Trichuris suis ova therapy for allergic rhinitis: A randomized, double-blind, placebo-controlled clinical trial

Peter Bager, PhD,[a] John Arnved, MD,[b] Steen Rønborg, PhD,[b] Jan Wohlfahrt, PhD,[a] Lars K. Poulsen, PhD,[c] Tine Westergaard, PhD,[a] Henning Willads Petersen, PharmD,[d] Bjarne Kristensen, MSc,[g] Stig Thamsborg, PhD,[e] Allan Roepstorff, PhD,[e] Christian Kapel, PhD,[f] and Mads Melbye, DMSc[a] *Copenhagen, Frederiksberg, and Allerød, Denmark*

Background: Parasitic helminth infections can protect against allergic airway inflammation in experimental models and have been associated with a reduced risk of atopy and a reduced course of asthma in some observational studies. Although no clinical evidence exists to support the use of helminth therapy for allergic disease, the helminth *Trichuris suis* has demonstrated efficacy in treatment of inflammatory bowel disease.

Objective: To determine efficacy of helminth therapy for allergic rhinitis.

Methods: We conducted a double-blind, placebo-controlled, parallel group trial in which 100 subjects age 18 to 65 years with grass pollen–induced allergic rhinitis were randomly assigned to ingest a total of 8 doses with 2500 live *T suis* ova or placebo with an interval of 21 days. The primary outcome was a change in mean daily total symptom score for runny, itchy, sneezing nose (maximum change, 9.0) or in percentage of well days during the grass pollen season.

Results: Treatment with *T suis* ova (N = 49) compared with placebo (N = 47) caused transient diarrhea peaking at day 41 in 33% of participants (placebo, 2%), and increased eosinophil counts ($P < .001$) and *T suis*–specific IgE ($P < .05$), IgG ($P < .001$), IgG_4 ($P < .003$), and IgA ($P < .001$), whereas there was no significant change in symptom scores (0.0; 95% CI, –0.5 to 0.4; $P = .87$), well days (3%; 95% CI, –9% to 14%; $P = .63$), total histamine ($P = .44$), grass-specific IgE ($P = .76$), or diameter of wheal reaction on skin prick testing with grass ($P = .85$) or 9 other allergens.

Conclusion: Repeated treatment with the helminth *T suis* induced a substantial clinical and immunologic response as evidence of infection, but had no therapeutic effect on allergic rhinitis. (J Allergy Clin Immunol 2010;125:123-30.)

Key words: *Allergic rhinitis, Trichuris suis, randomized clinical trial, parasite, helminth, allergy, IgE, skin prick test, total histamine, eosinophils*

The allergy epidemic in affluent countries remains largely unexplained.[1,2] So far, no effective cure for allergy has been identified, and available therapies have side effects.[3] A new therapeutic approach has been suggested in different experimental models of allergic disease showing that parasitic helminths can protect against allergic reactivity by helminth-induced regulatory T cells and cytokines.[4-20]

Unlike allergy, parasite infections are common in low-income countries and are often asymptomatic for years.[21] An intriguing hypothesis is that the immune system may have evolved to silence parasite infections, and has gone awry in their absence in affluent countries.[22-25] In line with this hypothesis, allergic diseases are T_H2 cytokine–mediated pathologies, whereas infections with helminths exhibit modified T_H2 cytokine responses in which the pathology is suppressed, possibly by regulatory T cells and the anti-inflammatory cytokines IL-10 and TGF-ß.[26] The hypothesis has gained support from cross-sectional studies showing reduced allergen skin reactivity associated with very different helminths such as *Trichuris trichiura*,[27-29] *Schistosoma* spp, hookworm, and *Ascaris lumbricoides* infections.[30,31] However, confounding by other exposures remains a possible bias in many of these studies.[31] To address such possibilities, the effect of parasite control on prevalence of allergic conditions in helminth-infected populations has been investigated. A study of antihelminthic therapy in 375 Venezuelan children,[32] a single-blind antihelminthic trial in 317 children in Gabon,[33] and an individually randomized placebo-controlled antihelminthic trial among 1566 Vietnamese school children[31,34] all reported significant increases in allergen skin sensitization after antihelminthic therapy. However, no effect on atopy was shown after school-randomized antihelminthic treatment in 2372 Ecuadorian children.[35] An overall conclusion from these studies is complicated by effects of substances from killed helminths, coinfections with other parasites, the initial duration of infection, and variable worm burdens. However, in studies including only subjects with asthma, schistosomiasis-infected subjects

From [a]the Statens Serum Institut, Department of Epidemiology Research, Copenhagen [b]the Pulmonology and Allergy Clinic of Copenhagen; [c]the Allergy Clinic, National University Hospital, Copenhagen; [d]Pharmacy Services, [e]the Department of Veterinary Disease Biology, and [f]the Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg; and [g]Phadia ApS, Allerød.

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Reprint requests: Peter Bager, PhD, Statens Serum Institut, Department of Epidemiology Research, Artillerivej 5, DK-2300 Copenhagen C, Denmark. E-mail: pbg@ssi.dk.

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Abbreviations used
ES: Excretory-secretory
mgA/L: Milligrams *Trichuris suis*–specific antibodies per liter serum
NO: Nitric oxide
SPT: Skin prick test
TSO: *Trichuris suis* ova

were shown to have a reduced course of asthma and skin reactivity to house dust mites associated with IL-10 production.[36-38]

In the 1990s, 3 clinical studies showed that ingestion of 2500 live ova from *Trichuris suis*, an intestinal helminth of pigs, was effective therapy for both the T_H1-mediated Crohn's disease and the T_H2-mediated disease ulcerative colitis.[39-41] The authors later showed in rodent models of colitis that helminth-induced regulatory T cells and cytokines could explain this therapeutic effect.[42-44] The mechanism of action is thus compatible with findings in helminth experiments using models of allergic airway disease.[4-6,8-10,12-16,19,20,45] In addition, patients with allergy undergoing effective allergen-specific immune therapy also exhibit regulatory T-cell proliferation and increased IL-10 levels.[46-48]

To assess the prospects of helminth therapy for allergies in human beings, we initiated a clinical trial of *T suis* ova (TSO) in the most common allergic disease in affluent countries: grass pollen–induced allergic rhinitis. Our primary objective was to test a claim for efficacy of TSO defined as an overall statistically significant reduction in total daily score for runny, itchy, sneezing nose, or in the percentage of well days during the grass season. To support this conventional self-reported outcome,[49] we performed measurements of allergen skin reactivity, grass-specific antibodies, basophil and eosinophil cells, and eosinophil inflammation of airways.

