

Worms as therapeutic agents for allergy and asthma: Understanding why benefits in animal studies have not translated into clinical success

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Helminth infections are associated with decreased rates of autoimmunity and allergy, and several clinical studies have demonstrated that intentional infection with helminths can reduce symptoms of autoimmune diseases. In contrast, though numerous animal studies have demonstrated that helminth infections ameliorate allergic diseases, clinical trials in humans have not shown benefit. In this article, we review in detail the 2 human studies that have prospectively tested whether helminth infections protect against allergy. We next review the research designs and results obtained from animal studies, and compare these to the human trials. We then postulate possible reasons for the lack of efficacy observed in clinical trials to date and discuss potential future areas of research in this field. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: Allergy, asthma, atopy, helminths, therapeutics, clinical trials, animal models, hygiene hypothesis

The hygiene hypothesis was developed to explain the high prevalence of allergy and autoimmune disorders in industrialized countries.^{1,2} The hypothesis proposes that an absence of childhood infections, especially parasitic infections,^{3,4} can lead to immune dysregulation and allergic disease.^{1,5} Case studies and prospective clinical trials have already shown that helminth infections can protect against autoimmune diseases, including multiple sclerosis,⁶ ulcerative colitis,^{7,8} and Crohn disease.⁹ On the heels of these trials, groups have moved forward to determine whether helminths can also be used as therapeutic agents for allergic diseases. Despite substantial laboratory and epidemiologic evidence suggesting helminths are protective against allergy, prospective double-blind studies have not demonstrated clinical benefit in human subjects. This article seeks to understand why the results of these trials were negative by reviewing the clinical studies in detail and comparing their experimental designs with

Abbreviations used

AHR: Airway hyperresponsiveness
As-MIF: *Anisakis simplex* homologue for macrophage migration inhibitory factor
BALF: Bronchoalveolar lavage fluid
Cs-TP: *Clonorchis sinensis* total worm antigen
NES: *Nippostrongylus brasiliensis* excretory-secretory products
OVA: Ovalbumin
TSO: *Trichuris suis* ova

those used in prior animal studies. On the basis of this review, we provide possible reasons for the lack of efficacy observed in clinical trials and discuss potential future work in this field.

HUMAN CLINICAL TRIAL: *TRICHURIS SUI*S FOR ALLERGIC RHINITIS

Administration of *Trichuris suis* ova (TSO; pig whipworm eggs) has been demonstrated to improve symptoms of patients with ulcerative colitis and Crohn disease.^{8,9} After oral ingestion of embryonated TSO, worms hatch in the small bowel and migrate to the proximal colon. Colonization is localized to the colon and self-limited,¹⁰ with worms cleared after several weeks by human subjects with normal immune systems.¹¹ With respect to Crohn disease, 2500 TSO given every 21 days resulted in greater than 72% improvement in symptom scores over 24 weeks, with benefits observed as early as 12 weeks.⁹ Similar results were found in a double-blind, randomized, placebo-controlled trial for patients with ulcerative colitis. Patients who received 2500 TSO every 14 days for 12 weeks reported greater than 40% improvement in symptom scores compared with 16% improvement for subjects receiving placebo.⁸

Bager et al¹² sought to expand on these findings by testing whether TSO would also be helpful for patients with allergic rhinitis. Performed in Denmark, the study recruited patients with allergic rhinitis during the prior 2 pollen seasons who had grass-specific IgE and positive skin prick test responses for grass pollen. Modeled after the protective Crohn disease and ulcerative colitis trials, participants in the double-blind, randomized, placebo-controlled study received either 2500 TSO or placebo every 21 days for a total of 24 weeks. Patients were exposed to helminths for at least 1 month before the peak of the pollen season.

The outcomes assessed by the study included self-reported rhinitis symptoms, use of allergy medication, improvement of allergy symptoms compared with previous pollen seasons, and changes in immune responses to TSO and grass allergen.

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Measures of mean daily symptom scores demonstrated that treatment with TSO did not reduce allergic rhinitis symptoms during the pollen season. Additionally, skin prick test response positivity, total histamine levels, and grass pollen IgE levels did not change as a result of TSO treatment. The only improvement observed was a reduction in the use of tablet medication during the study, although the use of nasal spray and eye drops remained the same.¹² Although subjects in both groups experienced mild adverse gastrointestinal symptoms, the group that received TSO had higher reports of flatulence and upper abdominal pain, as well as diarrhea, during days 30 to 41 of the study. Patients who received TSO had immunologic responses to the parasite, including *T suis*-specific antibody titers and increased eosinophil levels.¹²

HUMAN CLINICAL TRIAL: *NECATOR AMERICANUS* FOR ASTHMA

In a nested case-control study carried out in Jimma, Ethiopia, Scrivener et al¹³ found that hookworm infection with a parasite burden resulting in more than 50 eggs per gram of feces was associated with a reduced risk of wheeze. This led investigators to ask whether hookworm infection could be used as a therapeutic for asthmatic patients.

