PLOS PATHOGENS

Harnessing helminth-driven immunoregulation in the search for novel therapeutic modalities

Stephanie M. Ryan, Ramon M. Eichenberger [ID], Roland Ruscher [ID], Paul R. Giacomin, Alex Loukas [ID]*

Centre for Molecular Therapeutics, Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Queensland, Australia
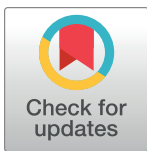
* alex.loukas@jcu.edu.au

Abstract

Parasitic helminths have coevolved with humans over millennia, intricately refining and developing an array of mechanisms to suppress or skew the host's immune system, thereby promoting their long-term survival. Some helminths, such as hookworms, cause little to no overt pathology when present in modest numbers and may even confer benefits to their human host. To exploit this evolutionary phenomenon, clinical trials of human helminth infection have been established and assessed for safety and efficacy for a range of immune dysfunction diseases and have yielded mixed outcomes. Studies of live helminth therapy in mice and larger animals have convincingly shown that helminths and their excretory/secretory products possess anti-inflammatory drug-like properties and represent an untapped pharmacopeia. These anti-inflammatory moieties include extracellular vesicles, proteins, glycans, post-translational modifications, and various metabolites. Although the concept of helminth-inspired therapies holds promise, it also presents a challenge to the drug development community, which is generally unfamiliar with foreign biologics that do not behave like antibodies. Identification and characterization of helminth molecules and vesicles and the molecular pathways they target in the host present a unique opportunity to develop tailored drugs inspired by nature that are efficacious, safe, and have minimal immunogenicity. Even so, much work remains to mine and assess this out-of-the-box therapeutic modality. Industry-based organizations need to consider long-haul investments aimed at unraveling and exploiting unique and differentiated mechanisms of action as opposed to toe-dipping entries with an eye on rapid and profitable turnarounds.

Introduction

Parasitic worms (helminths) infect approximately 2 billion people worldwide, predominantly children in rural subtropical and tropical areas with inadequate sanitation [1]. Helminths have struck a balance with their hosts, refined by millennia of coevolution, to meet their needs for propagation and transmission while minimizing pathology [2]. They promote wound healing and tissue repair and skew distinct immune processes to improve their long-term survival.

Arguably, the most masterful trait of parasitic helminths, from a drug development perspective, is their ability to potently regulate host inflammatory responses.

## Helminth-mediated prevention of inflammatory and metabolic disorders in people

In industrialized nations, there has been a reduction in exposure to infectious agents because of vaccination, increased sanitation, improved hygienic standards, and widespread use of antibiotics. The vast decline in the prevalence of contagious diseases from these communities, helminthiases, in particular, is inversely associated with an alarming increase in the incidence of inflammatory and metabolic disorders [3]. For example, the Western world is experiencing an increasing rate of inflammatory bowel disease (IBD), and at present, there is no available cure. Compounding matters, there has been a sharp rise in the incidence of IBD and allergies in the newly industrialized nations of Asia and Latin America [4]. This increase in noncommunicable diseases is, at least in part, a result of diets becoming westernized, decreased exposure to infections, large scale deworming programs, and mass migration [5].

Although the association between helminths and inflammatory diseases is multifactorial, selective pressure placed on the human genome by historically widespread helminth infection has driven various polymorphisms, including at loci associated with predispositions to inflammatory diseases [6]. Moreover, minimal exposure to pathogens results in an underdeveloped regulatory immune network, culminating in an increased prevalence of disorders that result from immune dysfunction [7, 8]. Helminth-mediated protection is not, however, restricted to autoimmune and allergic diseases. There is an inverse relationship observed between human helminth infection, insulin resistance, and type 2 diabetes (T2D) [9, 10]. It has been proposed that chronic helminth infection results in long-term beneficial effects on host metabolism, especially on white adipose tissue (WAT), intestines, and liver [11]. Understanding the molecular mechanisms of WAT inflammation is topical in drug development given the epidemic of metabolic diseases, and we will touch upon this again later in the review.

The mechanisms by which parasitic helminths regulate inflammation and metabolism are diverse and complex and have been reviewed extensively [11–15]. Helminths are potent drivers of T helper type 2 (T_H2) immune responses, characterized by eosinophilia, mast cell mastocytosis, type 2 innate lymphoid cells (ILC2s), tuft cells, and mucus production. Overlaid on this T_H2 response, however, is a predominant state of immune tolerance, characterized by an abundance of IL-10 produced by regulatory cell populations such as regulatory T cells (Tregs), regulatory B cells (Bregs), tolerogenic dendritic cells (DCs), and alternatively activated macrophages. Furthermore, the interactions between helminths, the microbiome and its attendant metabolites [16–21], and the nervous system have become increasingly important (Fig 1).

Clinical trials of experimental human helminth infections for treating inflammatory diseases

Experimental helminth infections have been used in the treatment of allergic and autoimmune diseases in human clinical trials for over 15 years and have yielded promising results (Table 1). Two helminth species have been used in clinical trials for treating inflammatory diseases—the pig whipworm *Trichuris suis* and the human hookworm *Necator americanus*. The therapeutic potential of orally administered *T. suis* ova (TSO) has been assessed in phase 1 trials in patients with the 2 primary forms of IBD—Crohn's disease and ulcerative colitis. After 12 weeks of therapy, significant improvement according to the intent-to-treat principle occurred in patients receiving TSO compared with those who received placebo [22]. TSO was also assessed in an open-label clinical trial in Crohn's disease patients, in which 72% of subjects were in remission after 24 weeks [23].
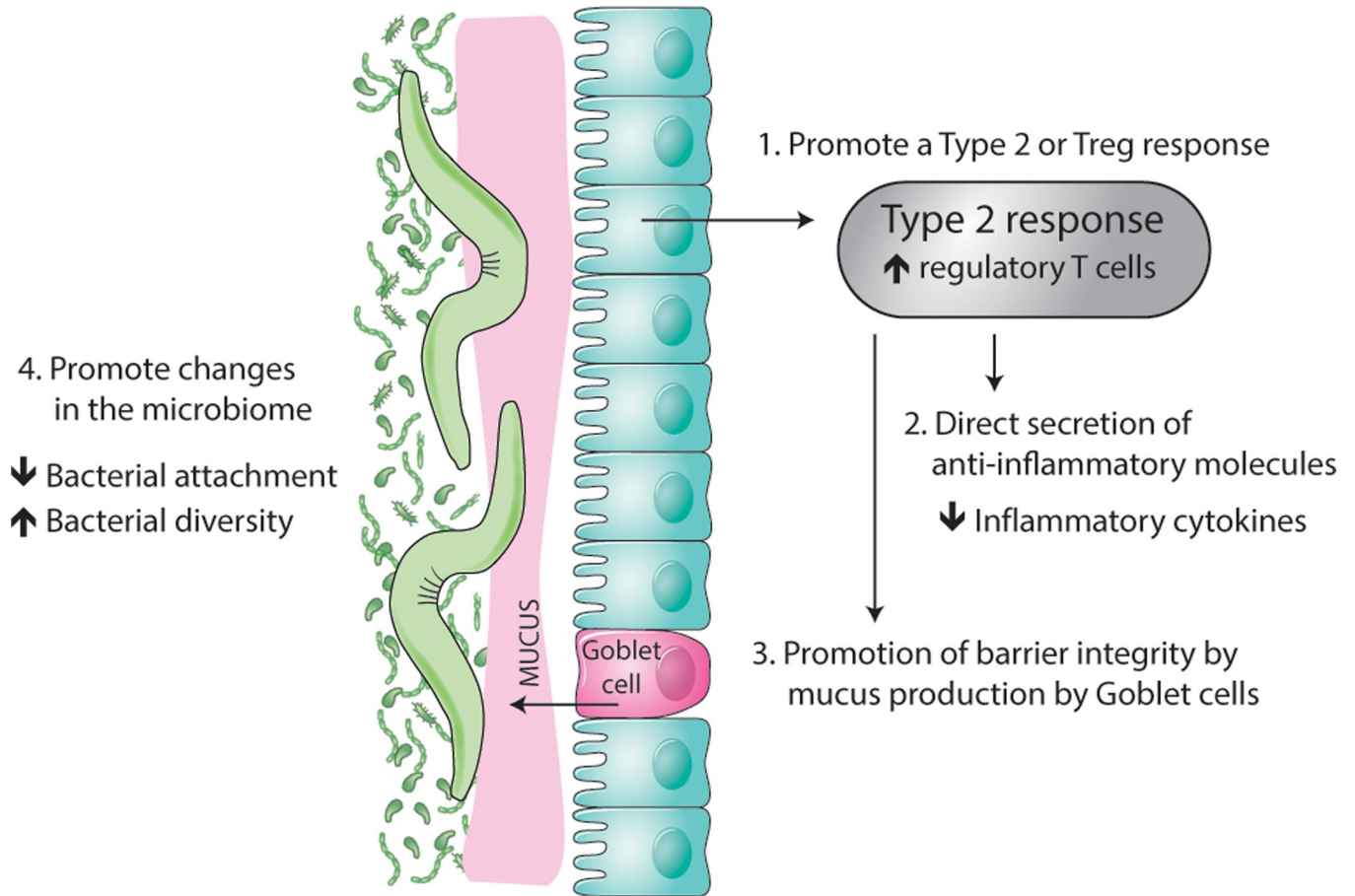
Fig 1. "Worm therapy" for immune dysregulation diseases. The range of host physiological factors impacted by gastrointestinal helminth infection could alleviate inflammatory disease. (1) Parasite-derived factors drive an inclusive or exclusive polarized regulatory or type 2 response, which is responsible for (2) direct secretion of anti-inflammatory molecules from the host immune system (3) and the promotion of barrier integrity, which is often compromised in the pathophysiology of IBD and foodborne incompatibilities. Furthermore, (4) helminth colonization provides factors for a diverse bacterial environment that protects against gut inflammation. IBD, inflammatory bowel disease; T_H2, T helper type 2; Treg, regulatory T cell.

https://doi.org/10.1371/journal.ppat.1008508.g001

Disappointingly, although early trials with *T. suis* showed promise, subsequent phase 2 trials failed to reach their clinical endpoints in both IBD [24] and multiple sclerosis [25, 26].

T. suis establishes chronic infections in pigs but is expelled from the human body within weeks and therefore requires frequent dosing. On the other hand, *N. americanus* is primarily a human parasite and can survive for years in infected individuals [27]. Experimental infection with *N. americanus* is safe and well tolerated in human volunteers [28–30]. In a small phase 1 clinical trial for Crohn's disease, percutaneous administration of *N. americanus* in relatively low doses was well tolerated, and patients who remained in the trial for 1 year were in disease remission [31]. A subsequent trial targeted celiac disease because of its histopathological similarities to IBD. Celiac subjects on a gluten-free diet who were experimentally infected with *N. americanus* displayed improved tolerance to escalating gluten micro-challenge, a decreased presence of inflammatory cytokine-producing T cells in the gut, and corresponding increases in mucosal Treg numbers [30]. Indeed, with the growing body of literature supporting a role for inflammation in driving T2D [32], a clinical trial using experimental *N. americanus* infection is currently being conducted to investigate the therapeutic effect of helminth infections in metabolic disorders (Fig 2) [33]. Most of these early-phase clinical trials were impaired by the absence of a

Table 1. Completed and ongoing therapeutic clinical trials using helminth products in humans in disease settings. These trials are rigorously assessing the safety and tolerability of experimental helminth infection and therapeutic efficacy of infections in disease indications.

