Hookworm infection

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Abstract | Hookworms are soil-transmitted nematode parasites that can reside for many years in the small intestine of their human hosts; *Necator americanus* is the predominant infecting species. Adult worms feed on the blood of a host and can cause iron deficiency anaemia, especially in high-risk populations (children and women of childbearing age). Almost 500 million people in developing tropical countries are infected, and simulation models estimate that hookworm infection is responsible for >4 million disability-adjusted life years lost annually. Humans mount an immune response to hookworms, but it is mostly unsuccessful at removing adult worms from the bowel. Accordingly, the host switches to an immune-tolerant state that enables hookworms to reside in the gut for many years. Although anthelmintic drugs are available and widely used, their efficacy varies and the drugs do not prevent reinfection. Thus, other control strategies aimed at improving water quality, sanitation and hygiene are needed. In addition, efforts are underway to develop a human hookworm vaccine through public–private partnerships. However, hookworms could also be a resource; as hookworms have the capability to regulate the host's inflammation, researchers are experimentally infecting patients to treat some inflammatory diseases as an approach to discover new anti-inflammatory molecules. This area of endeavour might well yield new biotherapeutics for autoimmune and allergic diseases.

Hookworms are soil-transmitted nematode parasites that can reside for many years in the small intestine of their human hosts, where they suck blood and can cause iron deficiency anaemia (IDA) in individuals who harbour moderate and high numbers of adult worms, which is known as hookworm disease. Hookworm infection affects almost 500 million people in the tropical regions of the world, accounting for 3.2 million disability-adjusted life years (DALYs) lost annually and ranking among the most important of the neglected tropical diseases in terms of causes of morbidity[1]. Alternative and newer estimates indicate that hookworm infection results in 4.1 million DALYs lost, as well as possibly over US$100 billion in global economic losses[2]. Indeed, hookworm infection, of which *Necator americanus* infection is the predominant human disease, has had a major influence on human history; in the early 1900s, hookworm infection was recognized as a major cause of anaemia and lost productivity in the southern United States. This understanding resulted in the formation of the Rockefeller-funded Human Hookworm Eradication Campaign[3], which is the first example of modern-day public health philanthropy (the original 1920 silent film can be viewed online[4]). Although hookworm infection has mostly been eliminated through economic development (rather than mass treatment) in western European countries, the United States, South Korea and Japan, the disease burden remains unacceptably high in many low-income and middle-income countries, despite implementation of mass drug administration (MDA) programmes[5]. Safe drugs are available to combat human hookworm infection, but their efficacies are variable to the point that some MDA campaigns are rendered ineffective owing to outright drug failure[6,7]. Moreover, in the absence of a protective immune response, reinfection can rapidly occur, sometimes within 4–6 months in areas of high transmission[8].

Although hookworms are clearly a major human pathogen, recent investigations into the immunobiology of hookworm infection have highlighted the 'Jekyll and Hyde' nature of this parasite. Indeed, the ability of hookworms and other helminths to modulate inflammation is so effective that some researchers are intentionally infecting human participants with low subclinical doses of larvae to treat a range of inflammatory diseases[9–12], spurring discovery efforts to mine hookworm genomes and proteomes for new macromolecules to be used in the treatment of some autoimmune and allergic disorders that plague industrialized countries.

In this Primer, we review the current state of the field in hookworm research, focusing on the epidemiology; pathobiology and immunobiology; methods of diagnosis, screening and prevention; and management of hookworm infection and disease. We also provide a final

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outlook, in which we speculate on future directions for a hookworm research agenda, emphasizing the expanding efforts in improving control strategies and exploiting the therapeutic properties of hookworms for treating inflammatory diseases.

Epidemiology

Global burden of disease

Prevalence. The most prevalent species of soil-transmitted helminths (STHs) are *Ascaris lumbricoides*, *Trichuris trichiura* and the three main hookworm species that infect humans (*N. americanus*, *Ancylostoma duodenale* and *Ancylostoma ceylanicum*). *N. americanus* is the predominant human hookworm that globally accounts for the majority of human hookworm cases; it is especially common in southern China, Southeast Asia, the Americas and most of Africa. *A. duodenale* is more focally endemic in the Mediterranean region, in northern regions of India and China and in North Africa. In some parts of Africa, China, India and elsewhere, it is not uncommon to find mixed human infections with *N. americanus* and *A. duodenale*. A third minor species, *A. ceylanicum*, which was thought to be primarily a canine parasite, has recently been identified as a highly prevalent species of hookworm in humans in a few focal regions in Southeast Asia, such as Malaysia and Indonesia, where it is also often co-endemic with *N. americanus*[13,14].

Climate and soil structure are crucial determinants of hookworm prevalence, as the parasite thrives in tropical and subtropical zones, where moisture and temperature are ideal for larval development outside the host. The different distribution of the various hookworm species is not absolute, with mixed infections often occurring in individuals. An estimated 438.9 million people (95% credible interval: 406.3–480.2 million) were infected with hookworm in 2010 (REF. 1), with the largest concentration of hookworm cases in Southeast Asia, followed by sub-Saharan Africa (FIG. 1). Hookworm infection tends to be more prevalent in rural areas, where the favourable tropical or subtropical ecologies converge with poverty and weak sanitary infrastructures. The decline in the prevalence of hookworm infection in the tropical regions of middle-income countries, such as China, was primarily achieved owing to newly urbanized economies, rapid economic development (especially in eastern China) and, in some areas, wide distribution of anthelmintic drugs. These advances were also the crucial factors in decreasing the prevalence of hookworm infection in the southern United States during the early twentieth century[15].

Morbidity and mortality. The prevalence of hookworm infection does not reflect its morbidity, which is directly correlated with the intensity of hookworm infection, generally expressed in eggs per gram of faeces (EPG)[16]. The WHO categorizes hookworm infections as light (≤1,999 EPG), moderate (2,000–3,999 EPG) and heavy (≥4,000 EPG)[17]. However, heavy infections are more likely to occur in areas that also exhibit high hookworm prevalence. Adult hookworms that feed in the host's gut cause blood loss, which can lead to IDA and hookworm disease. Hence, the greater the worm burden, the greater the blood loss. The extent of blood loss also depends on the species of infecting hookworm; *A. duodenale* is thought to be a wasteful feeder (not all the blood it ingests is digested) and is responsible for blood loss that is as much as 10-fold heavier than that caused by *N. americanus*[18]. Moreover, *A. duodenale* infection is associated with increased prevalence of IDA compared with *N. americanus* infection[19]. However, as *N. americanus* globally is the predominant hookworm on a population basis, it remains the leading cause of hookworm disease.

It is difficult to estimate the burden of disease from hookworm infection; the most common complications of this disease (IDA and poor birth outcomes, such as reduced birth weight and increased infant mortality) are often underreported and, when they are, they are not specifically associated with hookworm infection. Moreover, as hookworm infection often occurs in geographical areas where other conditions that are considered of greater public health importance, such as malaria and malnutrition[20], are endemic, the morbidity from hookworm infection can be overlooked or even attributed to these conditions. The 2013 Global Burden of Disease Study indicated that IDA is a major cause of mortality, resulting in approximately 180,000 deaths worldwide[21]. However, this study does not ascribe any of this mortality to hookworm infection, although it does indicate that a substantial percentage of IDA in Africa and Oceania is due to hookworm infection[22,23]. More recently, hookworm disease was uncoupled from general IDA and shown to be second only to malaria as an underlying cause of global anaemia caused by parasitic infections[23]. Hookworm infection is a substantial cause of IDA in Oceania, tropical Latin America, southern sub-Saharan Africa and Southeast Asia in particular (FIG. 1).

At-risk populations

In most protozoan and helminth parasitic infections, prevalence peaks in childhood. However, the age-specific epidemiology of hookworm infection is different: although prevalence is high among children, it increases

until it plateaus in adulthood[24]. Studies from China and Brazil show an increase from approximately 15% at 10 years of age to approximately 60% at 70 years of age[25].

Moderate or heavy infections can manifest as IDA (reviewed in REF. 26) in populations with low underlying iron reserves, such as children and women of childbearing age. Evidence strongly links chronic IDA in children with hookworm infection[27] and IDA can result in long-term poor health outcomes, including reduced cognitive, intellectual and physical development[28], reduced fertility among women and even reduced future wage-earning[29]. Patients with heavy infections often present with a microcytic hypochromic anaemia, which, in children, can lead to stunted growth, which is especially noticeable around puberty when infected adolescents fail to achieve their expected growth spurt. Intellectual and cognitive delays are also possible, but these can be subtle and difficult to measure[30,31]. For example, hookworm infection in children 6–11 years of age in Brazil was associated with reduced concentration and information processing skills[32].

Women of childbearing age are also at greater risk of hookworm disease, which can be worsened by a combination of low dietary iron intake in resource-poor settings and blood loss either from menstruation or pregnancy (owing to the iron demands of the fetus). Pregnant women with hookworm disease — notably, across Africa, where more than one-quarter of pregnant women are infected[33] — are at risk of higher maternal and neonatal issues (including maternal anaemia, low birth weight and infant mortality[34]), particularly when hookworm and malaria co-infections occur. Overall, it is estimated that 44 million pregnancies globally are complicated by hookworm infection[35]. For these reasons, women of childbearing age and children have been identified by the WHO as being at special risk for STH infections, but especially hookworm infection[36,37].

Mechanisms/pathophysiology

Host invasion

Of the stages of the hookworm life cycle (FIGS 2,3), third-stage larvae (L3) can infect the human host through percutaneous invasion (*N. americanus* and *A. duodenale*) or oral ingestion (*A. duodenale*). L3 are approximately 0.5–0.6 mm in length, and although initial skin invasion might go unnoticed, it can result in a local pruritic, erythematous rash (FIG. 3a), referred to as 'ground itch'. Ground itch typically appears on the hands and feet, which are the main sites of L3 percutaneous entry (via hair follicles, which are the path of least resistance).

