude extracts	Unknown	Drake *et al.* (1994), Lillywhite *et al.* (1995)
TM-WAP49	Adult	Whey acidic protein, pore forming	Recombinant protein	ISA720	SC	48%	Th2, IL9/13	Briggs *et al.* (2018)
			Domain-GST fusion protein	ISA720	SC	33%	Th2, IL9/13	Briggs *et al.* (2018)
PP2A	Adult	Serine/threonine protein phosphatase from *A. costaricensis*	Recombinant protein	Oleic-vinyl sulfone (OVS)	Intranasal	99.01[a] egg reduction 97.90% worm reduction	Th17/Th9, CCL20 and CCL11, mucus	Gomez-Samblas *et al.* (2017)
				Bacterial wall (BW)	Intranasal	99.85% egg reduction; 59.88% worm reduction		
T-cell epitope vaccine	Unknown	MHC-II/T-cell epitopes	HBC/VLP	None	Unknown	~50%	Th2, goblet cell, IgM and IgG2c	Zawawi *et al.* (2020)

[a]add % after 99.01.

fluorescent assay showed that ES antigens were distributed in a patchy fashion throughout the cytoplasm of the stichocytes (Despommier and Muller, 1976; Briggs *et al.*, 2018). Subcutaneous immunization of ES product induced nearly complete protective immunity against challenge infection of *T. muris* infective eggs, with recovered worms significantly smaller than those from controls indicating their impact on worm maturation (Briggs *et al.*, 2018; Leroux *et al.*, 2018). The ES induced protection was associated with strong type-2 immune response (Dixon *et al.*, 2008, 2010), enhanced intestinal goblet cell hyperplasia and proliferation of M2 macrophages. However, unlike natural infection, vaccine-induced immunity did not enhance epithelial turnover rate (Dixon *et al.*, 2010). The use of ES products to induce immunity is not developmental stage dependent, however, L3 ES may contain unique antigens required for early stage maturation, could potentially aid in earlier parasitic eradication (Dixon *et al.*, 2008).

Identification of specific antigens in ES products that induce protective immunity against *Trichuris* challenge is crucial to develop vaccines against whipworm infection. Some high molecular weight antigens (80–85, 90–95, 105–110 kDa) are related to protective immunity with induction of IgG1 (Else and Wakelin, 1990). A total of 11 immunogenic proteins with vaccine potential were identified in *T. muris* ES products after selective depletion of the protein with molecular mass of 43 kDa (known as TM43) (Shears *et al.*, 2018). Using gel filtration chromatography and mass spectrometry analysis, the 11 selected proteins were able to induce antigen-specific IL-13 and IL-9 production (Shears *et al.*, 2018). These proteins, including independent phosphoglycerate mutase (iPGM), serpin and translationally controlled tumour protein (TCTP), are present in *T. trichiura* but also have been reported in vaccine studies against other parasites (Rao *et al.*, 2002; Singh *et al.*, 2014), indicating their potential as vaccine candidates (Shears *et al.*, 2018). In addition to the potent protective immunity induced by *Trichuris* ES product proteins, the extracellular vehicles (EVs) isolated from *T. muris* ES are also immunogens. C57BL/6 mice vaccinated subcutaneously with EVs isolated from *T. muris* ES without adjuvant produced more than 50% worm reduction against a low dose of *T. muris* egg (25 embryonated eggs) challenge, associated with strong IgG1 response. The protection is dependent on intact vesicles. Several immunodominant proteins within EVs, including VWD, vitellogenin N and DUF1943-domain-containing protein, vacuolar protein sorting-associated protein 52 and TSP-1 domain-containing protein, are recognized by immune sera after vaccination and subsequent infection with *T. muris*. These EV proteins have homologues in other parasites of medical and veterinary importance (Shears *et al.*, 2018). *Trichuris* worms interact with host cells and immune system through stichocytes secreted proteins, non-protein small polar metabolites or miRNA in the form of EVs, therefore these products may be important targets for developing new vaccine (Hansen *et al.*, 2015; Entwistle and Wilson, 2017; Eichenberger *et al.*, 2018; Shears *et al.*, 2018). However, it is not feasible to directly use ES and related products as vaccine to prevent *Trichuris* infection due to their difficulty in scale-up manufacture, high cost to produce enough worms in animal hosts and safety issue with complex of ES proteins. Identification of specific antigens in ES products that induce protective immunity, and large-scale production of these protective antigens as recombinant proteins or epitope-based vaccine will be an important strategy to develop vaccine against trichuriasis.