Mortimer et al¹⁴ performed a preliminary dosing study with healthy volunteers. It was determined that a dose of 10 infectious *N americanus* larvae was well tolerated and resulted in a productive infection with more than 50 eggs per gram of feces. Larvae were applied directly to the skin for 24 hours to infect study subjects. Upon entering the skin, larvae migrate into the vasculature and travel through the heart and into the lungs. Larvae reach the lungs by day 10 after infection, when they emerge into the alveolar airspaces and crawl up the airways.¹⁵ By day 28 after infection, larvae have left the lungs to be swallowed down into the intestinal tract.¹⁵ The period of time larvae reside in the lungs, termed "pulmonary hookworm infection," can result in eosinophilic pneumonitis with symptoms of wheezing and shortness of breath.¹⁵ A second study was performed in which patients given a diagnosis of airway hyperresponsiveness (AHR) but not clinical asthma received a dose of 10 infectious larvae to address this safety concern in patients with compromised lung function.¹⁶ Infection was well tolerated in the at-risk study group, with side effects comparable with those of healthy volunteers.¹⁶

A double-blind, randomized, placebo-controlled clinical trial was then performed to determine whether *N americanus* infection could reduce the symptoms of asthma.¹⁷ The study recruited subjects given a diagnosis of clinical asthma by a physician who were currently taking daily inhaled corticosteroids. Baseline measurements were taken for forced expiratory volume, bronchial hyperresponsiveness after AMP challenge, and skin prick tests for common allergens. After exposure to infectious larvae or histamine as a control, groups were asked to keep a daily diary for recording peak expiratory flow, symptoms of hookworm infection, asthma symptoms, and use of asthma medication. Participants were evaluated at study visits every 2 weeks for 8 weeks and then again at weeks 12 and 16.¹⁷

For the primary outcome of the study, bronchial hyperresponsiveness was measured through sequential inhalations of AMP at 2-minute intervals until forced expiratory volume decreased by at least 20% (PD₂₀AMP) or maximum AMP had been inhaled. At

the end of the 16-week study, there was no difference in PD₂₀AMP values between infected and uninfected subjects, nor were there improvements in self-reported asthma symptoms, the secondary outcome.¹⁷

ANIMAL STUDIES

To gain insights into why the 2 prospective double-blind trials of helminth infection and allergic disease in human subjects have not shown benefits, we reviewed studies that investigated the effects of helminth infections, helminth antigens, or both in animal models of allergy.

Infection with live helminths

A substantial amount of work has been performed with animal models to explore helminth infection and host protection from allergy, with more than 30 such studies published to date. However, instead of testing whether helminth infections can treat established allergy, the majority of research has addressed whether helminths protect the host against the development of allergy; that is, in most experiments animals were infected first and then sensitized to an allergen. The majority of these experiments, which were conducted with a wide range of hypersensitivity models, demonstrated that helminths protect against development of allergic disease (Table I).¹⁸⁻⁵²

Surprisingly, only 4 groups have performed experiments to determine whether helminth infections are capable of protecting the host from established allergy (Table II).^{48,50,51,53} In these experiments animals were sensitized first and then infected, with infection used as a therapeutic agent for pre-existing allergy. This study design more closely mimics methods used in the aforementioned clinical trials. Of the 4 therapeutic animal studies, only 2 found evidence of protection.

In 1980, Jarrett et al⁵¹ reported the effects of *Nippostrongylus brasiliensis* infection on type 1 hypersensitivity reactions in Lister rats. In this study rats were sensitized against egg albumin by using *Bordetella pertussis* as an adjuvant. Twenty days after sensitization, rats were infected by means of subcutaneous injection of 4000 *N brasiliensis* L3 stage larvae. Like hookworms, *N brasiliensis* worms undergo a migration through the lungs before reaching the intestinal tract. However, in contrast to most successful helminth infections of human subjects, *N brasiliensis* infection is short lived, with immunocompetent rodents typically expelling the worms between days 10 and 13 after infection.⁵⁴ In the study by Jarrett et al,⁵¹ rats were assessed for anaphylactic reactions 2 weeks after *N brasiliensis* infection. Local anaphylaxis was assessed by measurement of skin test reactions 20 minutes after intradermal injection of egg albumin, and systemic anaphylaxis by quantification of shock symptoms within minutes of intravenous injection of egg albumin. Results convincingly demonstrated that helminth infections did not diminish either local or systemic allergic type I hypersensitivity reactions in previously sensitized mice. Additionally, these authors found that infection administered 2 weeks before sensitization also did not protect against allergen challenge.

Similar to the study by Jarrett et al,⁵¹ Wohlleben et al⁴⁸ found that *N brasiliensis* was unable to protect the host from established allergy. Animals were given intraperitoneal injections of ovalbumin (OVA) adsorbed to alum on days 0 and 14 and then infected with 1000 *N brasiliensis* L3 larvae on day 17. For the

TABLE I. Animal models with live helminth infections: protection against development of allergy