METHODS

Subjects

The study was performed in accordance with the Declaration of Helsinki[50] and Good Clinical Practice (GCP) and was approved by an independent review board of the Danish Ethics Committees (no. H-KF-2006-4100). Written informed consent was obtained before enrollment.

Volunteers were recruited from the capital area of Denmark (study area) through advertisement in public media. During 5 months before Denmark's grass pollen season in the year 2008, one hundred sixty-two volunteers were recruited and screened for eligibility (January 14 to April 30), and 100 subjects enrolled (March 10 to May 15) at the Pulmonology and Allergy Clinic of Copenhagen. Subjects were men, or women not of childbearing potential, age between 18 and 65 years, who had symptoms of grass pollen–induced allergic rhinitis in the last 2 pollen seasons or more, a wheal diameter $\geq$3 mm on skin prick testing with grass allergen, a specific IgE level against grass allergen $\geq$0.7 kilo units antigen per liter (kUA/L), a spirometric FEV_1 $\geq$70% of predicted, and no significant asthma. The detailed eligibility criteria are shown in this article's Table E1 in the Online Repository at www.jacionline.org.

Study design

The study was a parallel-group, double-blind, placebo-controlled, single-center trial. The sponsor electronically randomized subjects 1 to 1 in blocks of 10 to receive 8 treatments of placebo or TSO with an interval of 21 days. The trial consisted of 9 visits including 8 treatment visits, of which 3 were sampling visits, and 1 final visit 21 days after last treatment. The follow-up period ran from March 10 to October 30, 2008.

Intervention. The active agent (TSO) was isolated from *T suis*–inoculated barrier-bred Göttingen minipigs (miniature pigs) by Parasite Technologies A/S, Copenhagen, Denmark, and processed to vials by Ovamed GmbH, Barsbüttel, Germany, according to Good Manufacturing Practice (GMP) for phase II trials. Vials with TSO and placebo were delivered in separate boxes to the Pharmacy Services, Copenhagen, Denmark, who allocated subject numbers by labeling vials according to a packing list received from the sponsor. The vials were then transported to the trial clinic.

T suis ova was supplied as a nonsterile aqueous suspension containing 2500 viable, purified, embryonated TSO in 15 mL sulfate-stabilized 0.05 mol/L H_2SO_4 (pH 1.0) contained in 60 mL vials made of dark-violet hydrolytic resistant (DIN ISO 719) type III glass and sealed with black screw-caps with polyethene gasket (Rosa Heinz GmbH, Fahrenzhausen-Grossnöbach, Germany). Placebo was identically supplied and formulated except that it contained no TSO. The shelf-life of TSO at 2 to 8 °C was tested and proven for 6 months. Monthly shelf-life testing started 2 months before the first treatment, and by using 5 timely supplies from the trial batch in Germany, all treatments were done before expiration. The vial storage temperature was logged daily and kept at 2 to 8 °C during the entire trial. The infectivity of the trial batch was tested in 5 helminth-free minipigs each inoculated orally with 2000 TSO. At necropsy 21 days later, 49% of the inoculated eggs were recovered as larvae from the pig intestines, thus demonstrating a high infectivity of TSO.

Administration was performed by neutralizing the sulfuric acid with 400 mg ($\pm$50) $NaHCO_3$ powder (batch 102713; Statens Serum Institut, Copenhagen, Denmark), and by drinking directly from the vial. The taste, smell, and appearance of TSO and placebo were similar. Administration was performed either by 2 trial nurses on visits or as scheduled home administration allowed after the first treatment if the planned visit was not one of the sampling visits. For home administration, subjects received a 330-mL thermal steel mug with 6 hours temperature isolation which contained 1 vial, $NaHCO_3$ powder, a leaflet, and authorization letters for travels abroad.

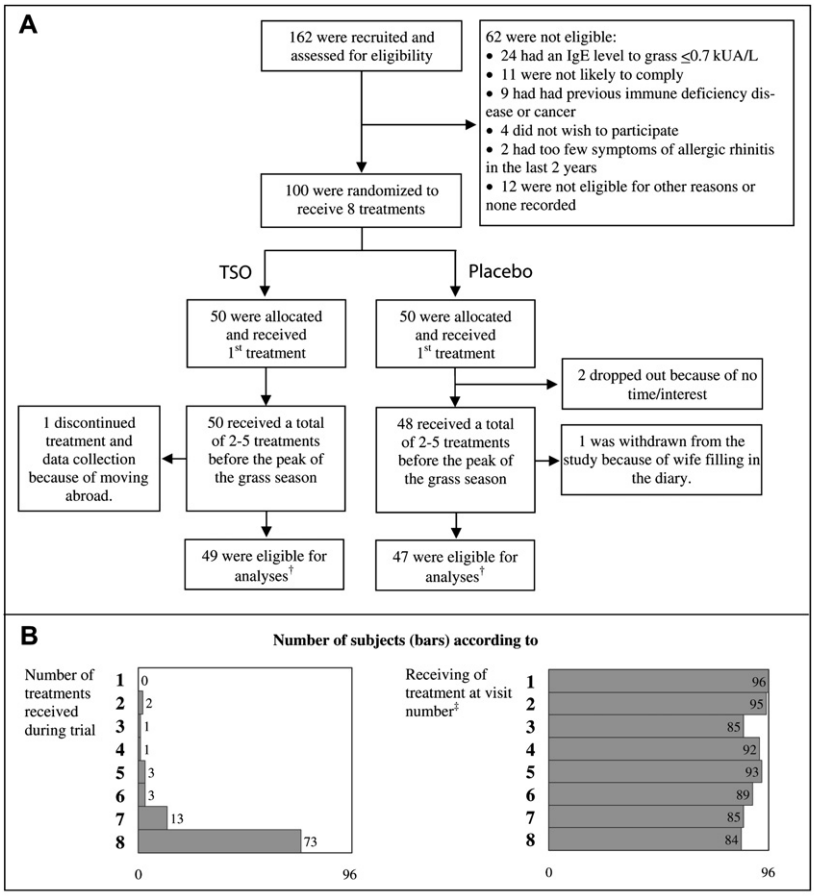
Data collection by subjects and safety monitoring. Subjects received a patient diary and a peak flow meter (Piko-1; nSpire Health Inc, Longmont, Colo). Safety was monitored on visits or via telephone and included daily records of FEV_1, asthma, diarrhea, flatulence, pruritus ani, and spontaneous reports of adverse events coded with MedDRA version 2.02 International Federation of Pharmaceutical Manufacturers and Associations, Geneva, Switzerland.