Trichuris *proteins of vaccine interest*

Tm43 and TT47

The most abundant component of *T. muris* adult ES is protein 43 kDa (TM43) (Drake *et al.*, 1994) and 47 kDa in *T. trichiura* (TT47) (Lillywhite *et al.*, 1995). TM43 is secreted by L3 and adult *T. muris* stages and detected in the mucus surrounding the worm head. Surprisingly, TM43 is produced by muscle cells beneath the cuticle in adult worms and is not a secretory product of stichocytes (Lillywhite *et al.*, 1995; Bancroft *et al.*, 2019). Further molecular cloning and genomic analysis have uncovered that TM43 is a poly-cysteine and histidine tailed protein with 36 cysteine residues, and a histidine-rich C-terminal region (Bancroft *et al.*, 2019). Similar to the *T. spiralis* homologue, TM43 may play a role in metal storage and as a transporter (Radoslavov *et al.*, 2010). Biophysical function assay have shown that TM43 protein can induce pore formation in planar phospholipid bilayers, potentially playing an additional functional role in facilitating worm to invade the host gut, establishing a syncytial environment in host caecal mucosa and promoting plasma protein leakage across the gut mucosal surface in disease pathogenesis of trichuriasis (Drake *et al.*, 1994, 1998). *In silico* docking analysis provides further insight into how this protein may interact with the host immune response. TM43 has a subdomain homologous to IL-13 receptor $\alpha 2$ suggesting its binding ability to IL-13 and inhibiting IL-13 function (Bancroft *et al.*, 2019). As IL-13 is a key effector cytokine during *T. muris* acute infection, this inhibitory binding activity to IL-13 suggests TM43 plays an important role in host immunomodulation during *Trichuris* infections, making it a potential target for vaccine development (Bancroft *et al.*, 2019). Mice immunized with HPLC-purified TM43 from ES product have comparable protective immunity to mice immunized with worm extracts (Drake *et al.*, 1994). Limitations for the use of the 43 kDa protein as a vaccine target centre around the possibility that TM43 is an IFN-γ homologue (Grencis and Entwistle, 1997), raising a concern for the potential production of auto-antibodies against host IFN-γ (Dixon *et al.*, 2008). Additionally, it was found that TM43 may not be produced by the early stages of larvae (L1 or L2) limiting its use to prevent early infection. It was also found that TM43 had overall low immunogenicity. These limitations down play this protein as an effective vaccine target (Dixon *et al.*, 2008).

Whey acidic protein

An immunodominant *T. trichiura* antigen with 50 kDa (TT50) was identified by immunological screening of a *T. trichiura* cDNA library with *T. trichiura* infection sera. Similar to TM43 or TT47, TT50 also induced pore formation in lipid bilayers, but in contrast to TM43 or TT47, TT50 contains repetitive nine four-disulphide-bonded core domains (Drake *et al.*, 1998). The four-disulphide-bonded core domain contains 50–51 amino acids with six highly conserved cysteine residues. Probing with anti-TT50 antibody recognized many bands in *T. trichiura* with high degree of cross-reactivity. Southern blot using TT55 DNA fragment recognized more than nine bands including a large gene product consisting of 904 aa and 95 kDa (TT95). Further analysis suggests TT95 may be a fusion product of two TT50 genes, therefore TT50 and TT95 are believed to be part of a four-disulphide-bonded core domain multigene family (Barker and Bundy, 1999). A multi-copy expression of this gene family and secreted property may reflect an adaptive and evolutionary response to the need for rapid synthesis of this essential protein (Barker and Bundy, 1999).

