Clinical reviews in allergy and immunology

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Trichuris suis ova: Testing a helminth-based therapy as an extension of the hygiene hypothesis

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Activity Objectives

1. To understand the evidence supporting the safety of helminthic therapies in patients with allergic disease.
2. To discuss the possible mechanisms for the therapeutic effects of helminths.
3. To briefly explain the hygiene hypothesis and its role in allergy/immunology.

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The hygiene hypothesis, which was put forward more than 20 years ago by Strachan, proposes that the recent increase in allergic and autoimmune diseases is due to increasing hygiene standards. Since then, numerous epidemiologic and animal studies have provided support for this hypothesis and showed that certain microorganisms, helminths in particular, have immunomodulatory effects. More recently, studies have led to the identification of some of the mechanisms underlying these immunomodulatory effects. Substances, or crude extracts, produced by worms and responsible for these effects have been analyzed. Clinical trials have been performed mainly with pig whipworm, which was chosen because it is likely to be nonpathogenic in human subjects. Eggs of the pig whipworm (*Trichuris suis* ova) have been shown to be safe in multiple studies. Efficacy has been demonstrated in patients with inflammatory bowel diseases and in 1 case of pecan allergy. Altogether, this information supports further investigation of *T suis* ova in patients with immune-mediated diseases, particularly in areas in which there is currently no therapy, such as food allergy. (J Allergy Clin Immunol 2012;130:3-10.)

Key words: *Hygiene hypothesis, helminth,* Trichuris suis*, peanut and tree nut allergy, mucosal immunity, immunomodulation, ES-62, clinical trial*

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Certain helminths have recently been proposed as therapies in patients with allergic diseases. Here we report some of the evidence supporting this approach. We will discuss the experience with the main helminths that have been proposed as therapy, analyze the possible mechanisms for the therapeutic effects of helminths, and discuss the future of helminthic therapies. Studies of helminthic therapies in patients with nonallergic diseases will also be discussed because helminths have broad immunomodulatory properties.

Helminths are parasitic worms. According to the classical definition of parasitism, it is a nonmutual relationship between 2 organisms belonging to different species in which the parasite but not the host benefits from the relationship. Although the benefit of parasitism for the parasite has been known for a long time, recent evidence indicates that the host might benefit as well. This new concept has emerged in the broader context of a

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Abbreviations used
AE: Adverse event
CD: Crohn disease
DC: Dendritic cell
ES: Excretory/secretory
IBD: Inflammatory bowel disease
MRI: Magnetic resonance imaging
MS: Multiple sclerosis
OR: Odds ratio
PC: Phosphorylcholine
RRMS: Relapsing-remitting multiple sclerosis
Treg: Regulatory T
TSO: *Trichuris suis* ova
UC: Ulcerative colitis

re-examination of the role of the microbiological environment in human health.

THE HYGIENE HYPOTHESIS

Throughout human evolution, the main threat to human health has been infectious disease. Mainly hygiene but also vaccination and antibiotics have limited this threat in a large part of the world. The increasing use of these tools in the developing world is having a major positive effect on survival and quality of life. Therefore it is counterintuitive that the progress of hygiene has coincided with an increased prevalence of allergic and autoimmune diseases in areas of the world where hygiene is the most widely used. The hygiene hypothesis proposes that a causal link exists between the adoption of modern hygiene and the increase in the prevalence of these immune dysfunctions.[1] A corollary of this hypothesis is that it is most relevant in children. Children are born with an immature immune system. Part of its maturation is initiated in the perinatal period with the colonization of respiratory and digestive mucosa with commensal microorganisms (microbiota). Simultaneously, secondary lymphoid structures develop in the intestinal mucosa. The mucosae themselves play an important role in the maturation process as a physical barrier and the source of cell contact–mediated signals and cytokines. Innate immune systems are at the forefront of the process and control the maturation of adaptive immune systems. The result of these maturation processes is multifaceted and combines the establishment of tolerance to food and harmless microorganisms, as well as that of defense mechanisms against pathogens. For a review of the perinatal maturation processes at the digestive and respiratory mucosa, see Renz et al.[2]