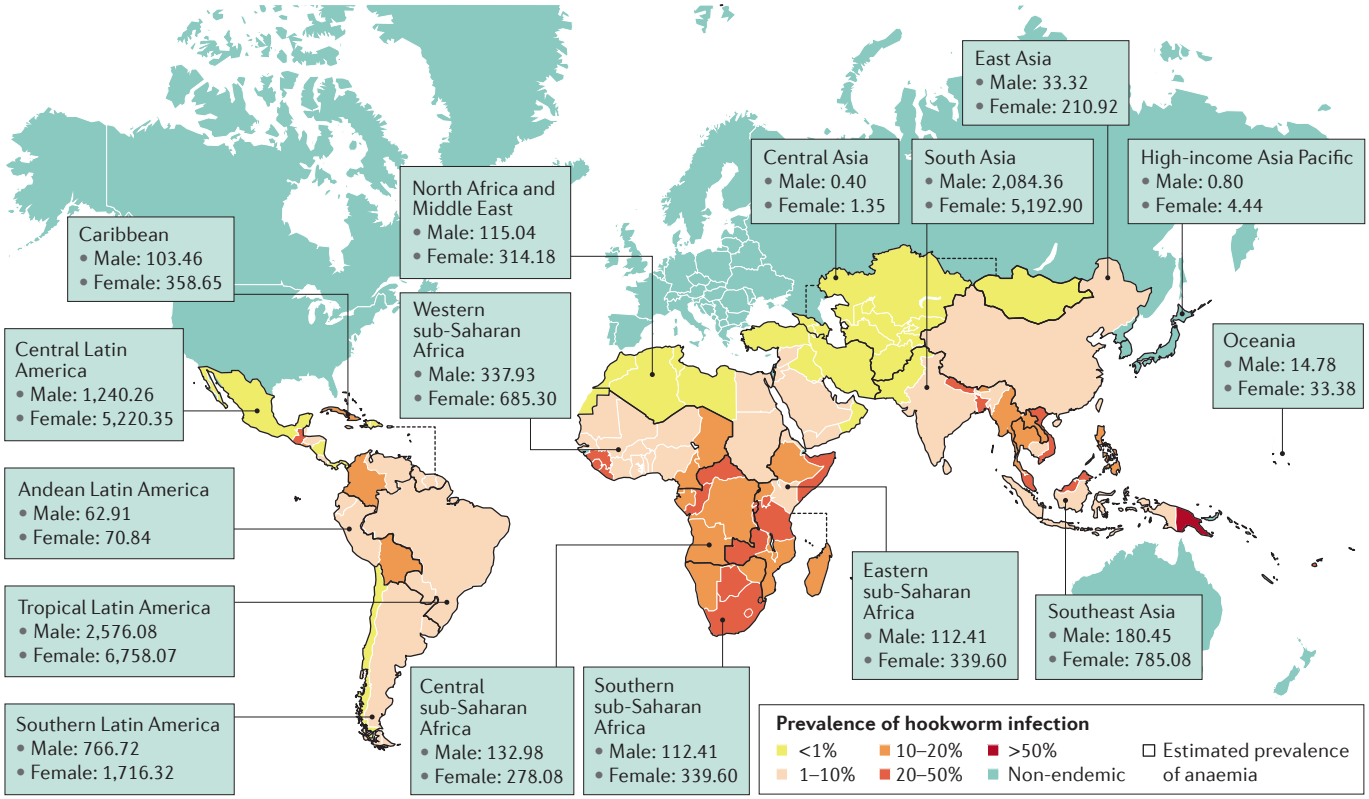

Figure 1 | **Distribution of hookworm infection and estimated prevalence of anaemia due to hookworm infection.** The prevalence data are for overall hookworm infection, regardless of the parasite species. In 2010, 140 million individuals in South Asia were estimated to be infected with hookworms, 117 million in sub-Saharan Africa, 77 million in Southeast Asia, 64 million in East Asia, 30 million in Latin America and the Caribbean, 10 million in Oceania and 4.6 million in the Middle East and North Africa. Oceania had the highest prevalence (49%), followed by sub-Saharan Africa (13%), Southeast Asia (12.6%), South Asia (8.6%), East Asia (5%), and Latin America and the Caribbean (5%)[1]. The regions delimited by black borders (the Global Burden of Disease world regions) show the estimated prevalence rate of anaemia per 100,000 population due to hookworm infection. Prevalence data from REF. 1, and based on geostatistical models for sub-Saharan Africa and available empirical information for all other regions. The anaemia data are courtesy of N. Kassebaum, University of Washington, Seattle, Washington, USA.

Upon penetrating human tissues and in response to host signals, such as insulin signalling molecules[38], the large secretory glands of infective L3 release macromolecules that facilitate parasite entry and invasion[39], including copious amounts of hydrolytic enzymes, such as proteases and hyaluronidases[40,41]. Prominent among the larval secretions is a family of cysteine-rich proteins that belong to the CAP family (cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins), which are often referred to as *Ancylostoma*-secreted proteins (ASPs)[42]. ASPs are crucial in the developmental stages of the parasite and represent roughly one-third of all secreted proteins[43]; the *N. americanus* genome contains 137 different genes encoding distinct ASPs[44].

At least one of these L3-secreted molecules, ASP-2, has been shown to be allergenic[45] and might explain why repeated hookworm infections produce ground itch and other forms of dermatitis, as well as hookworm pneumonitis and Wakana disease[26]. Once the L3 reach the bloodstream through skin capillaries, they begin the journey that eventually leads them to the small bowel (FIG. 3b), where they use their cutting plates (or 'teeth', as is the case for *Ancylostoma* spp.; FIG. 3c) to attach to the mucosa and start feeding.

Pathophysiology

Blood loss. The mechanisms by which hookworms induce blood loss are multifactorial. Substantial blood loss occurs as a direct result of actively feeding adult worms. The host's erythrocytes are digested in the gastrointestinal tract of the hookworm by a suite of mechanistically distinct proteolytic enzymes[46]. Haemoglobin is cleaved by proteases, and the by-product of this process, haem, is detoxified by the glutathione *S*-transferase GST-1 (REF. 47). However, most of the blood loss is a result of leakage around the attachment site of the hookworm

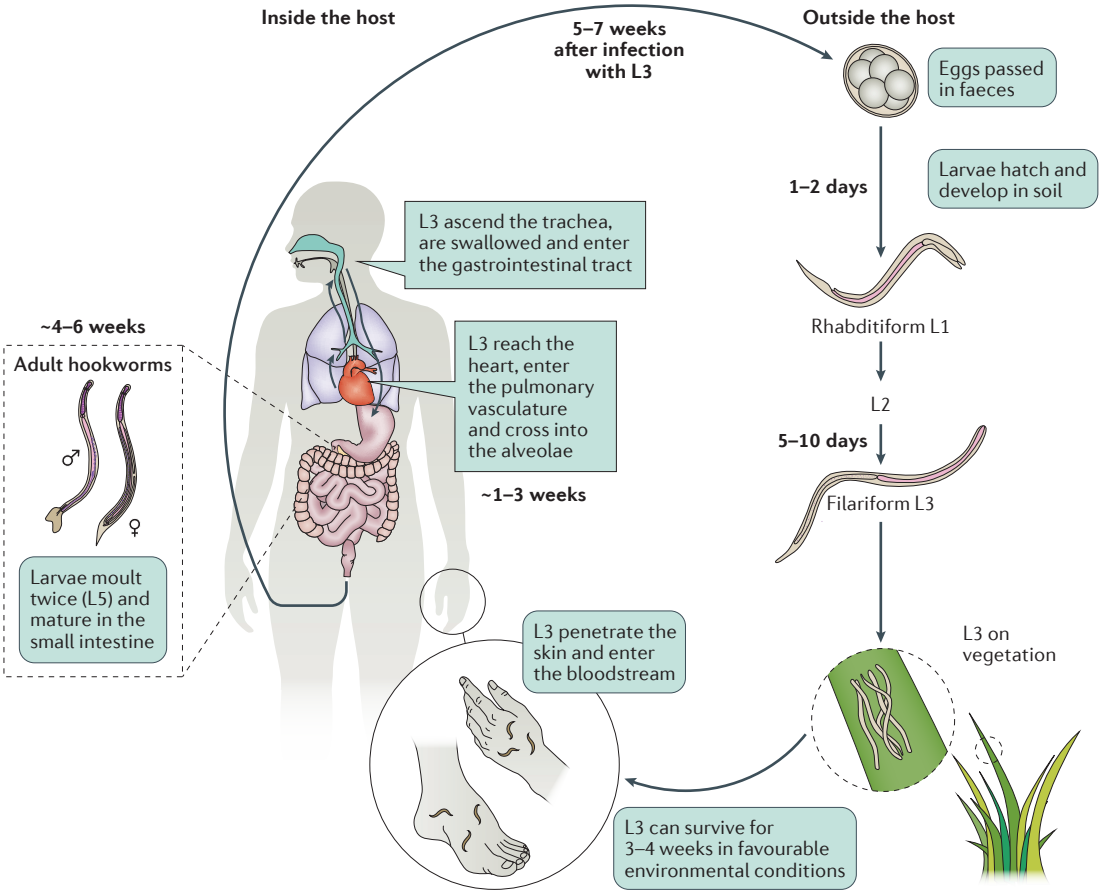

Figure 2 | **Life cycle of *Necator americanus*.** Hookworm eggs hatch in soil and rhabditiform (early) larvae moult twice (first-stage larvae (L1) and L2) before becoming infective (L3). L3 accumulate in soil or on grass awaiting exposure to human skin (often the hands, feet or buttocks), which they can penetrate. L3 then make their way to the peripheral vasculature, where they are passively swept within the bloodstream, first to the right side of the heart and then to the pulmonary vasculature. In the lungs, L3 exit from the alveolar capillaries into the bronchial tree, which they ascend to reach the pharynx, from which they enter the gastrointestinal tract to finally complete their migration to the small bowel. Once in the duodenum, immature L5 hookworms use 'teeth' (*Ancylostoma* spp.) or cutting plates (*Necator* spp.) that line their buccal capsule to lacerate the mucosa and anchor themselves in position to facilitate feeding and avoid being ejected by gut peristalsis. As they begin to feed on blood, juvenile worms mature into sexually dioecious adult parasites. Mature adult male and female hookworms mate, and female hookworms produce as many as 10,000 eggs per day. Eggs are evacuated from the host via the faecal stream. The process from L3 invasion to patency (egg production) takes approximately 6–8 weeks for *Necator americanus* and possibly a similar period of time for *Ancylostoma duodenale*.