Mice immunized with *T. muris* adult worm ES products produced more than 90% worm reduction against *T. muris* infection (Briggs *et al.*, 2018). The immune sera from the protected mice immunized with *T. muris* ES products were used to immunoscreen the cDNA library of *T. muris* adult worms. A total of 102 positive clones were obtained, 63 of them encode different sizes of a four-disulphide-bonded core domain protein. A protein with 49 kDa containing seven four-disulphide-bonded core

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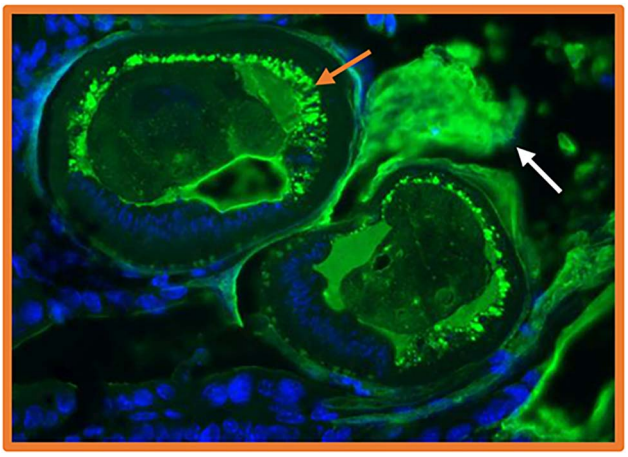

Fig. 4. Tm-WAP is located in stichosome (red arrow) of *T. muris* and secreted into the caecal lumen (white arrow) (adapted from Briggs *et al.*, 2018).

domains was cloned and expressed as a recombinant protein in yeast (Briggs *et al.*, 2018). Further structural and functional analysis identified this *T. muris* four-disulphide-bonded core domain belongs to a whey acid protein (WAP) family (Seki *et al.*, 2012; Foth *et al.*, 2014). This 49 kDa WAP protein was named as Tm-WAP49 that shares 54% and 47% amino acid sequence identity with TT95 and TT50, respectively. Native Tm-WAP49 is located in stichocytes as granules and secreted around the head of the worm embedded in caecal mucosa, assuming its potential functions *via* porin formation in the caecal epithelium (Briggs *et al.*, 2018) (Fig. 4). Tm-WAP49 likely plays an important role in the interaction with host cells and facilitates the establishment of parasitism in the host caecum. Mice immunized with yeast-expressed recombinant Tm-WAP49 protein formulated with ISA720 adjuvant produces protection of up to 48% against *T. muris* challenge infection and the protection is associated with a strong type-2 immune response including high levels of IL-4, IL-9, IL-13 and Th2-related IgG1 production. Mice immunized with only one four-disulphide-bonded core domain (50 amino acid) of Tm-WAP49 fused with *Na*-GST-1, a leading vaccine candidate for hookworm *N. americanus* (Zhan *et al.*, 2010), also produces 33% protection from *Trichuris* challenge (Briggs *et al.*, 2018). Fused with *Na*-GST-1 is to increase the immunogenicity of the 50 amino acid WAP domain, also to make the fusion possible as a bivalent vaccine against both trichuriasis and hookworm infection. Tm-WAP49 is highly immunodominant and homologues are found in *T. trichiura* (TT50 or TT95), making Tm-WAP49 a leading vaccine candidate against whipworm infection.

Protein serine/threonine protein phosphatase 2a (PP2A)

PP2A catalyses the dephosphorylation of phosphoserine/phosphothreonine side chains of proteins involved in many biochemical and cellular processes such as cell motility, embryogenesis, and differentiation and is highly conserved in nematodes (Janssens and Goris, 2001; Gomez-Samblas *et al.*, 2017; Briggs *et al.*, 2018). Immunoscreening a cDNA library of *Angiostrongylus costaricensis* adult worms with a pool of sera from patients with abdominal angiostrongylosis identified a positive clone encoding for the catalytic subunit of the serine/threonine protein phosphatase 2A (AcPP2A). Mice immunized with the recombinant catalytic region of AcPP2A provided complete protection against *A. costaricensis* challenge associated with increased levels of IFN-γ and IL-17 (Solano-Parada *et al.*, 2010). Importantly, significant cross protection was induced in lambs immunized intranasally with AcPP2A against two other intestinal nematodes, *Haemonchus contortus* and *Teladorsagia circumcincta*, challenge infections (Mohamed Fawzi *et al.*, 2013), suggesting mucosal immunization in nasal synergistically stimulates protective immunity in intestinal mucosa (Rhee *et al.*, 2012; Mohamed Fawzi *et al.*, 2013).