Through evolution, the immune system has developed from being composed only of cells of the innate immune system to acquiring the complex components of the acquired immune system. This evolution has taken place in response to the presence of various microorganisms, among which are helminths. The idea that the human immune system protects individuals and the human species against infection is a familiar one. Another aspect of this evolution is that interaction with certain microorganisms, including helminths, might be an important component of the normal maturation of the immune system.[3,4] Although this article will focus on the role of helminths, abundant evidence indicates that microorganisms other than helminths also confer protection against allergic diseases. This evidence stems from studies showing the protection conferred by growing up on a farm (reviewed in von Mutius and Vercelli[5]), with pets in the household, or in a crowded environment. More recently, studies have demonstrated an inverse association between the diversity of the gut microbiota early in life and the risk of allergic sensitization later in life (eg, see Bisgaard et al[6]).

Epidemiologic evidence from the developing world (observational studies) supporting the hygiene hypothesis

Numerous studies have found an inverse relationship between allergy (diversely defined) and the presence of a current or past helminth infection.[7,8] A recent meta-analysis of 21 studies showed that current parasitic infection was statistically significantly associated with a reduced risk of skin sensitization to allergens (odds ratio, 0.69; 95% CI, 0.60-0.79; $P < .01$).[9] Although this analysis shows a significant risk reduction, it relies on allergen skin reactivity, which is poorly correlated with clinically significant allergy.

Evidence in support of the hygiene hypothesis from therapeutic interventions in the developing world

Other studies have compared allergy status before and after anthelmintic treatment and generally showed an increased in allergen skin reactivity after anthelmintic treatment. For example, in a large (>1400 subjects) cohort study performed in rural Vietnam children (about two thirds of whom were infected with hookworm, mainly *Necator americanus*, and 7% with *Ascaris lumbricoides*) were randomized to anthelmintic treatment or placebo, followed over a 9-month period and assessed for exercise-induced bronchospasm, wheeze, and rhinitis (with a questionnaire). They also underwent skin tests to environmental allergens. Among these outcomes, only skin sensitization increased significantly in the treated group (adjusted odds ratio, 1.31; 95% CI, 1.02-1.67; $P = .03$). The incidence of the other outcomes (exercise-induced bronchospasm, wheeze, and rhinitis) was low (7%), which could have resulted in a lack of power of the study to detect any significant change.[10]

Another limitation of epidemiologic studies performed in the developing world is that maturation of the subject's immune system takes place while he or she is exposed to a parasitized world from birth. Therefore it is likely that the beneficial effect of this exposure, if it existed, would not have been completely reversed by the antiparasitic treatment administered during the study. This is suggested by a study showing that the immune profile of infants in Zanzibar was not significantly changed after periodic treatment with an antiparasitic drug.[11]

The Cochrane Collaboration recently published a protocol describing how they plan to review studies on helminths for asthma, which will assess the evidence on the safety and efficacy available from studies that meet certain criteria of methodological quality.[12]

Evidence in support of the hygiene hypothesis from animal studies

In these studies mice infected or not with gastrointestinal nematodes (*Heligmosomoides polygyrus*, *Trichuris muris*, or *Nippostrongylus brasiliensis*) were compared in models of allergic diseases (asthma or peanut allergy). Similar studies have been performed with murine models of autoimmune

diseases (collagen-induced arthritis or colitis). Overall, the effect of concomitant parasitic infection is to decrease the allergic or autoimmune phenotype. In asthma models decreased cellularity of the bronchoalveolar lavage fluid (particularly eosinophils and lymphocytes), decreased allergen-specific serum and/or bronchoalveolar lavage IgE levels, and reduced airway hyperreactivity have been reported.[13-15] In a model of peanut allergy, mice infected with *H polygyrus* failed to develop an anti-peanut IgE response after immunization and did not experience anaphylaxis on challenge with peanut.[16]

Overall, these epidemiologic and animal studies support the hygiene hypothesis in spite of the limitations inherent to each approach.