Protective effect	Helminth	Allergy model	Host	Time of sensitization	Notes	Reference
Infection protected against allergy development:						
Yes	<i>Angiostrongylus costaricensis</i>	AHR	BALB/c mice	5 d PI	Infected animals had fewer cells and IL-1 β in BALF.	36
Yes	<i>Heligmosomoides polygyrus</i>	AHR	BALB/c mice	14 d PI	Infection reduced airway eosinophilia and IL-5 levels and protected against airway hyperreactivity after methacholine challenge.	26
Yes	<i>H polygyrus</i>	AHR	BALB/c and C57BL/6 mice	28 d PI	Transfer of MLN B cells from infected animals to sensitized animals suppressed allergic responses.	47
Yes	<i>H polygyrus</i>	AHR	BALB/c and C57BL/6 mice	28 d PI	Suppression of AHR was dependent on CD25 ⁺ cells from MLNs.	50
Yes	<i>H polygyrus</i>	Food allergy	C3H/HeJ mice	5 d PI	Infection protected against production of peanut-specific IgE and symptoms of anaphylaxis in an IL-10–dependent manner.	19
Yes	<i>H polygyrus</i>	DTH	C57BL/6 mice	7 or 35 d PI	Mice infected for ≥ 7 days before sensitization had reduced splenocyte proliferation and secondary IgG responses.	38
Yes	<i>Nippostrongylus brasiliensis</i>	Local and systemic anaphylaxis	WAG rats	20 d PI	Infection reduced local and systemic anaphylaxis.	45
Yes	<i>Schistosoma japonicum</i>	AHR	BALB/c mice	24 d PI	Both unisexual (male) and bisexual infections reduced mucus production and cell infiltration after allergen challenge.	32
Yes	<i>S japonicum</i>	DTH	C57BL/6 mice	12 or 21 wk PI	Adoptive transfer of splenocytes from 12- or 21-wk infected animals protected against DTH.	24
Yes	<i>S japonicum</i>	AHR	BALB/c mice	8 wk PI	Infection reduced eosinophilia, mucus production, and allergen-specific IgE, IL-4, and IL-5 levels. Protection was also elicited through adoptive transfer of DCs from infected animals.	28
Yes	<i>S japonicum</i>	AHR	BALB/c mice	2 h after transfer	Transfer of DCs from infected animals resulted in expansion of IL-10–producing Foxp3 Treg cells in recipient mice.	27
Yes	<i>Schistosoma mansoni</i>	Systemic and passive anaphylaxis	BALB/c and C57BL/6 mice	7 wk PI	Infection protected against systemic and passive anaphylaxis in an IL-10– and B cell–dependent manner. However, the adoptive transfer of B cells from infected animals exacerbated anaphylaxis.	29
Yes	<i>S mansoni</i>	AHR	BALB/c and C57BL/6 mice	8 wk PI	Transfer of IL-10–producing B cells from infected animals to sensitized animals suppressed development of AHR and reversed inflammation present after challenge.	18
Yes	<i>S mansoni</i>	AHR	BALB/c mice	35 d PI	Infection reduced cell infiltration, eosinophilia, and IL-4 and IL-5 levels in BALF and OVA-specific IgE titers.	33
Yes	<i>Strongyloides stercoralis</i>	AHR	BALB/c mice	7 d PI	Infected animals had increased T _H 2 responses; however, they exhibited simultaneous suppression of allergen-specific responses.	46
Yes	<i>Trichinella spiralis</i>	AHR	C57BL/6 mice	4 wk PI	Infected animals were protected against AHR, with reduced cellular infiltrates and mucus production and an absence of goblet cell hyperplasia.	34

(Continued)

TABLE I. (Continued)

Protective effect	Helminth	Allergy model	Host	Time of sensitization	Notes	Reference
Infection protected against allergy development but only under specific conditions:						
Yes: <i>H polygyrus</i> No: <i>N brasiliensis</i>	<i>H polygyrus</i> , <i>N brasiliensis</i>	DTH	C57BL/6 mice	Multiple time points PI	Suppression of DTH was dependent on mice being infected ≥ 7 d before sensitization. Transfer of draining lymph node cells from infected animals to irradiated animals did not protect against DTH.	39
Yes: <i>T spiralis</i> No: <i>H polygyrus</i>	<i>H polygyrus</i> , <i>T spiralis</i>	DTH	BALB/c mice	<i>H polygyrus</i> : 13 d PI <i>T spiralis</i> : 2 d PI	T cells transferred from <i>T spiralis</i> -infected DO11.10 mice protected against DTH.	21
Yes: <i>H polygyrus</i> No: <i>N brasiliensis</i> No: <i>Trichuris muris</i>	<i>N brasiliensis</i> , <i>H polygyrus</i> , <i>T muris</i>	Antibody response to allergen	BALB/c, C57BL/6, and CBA mice	Multiple time points PI	<i>H polygyrus</i> decreased allergen-specific IgG titers when infection occurred before sensitization and when alum was used as an adjuvant for allergen immunization.	37
Yes: Unisex infection No: Natural infection	<i>S mansoni</i>	AHR	BALB/c mice	7-11 wk or 12-16 wk PI	Natural infection exacerbated AHR, but male-only infection was found to be protective in an IL-10-dependent manner.	30
Yes: Dependent on length of infection	<i>Ascaris suum</i>	Allergic eye disease	BALB/c and A/J mice	At time of infection or 44 d PI	Acute infection exacerbated allergy, causing eye inflammation and high levels of eotaxin, IL-6, and conjunctival eosinophils. Conversely, chronic infection protected against eye inflammation.	40
Yes: Dependent on length of infection	<i>Acanthocheilonema viteae</i> , <i>Brugia malayi</i> , <i>Litomosoides sigmodontis</i>	DTH	<i>Mastomys natalensis</i>	Multiple time points PI	Antibody titers against SRBCs were affected by chronicity of infection and microfilaremia. Protection against DTH was dependent on time of sensitization and species of helminth.	49
Yes: Dependent on length of infection	<i>S mansoni</i>	AHR	BALB/c mice	Multiple time points PI	Patent infection protected best against AHR and was associated with Treg cells. Protection waned after anthelmintic treatment.	52
Yes: Dependent on length of infection	<i>S mansoni</i>	AHR	C57BL/6 mice	8, 12, or 16 wk PI	Acute infection increased eosinophil numbers in BALF, whereas chronic infection reduced inflammation, eosinophil recruitment, and goblet cell hyperplasia. Intermediate and chronic infection protected against AHR.	41
Yes: Dependent on length of infection	<i>Trichinella pseudospiralis</i>	DTH	BALB/c and C57BL/6 mice	16 or 21 d PI	Animals were protected against DTH at 16 d after infection, during the migratory stage of the parasite, but had exacerbated DTH at 21 d after infection.	42
Yes: Dependent on length of infection	<i>T spiralis</i>	DTH	CD-1 mice	Multiple time points PI	Protection against DTH was observed at 0, 3, and 7 d after infection; however, protection waned at 14 d and was completely absent at 28 and 58 d after infection.	20
Yes: Dependent on host strain	<i>N brasiliensis</i>	Local and systemic anaphylaxis	WAG and BN rats	20 d PI	WAG strain was protected from allergy by infection, whereas BN strain was not.	44
Yes: Dependent on age of animal	<i>N brasiliensis</i>	Local and systemic anaphylaxis	WAG rats	20 d PI	Infected animals were protected against allergic responses; however, the level of protection was dependent on the age of the animal.	43
Yes: AHR No: AD	<i>H polygyrus</i>	AHR and AD	BALB/c mice	At time of infection	Infection inhibited airway inflammation but did not suppress AD responses; infection exacerbated thickness of skin lesions in AD allergy model.	25
Yes: AHR No: Local anaphylaxis	<i>N brasiliensis</i>	AHR and local anaphylaxis	BALB/c and C57BL/6 mice	4 or 8 wk PI	Four- and 8-wk infected mice were protected against AHR but not cutaneous allergen challenge.	48