Trial/phase	Helminth	Status (year range)	Study title and treatment	Results outcome	Reference
Phase I	TSO	2003 (complete)	**Crohn's disease** Initial safety studies of oral inoculation (2,500 ova) over 12 weeks ($n = 7$).	Patients displayed clinical improvements and no serious adverse events.	[128]
NCT01433471 Phase 1 and 2	TSO	2005 (complete)	**Ulcerative colitis** Open-label study randomized, double-blind, placebo-controlled oral inoculation (2,500 ova) over 12 weeks ($n = 30$).	Improvement in disease index by 43% in treatment cohort.	[23]
EUCTR2011-006344-71-DE Phase 1	TSO	2008 to 2011 (terminated)	**Rheumatoid arthritis** Oral inoculation (2,500 ova) in 2-week intervals for 24 weeks ($n = 50$).	Trial terminated and results unknown.	Immanuel Hospital Berlin, Germany
NCT00645749 Phase 2	TSO	2008 to 2015 (complete)	**Multiple sclerosis** (HINT) Oral inoculation (2,500 ova) 2-week intervals for 12 weeks ($n = 17$).	Trend toward 35% diminution in active lesions. Increase in T regulatory lymphocytes with treatment. Increase in serum levels of IL-4 and IL-10 during treatment. No serious adverse events.	[129]
NCT01006941 Phase 2	TSO	2009 to 2011 (complete)	**Multiple sclerosis** Nonrandomized, open-labeled oral inoculation (2,500 ova) 2-week intervals for 12 weeks ($n = 10$).	No obvious benefit observed in infection group. Mild to self-limiting adverse events.	[25]
EudraCT no. 2007-006099-12 Phase I	TSO	2007 to 2010 (complete)	**Allergic rhinitis** Randomized, double-blind, placebo-controlled, 2,500 ova administered ($n = 49$) and placebo ($n = 47$).	No therapeutic effect on allergic rhinitis of infection.	[130]
NCT01413243 Phase 2	TSO	2011 to 2016 (terminated)	**Multiple sclerosis** (TRIOMS) randomized control trial of oral inoculation (2,500 ova) 2-week intervals for 12 weeks ($n = 50$).	Unknown	[131]
NCT01434693 Phase 1	TSO	2011 to 2013 (complete)	**Crohn's disease** Randomized, double-blind, placebo-controlled sequential oral dose escalation (500, 2,500, 7,500 ova) ($n = 36$).	Placebo and treatment groups experience minor adverse events. No obvious improvement in pathology with infection.	[132]
NCT01576471 Phase 2	TSO	2013 (unknown)	**Crohn's disease** (TRUST-1) Randomized, double-blind, placebo-controlled oral inoculation of 7,500 ova in 2-week intervals. Placebo group included.	Unknown results of study	Coronado Biosciences, United Kingdom
NCT01279577 Phase 2	TSO	2011 to 2015 (complete)	**Crohn's disease** Randomized, double-blind, placebo-controlled, low, medium, high oral inoculation ova ($n = 254$) participants.	Unknown results of study	Dr. Falk Pharma, Germany
NCT01836939 Phase 1	TSO	2013 to 2015 (complete)	**Plaque psoriasis** Randomized, 2-arm trial of oral inoculation (2,500 ova) in 2-week intervals for 10 weeks and (7,500 ova) in 2-week intervals for 10 weeks ($n = 8$).	Unknown results of study	Icahn School of Medicine at Mount Sinai, US
NCT01948271 Phase 1	TSO	2013 to 2016 (terminated)	**Plaque psoriasis** Open-label, oral inoculation (7,500 ova) in 2-week intervals for 14-week duration ($n = 3$).	Trial terminated because of a lack of efficacy	Tufts Medical Center, US
NCT02011269 Phase 2	TSO	2013 to 2016 (withdrawn)	**Plaque psoriasis** Randomized, blinded, placebo-controlled, 3-arm trial of oral inoculation (7,500 ova) in 2-week intervals for 10 weeks, (15,000 ova) in 2-week intervals for 10 weeks.	Trial withdrawn and results unknown.	Coronado Biosciences, UK

(*Continued*)

Table 1. (Continued)

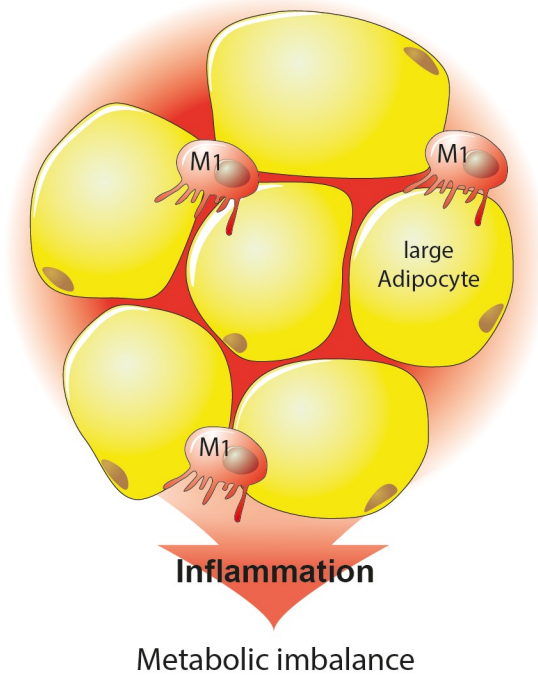| Trial/phase	Helminth	Status (year range)	Study title and treatment	Results outcome	Reference
Phase 1	*N. americanus* larvae	2006 (complete)	**Crohn's disease** Proof of concept: inoculation with larvae at week 0 (*n* = 9) and week between week 27 to 30 (*n* = 5)	Remission at week 45 observed in 5 patients inoculated in week 0. No serious adverse events.	[31]
	N. americanus larvae	2009	**Allergic rhinoconjunctivitis** 30 individuals with allergic rhinoconjunctivitis were randomized and inoculated with 10 larvae or placebo and followed for 12 weeks.	Hookworm infection did not induce clinically significant exacerbation of airway responsiveness.	[133]
NCT00469989	*N. americanus* larvae	2010	**Asthma** Randomized, placebo-controlled, inoculation with 10 larvae and followed for 16 weeks (*n* = 30).	Hookworm infection did not significantly improve bronchial responsiveness nor other measures of asthma control.	[28]
NCT00671138 Phase 2	*N. americanus* larvae	2007 to 2011 2011 to 2016 (completed)	**Celiac disease** Randomized, double-blinded, placebo-controlled trial. Part 1: inoculating celiac disease patients with the human hookworm *N. americanus* larvae at week 0 (*n* = 10) and week 12 (*n* = 10), followed by oral gluten challenge at week 20 of 16 g gluten per day for 5 days. Part 2: inoculating celiac disease patients with the *N. americanus* larvae at week 0 (*n* = 7) and week 12 (*n* = 7). At week 20, subjects were given an oral gluten challenge at week 20 of 16 g gluten per day for 5 days.	Infection conferred no obvious benefit to pathology. Mucosa of hookworm-infected subject maintained healthy appearance. No serious adverse events. Duodenal biopsy culture of hookworm-infected subjects had suppressed IL-17A and IFN-γ and increased levels of IL-10, IL-5 and regulatory T cells. No serious adverse events.	[30, 134]
NCT01661933 Phase 1 and 2	*N. americanus* larvae	2012 to 2014 (completed)	**Celiac disease** Inoculating celiac disease patients with the human hookworm *N. americanus* larvae at week 0 (*n* = 10) and week 4 (*n* = 10), followed by incremental gluten challenge (*n* = 12).	Ten patients successfully tolerated gluten challenge. No serious adverse events.	[135]
NCT02754609 Phase I	*N. americanus* larvae	2016 to 2020 (completed)	**Celiac disease** Hookworm therapy for celiac disease (NainCeD-3). Randomized, placebo (*n* = 10), inoculation week 0 and week 8 (*n* = 40) to assess safety and dose-ranging clinical trial examining sustained gluten challenge.	Manuscript in preparation	James Cook University, Australia
NCT00630383 Phase 2	*N. americanus* larvae	2008 to 2012 (withdrawn)	**Multiple sclerosis** Randomized, inoculation 25 larvae at week 0. Placebo group included.	Withdrawn—superseded by a similar study	University of Nottingham, UK
NCT01470521 Phase 2	*N. americanus* larvae	2011 to 2016 (complete)	**Multiple sclerosis** (WIRMS) Randomized, inoculation 25 larvae at week 0 (*n* = 36). Placebo group (*n* = 36).	Unknown study results	University of Nottingham, UK

HINT, Helminth-induced Immunomodulation Therapy; IFN-γ, interferon gamma; IL, interleukin; TRIOMS, *Trichuris Suis* Ova in Recurrent Remittent Multiple Sclerosis; TRUST-1, Treatment With Oral CNDO 201 *Trichuris Suis* Ova Suspension in Patients; TSO, *Trichuris suis* ova; WIRMS, Worms for Immune Regulation of Multiple Sclerosis.

standardized manufacturing protocol for *N. americanus*. However, methods for the production of cGMP human hookworms were recently described [34] and are essential advances if helminth therapy is to receive widespread acceptance by the medical fraternity [35].

Further to therapeutic trials of helminth infections in inflammatory disease settings, dose-escalation controlled helminth infections in healthy volunteers are currently ongoing (S1 Table), primarily intending to develop a platform to test anti-helminth vaccines and drugs [34, 36].

Obese adipose tissue: Lean adipose tissue:
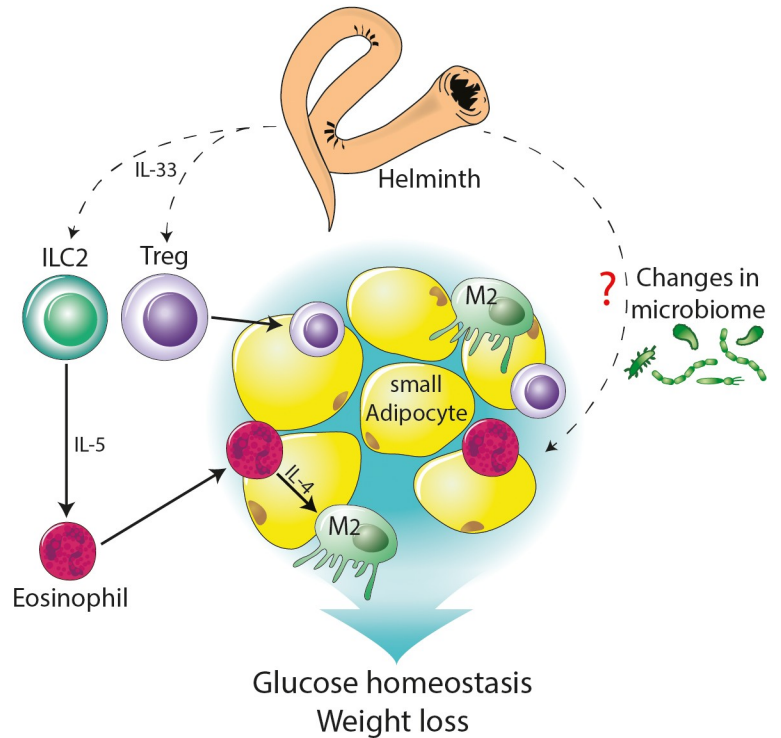
Fig 2. Inflammation and metabolic imbalance versus glucose homeostasis and weight loss, in response to infection with gastrointestinal nematodes and intravascular blood flukes and their ESPs. Chronic inflammation in adipose tissue is linked to a switch to M1 macrophages and the production of TNF-α and IL-1β. Helminth infection and helminth ESPs induce changes in the gut that lead to a regulatory/T_H2 milieu that results in reduced inflammation in adipose tissue, enhanced glucose homeostasis, and decreased weight gain in obese animals. Furthermore, this regulatory/T_H2 milieu increases IL-33 produced in adipose tissue by stromal cells within the progenitors of both adipocytes and mesenchymal cells. The production of IL-33 induces resident ILC2 to produce IL-5, which recruits eosinophils. Eosinophils in white adipose tissue secrete IL-4, which induces M2 macrophages. The production of IL-33 also induces regulatory T and B cells to produce IL-10, which sustains M2 macrophage activity. ESPs, excretory/secretory product; IL, interleukin; ILC2, type 2 innate lymphoid cell; M1, classically activated macrophage; M2, alternatively activated macrophage; T_H2, T helper type 2; TNF, tumour necrosis factor.

https://doi.org/10.1371/journal.ppat.1008508.g002

Insights into parasite–host interactions from animal models

Excretory-secretory products

Helminths secrete bioactive molecules that can suppress or skew host immune responses; collectively, this suite of molecules is referred to as excretory/secretory products (ESPs). ESPs are a complex mixture of proteins, peptides, nucleic acids, lipids, glycans, and small organic molecules. Administration of ESPs from many nematodes and platyhelminth species induces immune responses that reflect the active infections. Moreover, ESPs have therapeutic properties in a range of animal models of autoimmunity, allergy, and metabolic disease (see recent reviews [37–39]). Indeed, the use of ESPs instead of active helminth infection potentially addresses some of the drawbacks and obstacles currently faced by experimental helminth therapy [38].