TRICHURIS SUIS

When David Elliott, Robert Summers, and Joel Weinstock initiated the first clinical studies with eggs of *T suis* in the early 2000s, they reasoned that cases of inflammatory bowel diseases (IBDs) were concentrated in the Western world and rare in developing countries and that restoration of a less sanitized environment in the gut could be beneficial to patients with IBDs, as proposed by the hygiene hypothesis.[17] One major difference between individuals in Western countries and those in developing countries is the frequency of persistent parasitic infections. They reasoned that a parasite that would colonize the human gut without being invasive and causing symptoms could have therapeutic value in patients with IBDs. This is how they came up with the idea of using eggs of *T suis*.

Biology of *T suis*

T suis is a helminth of the nematode family.[18] When pigs, the preferred host, ingest embryonated eggs, larvae are released in the intestine and burrow in the superficial portion of the cecal and colonic mucosa. No systemic invasion occurs. The worms mature in the intestinal lumen. Eggs (ova) are produced and eliminated in the feces. Ova are not infective until they have incubated in the soil. *T suis* is frequently observed in nonindustrial farms but rare in intensive indoor production systems. In the pig *T suis* infection is mainly asymptomatic, except in piglets, in which it can cause diarrhea and prevent growth.[19]

Human subjects are not the natural host for *T suis*. Before the start of the use of *Trichuris suis* ova (TSO) as a therapeutic agent, only 1 report of human infection with *T suis* was published.[20] This report describes 2 volunteers who ingested 1000 or 5000 embryonated eggs. Both transiently passed eggs while remaining asymptomatic. Eggs were also found transiently in a systematic examination in the stool of a third subject, a laboratory technician who had handled TSO and was asymptomatic. Given the frequent presence of *T suis* in farms, the absence of report in the medical literature of cases of farmers found to be infected with *T suis* indicates that either *T suis* does not infect human subjects on farm exposure or that such an infection is asymptomatic.

Evidence of the safety of TSO

The first studies with TSO were performed in patients with IBDs. In the very first study 4 patients with active Crohn disease (CD) and 3 with ulcerative colitis (UC) were enrolled and received a single dose of 2500 TSO.[21] The next study enrolled 29 patients with CD who received 2500 eggs every 3 weeks for 24 weeks.[22] Finally, a randomized controlled trial was performed in 54 patients with severe UC (UC disease index ≥ 4) who received 2500 eggs or a placebo every other week for 12 weeks.[23] Most subjects were treated with various combinations of mesalamine, corticosteroid, azathioprine, and 6-mercaptopurine during the trial. In all 3 studies no adverse events (AEs) were reported. Some of the subjects underwent a colonoscopy during the trial. Rare helminths of variable size and maturity were seen. However, all systematic stool analyses performed during the studies were negative for ova and parasites in all the subjects. In a case report of a patient with CD resistant to thalidomide, azathioprine, and adalimumab, it is noted that the patient took 5 doses of TSO and that, sometime after this, a colonoscopy showed mild-to-severe lesions and the presence of helminthic forms below the ileocecal epithelium on a biopsy specimen. No result of stool analysis is reported, and the evolution is unknown.[24] Therefore it is not possible to assess the significance of the colonoscopy finding.

Evidence of safety is also available from a study performed in patients with multiple sclerosis (MS; Helminth-induced Immunomodulation Therapy study phase 1).[25] Five adults with newly diagnosed, treatment-naive, relapsing-remitting multiple sclerosis (RRMS) were treated with TSO, 2500 ova every other week, for a total of 6 doses over 3 months. No unexpected serious AEs were observed. Minor (grade 1) diarrhea or abdominal cramps that resolved spontaneously were noted in 3 subjects. Three subjects experienced transient grade 1 or lower eosinophilia that regressed while TSO therapy was continued. No worsening or relapse of MS was observed clinically or by using magnetic resonance imaging (MRI). One of the 26 stool samples collected for ova and parasite analysis during the entire study was positive with rare ova, and the subject from whom it came was asymptomatic. A control sample from the same subject was negative, and the subject was not treated with antiparasitic drugs. No subject dropped out or had to be removed from the study because of AEs.

This evidence of safety from the IBD studies and this MS study was considered sufficient for the US Food and Drug Administration to authorize the performance of a second open-label trial designed to provide proof of concept for the efficacy of TSO in patients with MS, which is currently underway at the University of Madison, Madison, Wisconsin.