(Continued)

TABLE I. (Continued)

Protective effect	Helminth	Allergy model	Host	Time of sensitization	Notes	Reference
Infection did not protect against allergy development:						
No	<i>Aspiculuris tetraptera</i> , <i>Syphacia muris</i>	Cytokine and antibody response to allergen	Wistar rat	NA	Infection did not reduce allergen-specific IgE, TNF- α , or IL-10 levels in sensitized animals.	22
No	<i>N brasiliensis</i>	Local and systemic anaphylaxis	Hooded Lister rats	20 d PI or at time of infection	Sensitization after infection did not protect against allergy; however, the group sensitized at the time of infection had the lowest anaphylaxis scores.	51
No	<i>Syphacia obvelata</i>	Systemic anaphylaxis	BALB/c and C57BL/6 mice	14 or 21 d PI	Pinworm infection exacerbated temperature decrease during anaphylaxis.	31
No	<i>Toxocara canis</i>	AHR	BALB/c mice	3 or 20 d PI	Infection exacerbated AHR response and lung pathology, including increased eosinophil counts and IL-4 levels in BALF.	35

AD, Atopic dermatitis; DC, dendritic cell; DTH, delayed-type hypersensitivity; *Foxp3*, Forkhead box protein 3; *MLN*, mesenteric lymph node; NA, not applicable; PI, postinfection; SRBC, sheep red blood cells; *Treg*, regulatory T.

AHR arm of the study, animals were given an intranasal challenge of OVA 1 week after infection. Cell types present in the airways and cytokine levels in bronchoalveolar lavage fluid (BALF) were assessed 6 days after challenge. There was no reduction in the number of eosinophils present in infected mice. Furthermore, there was actually an increase in levels of IL-5 and eotaxin present in the BALF compared with those seen in uninfected animals. For the local anaphylaxis arm of the study, mice were given a subcutaneous challenge of OVA 10 days after intranasal challenge. Both infected and uninfected animals exhibited strong local anaphylactic responses, as indicated by blue dye extravasation in the skin.

Negrão-Corrêa et al⁵³ tested whether helminth infection could be used as a therapeutic in a rat model of AHR. Wistar rats were sensitized by subcutaneous implantation of OVA. Animals then underwent intranasal challenges with OVA on days 14 and 21. As an experimental treatment, animals were infected with 5000 infective-stage L3 larvae of *Strongyloides venezuelensis* by subcutaneous injection on the day of the second OVA challenge. Like hookworms, *S venezuelensis* worms transit through the lungs before reaching the intestinal lumen. The authors found that animals infected at the time of the second challenge were protected against AHR 48 hours later, a time point when larvae are actively migrating through the lungs.⁵³

Finally, in 2005, Wilson et al⁵⁰ published a study evaluating the effects of *Heligmosomoides polygyrus* in a murine model of AHR.* *H polygyrus* is a chronic intestinal helminth infection of rodents that does not have a tissue-migratory phase. Although the majority of the publication is dedicated to determining whether infection can prevent the development of allergy, one experiment tested the therapeutic potential of *H polygyrus*. In this experiment animals were initially sensitized to OVA by intraperitoneal injection of OVA with alum as an adjuvant. Two weeks

after sensitization, animals were infected with *H polygyrus* by oral gavage with 200 L3 stage larvae. Fourteen and 16 days after infection, mice were challenged by intratracheal administration of OVA in PBS. Cell counts obtained from BALF of animals killed 1 day after the final OVA challenge demonstrated significantly reduced numbers of total cells and eosinophils in the airways of helminth-infected mice.