Figure 3 | **Developmental stages of the intra-host phase of the hookworm life cycle. a** | A few days after *Necator americanus* third-stage larvae (L3) infect the host, a rash known as 'ground itch' develops at the site of skin penetration. **b** | A longitudinal histological section of an adult *Ancylostoma caninum* attached to the duodenal wall of an infected dog. **c** | A scanning electron micrograph of *A. caninum*, with its 'teeth' clearly visible. **d** | An *N. americanus* female (arrow) and male in copula in the small bowel of a human volunteer[9]. **e** | Egg of *N. americanus* in human faeces.

in the gut of the host, rather than direct consumption by the parasite, and is caused by the secretion of parasite-derived anticoagulants, including factor Xa, factor XIa and factor VIIa–tissue factor inhibitors[48]. Adult hookworms are thought to ingest just 0.001 ml of blood per day[49], but the amount of blood loss at the site of attachment is much greater, such that moderate or heavy *N. americanus* infections can produce losses of >1 ml daily[28]. Heavy hookworm infections can lead to hypoproteinaemia, which can cause anasarca (oedema of the face and lower limbs) and abdominal distension from ascites. The skin of infected individuals acquires a sickly yellowish colour, sometimes referred to as 'chlorosis' in the older literature, accounting for historical references to yellowish pallor, geophagy (the practice of eating soil) and a yellow disease of laziness dating back to the third century BC (reviewed in REF. 50).

IDA. In the presence of moderate-to-heavy hookworm infections, when blood loss exceeds the host's intake and reserves of iron and proteins[51], chronic IDA and the accompanying hypoalbuminaemia occur. Very heavy hookworm burden in nutritionally deprived adults is sufficient to induce IDA with haemoglobin levels of <11 g per dl. A study of 1,449 individuals in Uganda ≥50 years of age revealed that the greatest risk factors for IDA were heavy hookworm burden (odds ratio: 3.45) and malaria (odds ratio: 3.49)[52]. In children, IDA can occur with even lower hookworm burdens than those observed in adults[53]. The degree of IDA also depends on the species of the infecting hookworm: *A. duodenale* causes greater blood loss than *N. americanus*[19]. A study of almost 3,600 children from Pemba Island, Zanzibar, found that infection with *N. americanus* resulted in a lower incidence of IDA than did co-infection with both *N. americanus* and *A. duodenale*[54]. Systematic reviews have confirmed the association between moderate and heavy hookworm burdens and IDA in children and adults[27,33]. Along with iron losses, moderate and heavy hookworm infections result in substantial protein losses, which lead to hypoalbuminaemia and hypoproteinaemia. In severe cases, this can result in a clinical picture that resembles kwashiorkor (a severe form of malnutrition). The combination of anaemia and protein losses, together with chlorosis, is a reason why, in early twentieth century China, chronic hookworm infection was sometimes known as the 'yellow puffy disease' (REF. 55).

Other features. Controlled human challenge infections with *N. americanus* of healthy volunteers who reside in countries where hookworms are no longer endemic (for example, Australia and the United Kingdom) have provided insight into the kinetics and manifestations of primary hookworm infection in the absence of other tropical pathogens and comorbidities[9,56,57]. Besides IDA and hypoalbuminaemia, the most prominent feature of hookworm infection is systemic[57] and mucosal[58] eosinophilia. Systemic eosinophilia can be detected within 4 weeks of *N. americanus* infection (that is, before arrival in the gut) and peaks at 6–12 weeks as the young adult hookworms arrive in the small bowel. Individuals with a light hookworm burden (infected with 10–20 L3) are often asymptomatic, although flatulence is common for the first 12 weeks and gastrointestinal disturbance is sometimes reported within the first 3–15 weeks[59]. The severity of gastrointestinal pain seems to be associated with the challenge inoculum[57,59].

Zoonotic hookworm infections

Zoonotic hookworms, particularly canine and feline parasites, can cause symptoms and pathological outcomes in humans that are not seen with anthropophilic hookworms. Perhaps the most frequently encountered zoonotic hookworm is *Ancylostoma braziliense*[60]. Percutaneous entry of *A. braziliense* L3 does not cause ground itch in humans but can cause cutaneous larva migrans (also known as 'creeping eruption'), which is a self-limiting condition that is characterized by serpiginous, 1–5-cm-long tunnels generated by the larvae migrating through the epidermis. Like ground itch,

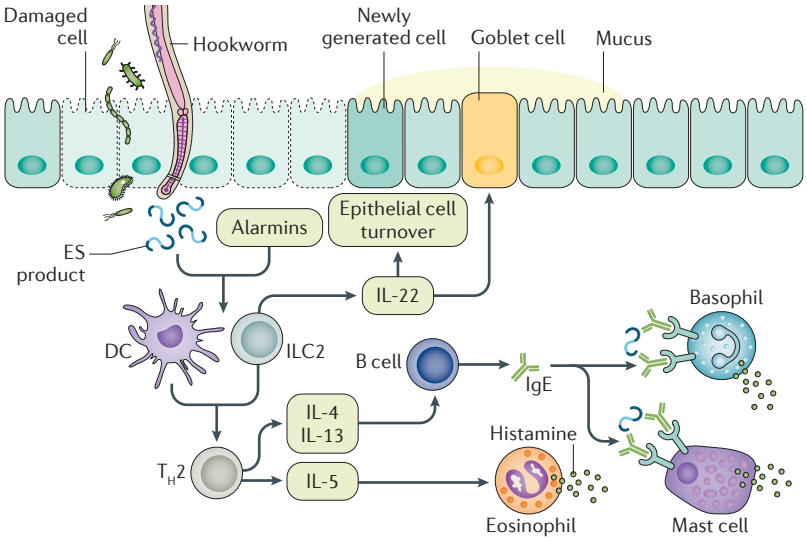

Figure 4 | **Hookworms induce a T helper 2 immune response in the early phase of intestinal infection.** Feeding worms bury their anterior ends in the wall of the small bowel, where they physically rupture cells within the mucosa and actively release excretory–secretory (ES) products. Damaged cells release alarmins, which, along with ES products, act directly on dendritic cells (DCs) and type 2 innate lymphoid cells (ILC2s), which in turn induce the development of T helper 2 (T_H2) cells. T_H2 cells, through secretion of IL-4 and IL-13, promote B cells to secrete IgE antibodies, which crosslink antigens on basophils and mast cells to trigger histamine release through degranulation. T_H2 cells also secrete IL-5, which activates eosinophils and promotes degranulation. ILC2, activated DCs and T cells release IL-22, which triggers epithelial cell turnover and mucus production by goblet cells. Disruption of the intestinal barrier can result in changes to the composition of the resident microbiota, and translocation of microbial products can exacerbate pathology.

cutaneous larva migrans usually occurs on the areas that are most likely to be in contact with contaminated soil. Cutaneous larva migrans is most frequently encountered in travellers who are returning from tropical locations and military personnel, and responds to treatment with oral anthelmintics (albendazole or ivermectin). In some cases, *A. braziliense* larvae may reach the lungs and cause pulmonary infiltrates[61], although, in the majority of cases, the infection does not progress beyond cutaneous larva migrans.

The common canine hookworm *A. caninum* is not thought to induce cutaneous larva migrans, possibly because *A. caninum* L3 rapidly reach the subcutaneous tissues where they undergo developmental arrest. However, gastrointestinal symptoms from human *A. caninum* infection can be severe. A series of 93 cases of eosinophilic gastroenteritis in adults living in urban northern Australia showed that enteric infection was linked to *A. caninum*[62]. Inflammation in some patients was so severe that surgical intervention was required[62,63]. Whether these reports indicate that *A. caninum* generates an excessive gastrointestinal inflammatory response in humans is unclear. It is also possible that *A. caninum* reaches the human gut more often than appreciated but goes unnoticed because it is usually expelled soon after arrival. In fact, after the description of the cases of eosinophilic gastroenteritis in northern Australia, similar cases were reported in the southern United States[64] and Egypt[65], as clinicians became aware of the differential diagnosis. In no case was more than a single adult worm identified in the patient, which accounts for the absence of eggs in the faeces[66].

Experimental models of hookworm infection

Animal models. Animal models for hookworm infection are available but have limitations, and each presents its own series of challenges, including cost, ethical and reproducibility issues[67,68]. The rodent strongyle nematode *Nippostrongylus brasiliensis* has a similar life cycle to *N. americanus* and induces a T helper 2 (T_H2)-type immune response that exhibits all the features of a human hookworm infection, including $CD4^+$ T cell-dependent IgE production, eosinophilia, mastocytosis and mucus production (see below, Immunopathology)[69]. *N. brasiliensis* reaches patency in the small bowel of mice, which has enabled researchers to conduct both mucosal and systemic immunological studies aimed at gaining mechanistic insight into human hookworm infection[70]. There is also some modest blood loss and drop in haemoglobin levels in *N. brasiliensis*-infected rats. However, *N. brasiliensis* is a phylogenetically distant nematode compared with human hookworms, belonging to the Trichostrongyloidea superfamily, as opposed to hookworms that belong to the Ancylostomatoidea superfamily. Moreover, rodents develop immunological resistance to *A. ceylanicum*[71], *N. americanus*[72] and *N. brasiliensis* (reviewed in REF. 70), a phenomenon that is much less apparent in human hookworm infections.