Definition of grass pollen season. Counts of grass pollen in Copenhagen were obtained from the Danish Asthma-Allergy Society. The start date of the grass season in 2008 was defined as first day of 3 consecutive days with counts of $\geq$10 pollen/m^3 (May 28).[51] The final date of the grass season was defined as the first day (after the date of the peak count, June 9) followed by 3 consecutive days with counts of <10 pollen/m^3 (July 27).[51]

Sampling plan. The 3 sampling visits included skin prick testing, measurement of exhaled nitric oxide (NO), and/or drawing of blood. The visits were scheduled at enrollment (skin prick test [SPT], NO, blood samples), between the peak and final date of the grass season (NO, blood samples), and at the final visit after the grass season (SPT, NO, blood samples). The blood drawn was analyzed within 24 hours for hematology and total histamine, whereas plasma was frozen for later antibody analyses.

Symptom scores and medication for allergic rhinitis. Each day, subjects scored their symptoms of allergic rhinitis on a scale from 0 to 3 (0, no symptoms; 1, mild; 2, moderate; 3, severe). The allergic rhinitis symptoms scored were runny nose, itchy nose, sneezing nose, blocked nose, red/itchy eyes, and watery eyes. Well days of allergic rhinitis were defined as days with a total symptom score no larger than 2 and no use of rescue medication against allergic rhinitis.[52]

In case of allergic symptoms, the subjects had free access to rescue medication in a stepwise fashion depending on the persistence and severity of symptoms. Rescue medication was scored according to predetermined criteria in this article's Table E2 in the Online Repository at www.jacionline.org.[51,52] The scoring scale was not seen by subjects. Subjects' assessment of overall improvement of pollen allergy symptoms compared with last year's season was dichotomized as improved (much better, better) or not improved (the same, worse, much worse).



* The treatment interval was 21 days (±3).
† Subjects were eligible for intention to treat analyses if their diary of symptom scores and medication usage for allergic rhinitis was completed for >50% of days during the grass pollen season, and they had a result on at least 2 subclinical measures – 1 from enrollment and 1 from another of the 2 sampling visit.
‡ Because of adverse events (gastrointestinal disorders) subsequently judged to be related to treatment, 4 subjects on TSO and 0 subjects on placebo discontinued treatment, and 8 subjects on TSO and 1 subject on placebo paused treatment number 3. Because of reasons unrelated to treatment, 4 subjects on TSO and 3 subjects on placebo discontinued treatment, and 2 subjects on TSO and 1 subject on placebo paused 1 treatment.

FIG 1. CONSORT study flow diagram **(A)** and treatment compliance* **(B)** for 100 subjects with grass pollen–induced allergic rhinitis in a clinical trial of TSO, Denmark, 2008.

Skin prick testing. Skin prick testing was performed according to the European guidelines (European Academy of Allergy and Clinical Immunology) using 1-mm lancets (ALK-Abelló A/S, Hørsholm, Denmark). All tests were performed in duplicate by 2 trained trial nurses to secure reproducibility. Soluprick SPT (ALK-Abelló) solutions with the following allergens were used: birch, grass, mugwort, horse, dog, cat, *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, *Alternaria alternata*, and *Cladosporium*; positive control, histamine chloride; negative control, isotonic saline. Skin wheal reactions were recorded after 10 minutes, marked with a roller tip pen, and an imprint transferred by Magic Tape (3M Corp, St Paul, Minn) to the record sheet. The largest wheal in each doublet was measured independently by 2 assistants using a special SPT wheal size ruler (ALK-Abelló). The mean difference between the 2 assistants' measurement was 0.3 mm (Q1, 0.0; Q3, 0.0).

Serum antibody titers. Serum grass-specific IgE and *T suis*-specific IgE, IgG, IgG_4, and IgA were measured by ImmunoCAP (ISO 13485; Phadia AB, Uppsala, Sweden). *T suis* excretory-secretory (ES) products were isolated *in vitro* from adult worms incubated in RPMI 1640 with 1% glucose, penicillin (500 U/mL), streptomycin (500 μg/mL), and Amphotericin B (1.25 μg/mL) for 24 hours at 38 °C in 5% CO_2. After centrifugation (50*g*, 5 minutes), the supernatant containing the ES products was filtered through a 0.45-μm membrane and stored at –80 °C. For ImmunoCAP, the ES products were concentrated on a 10,000 D membrane ultra filter Cat no. PBGC06210; Millipore Corporation, Billerica, Mass under pressure (60 psi).[53] In 14 nonatopic healthy donors, the normal *T suis* titers were 5.062 mg *T suis*–specific antibodies per liter serum (mgA/L; SD 2.059) for IgG and 0.0623 mgA/L (SD 0.0753) for IgG_4 (Phadia AB).