ESPs from many helminths, including at least 2 hookworm species (*Ancylostoma caninum* and *A. ceylanicum*), protected mice against T-cell-dependent trinitrobenzene sulfonic acid (TNBS)-induced colitis [39] and T-cell-independent dextran sulfate sodium (DSS) colitis [40, 41]. Prevention of immunopathology with ESPs is not only restricted to diseases driven by T_H1/T_H17 responses; both immuno-epidemiological observations [42] and mouse studies [43, 44] have shown that helminths and ESPs are also potent suppressors of T_H2-driven allergic

inflammation. The ability of ESPs to regulate all major forms of immunopathology is primarily attributed to its potent regulatory properties [45, 46]. Helminth ESPs drive a modified T_H2 response that is different from the canonical T_H2 response seen in allergies in which activated CD4+ cells secrete T_H2 cytokines, including interleukin (IL)-4, IL-5, and IL-13 [47]. ESPs instead induce a modified T_H2 response characterized by the secretion of regulatory cytokines such as IL-10 and the immunosuppressive transforming growth factor-beta (TGF-β) to ensure the containment of infection to manageable levels and that excessive T_H2 inflammation does not ensue [48].

ESPs target innate immune cell function

Parasitic helminths target several cell types that orchestrate a characteristic immune regulatory phenotype, notably, antigen-presenting cells such as DCs and macrophages. DCs possess intrinsic tolerance mechanisms that are integral initiators of the T_H2 response [49]. Regulatory or tolerogenic DCs expressing integrin alpha E (CD103) are located in the intestinal mucosa and mesenteric lymph nodes [50]. Mucosal CD103+ DCs are capable of antagonizing T_H2 induction in *Schistosoma mansoni* and *Heligmosomoides polygyrus* infections in mice. Mucosal CD103+ DCs secrete TGF-β, retinoic acid, and IL-2 and prevent immune-mediated pathology by promoting the expansion of Treg populations and maintaining intestinal homeostasis. Helminth ESPs target DCs and macrophage activation through suppression of pattern recognition receptor function, diminishing the ability of these innate sentinels to detect and respond to pathogen-associated molecular patterns (reviewed in [37]). Hookworm ESPs induce CD103+ DCs to express retinoic acid, which, in turn, facilitated the expansion of Tregs in a mouse model of asthma [51]. Moreover, helminth ESPs are known potent inducers of M2 macrophages [52], a cell population that drives T_H2 responses and promotes wound repair [2].

Recent studies have shed light on the mechanisms by which helminths initiate T_H2 responses by signaling through ILC2s (reviewed in [53]). ILC2s are enriched in the mucosa of gastrointestinal nematode–infected mice and arise in response to secretion of the alarmin IL-25 by intestinal tuft cells [54]. ILC2s respond rapidly to the presence of helminths by increasing in number and secretion of type 2 cytokines [54]. This cascade, in turn, results in the repair of epithelial barriers and recruitment of other innate cells, including eosinophils and culminates in the activation of T_H2 cells and regulatory pathways.

Helminths induce Treg activity

Tregs constitute around 5% of circulating CD4+ T cells and are identified by the lineage marker forkhead box P3 (FOXP3). Mutations in several genes that orchestrate Treg function, including the IL-2 receptor alpha subunit CD25 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) result in the development of severe autoimmune syndromes [55], so Tregs constitute a significant target for new therapeutic strategies. Such research is focusing on the ability of Tregs to mediate immune regulation via multiple mechanisms, including IL-2 deprivation, secretion of the regulatory cytokines IL-10 and TGF-β, and acquisition of costimulatory molecules from antigen-presenting cells through binding to CTLA-4.

Both nematodes and platyhelminths are potent drivers of Treg responses. In mice infected with either *Nippostrongylus brasiliensis* or *S. mansoni*, IL-4 receptor alpha-mediated signaling on Tregs was required in vivo for control of helminth-induced inflammation [56]. *H. polygyrus* ESPs directly induces Tregs in vitro using FOXP3-green fluorescent protein reporter mice [49], and more recently, several helminth recombinant proteins have been shown to drive Treg expansion [37]. Indeed, from a clinical perspective, molecules that drive expansion, mobilization, or increased mucosal homing of 9Tregs are the holy grail for many disorders that result

from immune dysregulation [57, 58], and we will provide defined examples later in this review. Regulatory B cells (Bregs) are also a feature of helminth infections [59] and are the primary source of IL-10 and other tissue-protective proteins, including (Resistin-like molecule-α) RELMα [60, 61]. Indeed, in mice infected with *H. polygyrus*, IL-10$^+$ Breg cells were able to promote expansion and maintenance of IL-10$^+$ FOXP3$^+$ Treg cell populations [61]. Fig 3 summarizes the multitude of cellular immune pathways upon which helminths and their ESPs impact, including the well-established influence of DCs, but also the emerging potential roles for factors produced by microbes, sensory neurons, and specialized intestinal epithelial cells that initiate the very early response to helminth exposure via ILC2s and innate granulocytes [62, 63]. These recently identified biological pathways may, therefore, be potential therapeutic targets for immunoregulation by helminths and their secreted products.

## Helminth therapeutic moieties

Despite the wealth of literature supporting the therapeutic use of helminth ESPs for treating inflammatory disorders, the capacity of a crude parasite-derived supernatant to be developed as a drug is limited. ESP proteomes from many distinct species of helminths have been characterized, and their annotation has been supported by the increasing number of draft genomes [64]. Although a handful of individual proteins with immunoregulatory properties have been identified from ESPs, helminth-secreted proteomes still present a relatively untapped pharmacopeia [65, 66]. Next, we highlight a select group of molecules with proven immunoregulatory roles and potential therapeutic properties.

Proteases and protease inhibitors

There are numerous examples of proteins from multicellular ecto- and endoparasites that have evolved from a protease or protease inhibitor scaffold but no longer possess canonical activity [65–67]. For example, 2 of the most abundant ESP proteins in *A. caninum* possess an ancestral netrin domain and are structural homologs of the tissue inhibitor of metalloprotease (TIMP) family [68]. Although *A. caninum* anti-inflammatory protein (*Ac*-AIP)-1 and *Ac*-AIP-2 possess a TIMP-like domain, they do not appear to have the ability to suppress matrix metalloprotease catalytic activity and instead have evolved a distinct anti-inflammatory function [67, 69]. Recombinant AIPs suppressed expression of DC activation and costimulation markers [52, 70] and drove the subsequent expansion and mucosal homing of Tregs, which protected against inducible asthma [51]. Prophylactic treatment of mice with recombinant *Ac*-AIP-1 in chemically induced colitis protected mice against weight loss, clinical disease, and intestinal histopathology and significantly reduced the expression of hallmark $T_H1/T_H2/ T_H17$ cytokines that drive inflammation in human IBD [71].

In similar fashion to the plasticity of the TIMP-like domain in hookworms, there is growing evidence that throughout their evolution, some cysteine protease inhibitor (cystatin) superfamily members have acquired novel roles that are independent of cysteine protease inhibition [72]. Filarial nematodes secrete cystatins that possess the canonical papain-like enzyme inhibitory activity but have also evolved a novel function that allows them to inhibit the catalytically distinct asparaginyl endopeptidase activity. This dual function assists in the inhibition of antigen processing in the major histocompatibility complex (MHC) class II pathway [73]. Cystatins from various helminth species suppress the secretion of inflammatory cytokines and promote IL-10 production by macrophages in particular [74, 75]. Cystatin from the filarial nematode *Acanthocheilonema viteae* suppressed inducible colitis and asthma in mice and displayed ex vivo bioactivity with human peripheral blood mononuclear cells from atopic patients with grass pollen allergy [76]. Moreover, oral delivery of *A. viteae* recombinant cystatin via
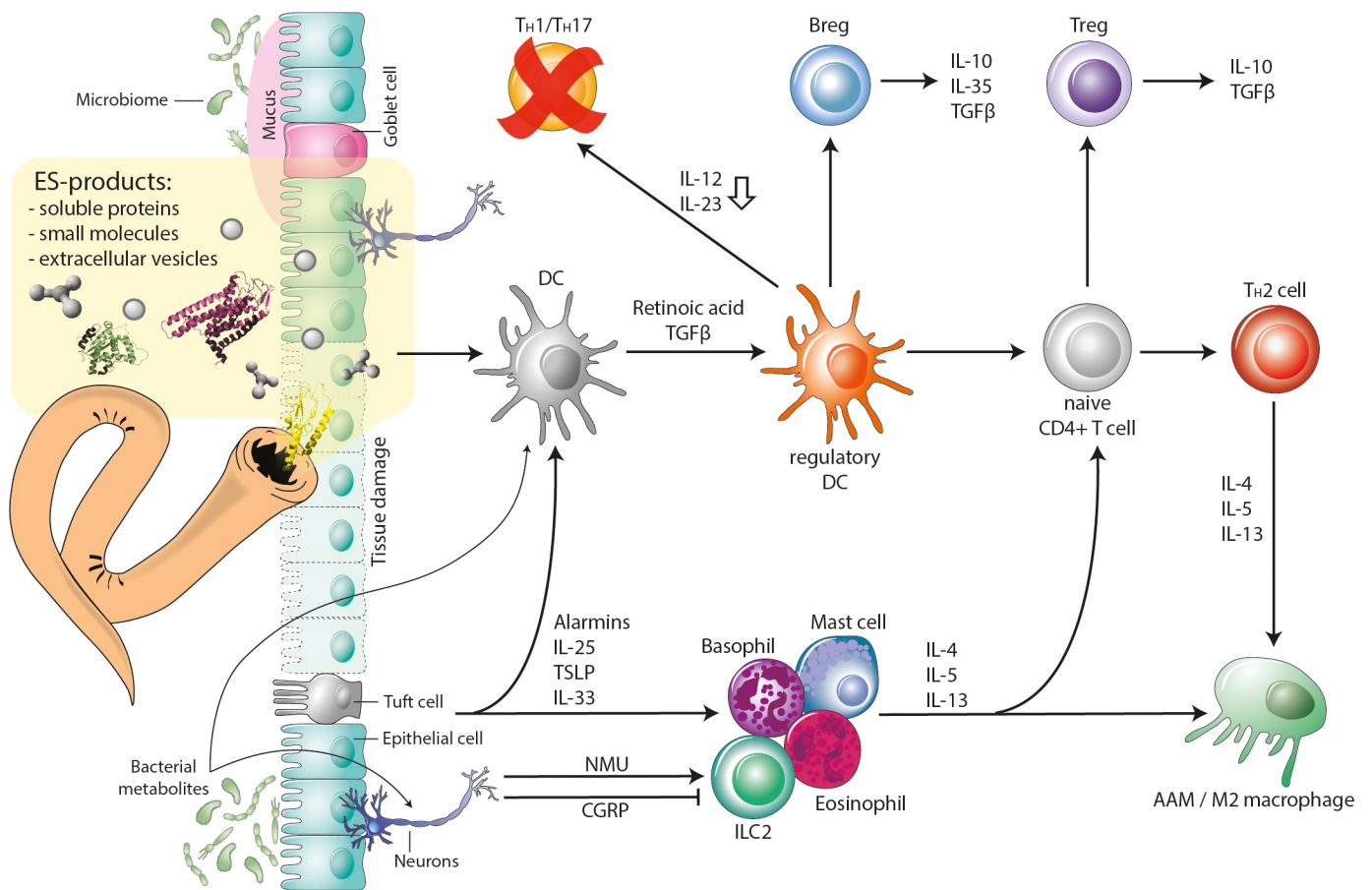
Fig 3. Helminths and their ESPs manipulate the host immune system. Helminth infection promotes T_H2 cell differentiation, Treg responses, macrophage polarization, and mucus production, which are regulated by multiple upstream events and stimulated by signals from the worm (ESPs), but also signals from the microbiome (metabolites) and tissue damage (alarmins). DCs are central to these processes and respond to alarmins, ESPs, and metabolites to adopt a regulatory phenotype that promotes Treg, Breg, and T_H2 cell development and suppress T_H17 and T_H1 cell responses. In addition, helminth-induced damage to the epithelium causes the release of alarmins such as TSLP, IL-25, and IL-33 from tuft cells and other epithelial cells, which can act on ILC2s and granulocytes to augment production of type 2 cytokines. This network is also influenced by sensory neurons within the gut that sense signals from helminths and microbes and elicit production of neuropeptides such as NMU and CGRP to regulate ILC2 responses directly. Breg, regulatory B cell; CGRP, calcitonin gene-related protein; DC, dendritic cell; ESPs, excretory/secretory product; ILC2, type 2 innate lymphoid cell; NMU, neuromedin U; T_H2, T helper type 2; TLSP, thymic stromal lymphopoietin; Treg, regulatory T cell.