An alternative and widely used model for human gastrointestinal nematode infections is mice infected with *Heligmosomoides polygyrus*. *H. polygyrus* has a different life cycle than hookworms and does not traverse the skin or lungs, relying on oral ingestion of infective larvae from the environment. Moreover, like *N. brasiliensis*, *H. polygyrus* is a member of the Trichostrongyloidea superfamly. However, like hookworm, *H. polygyrus* does induce chronic intestinal infections in some strains of mice that are not readily eliminated by a modified T_H2 cell response, which sheds light on protective immune mechanisms at play in gastrointestinal nematode infections[70,73].

Two other rodent models that have been developed are hamsters infected either with *A. ceylanicum* or *N. americanus*. Hamsters infected with *A. ceylanicum* develop patent infections and experience intestinal blood loss and anaemia[74], as well intestinal mucosal inflammation[75]. Acquired immunity can be elicited, so the model is suitable for vaccine development[71]. Similarly, it is possible to achieve patent infections in hamsters following *N. americanus* infection, although the numbers of larvae that become adult hookworms are lower than for *A. ceylanicum* and the infections are often short lived, but the model is still potentially useful for vaccine development studies[76].

Dogs develop both age-acquired and exposure-acquired immunity to canine hookworms[77]. However, even in the face of a robust immune response against many worm antigens[44,78], it is often elderly individuals in hookworm-endemic areas who harbour the heaviest hookworm burdens, which makes experimental and natural infections of rodents and dogs of limited value

in understanding the chronicity of and absence of protective immunity to hookworm in human populations[79]. A non-human primate model of *N. americanus* infection was established in marmosets using both field-adapted and laboratory-adapted strains of hookworms[80]. Infections became patent and animals developed disease and immune responses that were similar to those seen in human infections, including anaemia, eosinophilia and parasite-specific IgE and IgG antibody responses. Despite the apparent usefulness of this model, we could not identify additional publications since the original description in 2008, which perhaps reflects the expense and ethical concerns around the use of non-human primates. Nonetheless, consideration of more-widespread use of this model might be warranted, particularly when testing new drugs and vaccines before conducting expensive human clinical trials.

Controlled human challenge models. The human challenge model presents distinct advantages over studying hookworm infection in an endemic transmission setting, where co-infections are the norm, exposure history is sketchy at best and compliance is a challenge. However, some of these same advantages can be viewed as problematic, as the controlled setting may not reflect real-world hookworm infection, which usually occurs in developing countries and impoverished settings with poor hygiene. Thus, studying hookworm infection in isolation in developed countries with low incidence of infectious diseases might have reduced generalizability. Nonetheless, the increasing adoption of the human challenge model in various laboratories around the world will probably accelerate testing of new interventions to control hookworm disease, as well as provide proof of concept that the hookworm proteome is a veritable pharmacopoeia for inflammatory disease therapeutics[81]. Moreover, the ability to study human hookworm infections in a controlled setting offers substantial advantages over mouse model studies, particularly owing to the development of chronic infection and the immune markers that define it (some of which are not detected in mice), such as IgG4.

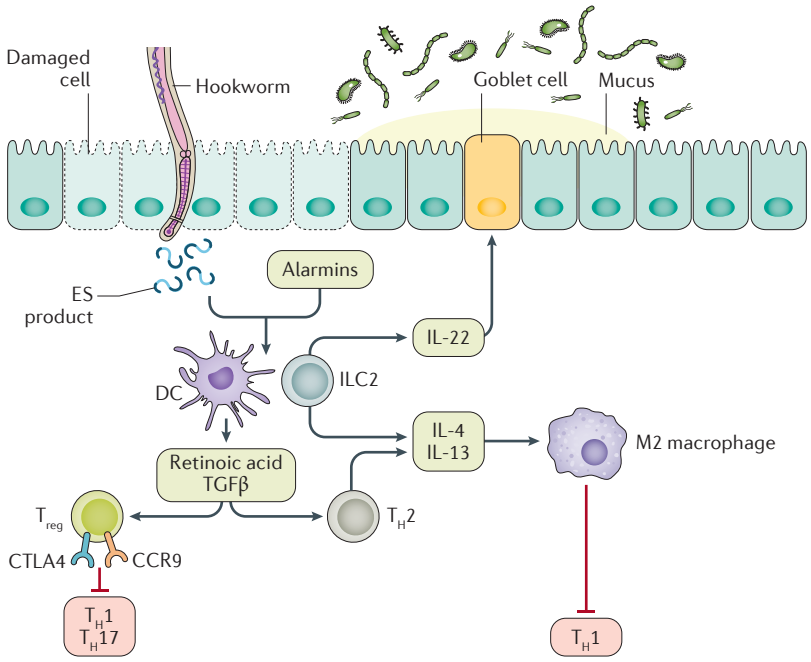

Figure 5 | **Immune pathways for parasite tolerance.** The secretion of excretory–secretory (ES) products from hookworms and the release of alarmins from damaged cells trigger the activation of type 2 innate lymphoid cells (ILC2s) and tolerogenic dendritic cells (DCs). As adult hookworms establish a chronic infection in the gut, tolerogenic pathways are triggered. DCs express reduced levels of activation and co-stimulatory markers and increased levels of molecules associated with tolerance, such as enzymes of the retinoic acid production pathway and transforming growth factor-β (TGFβ), which in turn generate anergic T helper 2 (T_H2) cells and regulatory T cells (T_{reg} cells) that express mucosal homing markers, such as CC-chemokine receptor 9 (CCR9) and the downregulatory checkpoint receptor cytotoxic T lymphocyte protein 4 (CTLA4) to prevent T_H1 cell-mediated and T_H17 cell-mediated damage. ILC2s and anergic T_H2 cells produce type 2 cytokines (IL-4 and IL-13) that promote alternative activation of M2 macrophages, which adopt a wound-healing phenotype and prevent T_H1-mediated damage. Increased mucus production triggered by IL-22, among other unknown factors, supports the maintenance of a species-rich microbiota, including bacteria that release short-chain fatty acids that promote T_{reg} cell function.

Immunopathology

Despite the high global prevalence of *N. americanus*, the immunopathology of human hookworm infection has not received as much attention as that of other human helminth infections, such as schistosomiasis and filariasis. This lack of information can be attributed to the difficulty and expense of maintaining the hookworm life cycle in a suitable animal model and the inability of any of the main species of hookworms to reach full maturity in mice in substantial numbers[82]. Nonetheless, mouse studies with hookworm-like gastrointestinal nematodes of rodents have revealed the predominant cellular mechanisms that drive the immunopathological responses to these parasites. Early host responses in a primary hookworm infection are aimed at eliminating the invading parasites. Migrating L3 and adult hookworms are the target of inflammatory responses that are characterized by systemic and localized eosinophilia and mastocytosis[58,78]. Experimental human hookworm infection is not associated with significant pulmonary inflammation or compromised airway responsiveness[11], but resulted in increased mucosal expression of T_H1 cytokines, such as interferon-γ (IFNγ), IL-2 and IL-15, and T_H2 cytokines, including IL-4, IL-5 and IL-13 (REF. 58), and infection intensity is negatively associated with parasite-specific IL-5 levels[83]. Despite the predominance of T_H2 cells, type 2 cytokines and parasite-killing antibody isotypes (such as IgE), attempts to dislodge adult hookworms from the gut are mostly unsuccessful. Moreover, despite the robust and enduring T_H2 cell response that is induced by hookworms, infected individuals show no signs of overt allergy to worms, and indeed are even protected from developing allergies to bystander antigens[84]. Thus, to limit the immunopathology caused by a primary hookworm infection, the phenotype of the immune response switches over time to become regulatory in nature (see section on Host tolerance), allowing the host and the parasite to reach a status quo, in which the parasite burden is regulated and tolerated by many infected individuals.

Immune clearance. In the *N. brasiliensis* experimental mouse model of human hookworm infection, acquired immunity manifests in the early stages of secondary infection. IgE-armed basophils are responsible for trapping larvae in the skin, thereby limiting their migration through the lungs and associated injury[83]. There is also evidence for clearance of parasites in the lungs, where long-lived M2 macrophages directly mediate rapid nematode damage and clearance[85]. In the gut, *N. brasiliensis* triggers T_H2 cell responses, including the secretion of type 2 cytokines, such as IL-4 and IL-13, and the induction of goblet cell hyperplasia and mucus production, all of which culminate in worm expulsion (FIG. 4). Moreover, the production of IL-25 by intestinal tuft cells was recently shown to be a key early event in triggering protective T_H2 cell responses in this model[86].

Unlike in the mouse model of *N. brasiliensis* infection, immunological clearance of hookworm infection in humans is less apparent, and, despite robust immune responses, substantial parasite numbers persist in the human host into old age. The kinetics of the antibody response has been addressed in controlled human challenge infections[57,59,87]. IgA, IgD, IgE, IgG1, IgG4 and IgM antibodies from individuals who live in hookworm-endemic settings have all been shown to bind to hookworm antigens[88,89]. IgE has a protective, anti-parasite role in many helminth infections[90] and has, therefore, been of particular interest in efforts to combat hookworm disease. In Papua New Guinea, it was shown that IgE, either parasite-specific or polyclonal, afforded partial protection against hookworm infection[91,92]. As with other helminth infections, IgE responses seem to develop over multiple exposures[56,93]. Despite the robust IgE response that is induced by hookworm infection, isotypes that counterbalance the protective nature of IgE, such as IgG4, are dominant in chronic human infections. In other human helminthiases, for example, filariasis and schistosomiasis, parasite-specific IgG4 correlates with a modified T_H2 immune response, which can be differentiated from the parasite-killing (and often more pathogenic) IgG1 and IgE isotypes[94]. A similar situation probably exists in hookworm infection, as a parasite-specific IgG4 response is a good predictor of hookworm infection[95,96]. Thus, if the immune response to hookworm is diverted from a modified T_H2–IgG4 response towards a potentially protective T_H2–IgE response, anti-parasite immunity might indeed be possible[97].