Amino acid sequence alignment confirms the catalytic region of PP2A from *A. costaricensis* (AcPP2A) shares high homology with those from whipworms (*T. trichiura, T. suis* and *T. muris*) (Gomez-Samblas *et al.*, 2017). AKR mice intranasally immunized with recombinant AcPP2A formulated with synthetic self-adjuvant oleic-vinyl sulphone (OVS) or with bacterial walls (BW) resulted in high faecal egg reduction (99.01% and 99.85%) and high reduction in adult worms collected from caecum (97.90% and 59.88%) against subsequent *T. muris* challenge. This protection was associated with the stimulation of a Th17/Th9 response and high levels of mucus secretion (Gomez-Samblas *et al.*, 2017). CCL20 and CCL11 chemokines are also highly elevated and serve as potent chemoattractants of effector immune cells and stimulators of goblet cells hyperplasia (Williams, 2006).

Macrophage migration inhibitory factor

Trichuris secreted ES products contain MIF that inhibits the migration of lymphocytes to tissue in a dose-dependent manner (Gaherwal and Prakash, 2011). Worm-secreted MIF is one of the immunomodulatory proteins that inhibits macrophage or other effector immune cells from migrating to the site of parasite infection (James and Nacy, 1993). Additionally, other parasitic MIF such as TsMIF cloned from *T. spiralis*, have structural, catalytic and cell-migration-inhibitory properties similar to mammalian MIF (Tan *et al.*, 2001). Mice immunized with a DNA vaccine with co-expression of TsMIF and *T. spiralis* cystatin-like domain protein (TsMCD-1) elicited 37.9% reduction of worm burden against *T. spiralis* larval challenge associated with specific type-1 immune responses such as increased IFN-γ and CD4+ and CD8+ T cells (Tang *et al.*, 2012, 2013). The homologue of MIF was also identified in human *T. trichiura* with 46% sequence identical to human MIT (Tan *et al.*, 2001). Due to the inhibition function for host immune effector cells and the vaccine efficacy in genetically related *T. spiralis*, MIF in *Trichuris* may likely be a good vaccine target.

T-cell epitope/VLP vaccine

Histocompatibility complex class II (MHC-II) T-cell epitopes were predicted from predicted ORFs in the *Trichuris* genome using *in silico* prediction tools. The coding proteins containing strong MHC-II T-cell epitopes were down-selected using criteria of containing signal peptide but without transmembrane domain, no mouse or human homology, no allergenic potential and with highest predicted solubility in virus-like particles (VLPs). Based on these criteria total four MHC-II T-cell epitopes with potential as vaccine candidates were selected from chymotrypsin-like serine protease and chitin-binding domain containing protein. These epitopes were incorporated into Hepatitis B core antigen virus-like particles (VLPs), which can be taken up and processed by antigen-presenting cells such as macrophages and dendritic cells. Mice immunized with pre-mixed four VLPs expressing each *Trichuris* T-cell epitope subcutaneously without adjuvant elicited significant protection against *T. muris* challenge (about 50% adult worm reduction). Additionally vaccinated mice had heightened production of type-2 cytokines produced by mesenteric lymph node, goblet cell hyperplasia, as well as high titres of serological IgM and IgG2c (Zawawi *et al.*, 2020). The epitope/VLP vaccine-based novel genomic and bioinformatic technologies provide a new era for vaccine design with integration of multiple vaccine candidates.

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Tm16

Tm16 is one of 20 immunodominant antigens identified in *T. muris* adult worm ES products using 2D-gel/Western blot and mass spectrometry screening protocols (Liu *et al.*, 2017). The crystal structure of Tm16 indicates Tm16 belongs to the phosphatidylethanolamine-binding-like protein family, possibly involved in regulatory functions (He *et al.*, 2016). Given that Tm16 is one of the immunodominant *T. muris* secreted proteins that induce protective immunity in immunized mice, and a homolog exists in human *T. trichiura*, Tm16 is putative vaccine candidate for preventing *Trichuris* infection; however more data are needed to determine the *in vivo* outcomes of Tm16 vaccines.