The safety of TSO was further confirmed in a phase 2b double-blind, randomized, placebo-controlled trial in 100 adults with seasonal allergic rhinitis.[26-29] The subjects received placebo or TSO, 8 doses of 2500 eggs, at 3-week intervals. Mild gastrointestinal side effects were reported in 47% of TSO-treated subjects but also in 32% of placebo-treated subjects. Transient and mild eosinophilia was observed in the TSO-treated subjects. No subject dropped out or had to be removed from the study because of AEs.

Finally, we performed a study in 6 adults aged 26 to 59 years with peanut or tree nut allergy. Peanut or tree nut allergy was chosen because it is the most common food allergy in the United States and because it resolves spontaneously in only about 20% of cases.[30] The end point was safety. Severity, as defined by the criteria of Ewan and Clark,[31] was mild in 1 subject and moderate in 5 subjects. Five of the 6 subjects were allergic to multiple foods, with peanut being the main food allergen in 4 subjects and cashew or hazelnut being the main food allergen in 1 subject each. All the subjects had other allergic manifestations, mainly allergic rhinitis (5 subjects) but also asthma (3 subjects) or eczema (1 subject).

Five of the subjects were women. The subjects received a total of 8 doses of 2500 ova every other week or at longer intervals. The full dose of TSO (2500 eggs) was reached progressively over 2 or 3 visits in an attempt at avoiding gastrointestinal side effects. This protocol did not seem to make a difference because most subjects experienced mild, transient, and spontaneously resolving gastrointestinal side effects. All the subjects had mild, transient, and asymptomatic eosinophilia that started in some subjects after the first dose of TSO and in others after a few doses. All the stool samples collected at every visit for TSO dosing and 2 weeks after the last dose was administered were negative for ova and parasites. No subject dropped out or had to be removed from the study because of AEs (Marie-Helene Jouvin and Jean-Pierre Kinet, unpublished data; funded by the Food Allergy Initiative).

Evidence of the safety of TSO is now available for more than 200 subjects enrolled in clinical trials. Two types of side effects observed in the MS and allergic rhinitis studies and our food allergy study, but not in the IBD studies, are mild-to-moderate gastrointestinal side effects and mild eosinophilia. Various factors could explain why these side effects, which were expected given the nature of TSO, were not noted in the IBD studies.

First, gastrointestinal side effects could have been missed in patients with IBDs because these patients experience frequent and often severe gastrointestinal symptoms caused by their IBDs. Second, the sources of TSO were different. The TSO preparations used in the IBD studies were made in the Immunology and Disease Resistance Laboratory, US Department of Agriculture, Beltsville, Maryland, whereas the subsequent studies used TSO prepared by Ovamed under standards of Good Medical Practice. Even though the same number of eggs were used in all studies (2500), it is possible that the potencies (viability and stability) of the preparations from the 2 sources were different. Third, most of the patients with IBD were receiving immunosuppressants at the same time as TSO, which might have blunted the eosinophilia.

One concern about the use of TSO as a therapy in humans is the possibility that egg preparations could contain other infectious agents often present in pigs, which are pathogenic in humans, such as hepatitis E virus. The Ovamed TSO preparations used in all the recent clinical trials were systematically tested for multiple infectious agents according to procedures and processes approved by the FDA. This guarantees their microbiological safety.

Another element contributing to the overall safety of using TSO as a therapy is the availability of anthelmintic drugs, such as albendazole or mebendazole, which are active against *T suis* and are generally considered safe, including in children. Such drugs could be used if a subject receiving TSO were to experience a TSO-related AE considered severe enough to require specific treatment.

Overall, the evidence of safety of TSO is strong. The side effects have been absent or mild and spontaneously resolving. TSO has been administered to subjects at risk, such as patients with IBDs receiving immunosuppressants concomitantly with TSO, without any adverse effects. Also, it should be noted that given the frequent presence of *T suis* in farms and the long history of pig farming all over the world, the absence of report in the medical literature of cases of farmers found to be infected with *T suis* represents a substantial, although indirect, evidence of safety.