Host tolerance. Co-evolution of helminths and humans has resulted in a status quo: on the one hand, anthelmintic, protective T_H2 immune responses limit hookworm numbers to manageable levels, whereas, on the other hand, over time, regulatory responses develop, which protect the host from excessive immunopathology but also prevent resident hookworms from being ejected by immune effector mechanisms. This phenomenon is exquisitely exemplified in the hookworm–human host relationship. The association between helminths and humans is so intimate that helminths have been credited with influencing entire arms of the human immune system. For example, the T_H2 cell response is thought to have evolved to fight helminths and mop up (or contain) the damage they induce during their migration and feeding[98]. Hookworms can regulate inflammation in a complex and multifactorial way. Like many other helminths, hookworms are potent inducers of regulatory immune responses that are aimed at promoting their own survival and reproductive capacity. In humans, the expansion of regulatory T cells (T_{reg} cells) has been reported in many helminth infections in endemic settings, including hookworm infection (reviewed in REF. 99). Peripheral blood mononuclear cells from individuals infected with hookworm that are pulsed with hookworm antigens had an impaired proliferative capacity, which was partially augmented by the depletion of T_{reg} cells[100]. Moreover, studies with low-dose experimental *N. americanus* infection in human volunteers showed a remarkable absence of inflammation surrounding feeding worms (FIG. 3d), an expansion of T_{reg} cell numbers in the gut[9,58] and upregulation of microRNAs that encode the regulatory cytokines IL-10 and transforming growth factor-β (TGFβ) and the intestinal wound-repair cytokine IL-22. In addition, intestinal tissue from humans who are experimentally infected with hookworms shows an increased expression of genes in the retinoic acid pathway, indicating the presence of tolerogenic dendritic cells, which might imprint gut homing on resident T cells[58] (FIG. 5).

Products from the resident gut microbiota, such as DNA and lipopolysaccharide (LPS), have been shown to cross the disrupted gastrointestinal endothelial barrier in diseases such as inflammatory bowel disease or upon treatment for HIV infection (reviewed in REF. 101). This translocation has immunological consequences: inflammation in the short term, but possibly the establishment of an anti-inflammatory state if there is chronic exposure to microbial products. In India, an area that is endemic for hookworm infection, the levels of LPS and other markers of microbial translocation were increased in individuals infected with hookworm compared with uninfected controls[102]. However, this

Box 1 | **Hookworms, microbiota and immunoregulation**

The rich variety of microbial species of the gut has been associated with a healthy gut and intestinal homeostasis[193]. Intriguingly, an increased bacterial richness in individuals with helminth infections has been observed in endemic areas[194]. A pilot study that explored the effect of experimental *Necator americanus* infection on the human gut microbiota[195] revealed an increase in the number of bacterial species 8 weeks after third-stage larvae (L3) infection[195]. When healthy human volunteers with coeliac disease and on a gluten-free diet were infected with *N. americanus*, the number of species in their already highly varied commensal microbiota increased and was further enriched after the volunteers received increasing doses of dietary gluten[182]. This observation represents a potential mechanism by which hookworms regulate gluten-induced inflammation and promote immune homeostasis in the gut, and has prompted research into the correlation between gut microbiota, macrobiota (for example, helminths) and inflammation in non-gastrointestinal organs and tissues. Chronic infection of mice with *Heligmosomoides polygyrus* increased microbial species richness and their subsequent production of immunoregulatory short-chain fatty acids in the gut and protection against inducible asthma, whereas antibiotic treatment ablated this protection[183]. Similarly, experimental human hookworm infection also resulted in increased production of short-chain fatty acids by intestinal commensal bacteria[183].

Box 2 | **Faecal examination for diagnosing hookworm infection**

- As hookworms are parasites of the digestive system, the diagnostic examinations use samples of fresh, fixed or frozen faeces, in which hookworm eggs (ova), larvae and whole or parts of the parasites can be detected.
- Faeces must be processed, fixed or frozen within 24 hours of collection, before the eggs can hatch into larvae, which are not detected by many quantitative methods.
- A negative concentration test should always be followed by a flotation test to ensure the absence of hookworm eggs, although pseudoparasites or artefacts, such as plant or fungi spores, plant cells and pollen or starch grains, might interfere with the microscopic identification of hookworm eggs in many flotation methods.
- No hookworm eggs are detected during the period before patent infection, while the larvae make their way through the vascular system to the small bowel to mate, a process that can take >5 weeks.
- Egg production does not occur continually and can be affected by the nutritional status of the host.
- The consistency of the faeces can also markedly affect the eggs per gram of faeces, which makes it an unreliable metric for calculating the number of worms in the gut.
- Besides egg-laying female hookworms, adult male worms as well as developing larval stages (pre-patent, fourth-stage larvae (L4) and L5) can be found in the human host, but cannot be detected by the methods described in TABLE 1 (which only detect L3 that hatched after the sample was collected).

increased microbial transfer was not associated with any acute-phase response, and indeed C-reactive protein levels were significantly decreased in these individuals. The lack of systemic inflammation was confirmed by unaltered levels of pro-inflammatory cytokines in the circulation, whereas the levels of anti-inflammatory cytokines, such as IL-10, were substantially increased and strongly correlated with the levels of LPS in circulation. Anthelmintic treatment lowered LPS levels, which indicates that hookworm infection was the cause of the increased microbial transfer. Dissecting the complex series of interactions between hookworms, microbiota and immunoregulation is now a priority, particularly in light of the current enthusiasm generated by the potential use of hookworms and their secreted products as the next generation of biologics to treat inflammation. The importance of the three-way relationship between microbiota, macrobiota (macroscopic residents of the gut, such as hookworms) and inflammation is only now being recognized (BOX 1).

Diagnosis, screening and prevention

Diagnostics

Hookworm diagnostics are used to confirm infection in clinical settings, map hookworm infections to identify 'hotspots' of transmission for targeted intervention and evaluate MDA campaigns. Examination (microscopic or molecular) of faecal samples is essential to diagnose hookworm infection[103] (BOX 2; TABLE 1).

As the prevalence of hookworm infection declines dramatically in locally defined settings, highly sensitive diagnostic methods are required to determine whether transmission has in fact been reduced after intensive MDA, particularly if other preventive measures have not been implemented[104]. In this regard, the detection of hookworm eggs in the faeces becomes imperative. The conventional egg-counting techniques (BOX 3) are inadequate in efforts to detect the extremely low egg counts that would be expected after intensive MDA has been implemented and transmission is 'rebounding' (REF. 104). As such, recent efforts have been devoted to the development of PCR-based methods (TABLE 1), which are still being validated for their ability to determine quantitative eggs counts. However, some of these tests have already proven to be more effective for the detection of hookworm infection than conventional diagnostics, which cannot identify extremely low numbers of hookworm eggs that are shed in faeces[105]. Indeed, one such multiplex quantitative PCR faecal assay can potentially differentiate hookworm species and obtain direct information on quantitative intestinal helminthic and protozoan infections[105].

In addition, when diagnostics are used to quantify EPG, the objective is to assess (by this proxy) the worm burden of an individual to determine if that individual is at risk of morbidity from hookworm infection. That is, measurements of EPG can identify individuals, especially children and women of childbearing age, who are likely to develop IDA and associated outcomes.

Prevention

The focus of most current control strategies is preventive chemotherapy through MDA (discussed below). However, complete elimination or at least long-term control and reduction in the prevalence of hookworm and other STH infections will probably require, besides MDA, general sanitary improvements, as shown in the Water, Sanitation and Hygiene (WASH) programme. This programme has been most promising as an integrated approach to the prevention of diarrhoeal diseases[106,107]. WASH interventions are diverse and include a range of improvements in water conditions (such as quality, quantity and access), sanitation (such as improved latrines and sewer maintenance) and hygiene (for example, adopting good personal hygiene habits, such as hand washing before eating and after defaecation, wearing shoes and safely storing water)[108–110]. Another key feature of WASH programmes is community engagement and awareness, with a particular focus on improving the general population's understanding of what constitutes unhygienic behaviour, such as open defaecation.

WASH programmes hold periodic community-based health education activities, such as the convening of community boards or community events, to discuss the sanitation and hygiene problems in the locale. WASH interventions also use innovative pedagogical methods, especially educational devices (for example, flashcards, games, songs and videogames), to teach children in endemic areas sanitation and hygiene[111]. Promoting the use of footwear has been a successful component of community engagement WASH programmes for the control of hookworm infection[107]. However, some studies suggest that the use of footwear to prevent hookworm infection is not supported by robust evidence[112]. Hookworm larvae can penetrate human skin anywhere in the body and exposure to hookworm larvae is not limited to the hands and feet; for example, during agricultural activities, such as dry rice cultivation, the naked chest and arms are also exposed.

There are few studies to date that look directly at the role of WASH programmes in controlling STH infections and even fewer that look solely at the effect of WASH programmes on hookworm infection. In the context of STH infections, WASH interventions have been associated with reduced odds of STH infection[107]. Individuals who had access to and used sanitation facilities were at lower risk of STH infections than individuals without sanitation[113]. Nevertheless, additional evidence of the benefits of WASH programmes in the reduction of STH infections is needed to improve the coordination between MDA and WASH interventions[114].