Studies with helminth antigen

In addition to live worms, the use of worm antigens to protect against allergy has been tested in 13 studies (Table III).^{33,55-66} Similar to combined allergy and infection models, the majority of the work with parasite antigens has focused on preventing the development of allergy. Overwhelming evidence has shown that specific antigens,^{55,60,62} pseudocoelomic fluids,^{61,65} excretory-secretory products,^{59,63,66} whole-worm antigen preparations,⁵⁶⁻⁵⁸ and egg antigen⁶⁴ are all capable of protecting the host against the development of allergy.

Three groups have shown that administration of parasite antigens after sensitization is capable of reducing the symptoms and allergic inflammation associated with established allergy.^{56,60,62} The use of worm antigen as a therapeutic for established allergy might be a useful alternative to natural infection because it bypasses some of the potentially harmful side effects associated with live worm infections. However, there are also potential disadvantages to using worm antigens. These include expenses incurred for antigen production and purification, the probable need for frequent administration, and the risk of allergic reactions to the worm antigens themselves.

Despite these potential pitfalls, studies with worm antigens have shown promising results in animal models. Park et al⁶⁰ sensitized mice to OVA and then administered an *Anisakis simplex* homologue for macrophage migration inhibitory factor (As-MIF). Varying concentrations of recombinant As-MIF were administered by intraperitoneal injection 30 minutes before allergen challenge in a murine model of AHR. As-MIF reduced cellular infiltration, goblet cell hyperplasia, and mucus

*Note: *Heligmosomoides polygyrus* was formerly known as *Nematospiroides dubius*. References that have used the former nomenclature have been updated to *H polygyrus* in this review.

production in the lungs in a dose-dependent manner. Additionally, As-MIF reduced levels of type 2 cytokines and increased levels of the regulatory cytokines IL-10 and TGF- β in BALF.

Jeong et al⁵⁶ obtained similar results in a murine AHR model by using *Clonorchis sinensis* total worm antigen (Cs-TP). In this study 100 μ g of worm antigen was administered as a crude homogenate by intraperitoneal injection 1 day before airway challenge in previously sensitized mice. Cs-TP administration reduced AHR, eosinophil numbers in BALF, mucus production, and T_H2 cytokine production in the lungs after methacholine challenge. Additionally, Cs-TP treatment induced expansion of forkhead box protein 3–positive regulatory T cells.

Finally, Schnoeller et al⁶² found that administration of recombinant *Acanthocheilonema viteae* cystatin also reduced airway inflammation when given after sensitization. Recombinant *A viteae* cystatin was administered by means of 4 intraperitoneal injections during the week between sensitization and challenge. Immunologic studies suggested macrophages, IL-10, and, to a lesser extent, regulatory T cells all played a role in protection.

Although these studies found worm antigens could be successful therapeutics, not all studies provided such promising outcomes. Trujillo-Vargas et al⁶³ had conflicting results with the use of *Nippostrongylus brasiliensis* excretory-secretory product (NES) to prevent allergic inflammation. When NES was administered with OVA during sensitization, there was a reduction in airway eosinophilia after challenge. However, that protection disappeared when NES was administered after OVA sensitization.⁶³ Furthermore, *Ascaris suum* body fluid only showed protection against a cutaneous model of delayed-type hypersensitivity when given during sensitization, whereas administration of *A suum* body fluid during challenge had no effect on foot pad swelling.⁶¹

Mechanisms of protection

One of the interesting aspects of chronic helminth infections is that they enhance immune regulatory mechanisms over time. Helminth-induced immunomodulation, as summarized by McSorley et al,⁶⁷ is multifactorial and causes suppression of both the innate and adaptive arms of the immune system. It has been shown that strong type 2 and innate lymphoid cell responses tend to confer resistance to infection in animal models.⁶⁸ There are multiple mechanisms by which worms are able to overcome host resistance to infection, including the induction of *de novo* forkhead box protein 3 expression on T cells⁶⁹ and IL-6 production.⁷⁰ Additionally, alterations in dendritic cell function, increased production of downregulatory cytokines (such as IL-10 and TGF- β) and increased frequencies of regulatory T and B cells have all been implicated as mechanisms underlying immune hyporesponsiveness. Immune suppression is likely due to the direct effect of worm products on immune cells, as well as chronic activation of the immune system with subsequent induction of negative feedback mechanisms.

AHR has been the most common type of allergy model used in animal studies that combine helminth infection and allergic disease. In most of these studies, helminths appear to protect against allergy by inhibiting the infiltration of inflammatory cells, especially eosinophils, into the airways. The majority of publications have linked reduced airway pathology to decreased levels of chemokines (including eotaxin 1) and type 2 cytokines (including IL-4, IL-15, and IL-13) in the BALF.[†]

Helminth excretory-secretory products have also been shown to block IL-33 release and suppress innate lymphoid cell responses, which results in suppression of allergic airway disease.⁶⁶

Although the transfer of cells from infected animals into sensitized naive animals has been successful at protecting the host from allergic inflammation,[‡] there is not yet a clear consensus on which cell type is most important for eliciting protection. At this time, cells that actively downregulate immune responses, including regulatory T and B cells, are the top contenders for inducing protection.

In studies that combined helminth infection with an allergy model, a reduction in allergen-specific antibody titers in the infected host was often observed. This phenomenon occurred with numerous species of helminths, including *H polygyrus*,^{19,37} *Litomosoides sigmodontis*,²³ *Schistosoma mansoni*,³³ *Schistosoma japonicum*,^{28,32} *Trichinella spiralis*,³⁴ and *Strongyloides stercoralis*.⁴⁶ Furthermore, immunization with parasite antigens in lieu of a natural infection also decreased allergen-specific antibody titers.^{55,56,58,59,62,63} The mechanism through which this occurs is not fully understood; however, clearance of IgE antibodies through CD23⁷¹ might be a plausible explanation that warrants further exploration. Although reduction in allergen-specific IgE levels does not always correlate with clinical benefit, it often occurs during allergen-specific immunotherapy,⁷² suggesting these data might prove to be an important base on which to frame future research endeavors.