Evidence of the efficacy of TSO

IBDs. The first study to assess the efficacy of TSO enrolled 29 patients with active CD (defined as a CD activity index score > 220) who received 2500 eggs every 3 weeks for 24 weeks. Seventy percent of the subjects experienced a remission (defined as a CD activity index score < 150) at the end of the study. No follow-up was performed after the end of the treatment period.[22] A randomized controlled trial was performed in 54 patients with severe UC (defined as a UC disease index score ≥ 4) who received 2500 eggs or a placebo every other week for 12 weeks. After 12 weeks, 43% of the subjects in the TSO group versus 17% of the subjects in the placebo group improved their disease activity index score.[23]

Overall, there is substantial preliminary evidence of efficacy for TSO in patients with IBDs. This evidence has been the basis for 3 new studies in patients with IBDs. A phase I dose-escalation study is being performed to test the safety and tolerability of single doses of placebo or TSO (500, 2500, and 7500 eggs) in patients with CD (ClinicalTrials.gov identifier NCT01434693). A phase II randomized controlled trial enrolling 212 subjects with CD is underway in Europe (ClinicalTrials.gov identifier NCT01279577). Finally, a study designed to investigate the immune effects of TSO in subjects with UC is underway (ClinicalTrials.gov identifier NCT01433471).

MS. The only study completed in the United States at this time had safety as its primary end point. Five adults with newly diagnosed, treatment-naive RRMS were treated with TSO, 2500 ova every other week, for a total of 6 doses for 3 months. A transient decrease in the number of new gadolinium-enhanced magnetic resonance imaging (MRI)–detected lesions was observed, with the mean number of lesions decreasing from 6.6 at baseline to 2 at the end of the TSO period and increasing to 5.8 two months after the end of TSO administration.[25]

In another trial 10 subjects with already established MS received 2500 TSO every other week for 12 weeks and underwent MRI every 3 weeks. Two subjects experienced relapses with TSO. New MRI-detected lesions appeared during TSO in 8 subjects. Therefore no evidence of efficacy was observed in this population.[32]

The evidence of safety provided by the study in patients with newly diagnosed RRMS described above was considered sufficient by the US Food and Drug Administration to allow the same team to conduct an efficacy study in the same population (Helminth-induced Immunomodulation Therapy study phase 2; ClinicalTrials.gov identifier NCT00645749). Another trial is underway in patients with RRMS. Fifty subjects will be enrolled in a randomized controlled trial to receive 2500 TSO or placebo every other week for 12 months. Efficacy will be assessed based on the cumulative number of new T2 hyperintense lesions on cerebral MRI (ClinicalTrials.gov identifier NCT01413243).

Allergic rhinitis. A phase 2b double-blind, randomized, placebo-controlled trial in 100 adults with seasonal allergic rhinitis was performed in Denmark.[26-29] The subjects received placebo or TSO, 8 doses of 2500 eggs each, at 3-week intervals. There was no difference in daily symptom scores (runny, itchy, and sneezing nose) or in the percentage of well days during the grass pollen season between the TSO and control groups. There was a nonstatistically significant trend for TSO-treated subjects to report more well days. Subjects receiving TSO reported significantly fewer days when they took antihistamine tablets ($P = .04$). The design of the study could explain this apparent lack of clinical efficacy. Twenty-three percent of the subjects had received only 2 doses when the pollen season started. On the basis of experience from the IBD trials, 2 doses are insufficient to observe any clinical efficacy. When subjects having received 3 to 5 doses before the

start of the pollen season were analyzed separately, no benefit of TSO was apparent, which could have been caused by statistical issues. The dose and schedule of administration of TSO have not yet been optimized in any of the pathologies in which TSO has been used. Therefore it is possible that the regimen used in this study is not appropriate for allergic rhinitis.