In the United States, South Korea, Australia and Japan[115,116], improvements in habitat and lifestyle similar to those advocated and implemented by WASH programmes led to the elimination of hookworm and other STH infections. These achievements demonstrate the potential effectiveness of WASH programmes. However, hygiene improvements in these countries were accompanied by improved economic conditions, and it is likely that general economic development and its accompanying forces of urbanization represent more-powerful forces for reducing hookworm prevalence and intensity than WASH programmes as an isolated measure. To date, MDA and WASH interventions in low-resource countries have not resulted in remarkable long-term declines in hookworm prevalence and intensity in the absence of simultaneous substantial economic development. Thus, WASH-related improvements might be more challenging to achieve in rural communities that are located in resource-limited settings, many of which still rely on subsistence economy. The construction of latrines in rural and remote schools and the use of household waste to produce biogas, a process that helps to reduce the pathogen load in night soil[117], are two modest but relevant changes that can contribute to the disruption of the life cycle of hookworms. Finally, all these considerations have stimulated interest in developing a hookworm vaccine (discussed below).

Management

Benzimidazole-based therapies

Currently, the two most commonly used drugs for the treatment of *N. americanus* and *A. duodenale* infections worldwide are mebendazole and albendazole, both of which are benzimidazole anthelmintic drugs (TABLE 2). These drugs act by inhibiting microtubule polymerization in invertebrates, therefore killing adult worms. Both *N. americanus* and *A. duodenale* have similar susceptibility to benzimidazoles; nevertheless, there are important differences in therapeutic efficacy between mebendazole and albendazole[118]. A single 400 mg dose of albendazole is more effective than a single 500 mg dose of mebendazole; a systematic review and meta-analysis of published randomized controlled trials of treatment for hookworm infection found an overall cure rate of 72% for a single dose of albendazole and 15% for a single dose of mebendazole[118]. Similarly, another study conducted in 1,845 schoolchildren at

Table 1 | **Microscopic-based and molecular-based faecal examination methods for the diagnosis of hookworm infection**

Test	Mechanism	Detects ova?	Detects larvae?	Sample requirements	Benefits	Limitations
Microscopic-based examinations						
FECT*	Concentration	Yes	Yes	10 g of fresh faeces	Sensitive, low technology and can detect other STHs	Only qualitative
Diethyl-acetate*	Concentration	Yes	Yes	20 g of fresh faeces	Sensitive, low technology and can detect other STHs	Only qualitative
Baerman*,‡	L3 migration	No	Yes	18 g of fresh faeces	Specific by morphological examination	L3 collection only
Harada–Mori*,‡	L3 migration	No	Yes	2 g of fresh faeces	Specific by morphological examination	L3 collection only
McMaster§ (REF. 196)	Flotation	Yes	No	2 g of fresh faeces	Sensitive, low technology and can detect other STHs	Trematodes‖
FLOTAC§ (REFS 197,198)	Flotation	Yes	No	2 g of fresh faeces	Sensitive, low technology and can detect other STHs	Availability of apparatus
Kato–Katz§	Glycerol	Yes	No	10, 20 or 50 mg¶ of fresh faeces	Standardized (templates) and can detect other STHs and trematodes	Desiccates eggs after 60 minutes
Molecular-based examinations						
Next-generation sequencing*	Genetic sequencing	Yes	Yes	Fresh, fixed or frozen faeces	Sensitive and can use frozen material	Availability of apparatus
PCR#	Primers	Yes	Yes	Fresh, fixed or frozen faeces	Sensitive and can use frozen material	Availability of apparatus
LAMP#	DNA	Yes	Yes	Frozen or fixed faeces	Sensitive and can use frozen material	Availability of apparatus

FECT, formalin-ether concentration technique; L3, third-stage larvae; LAMP, loop-mediated isothermal amplification; STH, soil-transmitted helminth. *Qualitative output. ‡Culture method for collecting hookworm larvae and is seldom used for diagnostic purposes (unless L3 are cultured to determine, based on their morphology, whether they are hookworms or another strongyle nematode). §Quantitative output. ‖Owing to the co-endemicity of many trematodes with STHs, including hookworms, a technique that does not identify both helminth infections doubles the work required for diagnosis. ¶Weight of faeces used in Kato–Katz depends on the size of the template in the kit. #Currently being validated as a quantitative method.

Box 3 | **Egg-counting methods**

Flotation methods

Two of the most popular egg-counting methods are the McMaster[196] and FLOTAC techniques[197,198]. In these methods, the faecal sample is mixed in a chambered device with a super-saturated saline solution; the density of the hookworm eggs is lower than the density of the saline, so the eggs rise to the surface, where they can be morphologically identified with low-magnification (40–100×) optical microscopy. The eggs are counted and eggs per gram of faeces are extrapolated[199]. These methods are extensively used because their components are low cost, the preparation is simple and does not require electricity and they are suitable for resource-limited settings, which is often the case of many hookworm infection-endemic settings.

Glycerol methods

Although not specifically designed for hookworms, the Kato–Katz faecal thick smear technique is the most common diagnostic method for hookworm infection. Here, homogenized faeces are covered by a glycerol solution on a glass slide and examined at 100× magnification after 'clearing' (when the glycerol turns from malachite green to transparent).The crucial drawback of using glycerol-based methods is that glycerol quickly desiccates the thin outer hyaline layer of hookworm eggs, which distorts and even dissolves eggs on the slide after only 60 minutes[200]. For accurate detection and quantification of hookworm eggs, slides must be read within 1 hour of preparation (maybe sooner in hotter climates), which is often difficult when the slides are prepared in the field. This limitation might be the reason for the underestimation of hookworm infection in national surveys, in which resources are devoted towards the elimination of the blood fluke *Schistosoma mansoni* and *Ascaris lumbricoides*, which are often considered of greater public health importance.

seven sites worldwide reported an overall cure rate of 87.8% for a single dose of albendazole, although this rate varied considerably across countries, age groups and different pretreatment intensity of infection[119]. Thus, despite their promise, the efficacies of these benzimidazole drugs in treating human hookworm infection can be highly variable, with surprisingly high rates of single-dose drug failure noted for both mebendazole and albendazole[6,7]. Three consecutive daily doses of either drug improve both cure and egg reduction rates, but this option is less convenient for MDA programmes, in which single-dose treatment is preferred[120]. Interestingly, the formulation of these drugs probably affects their bioavailability in the intestine, as different formulations of the same drug, which are produced by different manufacturers, result in different efficacies[121].

The reasons for the observed failures of mebendazole or albendazole to treat human hookworm infections are unclear. Repeated use of mebendazole in the same communities has been associated with diminishing efficacy over time, which suggests the emergence of drug resistance, although this hypothesis remains controversial[122]. However, resistance to benzimidazoles that are used in animal husbandry has been observed in intestinal nematodes infecting livestock and it has been linked to point mutations in the β-tubulin gene in the parasite genome[123]. Thus, research is ongoing to determine whether albendazole and mebendazole failure to treat hookworm and other STH infections are caused by similar resistance mechanisms[124].

Both albendazole and mebendazole have excellent safety profiles in the doses used to treat hookworm infection[125], although transient abdominal pain, diarrhoea, nausea, dizziness and headache might occur. As benzimidazoles are embryotoxic and teratogenic in pregnant rats and rabbits, there have been concerns about the use of these drugs in children <1 year of age and in pregnant women. To date, teratogenicity in humans has not been observed, and a study of >800 women who were treated with albendazole during the second and third trimesters demonstrated no adverse effects[126]. However, albendazole use in the first trimester is not recommended. Similarly, despite the lack of formal safety studies of albendazole or mebendazole in children <2 years of age, both drugs have been widely used to treat entire communities irrespective of the age of the individuals as part of MDA programmes[127], with no reports of adverse effects in children. As a result, the WHO recommends that both drugs can be used safely in children ≥1 year of age[128] (TABLE 2).

Alternative treatment approaches

Despite concerns about the sustainability of MDA with benzimidazoles and the potential emergence of resistance, few effective alternatives for the treatment of hookworm infection are available or being developed. Pyrantel pamoate and levamisole are alternative drugs, although neither is as effective as albendazole. Both drugs interfere with the function of nicotinic acetylcholine receptors on the body muscle of hookworms, which leads to muscle cell depolarization and spastic paralysis that result in expulsion of the worm from the gastrointestinal tract[129]. Tribendimidine, which is a synthetic derivative of amidantel that acts as a nicotinic acetylcholine receptor agonist, is also emerging as a treatment, and was highly active against hookworm infection in both animal models and human studies[130]. This drug was originally developed in the 1980s in China, where it was registered for human use in 2004, although no other country has approved it yet[131].

Pore-forming crystal (Cry) proteins, which are derived from *Bacillus thuringiensis*[132], show *in vivo* activity against *A. ceylanicum*[133]. In particular, Cry5B in combination with a nicotinic acetylcholine receptor agonist has synergistic activity against intestinal nematodes[132].

Finally, combination therapy using drugs with different mechanisms of action could improve treatment efficacy and stall the emergence of resistance, although it has not become common practice, as there is little data from well-designed clinical trials[134]. One trial combined single-dose albendazole with single-dose oxantel pamoate (an orthologue of pyrantel pamoate that is used in veterinary medicine) or ivermectin, but cure rates were only approximately 40–50% compared with 24% for single-dose mebendazole[135]. Most combination therapies would be expected to improve efficacy of trichuriasis treatments, but similar results might not be true for hookworm infection, as ivermectin or oxantel pamoate do not show substantial drug activity against it.

In industrialized countries, iron deficiency occurs only with chronic infections of moderate or heavy intensity. Thus, iron supplementation treatment is not routinely practiced in areas with access to benzimidazole anthelmintics. In resource-poor settings (where iron deficiency is most prevalent), no strong evidence

Table 2 | **Recommended drugs for the treatment of hookworm infections**

Patient population	Intestinal hookworm infection *(Necator americanus* and *Ancylostoma duodenale)*	Cutaneous larva migrans (zoonotic hookworms)
Non-pregnant adults	First line • Albendazole • Mebendazole	First line • Ivermectin
	Alternate • Pyrantel pamoate	
Pregnant women	First trimester • Treatment not recommended	Symptomatic relief • Topical corticosteroids • Antihistamines
	Second and third trimesters • Regimens as for non-pregnant adults*	
Children	12–24 months of age‡ • Albendazole • Mebendazole	12–24 months of age • Ivermectin
	>24 months of age • Regimens as for adults	>24 months of age • Albendazole

*Recommended by the WHO[37]. ‡Recommended by the WHO[128].

supports that iron supplementation provides added benefits, except possibly in pregnant women with severe anaemia due to hookworm infection[136,137]. Options for iron replacement include ferrous sulfate, ferrous gluconate or ferrous fumarate. To replete iron stores, treatment must continue for 3–6 months after haemoglobin concentration has been restored to physiological levels. The most common adverse effect of oral iron supplementation is gastrointestinal intolerance, including epigastric pain, nausea, vomiting, constipation and diarrhoea. Formulations with lower levels of elemental iron, liquid formulations or taking the formulation with food (although this option might reduce absorption) might reduce symptoms.