One of the most longstanding explanations for the mechanism by which helminths protect against allergy has been the IgE blocking hypothesis,² which states that high levels of IgE induced by helminth infections inhibit allergic sensitization by saturating IgE receptors. Although this is biologically plausible, a number of animal and *in vitro* studies suggest this phenomenon plays only a minor, if any, role in helminth-mediated protection against allergy.^{48,73,74} Furthermore, a study that surveyed different populations living in Saskatchewan also found no correlation between serum IgE levels and atopic disease in human subjects, signifying that IgE levels are likely due to genetic or environmental factors and might not be good predictors of allergic disease.⁷⁵

The extent to which the suppression of allergy effector cells is important for helminth-induced protection against allergy remains unknown. Recently, our group demonstrated that chronic helminth infection suppresses basophil activation in response to IgE receptor cross-linking.^{76,77} However, functionality of mast cells and basophils in a combined allergy/helminth model has yet to be studied.

DISCUSSION

After reviewing the literature from animal studies, we believe there are several key points to consider when trying to understand why clinical trials using helminth infections to treat allergic diseases have been unsuccessful.

1. It is not yet clear whether helminths are as effective at treating allergy as they are at preventing allergy

At this time, there are only limited animal data demonstrating that helminths can ameliorate allergic symptoms when administered after sensitization. Of 4 published animal studies

†References 25, 26, 28, 30, 32–34, 41, 46–48, 55–60, 63, and 65.

‡References 18, 24, 26–28, 40, 41, 47, and 50.

TABLE II. Animal models with live helminth infections: protection against established allergy

Protective effect	Helminth	Allergy model	Host	Notes	Reference
Infection protected against pre-existing allergy:					
Yes	<i>Heligmosomoides polygyrus</i>	AHR	BALB/c and C57BL/6 mice	Infected mice had reduced numbers of total cells and eosinophils in BALF.	50
Yes	<i>Strongyloides venezuelensis</i>	AHR	Wistar rats	Infection reduced AHR 48 h after challenge, even though larvae were migrating through the lungs at this time.	53
Infection did not protect against pre-existing allergy:					
No	<i>Nippostrongylus brasiliensis</i>	Local and systemic anaphylaxis	Hooded Lister rats	Allergic reactions were exacerbated by helminth infection. Parasite-induced IgE is not responsible for host protection from allergy.	51
No	<i>N. brasiliensis</i>	AHR and local anaphylaxis	BALB/c and C57BL/6 mice	Mice infected for 1 wk before challenge had high eosinophil numbers and high IL-5 levels in BALF. Mice were also not protected from local anaphylaxis.	48

*Note: *Heligmosomoides polygyrus* was formerly known as *Nematospiroides dubius*. References that have used the former nomenclature have been updated to *H. polygyrus* in this review.

involving infection after sensitization, 2 exhibited therapeutic efficacy.^{50,53} Given the tendency for investigators to publish positive findings (publication bias), we suspect that helminths might be better at preventing allergy than treating it. However, the hypothesis that prophylaxis is more effective than therapy will remain unproved until further research can be performed. Before embarking on further clinical trials, it might be worthwhile to test additional animal models of established allergy to better determine the therapeutic potential of helminths and helminth-derived products.

2. Location of helminth infection is likely important for parasites that do not induce systemic immunomodulatory effects

The effects helminths have on allergic disease are ultimately caused by changes in host immune function. Thus it is likely that immunologic changes need to occur at the site of the allergic reaction for protection to take place. For example, in the protective Crohn disease and ulcerative colitis clinical trials, TSO were located at the site of intestinal disease.^{8,9} In one of the 2 animal studies that showed a therapeutic benefit of live worms, the helminth used (*S. venezuelensis*) migrated through the same tissues as those involved in the allergic disease being evaluated (lungs in an AHR model).⁵³ Although the helminth used in the other successful therapeutic study of live worms in mice did not reside in the same tissue space as the allergic disease (*H. polygyrus*, an intestinal nematode, protected against AHR), the investigators did find that the infection induced systemic immunologic changes.⁵⁰ Similarly, all 3 of the protective helminth antigen studies administered antigen systemically.^{55,60,62}

In contrast to these studies, the negative clinical trial for allergic rhinitis used *T. suis*, a relatively noninvasive nematode that localizes to the intestinal tract. *T. suis* might not have protected against allergic rhinitis because it could not induce immunologic changes at the nasal mucosa. Thus localization of the helminth at the site of allergic disease or induction of systemic immune modulation by the infection might be necessary for helminths to protect against allergic disease.

3. High worm burdens might be necessary for optimal benefit

Although location could potentially explain the lack of efficacy for the *T. suis*/rhinitis trial, it does not apply to the *N. americanus*/

asthma study because hookworm larvae migrate through the lung, the anatomic site at which asthma occurs. For this study, one of the driving factors preventing benefit might have been the dose of larvae used. Only 10 *N. americanus* larvae were given to each study participant. This dose was chosen because it was found to sufficiently produce more than 50 eggs per gram of feces.¹⁴ However, when one looks at the initial epidemiologic article that described the association between hookworm and decreased wheeze, patients who exhibited significant protection had a hookworm infection that produced from 48 to 1458 eggs per gram of feces.¹³ For safety reasons, the trial used the minimum number of larvae necessary to produce more than 50 eggs per gram of feces. However, it could have missed potential clinical benefits that might be associated with higher doses of hookworms.