Case study in autism. The story of a boy with autism has raised much interest among parents of autistic children or children with food allergy. This boy had severe autism characterized by self-abuse, agitation, aggression, anxiety, obsessive/compulsive behavior, behavioral rigidity, impulsivity, "stimming" behavior, and extreme sensitivity to external stimuli. He was also allergic to pecan nuts and presented with seasonal allergic rhinitis. At the age of 15 years, he was started on TSO at a dose of 1000 ova every 3 weeks for 26 weeks. No clear effect was observed. The dose was increased to 2500 ova every 2 weeks. After 10 weeks at the higher dose, most of his autism symptoms had improved substantially. His seasonal and food allergies were gone, and he was able to eat pecan cookies without having any reaction. After 2 years, the dose of TSO was reduced to 1600 ova every 2 weeks, and the autism symptoms reappeared. The dose was increased back to 2500 ova every 2 weeks, and the autism symptoms improved again. He is now 21 years old. He has been on TSO continuously since 2008 and has not experienced any AEs. He is free of repetitive and disruptive behavior and cured of his allergies (http://autismtso.com/about/the_story/).

Peanut/tree nut allergy. In our study in adults with peanut or tree nut allergy, the only end point was safety. We assessed some standard allergy markers without knowing whether they would be predictors of TSO efficacy. We observed no significant change in allergen-specific serum IgE levels. There was no change in skin prick test reactivity, except in 1 subject who had a general decrease in reactivity and lost reactivity to peanut, which was the clinically dominant allergen for this subject. Four subjects reported a decrease in seasonal allergies while receiving TSO (Marie-Helene Jouvin and Jean-Pierre Kinet, unpublished data).

Two conclusions can be drawn from the studies performed with TSO. One is that TSO is safe, and the other is that the efficacy of TSO should be tested in diseases in which there is an unmet need, such as food allergy and autism, or when available therapies have significant side effects, such as IBDs.

Helminths other than *T suis*

***N americanus* (hookworm).** Hookworm has been tested in a few trials in healthy subjects, asthmatic patients, and patients with celiac disease with no clear clinical efficacy.[33-35] *N Americanus* as administered in these trials (staged cutaneous inoculations with a few infective third-stage larvae) appears safe. These trials were performed in the United Kingdom and Australia. Given that *N americanus* is a known human pathogen, clinical trials would likely face serious regulatory obstacles in the United States.

***Trichuris trichiura*.** A case report demonstrates the benefits of *Trichuris trichiura* and provides some information on their mechanisms.[36] One subject with severe and drug-resistant UC who chose to ingest eggs of *T trichiura*, was symptom free for 3 years without standard treatment, apart from 1 brief flare that responded to standard treatment. For a severe flare 3 years after the first *T trichiura* ingestion, he ingested *T trichiura* again, which made him symptom free. Clinical improvement correlated with improvement of the lesions in the colon assessed by means of colonoscopy. Remission was associated with a decrease in IL-17^+ T cells and the appearance of IL-22^+ T cells in the colonic mucosa. This case represents a clear demonstration of the benefit of helminths. However, as with *N americanus*, with *T trichiura* being a known human pathogen, clinical trials would likely face serious regulatory obstacles in the United States.

MECHANISMS RESPONSIBLE FOR THE PROTECTIVE ROLE OF HELMINTHS

The main approach to dissecting the mode of action of helminths has been to identify helminthic substances with immunomodulatory properties. Worms that had been shown to be effective in human subjects or in murine models were obtained, and extracts were made or excreted/secreted products were isolated from worm cultures.[37-39] More recently, the cloning of the genomes of some helminths has allowed a systematic search for substances with potential immunomodulatory activities (http://www.sanger.ac.uk/resources/downloads/helminths/trichuris-muris.html).[40]

ES-62

The most studied of the helminth products is ES-62. ES-62 is a 62-kDa phosphorylcholine (PC)–containing glycoprotein secreted by the filarial nematode *Acanthocheilonema viteae*.[41,42] ES-62 possesses many immunomodulatory activities that appear to be due mainly to its PC moiety. It inhibits B-cell activation and the signals that result from it (B-cell proliferation and cytokine secretion) by targeting various components of the signaling pathways downstream of the B-cell receptor. It skews the antigen-presenting cell (dendritic cells [DC] and macrophages) response toward hyporesponsiveness. ES-62 affects T-cell differentiation indirectly through its effect on antigen-presenting cells. However, it does not prevent T_H1 responses to BCG vaccine.[43] This suggests that ES-62 acts on T_H17 responses rather than T_H1 responses. It does not affect regulatory T (Treg) cells. ES-62 activity is dependent on the presence of Toll-like receptor 4 at the surface of the target cells. ES-62 also binds to poorly characterized molecular species on lymphocytes and macrophages.[44]