continual dosing of transgenic *Lactococcus lactis* prevented the onset of colitis in pigs [76]. Subsequent studies have shown that cystatins from other helminths, both nematodes, and platyhelminths, can suppress inducible colitis [77–79].

Cytokine mimics and cytokine-binding proteins

There is a growing body of literature on helminth ESPs that possess cytokine-like functions but not necessarily cytokine-like sequence homology or secondary structure. For example, *H. polygyrus* secretes a protein called *H. polygyrus* TGF-β mimic (*Hp*-TGM) that binds to the mammalian TGF-β complex and drives human and mouse Treg production but has no sequence homology to mammalian TGF-β. Instead, *Hp*-TGM is a member of the complement control protein (CCP) superfamily [80]. Recombinant *Hp*-TGM delayed allograft rejection in mice and increased Treg numbers in draining lymph nodes at the site of graft transplant, highlighting a potential use for this protein in a range of inflammatory settings. In like fashion, *N. americanus* activation-associated secreted protein (*Na*-ASP-2) secreted by *N. americanus*

infective larvae is a member of the sperm-coating protein (SCP/TAPS) family and has structural- and charge-mimicking features of CXC-chemokines that recruit Tregs [81]. *Na*-ASP-2 was shown to bind to CD79A on human B cells whereupon it affected the expression of genes involved in leukocyte transendothelial migration [82].

H. polygyrus secretes a cytokine-binding protein called *H. polygyrus* alarmin release inhibitor (*Hp*ARI). This protein also belongs to the CCP family and inhibits the release of alarmins [83]. *Hp*ARI binds directly to IL-33 and nuclear DNA, thereby tethering the alarmin within necrotic cells and preventing its release. Recombinant *Hp*ARI administered to mice intranasally suppressed ILC2s and eosinophil responses in the lungs of mice after administration of *Alternaria* allergen, highlighting its utility for treating inflammatory diseases in the lungs in particular.

Helminth molecules that accelerate wound healing

Helminths penetrate and migrate through skin and tissues without causing significant damage. The mechanisms employed by helminths to concurrently reduce injury and stimulate healing in the host are currently being explored [61, 84–86]. Most of the existing literature focuses on the role of the T_H2 response—type 2 macrophages in particular—in driving wound repair; however, a handful of helminth-secreted molecules with wound healing properties have been described. The liver fluke, *Opisthorchis viverrini*, secretes a granulin (GRN)-like growth factor, *Ov*-GRN-1, which promotes wound healing and angiogenesis [87]. Recombinant *Ov*-GRN-1 is challenging to express in scalable form, so a readily-synthesized bioactive peptide fragment of *Ov*-GRN-1 that retained both in vitro and in vivo wound healing properties was identified and retained the in vivo therapeutic properties of the parent protein [88]. Given the alarming incidence of T2D and associated comorbidities such as nonhealing diabetic foot ulcers, topical growth factor-like proteins and peptides from helminths address an area of great unmet need [89, 90].

Post-translational modifications

Many helminth immunomodulatory proteins are secreted and therefore undergo post-translational modifications (PTM). In some cases, the PTM are responsible for the addition of the regulatory moiety of interest. For example, the dominant ESP of *A. vitae* is ES-62. This glycoprotein that has therapeutic effects in a range of mouse models of inflammatory disorders such as arthritis [91], asthma [92], and even systemic lupus erythematosus [93]. ES-62 is an aminopeptidase that carries *N*-glycans decorated with phosphorylcholine (PC) [94, 95]. The fusion of PC to an unrelated carrier protein demonstrated that it retained its therapeutic properties, thus proving that PC is the bioactive moiety of ES-62 [96]. Small drug-like analogs of PC for the treatment of arthritis and chronic lung fibrosis have overcome immunogenicity concerns with the sizeable ES-62 protein [97, 98].

Chemical deglycosylation of ESPs from some helminths ablates protection against inflammatory diseases [99], highlighting the importance of glycans in driving regulatory responses. One such glycan that decorates schistosome soluble egg antigens (SEAs) and secreted egg proteins is the Lewis X-containing glycan found on dominant egg proteins such as omega-1 and interleukin-4-inducing principle (IPSE)/alpha-1 [100]. Recombinant IPSE/alpha-1 expressed in wild tobacco drives IL-10 production from Bregs [101] and suppresses inflammatory cytokine responses by skewing inflammatory monocytes toward anti-inflammatory M2 macrophages [102]. Administration of SEA to mice induced T_H2 immune responses characterized by M2 macrophages and eosinophils in WAT and liver and reduced fat mass gain and lowered insulin resistance and glucose intolerance [103, 104] (Fig 2). Protection against metabolic disease was dependent on the engagement of the mannose receptor CD206 and the release of IL-

33, inducing ILC2-dependent improvements in metabolic status [105]. The immunomodulatory Lewis X-containing glycan, lacto-N-fucopentaose III (LNFPIII) from the schistosome tegument also improves glucose tolerance and insulin sensitivity in diet-induced obese mice, at least in part via increased IL-10 production by macrophages and DCs, and resulted in reduced WAT inflammation [106]. LNFPIII treatment also up-regulated the expression of the multipurpose farnesoid X nuclear receptor (FXR)-α to reduce lipogenesis in the liver, thereby protecting against hepatosteatosis [107]. Producing appropriately glycosylated recombinant versions of schistosome glycoproteins has proven challenging. Nevertheless, recent efforts to glycoengineer Lewis X-containing proteins in wild tobacco with coexpression of defined glycosyltransferases proved successful [105] and opened up new possibilities for generating appropriately glycosylated immunotherapeutics.

Helminth metabolites—an untapped resource for small-molecule therapeutics

Although helminth proteins and their PTMs have been documented and discussed elsewhere in this review, much less is known about parasitic helminth metabolomes. The metabolomics revolution has begun to reveal small-molecule metabolites in parasitic helminth somatic extracts and ESPs [108–111]. Helminthology has been slow to adopt cutting edge metabolomics techniques [111], partly because of the difficulty in obtaining sufficient quantities of ESPs, but also because of the diversity in physicochemical properties of metabolites results in it being almost impossible for a single analytical method to provide the required full coverage of the metabolome of a given biological sample. As such, particular analyses are biased toward certain groups of metabolites. Only a handful of studies have characterized helminth metabolomes, and even fewer have addressed the anti-inflammatory properties of these molecules. *A. caninum* secretes metabolites that suppress both inducible colitis in mice and ex vivo production of inflammatory cytokines from human PBMCs [112]. The identity of the protective moieties is not yet known, but short-chain fatty acids secreted by the parasite (or the hookworm's resident microbiota) are prospective candidates. Another candidate is succinate, a metabolite that is produced by the intestinal microbiota that is critical for inducing intestinal tuft cells to initiate T_H2 responses. Succinate was recently shown to be a major component of metabolomes of gastrointestinal helminths [109, 112] and was significantly enriched in the ESP metabolome of hookworms compared to the somatic tissue metabolites.

The lipidome of different developmental stages of *S. mansoni* was recently described, and the egg stage was shown to be enriched in oxylipids with known immunoregulatory properties such as prostaglandins [113]. Indeed, prostaglandin E2 is secreted in high amounts by *T. suis* compared to other lipids and suppresses TNF and IL-12 secretion from lipopolysaccharide-activated human DCs [114]. Of course, prostaglandins are not unique to helminths and are a therapeutic target in their own right because of their up-regulation in some aggressive cancers [115]. Helminth metabolites that are likely to be candidate small-molecule drugs are those that are unique to parasites and have evolved to suppress defined immunopathological pathways safely and are readily synthesizable. We anticipate substantial growth in this area in the coming years given the increased access of researchers from diverse fields to metabolomics platforms.

Helminth-secreted extracellular vesicles in the treatment of inflammatory disorders

The discovery that parasitic helminths secrete extracellular vesicles (EVs) has spurred a new paradigm in the discovery of helminth-derived immunotherapeutics and antihelminth vaccines [116, 117]. EVs are a heterogeneous group of lipid-enclosed vesicles in the nano- to the

micrometer size range. There is increasing evidence that helminth EVs are essential players in regulating host inflammation and immunity, and their application as anti-inflammatory therapeutics has been considered. However, to date, the exact mechanisms by which helminth EVs polarize immune responses remain elusive.

In the *Alternaria* model of allergic asthma, administration of *H. polygyrus* EVs significantly reduced lung immunopathology via ILC2-mediated suppression of innate immunity [118]. The anticolitic therapeutic potential of helminth EVs has also been demonstrated in several recent reports. *N. brasiliensis* EV-treated mice were protected from T-cell-dependent acute colitis, specifically by the suppression of inflammatory cytokines and increased expression of IL-10 [119]. EVs from the liver fluke *Fasciola hepatica* can modulate T-cell-independent colitis [120], characterized by reduced expression of intestinal proinflammatory cytokines that suppress both mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer (NF-κB) signaling pathways.

Perhaps the most intriguing aspect of helminth EVs (from a drug development perspective) is the abundance of vesicular microRNAs (miRNAs) that are predicted to target mammalian host genes, particularly those involved in immune processes. Helminth EVs are actively internalized by host cells [119, 121], providing a mechanism by which the parasites transfer genetic material to the host in a bid to actively manipulate host gene expression [116, 118]. There is a growing body of literature demonstrating the presence of parasite-specific miRNAs secreted by worms, but little is known about their specific interactions with host genes. *H. polygyrus* miRNAs were shown to down-regulate the expression of the mouse phosphatase gene *dusp1* (which corresponds to MKP-1 in humans), a key regulator of MAPK signaling and T_H1 responses to toll-like receptor ligands using a luciferase reporter assay [118].