Future directions: from antigens to vaccines

Despite the promise of existing antigens so far, reverse vaccinology approaches have not been rigorously applied to *Trichuris* parasites, so that a multicentred bioinformatics initiative in this regard could potentially identify more suitable antigens in terms of their protective efficacy or durability of protection (Hotez, 2018*b*). Further, upon selection of antigens that are most effective in mouse or other animal model preclinical studies there are multiple steps required before they can be considered suitable for clinical trials. Some of these critical pathways were outlined earlier, but there still is a lack of consensus of what is the ideal product development strategy and a minimal or preferred target product profile (Diemert *et al.*, 2018). For example, within the product strategy, an important gap is the selection of suitable adjuvants. Because many of the protective immune responses to selected antigens rely on Th2 humoral immunity, it is likely that alum formulations may be suitable as they have been for experimental human hookworm vaccines (Adegnika *et al.*, 2021). Based on the hookworm experience, second immunostimulants will likely also be required, such as synthetic Toll-like receptor-4 (TLR-4) agonists or oligonucleotide CpG molecules (Bottazzi, 2015). The epidemiologic modelling assessments are also needed to determine how a trichuriasis vaccine will fit into ongoing deworming and preventive chemotherapy programmes, whether a vaccine specific for trichuriasis will be cost effective when linked to deworming or whether it will be necessary to combine *Trichuris* antigens with hookworm and *Ascaris* antigens in a pan-anthelminthic approach as outlined previously (Zhan *et al.*, 2014; Bartsch *et al.*, 2016). Still another consideration is whether recombinant proteins are best suited for polyvalent vaccine approaches given the cost and complexity of testing multiple candidates, *vs* employing one of the newer mRNA vaccine platform approaches that are evaluated for some parasitic infections (Versteeg *et al.*, 2019). The concurrent success of COVID-19 mRNA vaccine has provided proof-of-concept for the suitability of this approach in North America and Europe (Polack *et al.*, 2020), but this approach has not yet been shown to be suitable to be applied widely in low- and middle-income countries (LMICs). Finally, there is an urgent need to shape sustainable financing for the advancement of anthelminthic vaccines and these mechanisms do not yet exist.

Conclusions and challenges

Trichuriasis remains a global threat in poverty-stricken areas around the world, leading to significant morbidity and long-term social and economic consequences. Despite targeted efforts at disease control through mass drug administration policies, elimination remains elusive due to high rates of re-infection and poor efficacy of anthelminthic drugs. As a result, there is significant urgency to develop a preventive and/or therapeutic vaccine to aid *Trichuris* control efforts.

A trichuriasis vaccine and related technologies offers the potential promise of parasite elimination. However, as outlined above, future directions for *Trichuris* vaccine development will need to focus on (1) identifying homologous vaccine targets across helminth species for the development of panhelminthic vaccines, (2) imploring new technologies, including mRNA approaches, to advance vaccine development for trichuriasis, (3) epidemiologic models to confirm the benefit of vaccines linked to preventive chemotherapy and (4) reaching a clear consensus of what is a suitable product development strategy, what is the preferred target product profile and what would be the financing mechanisms to bring such a vaccine towards licensure.

The global policymakers have not widely accepted the urgency or potential benefits of anti-parasitic disease vaccines in lieu of less expensive but not necessarily cost-effective mass treatment interventions. This situation partly stems from first-generation efforts to develop anti-parasitic disease vaccines that did not benefit from safer and more effective adjuvants and immunostimulants, nor increasing sophistications in epidemiologic modelling. Since then, both fields have advanced and could be leveraged for a new generation of vaccines for parasitic infections, which are sometimes referred to as 'antipoverty' vaccines for their potential impact on promoting both public health and economic development (Hotez, 2018*a*).

For this to occur the essence of global health policy must shift as it pertains to neglected tropical diseases (NTDs). There must be recognition that trichuriasis and related STH infections or NTDs would benefit from vaccines, as well as new drugs and diagnostics, just as HIV/AIDS, malaria, and tuberculosis (Hotez, 2018*a*). With regards to the STH infections, such recognition should not have to await the results of a Deworm3 initiative that seeks to determine the feasibility of helminth elimination through community-based preventive chemotherapy. In our view, doing so represents a double standard that we would never consider for populations in North America and Europe. The world's poorest people in LMICs deserve the fundamental right of access to innovation as represented by vaccines to combat trichuriasis and other helminth infections (Hotez, 2019).

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