Total histamine. Total histamine was measured as a proxy for the number of basophils in blood. The total content of histamine in cells was determined by cell lysis with perchloric acid (7% $HClO_4$), and the amount of histamine was measured spectrofluorometrically according to the glass microfiber method (RefLab ApS, Copenhagen, Denmark).[54]

Hematology. Hematology analyses included hemoglobin and differential counts of leucocytes and erythrocytes performed by the Copenhagen G. P. Laboratory, Copenhagen, Denmark (ISO 17025).

Exhaled NO. Exhaled NO was measured as a proxy for eosinophil inflammation in the airways. Measurements were performed at a visually controlled flow level range of 10 to 330 mL/s by using an Aerocrine NIOX Flex FlexFlow (CE 0413, FDA KO21133; IntraMedic A/S, Copenhagen, Denmark).

Blinding

Treatment assignment was blinded to all personnel at the trial clinic, subjects, and data management personnel for the duration of the trial. The statistical protocol was finalized before unblinding. Antibody results were received on the day of unblinding and execution of analyses.

TABLE I. Subject characteristics by treatment group in 100 subjects with grass pollen–induced allergic rhinitis in a randomized clinical trial of TSO, Denmark, 2008

	TSO N = 50	Placebo N = 50
Sex, n (%)		
Male	48 (96)	47 (94)
Female	2 (4)	3 (6)
Age (y)		
Mean (SD)	35 (10)	39 (10)
Male	34 (9)	38 (10)
Female	60 (3)	51 (13)
Minimum-maximum	20-61	19-63
Caucasian, n (%)	50 (100)	50 (100)
Symptoms of allergic rhinitis,* n (%)		
Persistent	49 (98)	48 (96)
Intermittent	1 (2)	2 (4)
Severity of allergic rhinitis,* n (%)		
Moderate-severe	47 (94)	41 (82)
Mild	3 (6)	9 (18)
Years with significant grass pollen–induced allergic rhinitis the last 4 y,† n (%)		
4 y	46 (92)	48 (96)
1-3 y	4 (8)	2 (4)
Duration of allergic rhinitis,‡ y (SD)	20 (11)	24 (12)
Pets ever in household, n (%)	28 (56)	33 (66)

*Allergic rhinitis were classified according to the Allergic Rhinitis and its Impact on Asthma workshop report[62] by using a standard screening interview at enrollment about symptoms in the past. Symptoms were classified as intermittent (present less than 4 days a week or for less than 4 weeks) or persistent (present more than 4 days a week, and for more than 4 weeks), and severity was classified as moderate-severe if 1 of the following items were present during symptom periods, and as mild if they were absent: sleep disturbance; impairment of daily activities, leisure, and/or sport; impairment of school or work; or troublesome symptoms.

†Subjects were asked during screening where they would score the severity of their grass pollen symptoms in the last 1, 2, 3, and 4 years before the trial by indicating a point on a continuous visual analog scale ranging from not bothering (0.0 cm) to worst thinkable (10.0 cm). Significant symptoms were defined as a score ≥5 cm.

‡Subjects were asked at enrollment which year their symptoms of rhinoconjunctivitis started.

Statistical methods

Differences in outcome between the TSO and placebo group were estimated and evaluated by *t* tests for continuous variables and by logistic regression for binary variables. For symptom and medication scores and well days, the mean during the grass season (May 28 to July 27, 2008) was the outcome used in the analyses. Missing values were disregarded. As sensitivity analyses, the *t* tests were supplemented by nonparametric Wilcoxon tests. Estimations were performed by PROC GENMOD and PROCNPAR1WAY in SAS (version 9.1.3; Cary, NC), and all tests were 2-sided using a significance level of 5%.

RESULTS

A total of 100 subjects were randomized and completed the first treatment. In total, 98 subjects were followed up, and 96 subjects received 2 to 5 treatments (median, 3) before the observed peak of the grass season. The study flow is presented in Fig 1, *A*, and treatment compliance in Fig 1, *B*. In total, 98% of treatments and 100% of sampling visits were performed as scheduled. Subjects were located within the study area for, on average, 87% (Q1, 82%; Q3, 100%) of days in the grass season. Overall, subject characteristics were similar between the treatment groups (Table I).

Adverse clinical response to *T suis*

Treatment-emergent adverse events occurred in 83 (86%) of the 96 subjects, and the most frequent events were gastrointestinal disorders (228/514 events; 44%). Significantly more subjects in the TSO group experienced gastrointestinal disorders (76% vs 49% for placebo; $P = .007$). The most frequent type experienced was diarrhea (47% vs 32% for placebo; $P = .13$), whereas only upper abdominal pain (37% vs 4% for placebo; $P < .001$) and flatulence (43% vs 17% for placebo; $P = .005$) occurred in significantly more subjects in the TSO group. The percentage of subjects with diarrhea in the TSO group peaked at 33% on day 41 (placebo, 2%; Fig 2). No subjects were hospitalized due to gastrointestinal disorders caused by TSO.

Immunologic response to *T suis*

The immunological response to *T suis* is presented in Fig 3. Subjects treated with TSO had a significantly larger increase in counts of eosinophil cells from enrollment to grass season ($P < .001$) and from enrollment to the final visit after the last treatment ($P < .001$) compared with subjects on placebo. Furthermore, the increase in *T suis*–specific antibody titers was significantly higher in the grass season (IgG, $P < .001$; IgG_4, $P = .002$; IgE, $P = .049$; IgA, $P < .001$) and after the last treatment (IgG, $P < .001$; IgG_4, $P < .001$; IgE, $P = .001$; IgA, $P < .001$) compared with titers in the placebo group. The percentages of subjects on TSO and placebo who had *T suis*–IgG or *T suis*–IgG_4 titers above normal values were 86% versus 13% during the grass season and 92% versus 13% after the last treatment.

