Current perspectives

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*

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

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 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 SUIS* 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 al[12] 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 al[13] 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 al[14] 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% (PD_{20}AMP) or maximum AMP had been inhaled. At the end of the 16-week study, there was no difference in PD_{20}AMP 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 al[51] 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 al[48] 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