In contrast to this clinical trial, protective animal studies with live worms in animals have administered much higher doses of infectious larvae. Specifically, mice were given 5000 *S. venezuelensis* L3 larvae or 200 *H. polygyrus* L3 larvae to show a protective effect in models of AHR. Given the enormous difference in size between human subjects and mice, for 10 *N. americanus* larvae to be effective in human subjects, one would expect that a dose of only 1 or 2 larvae would be effective in mice. To date, studies with such low numbers of worms in animal models have not been conducted. Evidence suggesting that the dose of larvae used in the *N. americanus* trial might have been too low comes from the immunologic assays performed in that study. Infection with 10 larvae altered neither levels of allergen-specific IgE nor markers for systemic immune regulation, such as IL-10 or regulatory T cells.⁷⁸ Given the lack of efficacy in the clinical trial, it might be helpful to use animal models to test the extent to which protection against allergy is a dose-dependent phenomenon.

4. Duration of infection might be important

Recently, Layland et al⁵² performed a series of experiments designed to address the importance of timing in protecting the host against the development of allergic disease. They found that *S. mansoni* infection could reduce allergic airway inflammation when animals were sensitized 5 weeks into infection but not when animals had only been infected for 1 week. Similarly, Schopf et al⁴⁰ showed that sensitization during acute *A. suum* infection (days 0–4) exacerbated allergic eye disease, whereas sensitization during chronic infection (days 44–48) resulted in protection. Other investigators have also demonstrated that acute infection had either no effect or exacerbated allergic disease, whereas chronic infection

TABLE III. Animal models with helminth antigens: protection against development of allergy and established allergy

Protective effect	Helminth	Antigen	Allergy model	Host	Notes	Reference
Treatment protected against development of allergy and established allergy:						
Yes: Therapeutic	<i>Anisakis simplex</i>	As-MIF	AHR	BALB/c mice	Treatment with As-MIF before challenge reduced airway inflammation in a dose-dependent manner.	60
Yes: Prevent development Yes: Therapeutic	<i>Acanthocheilonema viteae</i>	Av17	AHR	BALB/c mice	Cystatin administered during or after sensitization suppressed airway inflammation, with a reduction in lung infiltrates and eosinophil counts. Macrophages, Treg cells, and IL-10 played a role in protection.	62
Yes: Prevent development Yes: Therapeutic	<i>Clonorchis sinensis</i>	TP	AHR	BALB/c mice	Antigens prevented sensitization, as well as reduced AHR, when given at the time of challenge.	56
Treatment protected against development of allergy but not established allergy:						
Yes: Prevent development No: Therapeutic	<i>Ascaris suum</i>	ABF	DTH	BALB/c, B10.BR, and B10.S mice	Sensitizing in the presence of ABF significantly reduced foot pad swelling after OVA challenge. However, treating with ABF at the time of challenge did not reduce allergic responses. Protection was mediated by heat-stable factor(s).	61
Yes: Prevent development No: Therapeutic	<i>Nippostrongylus brasiliensis</i>	NES	AHR	BALB/c, C57BL/6, and C3H/HeJ mice	NES administered during sensitization reduced airway responses. However, NES given after OVA sensitization did not protect against allergic inflammation. Protection was mediated by heat-stable factor(s).	63
Treatment protected against allergy development; protection against established allergy was not tested:						
Yes: Prevent development	<i>A suum</i>	ASC	AHR	C57BL/6 and B10.A mice	Antigen administration reduced cellular infiltration, eosinophil peroxidase levels, eotaxin levels, and T _H 2 cytokine production in BALF.	58
Yes: Prevent development	<i>A suum</i>	PCF	AHR	BALB/c, C57BL/6, and A/J mice	Animals sensitized simultaneously with ragweed and PCF had reduced allergic conjunctivitis and pulmonary inflammation. Systemic administration of PCF appeared to reduce allergic inflammation at multiple challenge sites.	65
Yes: Prevent development	<i>Heligmosomoides polygyrus</i>	HES	AHR	BALB/c mice	HES suppressed airway inflammation and decreased OVA-specific IgG ₁ , IgG _{2a} , and IgE levels. Protection was mediated by heat-stable factor(s).	59
Yes: Prevent development	<i>H polygyrus</i>	HES	AHR	BALB/c mice	HES suppressed AHR by blocking IL-33 release and ILC function.	66
Yes: Prevent development	<i>Schistosoma japonicum</i>	SEA	AHR	BALB/c mice	SEA administration reduced airway inflammation and increased expression of Foxp3 on CD4 ⁺ CD25 ⁺ cells.	64
Yes: Prevent development	<i>Schistosoma mansoni</i>	Whole eggs*	AHR	BALB/c mice	Egg treatment reduced cell infiltration, eosinophilia, and IL-4 and IL-5 levels in BALF and OVA-specific IgE titers. Treatment also increased IL-10 levels and Foxp3 ⁺ T-cell numbers.	33
Yes: Prevent development	<i>S mansoni</i>	Sm22.6, PIII, Sm29	AHR	BALB/c mice	<i>S mansoni</i> antigens reduced airway inflammation and T _H 2 cytokine levels in BALF and OVA-specific IgE levels.	55
Yes: Prevent development	<i>Toxascaris leonina</i>	TP, ES	AHR	BALB/c mice	Antigens reduced airway inflammation, goblet cell numbers, sensitivity to methacholine, influx of neutrophils and lymphocytes, and T _H 2 cytokine production in the lung.	57