Efficacy

Table II presents symptom and medication scores and well days for allergic rhinitis. A higher score indicates a higher level of symptoms or use of medication for allergic rhinitis. Subjects treated with TSO and placebo had similar symptom scores ($P = .63$), percentage of well days ($P = .88$), and medication scores ($P = .11$). Days with use of nasal spray ($P = .63$) and eye drops ($P = .74$) were equally frequent in the treatment groups; however, subjects treated with TSO had fewer days with tablet usage compared with subjects on placebo ($P = .04$). The percentage of subjects assessing an overall improvement of pollen allergy symptoms compared with last year's season was similar in the treatment groups ($P = .86$).

Table III presents subclinical measures of allergic reactivity. There was no difference between treatment groups in the mean number of skin wheals with diameter 0 mm on prick testing with 9 allergens excluding grass ($P = .85$) when comparing skin prick testing after the last treatment and at enrollment. Neither was there any difference in the change since enrollment in diameter of wheal in reaction to grass allergen after the last treatment ($P = .85$) nor in titers of grass-IgE during the grass season ($P = .40$) and after the last treatment ($P = .76$). The change in amount of total histamine in blood and of exhaled NO was not different between the treatment groups at any sampling point during the trial. Among all subjects, the mean value of grass-IgE, total histamine, and NO increased from enrollment to grass season ($P < .0001$, $P = .007$, $P < .0001$, respectively), and a similar increase was observed separately in the placebo group, as well as in the TSO group.

DISCUSSION

Repeated treatment of human beings with ova from the pig parasite *T suis* induced a substantial clinical and systemic

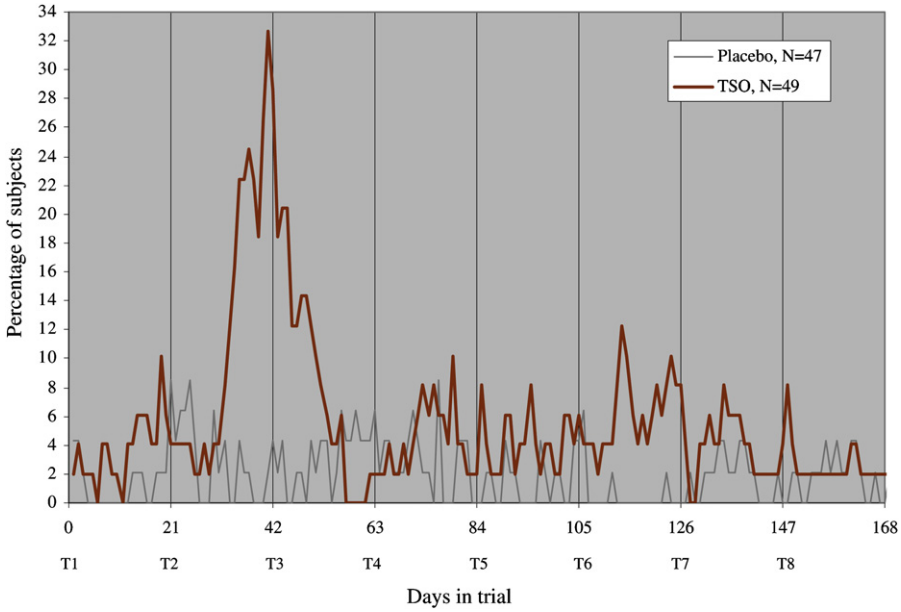


FIG 2. Percentage of subjects who reported treatment-emergent diarrhea by number of days enrolled in a randomized clinical trial of TSO, Denmark, 2008. *Tx,* Treatment no. x.

immunologic response as evidence of infection. However, the infection had no therapeutic effect on allergic rhinitis.

The clinical response to TSO consisted mainly of diarrhea. It began approximately 30 days after first exposure to TSO and peaked in incidence at day 41. The diarrhea in the TSO group likely reflects the result of superficial damage to the intestinal mucosa by *T suis* larvae after hatching.[55,56] Mucosal damage from adult *T suis* worms or persistent infection is unlikely, because, as reviewed elsewhere,[57] *T suis* has a very limited life cycle in human beings. No intestinal side effects were documented in the previous studies of T. suis ova as therapy for inflammatory bowel disease.[39-41]

During the grass season, subjects exhibited an expected increase in symptom scores, use of relief medication, grass-specific IgE, eosinophil and basophil cells, and eosinophil inflammation of airways. However, there was no difference between the treatment groups in any of these parameters except the eosinophil counts, and there was no significant loss of skin reactivity to allergens after the 6 months of treatment. The observation of increased eosinophil counts in the TSO group is a common phenomenon in helminth infections. Antihistamine tablets were used slightly fewer days by subjects on TSO, and this observation might be a result of *T suis*–induced diarrhea affecting the blinding of a minority of subjects.

We achieved a preseasonal treatment period of 6 to 15 weeks for 77% of subjects (3-5 treatments) and 3 to 5 weeks for 23% of subjects (2 treatments). In a previous study of Crohn's disease, use of the same dose, interval, and treatment duration resulted in disease remission in 66% of patients after 12 weeks and in 72% after 24 weeks.[41] Two other studies on *T suis* treatment in Crohn's disease and ulcerative colitis used more frequent disease assessments and reported responses after 4 to 6 weeks of treatment.[39,40] Thus, notwithstanding the different diseases under study compared with allergic rhinitis, the duration of our preseasonal treatment was close to optimal for detection of an effect. Furthermore, results were the same in the analyses of subjects treated 6 to 15 weeks versus 3 to 5 weeks before the peak of the grass season.