Looking forward, a deeper understanding of helminth miRNA immunobiology may lead to advances in miRNA-based therapeutics for a whole host of immune dysfunction diseases, including conditions like scleroderma, in which defined miRNAs (of host origin) are already in preclinical and clinical development (reviewed in [122]). EV research has been critical for demonstrating how miRNAs could be used to treat disease, but some practical challenges and obstacles need to be overcome before this type of therapy enjoys mainstream application [123].

Turning worm proteins into conventional drugs

The molecular diversity of known helminth ESPs with therapeutic properties is impressive, and considering that we have only dipped our toes in the water, the potential breadth of moieties is prodigious. Each family of molecules and even individual members within those families present unique and shared challenges. Moreover, the pharmacokinetic (PK) properties of a helminth-derived therapeutic protein and how best to deliver it to the target tissue/organ are challenging. For example, an ideal IBD drug would be delivered orally, and although some helminth ESPs have likely evolved to be stable in acidic environments, ensuring that a protein enjoys safe passage through the stomach and delivery to the specific cell type in the gut is not straightforward. Moreover, what does the optimal PK profile of a helminth protein look like? Unlike most biologics (e.g., monoclonal antibodies) in which plasma half-life can be measured in weeks, a helminth protein might only be present for hours. This longer half-life might, however, be sufficient for enduring functional activity via impact on defined cell types and mobilization of those cells to inflamed tissues [51]. The foreignness of a helminth protein also poses potential immunogenicity concerns, and the development of antidrug antibodies could be problematic.

Furthermore, the recombinant expression platform is of paramount importance for the production of helminth biologics. Many groups are expressing recombinant helminth proteins in cell lines that are used for industrial-scale production of antibodies and other therapeutic proteins. Levels of endotoxin and other host cell-derived contaminants need to be strictly controlled for cGMP grade production of biologics, particularly for immunotherapeutics that aim to wind down inflammatory responses (as opposed to promoting them with vaccines), so an emphasis on eukaryotic cell expression platforms, such as HEK293, is recommended.

Helminth-derived miRNAs are predicted to target an impressive array of mammalian host genes involved in inflammation, but how those miRNAs might be therapeutically delivered to target cells, internalized, and then encounter their target gene is not clear [124, 125]. In the natural setting, many miRNAs are delivered to target cells in the sheltered environment of EVs, but recapitulating this process for a therapeutic purpose requires novel approaches, such as the use of synthetic exosomes [126].

Despite these challenges, evolutionary selection pressure has tailored helminth ESPs to be efficacious and safe, at least in the setting of active helminth infection. Of course, in a therapeutic setting, the dose, the frequency, and route of administration are likely to be different. Furthermore, it is important to note that numerous nature-inspired drugs, sourced from the venom of various invertebrates and vertebrates, are commercially available for treating a range of disorders [127]. It is timely that helminths now join this list and that drugs inspired by these exquisitely adapted parasites get the attention they deserve. The scientific community implores industry-based organizations to make long-term investments intended at deciphering and capitalizing on the extraordinary and diverse modes of action of these products to unearth the next generation of novel therapeutics.

Supporting information

S1 Table. Completed and ongoing experimental helminth infection studies in healthy volunteers.
(DOCX)

References

1. Hotez PJ, Alvarado M, Basanez MG, Bolliger I, Bourne R, Boussinesq M, et al. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. PLoS Negl Trop Dis. 2014; 8(7):e2865. https://doi.org/10.1371/journal.pntd.0002865 PMID: 25058013; PubMed Central PMCID: PMC4109880.

2. Chen F, Liu Z, Wu W, Rozo C, Bowdridge S, Millman A, et al. An essential role for TH2-type responses in limiting acute tissue damage during experimental helminth infection. Nature medicine. 2012; 18 (2):260–266. https://doi.org/10.1038/nm.2628 PMID: 22245779; PubMed Central PMCID: PMC3274634.

3. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med. 2002; 347(12):911–920. https://doi.org/10.1056/NEJMra020100 PMID: 12239261.

4. Windsor JW, Kaplan GG. Evolving Epidemiology of IBD. Curr Gastroenterol Rep. 2019; 21(8):40. https://doi.org/10.1007/s11894-019-0705-6 PMID: 31338613.

5. Biagioni B, Vitiello G, Bormioli S, Tarrini D, Lombardi C, Rossi O, et al. Migrants and allergy: a new view of the atopic march. Eur Ann Allergy Clin Immunol. 2019; 51(3):100–114. https://doi.org/10.23822/EurAnnACI.1764-1489.96 PMID: 30983310.

6. Fumagalli M, Pozzoli U, Cagliani R, Comi GP, Riva S, Clerici M, et al. Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions. J Exp Med. 2009; 206(6):1395–1408. https://doi.org/10.1084/jem.20082779 PMID: 19468064; PubMed Central PMCID: PMC2715056.

7. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. Science. 2002; 296(5567):490–494. https://doi.org/10.1126/science.296.5567.490 PMID: 11964470.

8. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989; 299(6710):1259–1260. https://doi.org/10.1136/bmj.299.6710.1259 PMID: 2513902; PubMed Central PMCID: PMC1838109.

9. Wiria AE, Sartono E, Supali T, Yazdanbakhsh M. Helminth infections, type-2 immune response, and metabolic syndrome. PLoS Pathog. 2014; 10(7):e1004140. https://doi.org/10.1371/journal.ppat.1004140 PMID: 24992724; PubMed Central PMCID: PMC4081794.

10. Hays R, Esterman A, Giacomin P, Loukas A, McDermott R. Does Strongyloides stercoralis infection protect against type 2 diabetes in humans? Evidence from Australian Aboriginal adults. Diabetes Res Clin Pract. 2015; 107(3):355–361. https://doi.org/10.1016/j.diabres.2015.01.012 PMID: 25656764.

11. van der Zande HJP, Zawistowska-Deniziak A, Guigas B. Immune Regulation of Metabolic Homeostasis by Helminths and Their Molecules. Trends Parasitol. 2019; 35(10):795–808. https://doi.org/10.1016/j.pt.2019.07.014 PMID: 31492623.

12. Harris NL, Loke P. Recent Advances in Type-2-Cell-Mediated Immunity: Insights from Helminth Infection. Immunity. 2017; 47(6):1024–1036. https://doi.org/10.1016/j.immuni.2017.11.015 PMID: 29262347.

13. Gazzinelli-Guimaraes PH, Nutman TB. Helminth parasites and immune regulation. F1000Res. 2018;7. https://doi.org/10.12688/f1000research.13350.2 PMID: 29527296; PubMed Central PMCID: PMC6206608.

14. Maizels RM. Regulation of Immunity and allergy by helminth parasites. Allergy. 2019; 75(3):524–534. https://doi.org/10.1111/all.13944 PMID: 31187881.

15. Grencis RK, Humphreys NE, Bancroft AJ. Immunity to gastrointestinal nematodes: mechanisms and myths. Immunol Rev. 2014; 260(1):183–205. https://doi.org/10.1111/imr.12188 PMID: 24942690; PubMed Central PMCID: PMC4141702.

16. Lee SC, Tang MS, Lim YA, Choy SH, Kurtz ZD, Cox LM, et al. Helminth colonization is associated with increased diversity of the gut microbiota. PLoS Negl Trop Dis. 2014; 8(5):e2880. https://doi.org/10.1371/journal.pntd.0002880 PMID: 24851867; PubMed Central PMCID: PMC4031128.

17. Giacomin P, Croese J, Krause L, Loukas A, Cantacessi C. Suppression of inflammation by helminths: a role for the gut microbiota? Philos Trans R Soc Lond B Biol Sci. 2015; 370(1675). https://doi.org/10.1098/rstb.2014.0296 PMID: 26150662; PubMed Central PMCID: PMC4528494.

18. Giacomin P, Zakrzewski M, Croese J, Su X, Sotillo J, McCann L, et al. Experimental hookworm infection and escalating gluten challenges are associated with increased microbial richness in celiac subjects. Sci Rep. 2015; 5:13797. https://doi.org/10.1038/srep13797 PMID: 26381211; PubMed Central PMCID: PMC4585380.

19. Brosschot TP, Reynolds LA. The impact of a helminth-modified microbiome on host immunity. Mucosal Immunol. 2018; 11(4):1039–1046. https://doi.org/10.1038/s41385-018-0008-5 PMID: 29453411.

20. Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, et al. The Intestinal Microbiota Contributes to the Ability of Helminths to Modulate Allergic Inflammation. Immunity. 2015; 43(5):998–1010. https://doi.org/10.1016/j.immuni.2015.09.012 PMID: 26522986; PubMed Central PMCID: PMC4658337.

21. Ramanan D, Bowcutt R, Lee SC, Tang MS, Kurtz ZD, Ding Y, et al. Helminth infection promotes colonization resistance via type 2 immunity. Science. 2016; 352(6285):608–612. https://doi.org/10.1126/science.aaf3229 PMID: 27080105; PubMed Central PMCID: PMC4905769.

22. Summers RW, Elliott DE, Urban JF Jr., Thompson RA, Weinstock JV. Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology. 2005; 128(4):825–832. https://doi.org/10.1053/j.gastro.2005.01.005 PMID: 15825065.

23. Summers RW, Elliott DE, Urban JF Jr., Thompson R, Weinstock JV. Trichuris suis therapy in Crohn's disease. Gut. 2005; 54(1):87–90. https://doi.org/10.1136/gut.2004.041749 PMID: 15591509; PubMed Central PMCID: PMC1774382.

24. Scholmerich J, Fellermann K, Seibold FW, Rogler G, Langhorst J, Howaldt S, et al. A Randomised, Double-blind, Placebo-controlled Trial of Trichuris suis ova in Active Crohn's Disease. J Crohns Colitis. 2017; 11(4):390–399. https://doi.org/10.1093/ecco-jcc/jjw184 PMID: 27707789; PubMed Central PMCID: PMC5881737.

25. Voldsgaard A, Bager P, Garde E, Akeson P, Leffers AM, Madsen CG, et al. Trichuris suis ova therapy in relapsing multiple sclerosis is safe but without signals of beneficial effect. Multiple sclerosis (Houndmills, Basingstoke, England). 2015; 21(13):1723–1729. Epub 2015/02/24. https://doi.org/10.1177/1352458514568173 PMID: 25698173.

26. Fleming J, Hernandez G, Hartman L, Maksimovic J, Nace S, Lawler B, et al. Safety and efficacy of helminth treatment in relapsing-remitting multiple sclerosis: Results of the HINT 2 clinical trial. Multiple sclerosis (Houndmills, Basingstoke, England). 2017:1352458517736377. https://doi.org/10.1177/1352458517736377 PMID: 29064315; PubMed Central PMCID: PMC5878983.

27. Loukas A, Hotez PJ, Diemert D, Yazdanbakhsh M, McCarthy JS, Correa-Oliveira R, et al. Hookworm infection. Nat Rev Dis Primers. 2016; 2:16088. https://doi.org/10.1038/nrdp.2016.88 PMID: 27929101.

28. Feary JR, Venn AJ, Mortimer K, Brown AP, Hooi D, Falcone FH, et al. Experimental hookworm infection: a randomized placebo-controlled trial in asthma. Clin Exp Allergy. 2010; 40(2):299–306. https://doi.org/10.1111/j.1365-2222.2009.03433.x PMID: 20030661; PubMed Central PMCID: PMC2814083.

29. Blount D, Hooi D, Feary J, Venn A, Telford G, Brown A, et al. Immunologic profiles of persons recruited for a randomized, placebo-controlled clinical trial of hookworm infection. Am J Trop Med Hyg. 2009; 81 (5):911–916. https://doi.org/10.4269/ajtmh.2009.09-0237 PMID: 19861631.