MDA

Goals. Chronic infection with hookworm or other STHs leads to undernutrition and impairs physical and cognitive development in children. Thus, control programmes based on frequent and periodic MDA to children in endemic areas have been implemented, with the goal of large-scale reduction in the burden of these diseases. In 2001, a WHO resolution urged member states to regularly treat high-risk groups and ≥75% of at-risk preschool and school-aged children by 2020. Unfortunately, it does not seem likely that this goal will be achieved despite substantial expansion of MDA programmes over the past decade[138,139].

Good-quality, non-proprietary, inexpensive benzimidazoles are widely available. Treating children with benzimidazoles regularly diminishes hookworm burdens, maintaining them below the disease-causing threshold[140,141]. Studies in preschool children have shown that anthelmintic treatment improved nutritional indicators, including stunted growth and muscle wasting[142,143]. MDA treatment also decreases the number of hookworm eggs that are shed into the environment, therefore contributing to reduced transmission. However, elimination in endemic countries by MDA alone is unlikely, as large numbers of infected adults are not currently targeted by MDA programmes[5,139].

Limitations. The principal drawback of MDA programmes is that re-infection often occurs rapidly once treatment stops[8,144]. Hookworm re-infection can reach 25% of pretreatment levels as early as 18 weeks after albendazole treatment[135]. Annual treatment rounds of albendazole MDA in eastern Indonesia reduced the prevalence of hookworm infection from 28% to 4% after 5 years, but prevalence rebounded to near the initial rate within 3 years after cessation of MDA, although infection intensities were lower[145].

Another concern regarding MDA interventions is the findings of unusually high rates of outright drug failure with either mebendazole or albendazole[6,7] as well as the potential emergence of drug resistance, which is already widespread in livestock nematodes because of ubiquitous benzimidazole use[146]. In fact, several reports of reduced cure rates of human hookworm infection have been published[7,147,148]. In addition, the efficacy of single-dose albendazole and mebendazole can be highly variable, with frequent episodes of drug failure[149,150].

Despite MDA successfully lowering the prevalence of ascariasis, re-infection and variable drug efficacies might have contributed to MDA failure of reducing global hookworm prevalence over the past 23 years. For instance, the 2013 Global Burden of Disease Study found that hookworm prevalence has decreased by only 5% compared with a 25% decrease for ascariasis[21]. Thus, eliminating the public health problem that endemic hookworm infection causes will probably also require simultaneous and substantial economic development and sanitation improvements in affected areas.

Quality of life

Compared with other neglected tropical diseases[151], there is a dearth of qualitative or social science studies on hookworm infection. The main consequence of parasite feeding and intestinal blood loss is iron deficiency, which eventually leads to IDA. Hookworm disease especially affects populations with low underlying iron reserves (for example, children and women of childbearing age) with moderate or heavy infections.

Women and children

Moderate and heavy hookworm infections can cause anaemia in pregnant[33] and non-pregnant women[27]. IDA in pregnancy can lead to poor outcomes for both the mother and the baby in resource-limited countries[33,152], including reduced fertility[29], maternal morbidity and even mortality and reduced child survival[34,152].

Systematic evidence also links anaemia to moderate and heavy hookworm infections in children[27]. The paediatric physical consequences of hookworm disease include reduced pubertal and post-pubertal growth and fitness[153] and were first described in the 1920s[154]. Similarly, by the 1920s, moderate and heavy hookworm infections were linked to reduced intelligence ('mental sluggishness')[155], and more-recent studies have supported an association between hookworm infection and cognitive delays in children[31]. However, direct measurement of health-related quality of life among

school-aged children using generic instruments was shown not to be sufficiently sensitive to measure the effect of parasitic infections (including hookworm infection) in Cote d'Ivoire[156] and Yunnan Province, China[157]. Thus, more-precise and tailored instruments to assess quality of life are needed.

Productivity and poverty

As highlighted above, the most recent estimates indicate that hookworm infection globally is linked to 4.1 million DALYs lost, ranking it among the most serious of the neglected tropical diseases along with schistosomiasis and leishmaniasis[2]. Chronic hookworm infection also reinforces poverty in low-income and middle-income countries through its long-term debilitating effects[15]. For instance, an analysis of paediatric hookworm infection in the southern United States in the early twentieth century showed that it resulted in a substantial drop in future wage-earnings[158]. Additional (mostly anecdotal) evidence shows the long-term damage of hookworm infection to worker productivity, especially in agricultural activities[159]. Anaemia associated with helminth infections (presumably mostly hookworm infection) among female tea pluckers in Bangladesh decreased labour productivity and increased sick days[160], although among anaemic Sri Lankan tea pluckers, low dietary iron intake was a more important determinant than hookworm disease[161]. It is difficult to attribute these adverse effects on productivity specifically to hookworm infection rather than to other STH infections or alternative causes of undernutrition. Recently, the Public Health Computational and Operational Research (PHICOR) group at Johns Hopkins University provided new economic impact assessments of hookworm infection, finding that total productivity losses were as high as US$139 billion annually[2].

Effects of treatment

In a systematic analysis, single-dose mebendazole in MDA programmes had no effect on improving hookworm disease[27], which is consistent with the low efficacy and occasional failure of this drug[6]. Conversely, albendazole improved anaemia in doses that are commonly used during MDA programmes[27]. However, other studies have indicated that albendazole often fails to adequately treat hookworm infection in MDA programmes in low-income settings[7,150], findings that might partly explain why a 2015 *Cochrane* analysis could not always find strong evidence for the therapeutic effect of deworming interventions[149]. In terms of quality of life, there are minimal studies available, but in at least one case, anthelmintic treatment does not show a direct effect on worker productivity[160]. Interestingly, treatment of cutaneous larva migrans resulted in substantial improvements in quality of life[162].

Comorbidities

In Africa, hookworm and malaria co-infections are widespread and can lead to severe anaemia[20,163]. Indeed, regions of predicted hookworm prevalence (based on both the observed prevalence among school-aged children and the satellite-derived environmental data) mirror the areas that have climatic suitability for *Plasmodium falciparum* malaria transmission[163]. Systematic reviews confirm that school-aged children and pregnant women in particular are at risk for malaria and hookworm co-infections[20,163]. However, whereas hookworm disease is due to intestinal blood loss, malaria-induced anaemia results from haemolysis, splenic sequestration and dyserythropoiesis. Thus, the two infections can produce comorbidities that lead to anaemia, but arrive at this clinical sequela through different mechanisms. Similarly, co-infections with hookworms and schistosomes (especially *Schistosoma mansoni*) are also common in Africa[164]. Schistosomiasis anaemia is a result of chronic inflammation and some blood loss. Triple infection with hookworms, schistosomes and *P. falciparum* are not uncommon on the African continent and might combine (along with high rates of haemoglobinopathies and inadequate diet) to produce additive haematological effects — a 'perfect storm' of anaemia, which is a major issue, especially in rural Africa[165].

Box 4 | **Hookworm vaccine development**

Helminths produce a vast array of proteases and other enzymes[44] to facilitate tissue penetration, migration, feeding and immunoregulation. Thus, theses enzymes are prime candidate antigens for a vaccine[201,202]. For hookworm vaccines, recombinant forms of two parasite-derived enzymes are under product and clinical development[170]. The first is a haemoglobin-digesting aspartic protease, APR-1 (REF. 46), which has been tested as a subunit vaccine in animal models of hookworm infection and is now produced as a recombinant protein under current good manufacturing practices (cGMPs) that is suitable for clinical testing[203,204]. The second is a recombinant form of glutathione *S*-transferase 1 that confers partial protection in canine and hamster models and has also now been produced under cGMPs[47,205]. Both vaccines have been proven safe and immunogenic in phase Ia trials in hookworm-naive volunteers (D.D., unpublished observations) and are progressing to phase Ib trials in endemic countries. The ultimate goal is to combine these adult hookworm antigens that are required for parasite blood digestion and haem detoxification[172] into a multivalent vaccine[204]. This human hookworm vaccine has been modelled and shown to be both cost-effective and cost-saving, while also representing a key biotechnology for the elimination of hookworm infection[5]. In a phase I clinical trial in Brazil, an older first-generation vaccine that comprised a single dose of the *Na*-ASP-2 vaccine (based on a protein secreted exclusively by L3) induced generalized urticarial reactions associated with increased levels of anti-ASP-2 IgE antibodies, which were already present in the individuals' sera, most likely the result of a previous infection[45]. Thus, some antigen discovery programmes now introduce steps to avoid selecting antigens that are the targets of IgE in natural infections[206]. This problem has now been circumvented with the new bivalent vaccine that comprised adult hookworm antigens.

Developing an anthelmintic vaccine that induces sterilizing immunity (that is, complete prevention of persistent infection) is considered unattainable[172]. However, because morbidity is proportional to infection intensity, a vaccine that provides protective immunity (decreased blood loss and hookworm numbers in the intestine) and prevents most moderate and heavy infections would have a major effect on the global burden of disease. Models show that even a partially efficacious paediatric vaccine could drastically decrease hookworm prevalence in children to 14.6% in 20 years, compared with 54.1% and 57.2% with mass drug administration and no intervention, respectively[5]. The vaccine could be administered to preschool children before they are first exposed to hookworm larvae or to both preschool and school-aged children (who might have already been exposed or infected) via a combination of vaccine and chemotherapy. A booster during adulthood is predicted to almost halve the overall prevalence[5].