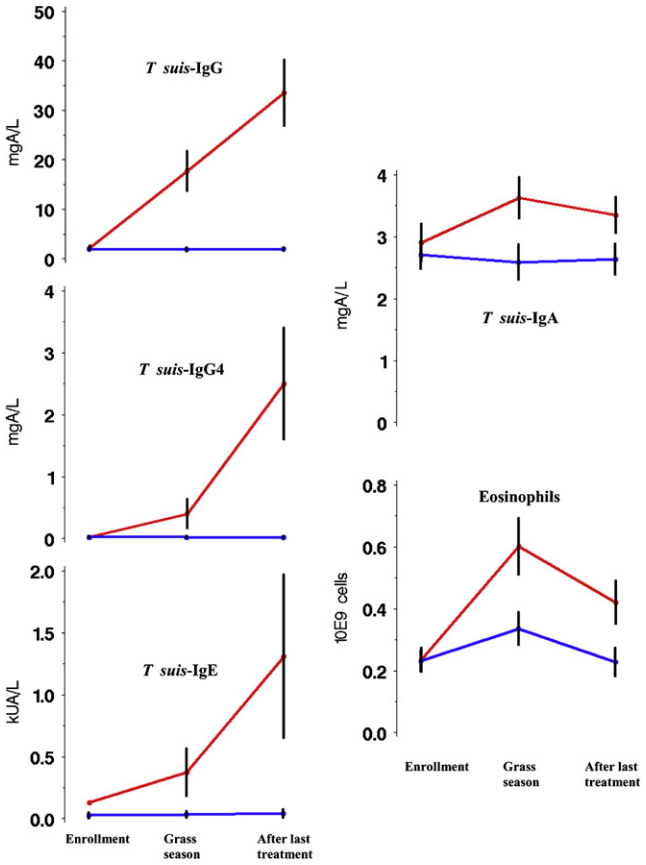


FIG 3. Eosinophil counts and *T suis*–specific antibody titers measured at 3 sampling visits in 96 adults with grass pollen–induced allergic rhinitis in a randomized clinical trial of TSO *(red line)* and placebo *(blue line)*, Denmark, 2008.

The contrasting finding of a lack of effect of *T suis* on allergic rhinitis observed in this study and the effect on inflammatory bowel disease observed in previous studies is of

TABLE II. Allergic rhinitis symptoms and medication use reported in diary* during the grass season by treatment group in 96 subjects with grass pollen–induced allergic rhinitis in a randomized clinical trial of TSO, Denmark, 2008

	TSO	Placebo		
	Mean (SD) N = 49	Mean (SD) N = 47	Difference (95% CI) in mean during grass season between TSO and placebo	*P* value†
Mean daily symptom score	1.4 (1.2)	1.4 (1.1)	0.0 (–0.5, 0.4)	.87
Well days (% days)	36 (29)	33 (27)	3 (–9, 14)	.63
Mean daily medication score	4.0 (2.8)	5.1 (3.8)	−1.1 (–2.5, 0.2)	.11
Medication use‡ (% days)				
Tablets	31 (29)	45 (35)	−14 (–27, −1)	.04
Nasal sprays	22 (27)	25 (30)	−3 (–14, 9)	.63
Eye drops	23 (25)	25 (28)	−2 (–12, 9)	.74

*The 96 subjects completed their diary of symptom scores and use of medication for allergic rhinitis for on average 98% (Q1, 98%; Q3, 98%) of the 51-day duration of the grass season.

†The *P* values are based on *t* tests. Supplementary nonparametric Wilcoxon tests supported the same results except that use of tablets became insignificant ($P = .06$). Furthermore, the results were the same when including all days subjects were in the trial.

‡Self-reported medication in diaries accounted for use of an estimated average of 92% of tablets (Q1, 82%; Q3, 100%), 85% of nasal spray doses (Q1, 64%; Q3, 98%), and 110% of eye drops (Q1, 65%; Q3, 104%) that each subject received from the trial clinic. For details see this article's Table E3 in the Online Repository at www.jacionline.org.

TABLE III. Subclinical measures of allergic reactivity measured at different sampling visits by treatment group in 96 subjects with grass pollen–induced allergic rhinitis in a randomized clinical trial of TSO, Denmark, 2008

	TSO	Placebo		
	Mean (SD) N = 49	Mean (SD) N = 47	Difference (95% CI) between TSO and placebo in change since enrollment	*P* value*
Number of skin wheals of 0 mm in diameter on prick testing with 9 allergens excluding grass				
At enrollment	6.0 (1.9)	5.1 (2.4)		
After last treatment	6.0 (2.0)	5.6 (2.4)	−0.4 (–0.9, 0.0)	0.07
Diameter of skin wheal on prick testing with grass allergen (mm)				
At enrollment	9.3 (2.6)	8.7 (2.8)		
After last treatment	9.8 (2.7)	9.9 (2.5)	−0.1 (–1.2, 1.0)	0.85
Grass-specific serum IgE (kUA/L)				
At enrollment	16.5 (19.0)	13.9 (15.5)		
During grass season	23.9 (28.5)	24.2 (26.8)	−2.7 (–8.9, 3.6)	0.40
After last treatment	25.3 (28.6)	23.6 (24.5)	−0.9 (–6.2, 4.5)	0.76
Total histamine (ng/mL)				
At enrollment	122.0 (64.9)	117.3 (63.8)		
During grass season	147.6 (74.8)	138.6 (59.5)	4.3 (–29.4, 37.9)	.81
After last treatment	114.0 (57.9)	104.0 (51.9)	11.6 (–17.5, 40.7)	.44
Exhaled NO (parts per bilion by volume)				
At enrollment	30.4 (39.3)	24.3 (15.5)		
During grass season	52.1 (43.0)	44.1 (31.0)	1.8 (–16.4, 20.0)	.85
After last treatment	27.4 (12.4)	26.3 (11.7)	−7.7 (–20.6, 5.1)	.24

*The *P* values are based on *t* tests. Supplementary nonparametric Wilcoxon tests supported the same results.

particular interest.[39-41] An overall interpretation is that anti-inflammatory effects of *T suis* are limited to the intestine. Accordingly, beneficial effects of *T suis* on inflammatory bowel disease could be solely a result of local epithelial immune cell interactions and epithelial cell responses that affect mucous production, defensin release, and barrier function.[58] Our results are compatible with this interpretation, because we observed a persistent eosinophil response and antibody response to *T suis*, but no effect on allergic reactivity in blood, airways, and skin after 6 months.