30. Daveson AJ, Jones DM, Gaze S, McSorley H, Clouston A, Pascoe A, et al. Effect of hookworm infection on wheat challenge in celiac disease—a randomised double-blinded placebo controlled trial. PloS ONE. 2011; 6(3):e17366. https://doi.org/10.1371/journal.pone.0017366 PMID: 21408161; PubMed Central PMCID: PMC3050888.

31. Croese J, O'Neil J, Masson, Cooke S, Melrose W, Pritchard D, et al. A proof of concept study establishing Necator americanus in Crohn's patients and reservoir donors. Gut. 2006; 55(1):136–137. https://doi.org/10.1136/gut.2005.079129 PMID: 16344586; PubMed Central PMCID: PMC1856386.

32. Donath MY. Multiple benefits of targeting inflammation in the treatment of type 2 diabetes. Diabetologia. 2016; 59(4):679–682. https://doi.org/10.1007/s00125-016-3873-z PMID: 26868493.

33. Pierce D, Merone L, Lewis C, Rahman T, Croese J, Loukas A, et al. Safety and tolerability of experimental hookworm infection in humans with metabolic disease: study protocol for a phase 1b randomised controlled clinical trial. BMC Endocr Disord. 2019; 19(1):136. https://doi.org/10.1186/s12902-019-0461-5 PMID: 31829172.

34. Diemert D, Campbell D, Brelsford J, Leasure C, Li G, Peng J, et al. Controlled Human Hookworm Infection: Accelerating Human Hookworm Vaccine Development. Open forum infectious diseases. 2018; 5(5):ofy083. https://doi.org/10.1093/ofid/ofy083 PMID: 29780848; PubMed Central PMCID: PMC5952933.

35. Donath MY, Dinarello CA, Mandrup-Poulsen T. Targeting innate immune mediators in type 1 and type 2 diabetes. Nat Rev Immunol. 2019; 19(12):734–746. https://doi.org/10.1038/s41577-019-0213-9 PMID: 31501536.

36. Hoogerwerf MA, Coffeng LE, Brienen EAT, Janse JJ, Langenberg MCC, Kruize YCM, et al. New Insights Into the Kinetics and Variability of Egg Excretion in Controlled Human Hookworm Infections. The Journal of infectious diseases. 2019; 220(6):1044–1048. https://doi.org/10.1093/infdis/jiz218 PMID: 31077279.

37. Maizels RM, Smits HH, McSorley HJ. Modulation of Host Immunity by Helminths: The Expanding Repertoire of Parasite Effector Molecules. Immunity. 2018; 49(5):801–818. https://doi.org/10.1016/j.immuni.2018.10.016 PMID: 30462997; PubMed Central PMCID: PMC6269126.

38. Harnett MM, Harnett W. Can Parasitic Worms Cure the Modern World's Ills? Trends Parasitol. 2017; 33(9):694–705. https://doi.org/10.1016/j.pt.2017.05.007 PMID: 28606411.

39. Ruyssers NE, De Winter BY, De Man JG, Loukas A, Pearson MS, Weinstock JV, et al. Therapeutic potential of helminth soluble proteins in TNBS-induced colitis in mice. Inflamm Bowel Dis. 2009; 15 (4):491–500. https://doi.org/10.1002/ibd.20787 PMID: 19023900.

40. Cancado GG, Fiuza JA, de Paiva NC, Lemos Lde C, Ricci ND, Gazzinelli-Guimaraes PH, et al. Hookworm products ameliorate dextran sodium sulfate-induced colitis in BALB/c mice. Inflamm Bowel Dis. 2011; 17(11):2275–2286. https://doi.org/10.1002/ibd.21629 PMID: 21290484.

41. Ferreira I, Smyth D, Gaze S, Aziz A, Giacomin P, Ruyssers N, et al. Hookworm excretory/secretory products induce interleukin-4 (IL-4)+ IL-10+ CD4+ T cell responses and suppress pathology in a mouse model of colitis. Infect Immun. 2013; 81(6):2104–2111. https://doi.org/10.1128/IAI.00563-12 PMID: 23545299; PubMed Central PMCID: PMC3676036.

42. Wammes LJ, Mpairwe H, Elliott AM, Yazdanbakhsh M. Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. Lancet Infect Dis. 2014; 14(11):1150–1162. https://doi.org/10.1016/S1473-3099(14)70771-6 PMID: 24981042.

43. McSorley HJ, O'Gorman MT, Blair N, Sutherland TE, Filbey KJ, Maizels RM. Suppression of type 2 immunity and allergic airway inflammation by secreted products of the helminth Heligmosomoides polygyrus. Eur J Immunol. 2012; 42(10):2667–2682. https://doi.org/10.1002/eji.201142161 PMID: 22706967; PubMed Central PMCID: PMC4916998.

44. Shepherd C, Giacomin P, Navarro S, Miller C, Loukas A, Wangchuk P. A medicinal plant compound, capnoidine, prevents the onset of inflammation in a mouse model of colitis. J Ethnopharmacol. 2018; 211:17–28. https://doi.org/10.1016/j.jep.2017.09.024 PMID: 28942135.

45. Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE. Helminth parasites—masters of regulation. Immunol Rev. 2004; 201:89–116. https://doi.org/10.1111/j.0105-2896.2004.00191.x PMID: 15361235.

46. Hewitson JP, Grainger JR, Maizels RM. Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. Mol Biochem Parasitol. 2009; 167(1):1–11. https://doi.org/10.1016/j.molbiopara.2009.04.008 PMID: 19406170; PubMed Central PMCID: PMC2706953.

47. Jolink H, de Boer R, Willems LN, van Dissel JT, Falkenburg JH, Heemskerk MH. T helper 2 response in allergic bronchopulmonary aspergillosis is not driven by specific Aspergillus antigens. Allergy. 2015; 70(10):1336–1339. https://doi.org/10.1111/all.12688 PMID: 26179335.

48. Logan J, Navarro S, Loukas A, Giacomin P. Helminth-induced regulatory T cells and suppression of allergic responses. Curr Opin Immunol. 2018; 54:1–6. https://doi.org/10.1016/j.coi.2018.05.007 PMID: 29852470.

49. Grainger JR, Smith KA, Hewitson JP, McSorley HJ, Harcus Y, Filbey KJ, et al. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. J Exp Med. 2010; 207(11):2331–2341. https://doi.org/10.1084/jem.20101074 PMID: 20876311; PubMed Central PMCID: PMC2964568.

50. Wensveen FM, Jelencic V, Valentic S, Sestan M, Wensveen TT, Theurich S, et al. NK cells link obesity-induced adipose stress to inflammation and insulin resistance. Nat Immunol. 2015; 16(4):376–385. https://doi.org/10.1038/ni.3120 PMID: 25729921.

51. Navarro S, Pickering DA, Ferreira IB, Jones L, Ryan S, Troy S, et al. Hookworm recombinant protein promotes regulatory T cell responses that suppress experimental asthma. Sci Transl Med. 2016; 8 (362):362ra143. https://doi.org/10.1126/scitranslmed.aaf8807 PMID: 27797959.

52. Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. Nature reviews Immunology. 2011; 11(6):375–388. https://doi.org/10.1038/nri2992 PMID: 21610741.

53. Lewis G, Wang B, Shafiei Jahani P, Hurrell BP, Banie H, Aleman Muench GR, et al. Dietary Fiber-Induced Microbial Short Chain Fatty Acids Suppress ILC2-Dependent Airway Inflammation. Frontiers in immunology. 2019; 10:2051. https://doi.org/10.3389/fimmu.2019.02051 PMID: 31620118; PubMed Central PMCID: PMC6760365.

54. von Moltke J, Ji M, Liang HE, Locksley RM. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature. 2016; 529(7585):221–225. https://doi.org/10.1038/nature16161 PMID: 26675736; PubMed Central PMCID: PMC4830391.

55. Ferreira LMR, Muller YD, Bluestone JA, Tang Q. Next-generation regulatory T cell therapy. Nat Rev Drug Discov. 2019; 18(10):749–769. https://doi.org/10.1038/s41573-019-0041-4 PMID: 31541224.

56. Abdel Aziz N, Nono JK, Mpotje T, Brombacher F. The Foxp3+ regulatory T-cell population requires IL-4Ralpha signaling to control inflammation during helminth infections. PLoS Biol. 2018; 16(10): e2005850. https://doi.org/10.1371/journal.pbio.2005850 PMID: 30379806; PubMed Central PMCID: PMC6231676.

57. Bluestone JA, Trotta E, Xu D. The therapeutic potential of regulatory T cells for the treatment of autoimmune disease. Expert Opin Ther Targets. 2015; 19(8):1091–1103. https://doi.org/10.1517/14728222.2015.1037282 PMID: 25881491.

58. Ferreira LMR, Muller YD, Bluestone JA, Tang Q. Next-generation regulatory T cell therapy. Nat Rev Drug Discov. 2019; 18:749–769. https://doi.org/10.1038/s41573-019-0041-4 PMID: 31541224.

59. Hussaarts L, van der Vlugt LE, Yazdanbakhsh M, Smits HH. Regulatory B-cell induction by helminths: implications for allergic disease. The Journal of allergy and clinical immunology. 2011; 128(4):733–739. https://doi.org/10.1016/j.jaci.2011.05.012 PMID: 21684587.

60. Chen F, Wu W, Jin L, Millman A, Palma M, El-Naccache DW, et al. B Cells Produce the Tissue-Protective Protein RELMalpha during Helminth Infection, which Inhibits IL-17 Expression and Limits Emphysema. Cell reports. 2018; 25(10):2775–83.e3. https://doi.org/10.1016/j.celrep.2018.11.038 PMID: 30517865.

61. Gao X, Ren X, Wang Q, Yang Z, Li Y, Su Z, et al. Critical roles of regulatory B and T cells in helminth parasite-induced protection against allergic airway inflammation. Clinical and experimental immunology. 2019; 198(3):390–402. https://doi.org/10.1111/cei.13362 PMID: 31397879; PubMed Central PMCID: PMC6857085.

62. Nagashima H, Mahlakoiv T, Shih HY, Davis FP, Meylan F, Huang Y, et al. Neuropeptide CGRP Limits Group 2 Innate Lymphoid Cell Responses and Constrains Type 2 Inflammation. Immunity. 2019; 51 (4):682-95.e6. https://doi.org/10.1016/j.immuni.2019.06.009 PMID: 31353223; PubMed Central PMCID: PMC6801073.

63. Bouchery T, Le Gros G, Harris N. ILC2s-Trailblazers in the Host Response Against Intestinal Helminths. Frontiers in immunology. 2019; 10:623. https://doi.org/10.3389/fimmu.2019.00623 PMID: 31019505; PubMed Central PMCID: PMC6458269.

64. Stoltzfus JD, Pilgrim AA, Herbert DR. Perusal of parasitic nematode 'omics in the post-genomic era. Mol Biochem Parasitol. 2017; 215:11–22. https://doi.org/10.1016/j.molbiopara.2016.11.003 PMID: 27887974; PubMed Central PMCID: PMC5440216.

65. Holt DC, Fischer K, Allen GE, Wilson D, Wilson P, Slade R, et al. Mechanisms for a novel immune evasion strategy in the scabies mite sarcoptes scabiei: a multigene family of inactivated serine proteases. J Invest Dermatol. 2003; 121(6):1419–1424. https://doi.org/10.1046/j.1523-1747.2003.12621.x PMID: 14675192.

66. Zhu S. Did cathelicidins, a family of multifunctional host-defense peptides, arise from a cysteine protease inhibitor? Trends Microbiol. 2008; 16(8):353–360. https://doi.org/10.1016/j.tim.2008.05.007 PMID: 18632274.

67. Cantacessi C, Hofmann A, Pickering D, Navarro S, Mitreva M, Loukas A. TIMPs of parasitic helminths —a large-scale analysis of high-throughput sequence datasets. Parasit Vectors. 2013; 6:156. https://doi.org/10.1186/1756-3305-6-156 PMID: 23721526; PubMed Central PMCID: PMC3679795.