The inhibitory effect of ES-62 on mast cells and its relevance to allergy are especially clear. *In vitro* ES-62 inhibits mast cell activation induced by the high-affinity IgE receptor FcεRI, a critical receptor in patients with IgE-mediated allergy, by causing the sequestration and subsequent degradation of protein kinase Cα, thus decreasing sphingosine kinase mediated calcium signaling and blocking nuclear factor κB activation.[45] This effect translates *in vivo* into protection in an asthma model. Mice sensitized and challenged with ovalbumin and treated with ES-62 had no lung mast cell degranulation, no bronchial hyperresponsiveness to methacholine, and no inflammatory cell infiltrates in the lung in contrast to untreated mice. ES-62–treated mice were also protected in a cutaneous model of immediate-type hypersensitivity induced by oxazolone. It is likely that the effect of ES-62 on other target cells, such as DCs, also contributes to the protection observed after ES-62 treatment in these allergy models.

Other parasites, such as *Ascaris suum*, have been found to produce PC-containing immunomodulatory compounds that might act similarly.[41]

H polygyrus products

In worms with antiallergic activities, such as *H polygyrus*, multiple immunomodulatory activities have been demonstrated

in unpurified excretory/secretory (ES) products.[46] Among these activities is the induction of *de novo* forkhead box protein 3–positive Treg cells that can suppress anti-CD3–induced proliferation of $CD4^+$ naive T cells.[47] This activity is due to the presence of a TGF-β–like but antigenically different substance acting specifically on T cells. ES product–induced Treg cells are able to inhibit airway inflammation when injected into mice in which an allergic asthma model has been induced. In mice immunized orally with ova and infected with *H polygyrus* ova–specific forkhead box protein 3–positive Treg cells are detected in the gut-associated lymphoid tissue at a much higher frequency than in uninfected mice. It is likely that *H polygyrus* ES products have other immunomodulatory activities. For example, $CD4^-CD19^+CD23^{high}$ B cells isolated from mesenteric lymph nodes of *H polygyrus*–infected mice when injected into mice, can confer protection in an allergic asthma model.[48]

T suis products

In *T suis* serine protease inhibitors have been identified that inhibit important proteases in mast cell function, such as murine mast cell protease 1.[49] Other unpurified and unidentified soluble products extracted from *T suis* inhibit activation of human monocyte–derived immature DCs by LPS or polyinosine-polycytidylic acid, resulting in lower secretion of TNF-α and IL-12.[50] When *T suis* product–primed DCs are stimulated with LPS or polyinosine-polycytidylic acid, washed to remove *T suis* products, and then incubated with naive T cells, T-cell skewing is shifted away from a T_H1 phenotype toward a T_H2 phenotype. In addition, memory T cells induced by *T suis* product–primed DCs in T_H17 phenotype–inducing conditions do not produce T_H17A. These same soluble products protect mice against induction of experimental autoimmune encephalomyelitis, a classical model of MS.[50] Whether these *T suis* extracts could confer protection against an allergy model has not been reported.

Analysis of the *T suis* transcriptome has led to the identification of more than a hundred putative proteins containing conserved domains or signatures relevant to inflammation and immunity, such as proteases, protease inhibitors, kinases (serine/threonine kinase and calcium-calmodulin–dependent kinase), calcium channels, G protein–coupled receptors, and inositol triphosphate/ryanodine receptor. Whether these proteins are synthesized and what their activites are remain to be investigated.[40]

WHAT NOW?

The studies performed thus far have (1) demonstrated the safety and provided some evidence of efficacy for TSO and (2) shed some light on the mechanisms used by parasites to exert immunomodulatory effects.

In terms of clinical trials

Given the demonstrated safety of TSO, additional clinical trials should be performed with TSO in patients with allergic diseases.

Food allergies are an attractive indication. There is a clear need for therapeutic approaches that are not allergen specific because most patients are polysensitized. *T suis*, through its interactions with the gut epithelium and immune system, could affect mechanisms in the gut that are thought to contribute to or protect against food allergy.[2,51,52] Another indication worth exploring is eosinophilic esophagitis, in which the unmet need is clear.