ABF, *Ascaris suum* body fluid; ASC, *Ascaris suum* body extract; Av17, *Acanthocheilonema viteae* cystatin; DTH, delayed-type hypersensitivity; ES, excretory-secretory product; Foxp3, forkhead box protein 3; HES, *Heligmosomoides polygyrus* excretory-secretory product; ILC, innate lymphoid cell; PCF, *Ascaris suum* pseudocoelomic fluid; SEA, soluble schistosoma egg antigen; TP, total worm antigen; Treg, regulatory T.

*Administration of *S mansoni* eggs will not result in a live infection. Therefore *S mansoni* eggs are considered an antigen in the context of this review.

was protective.^{41,48} To date, no one has investigated whether chronicity affects the ability of helminth infections to protect against pre-existing allergy in human subjects. The aforementioned studies suggest that the therapeutic potential of helminths might be greatest in the setting of chronic infection.

5. Helminths might not be able to protect against all types of allergic disease

Animal studies demonstrating the successful use of helminths as therapeutic agents were primarily performed in airway

hypersensitivity models. In contrast, studies that did not find protection were conducted in cutaneous and systemic anaphylaxis models. The latter models of allergy are predominantly dependent on classical IgE-mediated type 1 hypersensitivity, whereas the pathogenesis of disease in airway hypersensitivity is multifactorial. Because of this, we believe it would be judicious to demonstrate that helminths can protect against type 1 hypersensitivity reactions in animals before proceeding to clinical studies. Furthermore, when considering asthma, it is important to note that our understanding of its cause is currently evolving. Not all forms of asthma are considered to be classic eosinophil-mediated responses,^{79,80}

and trials testing worms and worm products should take precautions to note the mechanistic endotype of asthma included in their studies.

6. Use of concomitant medications might prevent helminths from exerting their protective effects

Although the exact mechanism through which helminths protect against allergic disease in animal models is not fully understood, the preponderance of evidence strongly suggests that they do so by inducing host immunoregulatory mechanisms. A major difference between the negative clinical trials and the protective animal studies was the use of concomitant allergy medications. In the *T suis*/rhinitis trial subjects routinely used oral antihistamines and nasal corticosteroids for symptom relief. Similarly, all of the subjects in the *N americanus*/asthma study took daily inhaled corticosteroids. Because both corticosteroids and antihistamines can have broad effects on immune system function, it is possible that use of these medications might have impeded helminth-induced development of host immunoregulatory networks. We believe it would be worthwhile to test whether these medications attenuate helminth-induced protection in animal models of allergy. Positive findings would have important implications for the design of future clinical trials.

7. Host genetics might influence the therapeutic effects of helminth infection

It is well known that certain species of rodents are more susceptible to helminth infection than others,^{68,81,82} and this phenomenon is also observed with respect to host protection from allergy. Studies have reported that allergic responses,^{83,84} as well as the protective effects of helminth infection,⁴⁴ are host strain specific. It might be necessary to determine whether specific human genotypes are more receptive to helminth therapy to better translate positive results obtained from animal models into new therapies for allergic disease. The failure of current clinical trials could be attributed to the lack of stratification of patients into responder and nonresponder groups. Because the human genome and microbiome have only just begun to be explored in detail, our lack of understanding of the genetic factors contributing to allergic disease and the host response to parasitic infections is a major hurdle to overcome if we wish to improve our therapeutic repertoire.

8. Different helminth species will likely have varying potential to protect against allergy

When discussing the relationship between helminths and allergic disease, there is a tendency to lump all helminths together, despite the incredible variety that exists biologically and anatomically. Although the immune responses induced by helminths have similarities, their ability to protect against allergy likely varies. For example, when looking at protection against established allergy, animal studies that had negative results used *N brasiliensis*,^{48,51} whereas protective studies used *H polygyrus*⁵⁰ and *S venezuelensis*.⁵³ As such, we believe it is beneficial to consider the therapeutic potential of a particular helminth or related antigen solely based on experiments performed with that species of parasite; comparing data from one species with those from another might hinder our efforts to fully understand the phenomenon of host protection from allergy.

FUTURE DIRECTIONS

It is unlikely that a silver bullet exists when it comes to using helminth infections to treat allergy. Because the health care field is evolving toward personalized medicine, it might be worthwhile to entertain the idea that particular species of helminths, or specific antigens, are best suited for particular types of allergic diseases. Overall, the type of infection (localized vs systemic), intensity of infection (light vs heavy), duration of infection (acute vs chronic), type of allergic response (asthma vs atopy), use of concomitant medications, host genetics, and species of helminth might all play a role in determining whether the host will be protected from allergy.

The prevention of allergies might be a helpful goal in the future if we can predict which patients are most susceptible to allergy. However, at this time, the primary use for helminths and helminth products would be consideration for treatment. Despite the negative studies in human subjects to date, much of the preliminary evidence from animal studies is promising. Once more research is performed to confirm that protection is possible and to investigate the mechanisms through which this protection occurs, we will be better poised to succeed at developing helminth-based therapies for allergic diseases.

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