68. Zhan B, Badamchian M, Meihua B, Ashcom J, Feng J, Hawdon J, et al. Molecular cloning and purification of Ac-TMP, a developmentally regulated putative tissue inhibitor of metalloprotease released in relative abundance by adult Ancylostoma hookworms. Am J Trop Med Hyg. 2002; 66(3):238–244. https://doi.org/10.4269/ajtmh.2002.66.238 PMID: 12139214.

69. Kucera K, Harrison LM, Cappello M, Modis Y. Ancylostoma ceylanicum excretory-secretory protein 2 adopts a netrin-like fold and defines a novel family of nematode proteins. J Mol Biol. 2011; 408(1):9–17. https://doi.org/10.1016/j.jmb.2011.02.033 PMID: 21352830; PubMed Central PMCID: PMC3070796.

70. Cuellar C, Wu W, Mendez S. The hookworm tissue inhibitor of metalloproteases (Ac-TMP-1) modifies dendritic cell function and induces generation of CD4 and CD8 suppressor T cells. PLoS Negl Trop Dis. 2009; 3(5):e439. https://doi.org/10.1371/journal.pntd.0000439 PMID: 19468296; PubMed Central PMCID: PMC2678263.

71. Ferreira IB, Pickering DA, Troy S, Croese J, Loukas A, Navarro S. Suppression of inflammation and tissue damage by a hookworm recombinant protein in experimental colitis. Clin Transl Immun. 2017; 6(10): e157. https://doi.org/10.1038/cti.2017.42 PMID: 29114386; PubMed Central PMCID: PMC5671989

72. Ochieng J, Chaudhuri G. Cystatin superfamily. J Health Care Poor Underserved. 2010; 21(1 Suppl):51–70. https://doi.org/10.1353/hpu.0.0257 PMID: 20173285; PubMed Central PMCID: PMC2888135.

73. Murray J, Manoury B, Balic A, Watts C, Maizels RM. Bm-CPI-2, a cystatin from Brugia malayi nematode parasites, differs from Caenorhabditis elegans cystatins in a specific site mediating inhibition of the antigen-processing enzyme AEP. Mol Biochem Parasitol. 2005; 139(2):197–203. https://doi.org/10.1016/j.molbiopara.2004.11.008 PMID: 15664654.

74. Hartmann S, Lucius R. Modulation of host immune responses by nematode cystatins. Int J Parasitol. 2003; 33(11):1291–1302. https://doi.org/10.1016/s0020-7519(03)00163-2 PMID: 13678644.

75. Gregory WF, Maizels RM. Cystatins from filarial parasites: evolution, adaptation and function in the host-parasite relationship. Int J Biochem Cell Biol. 2008; 40(6–7):1389–1398. https://doi.org/10.1016/j.biocel.2007.11.012 PMID: 18249028.

76. Danilowicz-Luebert E, Steinfelder S, Kuhl AA, Drozdenko G, Lucius R, Worm M, et al. A nematode immunomodulator suppresses grass pollen-specific allergic responses by controlling excessive Th2 inflammation. Int J Parasitol. 2013; 43(3–4):201–210. https://doi.org/10.1016/j.ijpara.2012.10.014 PMID: 23174104.

77. Coronado S, Barrios L, Zakzuk J, Regino R, Ahumada V, Franco L, et al. A recombinant cystatin from Ascaris lumbricoides attenuates inflammation of DSS-induced colitis. Parasite Immunol. 2017; 39(4). https://doi.org/10.1111/pim.12425 PMID: 28295446.

78. Khatri V, Amdare N, Tarnekar A, Goswami K, Reddy MV. Brugia malayi cystatin therapeutically ameliorates dextran sulfate sodium-induced colitis in mice. J Dig Dis. 2015; 16(10):585–594. https://doi.org/10.1111/1751-2980.12290 PMID: 26358507.

79. Wang S, Xie Y, Yang X, Wang X, Yan K, Zhong Z, et al. Therapeutic potential of recombinant cystatin from Schistosoma japonicum in TNBS-induced experimental colitis of mice. Parasit Vectors. 2016; 9:6. https://doi.org/10.1186/s13071-015-1288-1 PMID: 26728323; PubMed Central PMCID: PMC4700642.

80. Johnston CJC, Smyth DJ, Kodali RB, White MPJ, Harcus Y, Filbey KJ, et al. A structurally distinct TGF-beta mimic from an intestinal helminth parasite potently induces regulatory T cells. Nat Commun. 2017; 8(1):1741. https://doi.org/10.1038/s41467-017-01886-6 PMID: 29170498; PubMed Central PMCID: PMC5701006.

81. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Sci. 2019; 110(7):2080–2089. https://doi.org/10.1111/cas.14069 PMID: 31102428; PubMed Central PMCID: PMC6609813.

82. Tribolet L, Cantacessi C, Pickering DA, Navarro S, Doolan DL, Trieu A, et al. Probing of a human proteome microarray with a recombinant pathogen protein reveals a novel mechanism by which hookworms suppress B-cell receptor signaling. J Infect Dis. 2015; 211(3):416–425. https://doi.org/10.1093/infdis/jiu451 PMID: 25139017.

83. Osbourn M, Soares DC, Vacca F, Cohen ES, Scott IC, Gregory WF, et al. HpARI Protein Secreted by a Helminth Parasite Suppresses Interleukin-33. Immunity. 2017; 47(4):739–51.e5. https://doi.org/10.1016/j.immuni.2017.09.015 PMID: 29045903; PubMed Central PMCID: PMC5655542.

84. Gause WC, Wynn TA, Allen JE. Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. Nat Rev Immunol. 2013; 13(8):607–614. https://doi.org/10.1038/nri3476 PMID: 23827958.

85. faz lopez B, Morales-Montor J, Terrazas L. Role of Macrophages in the Repair Process during the Tissue Migrating and Resident Helminth Infections. BioMed Research International. 2016; 2016:1–11. https://doi.org/10.1155/2016/8634603 PMID: 27648452

86. Ariyaratne A, Finney CAM. Eosinophils and Macrophages within the Th2-Induced Granuloma: Balancing Killing and Healing in a Tight Space. Infect Immun. 2019; 87(10). https://doi.org/10.1128/iai.00127-19 PMID: 31285249; PubMed Central PMCID: PMC6759305.

87. Smout MJ, Sotillo J, Laha T, Papatpremsiri A, Rinaldi G, Pimenta RN, et al. Carcinogenic Parasite Secretes Growth Factor That Accelerates Wound Healing and Potentially Promotes Neoplasia. PLoS Pathog. 2015; 11(10):e1005209. https://doi.org/10.1371/journal.ppat.1005209 PMID: 26485648

88. Dastpeyman M, Bansal PS, Wilson D, Sotillo J, Brindley PJ, Loukas A, et al. Structural Variants of a Liver Fluke Derived Granulin Peptide Potently Stimulate Wound Healing. Journal of medicinal chemistry. 2018; 61(19):8746–8753. https://doi.org/10.1021/acs.jmedchem.8b00898 PMID: 30183294.

89. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clinics in Dermatology. 2007; 25 (1):9–18. https://doi.org/https://doi.org/10.1016/j.clindermatol.2006.09.007 PMID: 17276196

90. Ibrahim NI, Wong SK, Mohamed IN, Mohamed N, Chin K-Y, Ima-Nirwana S, et al. Wound Healing Properties of Selected Natural Products. Int J Environ Res Public Health. 2018; 15(11):2360. https://doi.org/10.3390/ijerph15112360 PMID: 30366427.

91. Pineda MA, Lumb F, Harnett MM, Harnett W. ES-62, a therapeutic anti-inflammatory agent evolved by the filarial nematode Acanthocheilonema viteae. Mol Biochem Parasitol. 2014; 194(1–2):1–8. https://doi.org/10.1016/j.molbiopara.2014.03.003 PMID: 24671112.

92. Rzepecka J, Siebeke I, Coltherd JC, Kean DE, Steiger CN, Al-Riyami L, et al. The helminth product, ES-62, protects against airway inflammation by resetting the Th cell phenotype. Int J Parasitol. 2013; 43(3–4):211–223. https://doi.org/10.1016/j.ijpara.2012.12.001 PMID: 23291461; PubMed Central PMCID: PMC3584281.

93. Aprahamian TR, Zhong X, Amir S, Binder CJ, Chiang LK, Al-Riyami L, et al. The immunomodulatory parasitic worm product ES-62 reduces lupus-associated accelerated atherosclerosis in a mouse model. Int J Parasitol. 2015; 45(4):203–207. https://doi.org/10.1016/j.ijpara.2014.12.006 PMID: 25666929; PubMed Central PMCID: PMC4355381.

94. Deehan MR, Harnett MM, Harnett W. A filarial nematode secreted product differentially modulates expression and activation of protein kinase C isoforms in B lymphocytes. J Immun. 1997; 159 (12):6105–6111. PMID: 9550411.

95. Harnett W, Harnett MM. Immunomodulatory activity and therapeutic potential of the filarial nematode secreted product, ES-62. Adv Exp Med Biol. 2009; 666:88–94. https://doi.org/10.1007/978-1-4419-1601-3_7 PMID: 20054977.

96. Harnett W, Deehan MR, Houston KM, Harnett MM. Immunomodulatory properties of a phosphorylcholine-containing secreted filarial glycoprotein. Parasite Immunol. 1999; 21(12):601–608. https://doi.org/10.1046/j.1365-3024.1999.00267.x PMID: 10583862.

97. Doonan J, Lumb FE, Pineda MA, Tarafdar A, Crowe J, Khan AM, et al. Protection Against Arthritis by the Parasitic Worm Product ES-62, and Its Drug-Like Small Molecule Analogues, Is Associated With Inhibition of Osteoclastogenesis. Front Immun. 2018; 9:1016. https://doi.org/10.3389/fimmu.2018.01016 PMID: 29867986; PubMed Central PMCID: PMC5967578.

98. Suckling CJ, Mukherjee S, Khalaf AI, Narayan A, Scott FJ, Khare S, et al. Synthetic analogues of the parasitic worm product ES-62 reduce disease development in in vivo models of lung fibrosis. Acta Trop. 2018; 185:212–218. https://doi.org/10.1016/j.actatropica.2018.05.015 PMID: 29802846.

99. Matisz CE, Geuking MB, Lopes F, Petri B, Wang A, Sharkey KA, et al. Helminth Antigen-Conditioned Dendritic Cells Generate Anti-Inflammatory Cd4 T Cells Independent of Antigen Presentation via Major Histocompatibility Complex Class II. Am J Pathol. 2018; 188(11):2589–2604. https://doi.org/10.1016/j.ajpath.2018.07.008 PMID: 30121255.

100. Wuhrer M, Balog CI, Catalina MI, Jones FM, Schramm G, Haas H, et al. IPSE/alpha-1, a major secretory glycoprotein antigen from schistosome eggs, expresses the Lewis X motif on core-difucosylated N-glycans. The FEBS journal. 2006; 273(10):2276–2292. https://doi.org/10.1111/j.1742-4658.2006.05242.x PMID: 16650003.

101. Haeberlein S, Obieglo K, Ozir-Fazalalikhan A, Chaye MAM, Veninga H, van der Vlugt L, et al. Schistosome egg antigens, including the glycoprotein IPSE/alpha-1, trigger the development of regulatory B

cells. PLoS Pathog. 2017; 13(7):e1006539. https://doi.org/10.1371/journal.ppat.1006539 PMID: 28753651; PubMed Central PMCID: PMC5550006.