TSO has been shown to be efficient in diseases of the digestive track (IBDs). Whether this indicates that the effects of TSO are limited to or predominant in the digestive immune system remains to be investigated.

The doses, schedules of administration, and duration of treatment used thus far with TSO have been entirely empiric. They should be optimized. A trial is underway comparing 3 doses (including 7500 eggs) in patients with CD (NCT01279577), which should provide valuable information.

Clinical trials should be performed in children. This is obvious for food allergies because the effect of the disease is most severe in children. In addition, it is likely that TSO will be more efficient in young children with a maturing immune system than in adults.

In terms of basic research

In parallel with clinical trials, more basic research should be initiated to identify the mechanisms of the effects of TSO. A clinical trial is underway in patients with UC in which subjects receive TSO or a placebo in a crossover design and undergo colonoscopies with immunologic and transcriptome analyses of colon biopsy specimens (NCT01433471). This study should provide valuable information on mucosal immunity in subjects receiving TSO. For example, TSO might promote the development of Treg cells that are associated with recovery from food allergy[53] or affect DC maturation, which is considered a critical control point in T_H2 responses and tolerance.[54] Systematic investigations should be conducted to assess whether *T suis* or its products affect mechanisms in the gut that are thought to contribute to or protect against food allergy.[2,51,52] In addition, it is likely that TSO modifies the gut microbiota. This would represent an attractive mechanism given the evidence that reduced diversity of the gut microbiota in infancy is associated with an increased risk of allergies in later childhood.[2,6]

T suis products could be obtained from cultures or identified by mining its genome and then tested *in vitro* and in animal models of allergic diseases. However, it should be noted that, given the safety record and ease of conservation and administration of TSO, identifying active substances in *T suis* that could be used as a classical drug is not required to further the development of TSO as a possible treatment.

H polygyrus also appears to be a possible source of immunomodulatory drugs and should be investigated further.

Biomarkers should be developed. Eosinophilia is a classical marker of helminth infection. It was observed in many of the subjects in the TSO trials. However, the absence of correlation between eosinophilia and clinical efficacy observed in the allergic rhinitis study indicates that eosinophilia cannot be used as a surrogate marker of efficacy. Therefore surrogate markers of clinical efficacy need to be developed. Some of these markers should be selected by using the future results of the UC trial mentioned above, which might point toward mechanisms that could be investigated noninvasively. In parallel, a systematic exploration of the immune system before, during, and after treatment with TSO should be deployed that should combine (1) cytometry by time-of-flight to analyze extensively the cell-surface markers, cytokines, and antigen specificity of inflammatory and immune cells from the blood and nasal cavities (in subjects with allergic rhinitis) on resting cells and after *in vitro* stimulation[55,56] and (2) analysis of cytokines, chemokines, and growth factors in serum and nasal secretions by means of multiplex analysis.[57]

It is clear that microbiota of the epithelia and mucosa are implicated in maintaining health and that their alterations are present in numerous diseases. This is especially true in patients with allergic diseases in whom cohort studies have shown that the gut microbiota profile of children at 1 and 12 months of age was indicative of the risk of having allergies at age 6 years.[6] The microbiota and microbiome in the guts and mouths of allergic subjects before, during, and after treatment with TSO should be studied. They could represent a source of valuable biomarkers that can be obtained noninvasively.

What do we know?

- **Ova (eggs) of the parasite *Trichuris suis* (TSO) are safe.**
- **Preliminary evidence of the efficacy of TSO in patients with IBDs has been obtained.**
- **Related parasites and substances produced by these parasites have substantial effects on various components of the immune system (mast cells, Treg cells, and DCs) *in vitro* and in animal models.**

What is still unknown?

- **What are the optimal doses, frequency of administration, and duration of treatment with TSO?**
- **Among allergic diseases, what are the best indications for TSO?**
- **What are the effects of TSO on the immune system at the molecular and cellular levels?**
- **Should active substances produced by worms be used instead of the eggs?**
- **What biomarkers can be used to predict which patient will respond to TSO and to monitor treatment?**

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