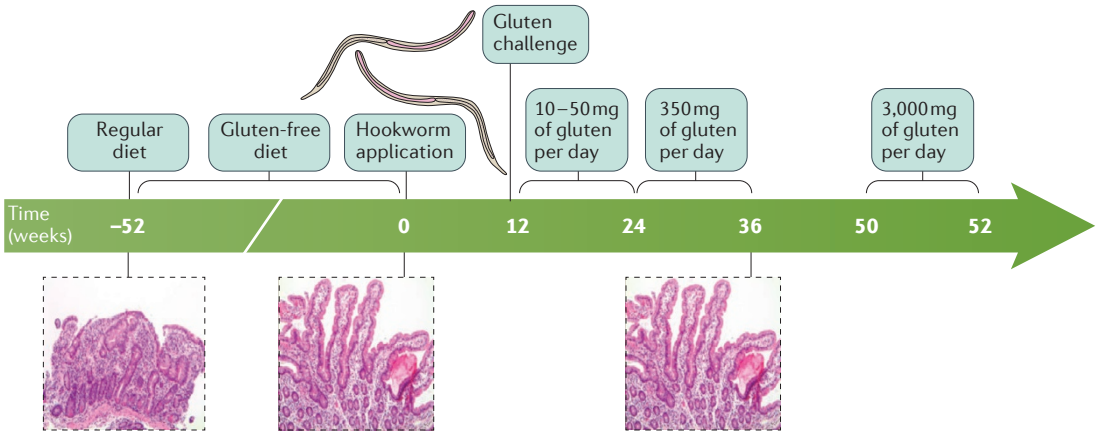

Figure 6 | **Controlled human hookworm infection restores gluten tolerance.** Individuals affected by coeliac disease who are on a regular diet that contains gluten develop inflammation in the gut, which leads to blunting of the villi and hyperplasia of the crypts (inset, left). In healthy individuals and in diet-treated patients with coeliac disease, the villus-height-to-crypt-depth ratio exceeds 3:1. After commencing a strict gluten-free diet, it takes approximately 1 year for the morphology of the duodenal epithelium to be restored to a healthy state (inset, middle), but much less time for its appearance to deteriorate when gluten is re-introduced in the diet. Individuals affected by coeliac disease and on a strict long-term gluten-free diet were infected with 20 *Necator americanus* third-stage larvae and were fed increasing amounts of pasta (oral gluten) over a 52-week period[9]. Controlled hookworm infection resulted in protection against villous blunting and other inflammatory changes that characterize coeliac disease (inset, right).

Such findings have generated an interest in simultaneously preventing all three infections by linking intermittent preventive therapy for malaria with anthelminthic MDA[165] or by co-formulating hookworm, schistosome and *P. falciparum* antigens in a multivalent vaccine[166]. As the malaria vaccine RTS,S advances towards licensure, it would be interesting to explore how it could be combined with one or more hookworm or schistosome antigens that are currently under development.

Outlook

Prospects for a hookworm vaccine

Hookworm infection, like most other neglected tropical diseases, is a disease of impoverished people. The WHO has proposed annual albendazole or mebendazole MDA of entire populations or age strata within an endemic area as the most cost-effective means of reducing morbidity caused by chronic intestinal helminth infections[167]. However, annual deworming might be less effective for hookworm infection than for other STH infections[139,168], possibly owing to benzimidazole drug failures and post-treatment reinfections. Indeed, the 2013 Global Burden of Disease Study found that the global prevalence of hookworm infection is nearly unchanged despite more than two decades of deworming[21,169]. Such concerns about the sustainability of MDA programmes prompted the establishment of the Human Hookworm Vaccine Initiative (HHVI)[170–172], an initiative of the non-profit product development partnership of the Sabin Vaccine Institute. The goal of the HHVI is to develop a subunit vaccine that prevents moderate and heavy hookworm infections, therefore minimizing intestinal blood loss caused by feeding adult worms (BOX 4).

The development of vaccines for neglected tropical diseases is being led by non-profit product development partnerships in collaboration with academic and industrial partners, including vaccine manufacturers based in resource-limited countries. Thus, issues that are often taken for granted in vaccine development (such as manufacturing cost and cold chain logistics) combined with insufficient financial investment are major hurdles in the development of neglected tropical disease vaccines — and a pressing issue for public health in general.

Hookworm infection to treat inflammatory diseases

Hookworm infection has been viewed in an entirely different light since the publication of the first clinical trial on the therapeutic use of experimental human hookworm infection to treat inflammatory diseases[10]. The epidemiological association between helminth infection and reduced susceptibility to inflammatory diseases has been proposed[173,174], with compelling examples of hookworm infection protecting against the onset of allergies. Indeed, negative associations have been reported between hookworm infection and the risk of wheeze[84], dust mite allergy[175] and even metabolic diseases as determined by insulin sensitivity[176]. Furthermore, studies in populations from poor-hygiene areas migrating to high-sanitation regions have shown that these individuals have an increased risk of inflammatory diseases[177], although there might be other contributing factors (such as changes in the host microbiota). The lack of exposure to pathogens, such as hookworms, often combined with genetic predisposition, can uncover underlying inflammatory diseases[178]. However, it has also been pointed out that elements of the helminth-associated 'hygiene hypothesis' are often not so clear-cut. For example, helminth infections that lead to inflammatory diseases are surprisingly prevalent in high-income countries, whereas some helminth species can actually promote inflammatory states[179].

Nevertheless, some of the observations highlighted above have led to clinical trials using iatrogenic helminth infections to treat inflammatory diseases, which have had mixed results[180]. Experimental human hookworm infection exhibited no effect on asthma or allergic rhinitis[11,181]. However, experimental hookworm infection restored gluten tolerance in individuals affected by coeliac disease when coupled with increasing dietary gluten. Infection with 20 worms enabled patients to tolerate gluten doses corresponding to a medium-sized bowl of pasta every day for 2 weeks, with no clinical, histological or immunological evidence of inflammation[9] (FIG. 6).

Although the mechanisms behind the therapeutic benefits of hookworm infection are unclear, both infected healthy individuals and patients with coeliac disease had reduced levels of inflammatory cytokines and increased levels of regulatory cytokines and T_{reg} cells in the gut[9,58]. Moreover, infection of patients with coeliac disease with hookworms resulted in an increased richness of commensal microbial species in the gut[182] and increased concentrations of anti-inflammatory short-chain fatty acids derived from the microbiota[183].

The notion of live helminth therapy is unpalatable to many people, so the development of the worm itself as a marketable drug product would face substantial regulatory hurdles. However, the immunoregulatory prowess of hookworms seems to reside within their secretomes. Adult-stage hookworms that are recovered from definitive hosts after euthanasia can be cultured *in vitro* whereupon they secrete various molecules, including proteins, peptides, glycans and small molecules, which are collectively referred to as excretory–secretory (ES) products. ES products of *A. caninum* have been shown to protect mice against chemically induced colitis[184–186]. Defined synthetic ES peptides have been shown to suppress T cell proliferation[187], and a recombinant hookworm protein with sequence identity to mammalian tissue inhibitor of metalloproteinase protected mice against asthma by inducing tolerogenic dendritic cells and expanding T_{reg} cell numbers that homed to mucosal sites of inflammation[188]. Moreover, a recombinant ES protein that binds to the CD11b integrin prevents neutrophil accumulation and is neuroprotective after focal ischaemia in rats[189], and even underwent phase IIa clinical trials for stroke[190]. The unique specificity and potency of hookworm antithrombotics have also generated substantial interest in their use for treating a range of vascular pathologies, including heart disease, stroke and even cancer[191]. The ES proteome of *A. caninum* has been characterized using tandem mass spectrometry[43], and the recent publication of the *N. americanus* genome and immunome[44] will facilitate the discovery of new proteins with potential as novel biologics, as well as vaccine and diagnostic antigens to control and ultimately eradicate hookworm disease.

Final words

Hookworm infection has plagued humans for millennia and there are descriptions of helminths in the earliest medical texts from 1500 BC[192]. Hookworms are one of the most exquisitely adapted human parasites and are capable of overcoming the barrage of physical and physiological challenges they encounter along their migratory route within the host. They can survive for decades in the gastrointestinal tract despite an immune onslaught targeting hundreds of antigens[44] (A.L., unpublished observation). Indeed, the ability of this ancient parasite to avoid the immune system might well hold the key to the control of modern-day immune and metabolic diseases. Despite huge international efforts to control hookworm and other STH infections, little progress has been made. Now more than ever, new strategies are needed. The continuous use of currently available drugs and the development of new ones are fundamental, and so are substantial investments towards economic development and improved sanitation in endemic, resource-limited regions. However, drugs alone are not likely to eliminate hookworm infection, especially given the variability of mebendazole and albendazole efficacies. Thus, a human hookworm vaccine would represent a leap towards global elimination. Much work lies ahead for public health workers, clinicians and biomedical scientists to eradicate hookworm infection from endemic countries while harnessing its untapped therapeutic properties.

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Introduction (A.L.); Epidemiology (J.M.B. and P.J.H.); Mechanisms/pathophysiology (A.L., M.Y. and J.C.); Diagnosis, screening and prevention (J.M.B., R.C.-O., P.J.H., J.C., D.D. and J.S.M.); Management (D.D., P.J.H. and J.S.M.); Quality of life (P.J.H.); Outlook (A.L.); Overview of Primer (A.L.).

Competing interests
A.L., P.J.H. and J.M.B. are co-inventors on patents for the use of hookworm proteins as subunit vaccines against human hookworm disease. A.L. is an inventor on patents for the use of hookworm proteins as therapeutics for the treatment of inflammatory diseases and has received funding for such projects from Janssen R&D, USA. All other authors declare no competing interests.