102. Knuhr K, Langhans K, Nyenhuis S, Viertmann K, Kildemoes AMO, Doenhoff MJ, et al. Schistosoma mansoni Egg-Released IPSE/alpha-1 Dampens Inflammatory Cytokine Responses via Basophil Inter-leukin (IL)-4 and IL-13. Frontiers in immunology. 2018; 9:2293. https://doi.org/10.3389/fimmu.2018.02293 PMID: 30364177; PubMed Central PMCID: PMC6191518.

103. Hussaarts L, Garcia-Tardon N, van Beek L, Heemskerk MM, Haeberlein S, van der Zon GC, et al. Chronic helminth infection and helminth-derived egg antigens promote adipose tissue M2 macro-phages and improve insulin sensitivity in obese mice. Faseb j. 2015; 29(7):3027–39. Epub 2015/04/09. https://doi.org/10.1096/fj.14-266239 PMID: 25852044.

104. Kahl J, Brattig N, Liebau E. The Untapped Pharmacopeic Potential of Helminths. Trends Parasitol. 2018; 34(10):828–842. https://doi.org/10.1016/j.pt.2018.05.011 PMID: 29954660.

105. Hams E, Bermingham R, Wurlod FA, Hogan AE, O'Shea D, Preston RJ, et al. The helminth T2 RNase omega1 promotes metabolic homeostasis in an IL-33- and group 2 innate lymphoid cell-dependent mechanism. Faseb j. 2016; 30(2):824–835. https://doi.org/10.1096/fj.15-277822 PMID: 26490658; PubMed Central PMCID: PMC4973506.

106. Tang CL, Liu ZM, Gao YR, Xiong F. Schistosoma Infection and Schistosoma-Derived Products Modu-late the Immune Responses Associated with Protection against Type 2 Diabetes. Frontiers in immu-nology. 2017; 8:1990. https://doi.org/10.3389/fimmu.2017.01990 PMID: 29387059; PubMed Central PMCID: PMC5776330.

107. Bhargava P, Li C, Stanya KJ, Jacobi D, Dai L, Liu S, et al. Immunomodulatory glycan LNFPIII allevi-ates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. Nature medicine. 2012; 18(11):1665–1672. https://doi.org/10.1038/nm.2962 PMID: 23104131; PubMed Central PMCID: PMC3493877.

108. Becker AC, Willenberg I, Springer A, Schebb NH, Steinberg P, Strube C. Fatty acid composition of free-living and parasitic stages of the bovine lungworm Dictyocaulus viviparus. Mol Biochem Parasitol. 2017; 216:39–44. https://doi.org/10.1016/j.molbiopara.2017.06.008 PMID: 28651962.

109. Wangchuk P, Kouremenos K, Eichenberger RM, Pearson M, Susianto A, Wishart DS, et al. Metabolo-mic profiling of the excretory-secretory products of hookworm and whipworm. Metabolomics. 2019; 15 (7):101. https://doi.org/10.1007/s11306-019-1561-y PMID: 31254203.

110. Wangchuk P, Constantinoiu C, Eichenberger RM, Field M, Loukas A. Characterization of Tapeworm Metabolites and Their Reported Biological Activities. Molecules. 2019; 24(8). https://doi.org/10.3390/molecules24081480 PMID: 30991712; PubMed Central PMCID: PMC6514793.

111. Kokova D, Mayboroda OA. Twenty Years on: Metabolomics in Helminth Research. Trends Parasitol. 2019; 35(4):282–288. https://doi.org/10.1016/j.pt.2019.01.012 PMID: 30824203.

112. Wangchuk P, Shepherd C, Constantinoiu C, Ryan RYM, Kouremenos KA, Becker L, et al. Hookworm-Derived Metabolites Suppress Pathology in a Mouse Model of Colitis and Inhibit Secretion of Key Inflammatory Cytokines in Primary Human Leukocytes. Infection and immunity. 2019; 87(4). https://doi.org/10.1128/iai.00851-18 PMID: 30670556; PubMed Central PMCID: PMC6434117.

113. Giera M, Kaisar MMM, Derks RJE, Steenvoorden E, Kruize YCM, Hokke CH, et al. The Schistosoma mansoni lipidome: Leads for immunomodulation. Anal Chim Acta. 2018; 1037:107–118. https://doi.org/10.1016/j.aca.2017.11.058 PMID: 30292284.

114. Laan LC, Williams AR, Stavenhagen K, Giera M, Kooij G, Vlasakov I, et al. The whipworm (Trichuris suis) secretes prostaglandin E2 to suppress proinflammatory properties in human dendritic cells. Faseb J. 2017; 31(2):719–731. https://doi.org/10.1096/fj.201600841R PMID: 27806992; PubMed Central PMCID: PMC5240662.

115. Karpisheh V, Nikkhoo A, Hojjat-Farsangi M, Namdar A, Azizi G, Ghalamfarsa G, et al. Prostaglandin E2 as a potent therapeutic target for treatment of colon cancer. Prostaglandins Other Lipid Mediat. 2019:106338. https://doi.org/10.1016/j.prostaglandins.2019.106338 PMID: 31100474.

116. Coakley G, Buck AH, Maizels RM. Host parasite communications-Messages from helminths for the immune system: Parasite communication and cell-cell interactions. Mol Biochem Parasitol. 2016; 208 (1):33–40. https://doi.org/10.1016/j.molbiopara.2016.06.003 PMID: 27297184; PubMed Central PMCID: PMC5008435.

117. Eichenberger RM, Sotillo J, Loukas A. Immunobiology of parasitic worm extracellular vesicles. Immu-nol Cell Biol. 2018; 96(9)704–713. https://doi.org/10.1111/imcb.12171 PMID: 29808496.

118. Buck AH, Coakley G, Simbari F, McSorley HJ, Quintana JF, Le Bihan T, et al. Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. Nat Com-mun. 2014; 5:5488. https://doi.org/10.1038/ncomms6488 PMID: 25421927; PubMed Central PMCID: PMC4263141.

119. Eichenberger RM, Ryan S, Jones L, Buitrago G, Polster R, Montes de Oca M, et al. Hookworm Secreted Extracellular Vesicles Interact With Host Cells and Prevent Inducible Colitis in Mice. Frontiers in immunology. 2018; 9:850. https://doi.org/10.3389/fimmu.2018.00850 PMID: 29760697; PubMed Central PMCID: PMC5936971.

120. Roig J, Saiz ML, Galiano A, Trelis M, Cantalapiedra F, Monteagudo C, et al. Extracellular Vesicles From the Helminth Fasciola hepatica Prevent DSS-Induced Acute Ulcerative Colitis in a T-Lymphocyte Independent Mode. Front Microbiol. 2018; 9:1036. https://doi.org/10.3389/fmicb.2018.01036 PMID: 29875750; PubMed Central PMCID: PMC5974114.

121. Marcilla A, Trelis M, Cortes A, Sotillo J, Cantalapiedra F, Minguez MT, et al. Extracellular vesicles from parasitic helminths contain specific excretory/secretory proteins and are internalized in intestinal host cells. PloS ONE. 2012; 7(9):e45974. https://doi.org/10.1371/journal.pone.0045974 PMID: 23029346; PubMed Central PMCID: PMC3454434.

122. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov. 2017; 16(3):203–222. Epub 2017/02/18. https://doi.org/10.1038/nrd.2016.246 PMID: 28209991.

123. Samanta S, Rajasingh S, Drosos N, Zhou Z, Dawn B, Rajasingh J. Exosomes: new molecular targets of diseases. Acta Pharmacol Sin. 2018; 39(4):501–513. https://doi.org/10.1038/aps.2017.162 PMID: 29219950; PubMed Central PMCID: PMC5888687.

124. Pottoo FH, Barkat MA, Harshita, Ansari MA, Javed MN, Sajid Jamal QM, et al. Nanotechnological based miRNA intervention in the therapeutic management of neuroblastoma. Semin Cancer Biol. Forthcoming 2019. https://doi.org/10.1016/j.semcancer.2019.09.017 PMID: 31562954.

125. Sil S, Dagur RS, Liao K, Peeples ES, Hu G, Periyasamy P, et al. Strategies for the use of Extracellular Vesicles for the Delivery of Therapeutics. J Neuroimmune Pharmacol. 2019. https://doi.org/10.1007/s11481-019-09873-y PMID: 31456107.

126. Li SP, Lin ZX, Jiang XY, Yu XY. Exosomal cargo-loading and synthetic exosome-mimics as potential therapeutic tools. Acta Pharmacol Sin. 2018; 39(4):542–551. https://doi.org/10.1038/aps.2017.178 PMID: 29417947; PubMed Central PMCID: PMC5888690.

127. Pennington MW, Czerwinski A, Norton RS. Peptide therapeutics from venom: Current status and potential. Bioorg Med Chem. 2018; 26(10):2738–2758. https://doi.org/10.1016/j.bmc.2017.09.029 PMID: 28988749.

128. Summers RW, Elliott DE, Qadir K, Urban JF Jr., Thompson R, Weinstock JV. Trichuris suis seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am J Gastroenterol. 2003; 98(9):2034–2041. https://doi.org/10.1111/j.1572-0241.2003.07660.x PMID: 14499784.

129. Fleming J, Hernandez G, Hartman L, Maksimovic J, Nace S, Lawler B, et al. Safety and efficacy of helminth treatment in relapsing-remitting multiple sclerosis: Results of the HINT 2 clinical trial. Mult Scler. 2019; 25(1):81–91. https://doi.org/10.1177/1352458517736377 PMID: 29064315; PubMed Central PMCID: PMC5878983.

130. Bager P, Arnved J, Ronborg S, Wohlfahrt J, Poulsen LK, Westergaard T, et al. Trichuris suis ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. J Allergy Clin Immunol. 2010; 125(1):123-30.e1-3. https://doi.org/10.1016/j.jaci.2009.08.006 PMID: 19800680.

131. Rosche B, Wernecke KD, Ohlraun S, Dorr JM, Paul F. Trichuris suis ova in relapsing-remitting multiple sclerosis and clinically isolated syndrome (TRIOMS): study protocol for a randomized controlled trial. Trials. 2013; 14:112. https://doi.org/10.1186/1745-6215-14-112 PMID: 23782752; PubMed Central PMCID: PMC3680966.

132. Sandborn WJ, Elliott DE, Weinstock J, Summers RW, Landry-Wheeler A, Silver N, et al. Randomised clinical trial: the safety and tolerability of Trichuris suis ova in patients with Crohn's disease. Aliment Pharmacol Ther. 2013; 38(3):255–263. https://doi.org/10.1111/apt.12366 PMID: 23730956.

133. Feary J, Venn A, Brown A, Hooi D, Falcone FH, Mortimer K, et al. Safety of hookworm infection in individuals with measurable airway responsiveness: a randomized placebo-controlled feasibility study. Clin Exp Allergy. 2009; 39(7):1060–1068. https://doi.org/10.1111/j.1365-2222.2009.03187.x PMID: 19400893; PubMed Central PMCID: PMC2728895.

134. Gaze S, Driguez P, Pearson MS, Mendes T, Doolan DL, Trieu A, et al. An immunomics approach to schistosome antigen discovery: antibody signatures of naturally resistant and chronically infected individuals from endemic areas. PLoS Pathog. 2014; 10(3):e1004033. https://doi.org/10.1371/journal.ppat.1004033 PMID: 24675823; PubMed Central PMCID: PMC3968167.

135. Croese J, Giacomin P, Navarro S, Clouston A, McCann L, Dougall A, et al. Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease. J Allergy Clin Immunol. 2015; 135(2):508–516. https://doi.org/10.1016/j.jaci.2014.07.022 PMID: 25248819.

136. Langenberg MCC, Hoogerwerf MA, Koopman JPR, Janse JJ, Kos-van Oosterhoud J, Feijt C, et al. A controlled human Schistosoma mansoni infection model to advance novel drugs, vaccines and diagnostics. Nat Med. 2020; 26:326–332. https://doi.org/10.1038/s41591-020-0759-x PMID: 32066978.