In experimental studies supporting our treatment rationale,[4-20] 7 different helminths were studied, and all reduced allergen-specific IgE[4-8,11,15,17,19,20] and protected against allergic airway inflammation, asthma, airway hyperreactivity, anaphylaxis, or food allergy by induction of regulatory T cells or cytokines.[4-6,8-10,12-16,19,20,45] In view of the increasingly important role of regulatory T cells and cytokines as a treatment rationale,[59] as well as the safety concerns associated with invasive helminths as therapy,[60,61] we felt our trial was well placed. In the experimental studies, helminths were administered mostly before or during sensitization with

allergen, and could in all studies have exerted their protective effect on airway reactivity *after* sensitization. Accordingly, a protective effect induced solely by helminth exposure between allergen sensitization and subsequent allergen challenge, was demonstrated by intravenous injection of mesenteric or splenic lymphocytes from helminth-infected mice into uninfected mice *after* sensitization (*Heligmosomoides polygyrus* and Schistosomiasis model),[9,14] and by intraperitoneal injection of a helminth ES compound *after* sensitization (filarial cystatin).[4] However, the positive results using these 2 approaches may be explained by a high systemic exposure to helminth-induced regulatory T cells and cytokines, which may or may not be achievable in a safe way by using helminth therapy in human beings. In *T suis* therapy, a gradual up-dosing beyond 2500 TSO might be safe. However, it is clearly necessary first to address limitations in induction and effects of regulatory T cells and cytokines.

In conclusion, we have shown that repeated treatment with *T suis* induces a substantial clinical and immunologic response as evidence of infection, but has no therapeutic effect on allergic rhinitis.

The study was initiated by researchers and supported by unrestricted grants. Statens Serum Institut took on the role of GCP as (noncommercial) sponsor: Peter Bager, Study Director and author of the first Investigational Medicinal Product Dossier and Investigator's brochure for TSO leading to the first clinical trial authorization according to the standards of the International Committee for Human Medicines (EudraCT no. 2007-006099-12; Danish Medicine Agency no. 2612-3616); Mads Melbye, Sponsor; Stella Hounsgaard, Quality Coordinator, quality control of GMP, trial clinic, and electronic generation of the randomization sequence by the Quality Assurance Department (biostatistician Anders Mørup Jensen); Jan Wohlfahrt, Data Manager; and Camilla Panduro Pretzmann, Safety Regulatory Officer. John Arnved, MD (internal and pulmonary medicine), Head of trial clinic, and Steen Rønborg, MD (internal and pulmonary medicine), PhD, conducted the screening, enrollment, and clinical visits at the Pulmonology and Allergy Clinic of Copenhagen, and the nurses Eva Kondrup and Helle Arnved carried out invaluable patient care during 852 visits. The GCP unit of the National University Hospital monitored the trial clinic and the data management at the sponsor's site. *T suis* antigen was kindly provided by Dr Falk Pharma GmbH, Freiburg, Germany. We thank the participants who enrolled in this study.

Clinical implications: Nonspecific immune therapy with live ova from the helminth parasite *Trichuris suis* had no effect on allergic rhinitis but induced an appropriate clinical and immunologic response consistent with helminth infection.

REFERENCES

1. Butland BK, Strachan DP, Lewis S, Bynner J, Butler N, Britton J. Investigation into the increase in hay fever and eczema at age 16 observed between the 1958 and 1970 British birth cohorts. BMJ 1997;315:717-21.
2. Linneberg A, Gislum M, Johansen N, Husemoen LL, Jorgensen T. Temporal trends of aeroallergen sensitization over twenty-five years. Clin Exp Allergy 2007;37:1137-42.
3. Plaut M, Valentine MD. Clinical practice: allergic rhinitis. N Engl J Med 2005;353:1934-44.
4. Schnoeller C, Rausch S, Pillai S, Avagyan A, Wittig BM, Loddenkemper C, et al. A helminth immunomodulator reduces allergic and inflammatory responses by induction of IL-10-producing macrophages. J Immunol 2008;180:4265-72.
5. Hartmann S, Schnoeller C, Dahten A, Avagyan A, Rausch S, Lendner M, et al. Gastrointestinal nematode infection interferes with experimental allergic airway inflammation but not atopic dermatitis. Clin Exp Allergy 2009. Epub ahead of print, June 8.
6. Pacifico LG, Marinho FA, Fonseca CT, Barsante MM, Pinho V, Sales-Junior PA, et al. Schistosoma mansoni antigens modulate experimental allergic asthma in a murine model: a major role for CD4 + CD25 + Foxp3 + T cells independent of interleukin-10. Infect Immun 2009;77:98-107.
7. Fenoy I, Giovannoni M, Batalla E, Martin V, Frank FM, Piazzon I, et al. Toxoplasma gondii infection blocks the development of allergic airway inflammation in BALB/c mice. Clin Exp Immunol 2009;155:275-84.
8. Dittrich AM, Erbacher A, Specht S, Diesner F, Krokowski M, Avagyan A, et al. Helminth infection with Litomosoides sigmodontis induces regulatory T cells and inhibits allergic sensitization, airway inflammation, and hyperreactivity in a murine asthma model. J Immunol 2008;180:1792-9.
9. Smits HH, Hammad H, van NM, Soullie T, Willart MA, Lievers E, et al. Protective effect of Schistosoma mansoni infection on allergic airway inflammation depends on the intensity and chronicity of infection. J Allergy Clin Immunol 2007;120:932-40.
10. Yang J, Zhao J, Yang Y, Zhang L, Yang X, Zhu X, et al. Schistosoma japonicum egg antigens stimulate CD4 CD25 T cells and modulate airway inflammation in a murine model of asthma. Immunology 2007;120:8-18.
11. Trujillo-Vargas CM, Werner-Klein M, Wohlleben G, Polte T, Hansen G, Ehlers S, et al. Helminth-derived products inhibit the development of allergic responses in mice. Am J Respir Crit Care Med 2007;175:336-44.
12. Kitagaki K, Businga TR, Racila D, Elliott DE, Weinstock JV, Kline JN. Intestinal helminths protect in a murine model of asthma. J Immunol 2006;177:1628-35.
13. Mangan NE, Van RN, McKenzie AN, Fallon PG. Helminth-modified pulmonary immune response protects mice from allergen-induced airway hyperresponsiveness. J Immunol 2006;176:138-47.
14. Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med 2005;202:1199-212.
15. Wohlleben G, Trujillo C, Muller J, Ritze Y, Grunewald S, Tatsch U, et al. Helminth infection modulates the development of allergen-induced airway inflammation. Int Immunol 2004;16:585-96.
16. Mangan NE, Fallon RE, Smith P, Van RN, McKenzie AN, Fallon PG. Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. J Immunol 2004;173:6346-56.
17. Wang CC, Nolan TJ, Schad GA, Abraham D. Infection of mice with the helminth Strongyloides stercoralis suppresses pulmonary allergic responses to ovalbumin. Clin Exp Allergy 2001;31:495-503.
18. Turner KJ, Shannahan K, Holt PG. Suppression of allergic reactivity by intestinal helminths: susceptibility is a function of IgE responder phenotype. Int Arch Allergy Appl Immunol 1985;78:329-31.
19. Bashir ME, Andersen P, Fuss IJ, Shi HN, Nagler-Anderson C. An enteric helminth infection protects against an allergic response to dietary antigen. J Immunol 2002;169:3284-92.
20. Nagler-Anderson C. Helminth-induced immunoregulation of an allergic response to food. Chem Immunol Allergy 2006;90:1-13.
21. Ndiaye M, Bousquet J. Allergies and parasitoses in sub-Saharan Africa. Clin Rev Allergy Immunol 2004;26:105-13.
22. Wickelgren I. Immunotherapy: can worms tame the immune system? Science 2004;305:170-1.
23. Schubert C. The worm has turned. Nat Med 2004;10:1271-2.
24. Wilson MS, Maizels RM. Regulation of allergy and autoimmunity in helminth infection. Clin Rev Allergy Immunol 2004;26:35-50.
25. Jackson JA, Friberg IM, Little S, Bradley JE. Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? Immunology 2009;126:18-27.
26. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 2003;3:733-44.
27. Cooper PJ, Chico ME, Bland M, Griffin GE, Nutman TB. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. Am J Respir Crit Care Med 2003;168:313-7.
28. Cooper PJ, Chico ME, Rodrigues LC, Ordonez M, Strachan D, Griffin GE, et al. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. J Allergy Clin Immunol 2003;111:995-1000.
29. Rodrigues LC, Newcombe PJ, Cunha SS, Cantara-Neves NM, Genser B, Cruz AA, et al. Early infection with Trichuris trichiura and allergen skin test reactivity in later childhood. Clin Exp Allergy 2008;39:1769-77.
30. Leonardi-Bee J, Pritchard D, Britton J. Asthma and current intestinal parasite infection: systematic review and meta-analysis. Am J Respir Crit Care Med 2006;174:514-23.
31. Flohr C, Quinnell RJ, Britton J. Do helminth parasites protect against atopy and allergic disease? Clin Exp Allergy 2009;39:20-32.

32. Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. J Allergy Clin Immunol 1993;92:404-11.
33. van den Biggelaar AH, Rodrigues LC, van RR, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004;189:892-900.
34. Flohr C, Tuyen LN, Lewis S, Minh TT, Campbell J, Britton J, et al. Low efficacy of mebendazole against hookworm in Vietnam: two randomized controlled trials. Am J Trop Med Hyg 2007;76:732-6.
35. Cooper PJ, Chico ME, Vaca MG, Moncayo AL, Bland JM, Mafla E, et al. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 2006; 367:1598-603.
36. Oliveira RR, Gollob KJ, Figueiredo JP, Alcantara LM, Cardoso LS, Aquino CS, et al. Schistosoma mansoni infection alters co-stimulatory molecule expression and cell activation in asthma. Microbes Infect 2009;11:223-9.
37. Medeiros M Jr, Almeida MC, Figueiredo JP, Atta AM, Mendes CM, Araujo MI, et al. Low frequency of positive skin tests in asthmatic patients infected with Schistosoma mansoni exposed to high levels of mite allergens. Pediatr Allergy Immunol 2004;15:142-7.
38. Medeiros M Jr, Figueiredo JP, Almeida MC, Matos MA, Araujo MI, Cruz AA, et al. Schistosoma mansoni infection is associated with a reduced course of asthma. J Allergy Clin Immunol 2003;111:947-51.
39. Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R, Weinstock JV. Trichuris suis seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am J Gastroenterol 2003;98:2034-41.
40. Summers RW, Elliott DE, Urban JF Jr, Thompson RA, Weinstock JV. Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology 2005;128:825-32.
41. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. Trichuris suis therapy in Crohn's disease. Gut 2005;54:87-90.
42. Elliott DE, Li J, Blum A, Metwali A, Qadir K, Urban JF Jr, et al. Exposure to schistosome eggs protects mice from TNBS-induced colitis. Am J Physiol Gastrointest Liver Physiol 2003;284:G385-91.
43. Elliott DE, Setiawan T, Metwali A, Blum A, Urban JF Jr, Weinstock JV. Heligmosomoides polygyrus inhibits established colitis in IL-10-deficient mice. Eur J Immunol 2004;34:2690-8.
44. Weinstock JV, Summers RW, Elliott DE. Role of helminths in regulating mucosal inflammation. Springer Semin Immunopathol 2005;27:249-71.
45. Wilson MS, Maizels RM. Regulatory T cells induced by parasites and the modulation of allergic responses. Chem Immunol Allergy 2006;90:176-95.
46. Burastero SE, Mistrello G, Falagiani P, Paolucci C, Breda D, Roncarolo D, et al. Effect of sublingual immunotherapy with grass monomeric allergoid on allergen-specific T-cell proliferation and interleukin 10 production. Ann Allergy Asthma Immunol 2008;100:343-50.
47. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4 + CD25+ cells in the nasal mucosa. J Allergy Clin Immunol 2008;121:1467-72, 1472.el.
48. Francis JN, Till SJ, Durham SR. Induction of IL-10 + CD4 + CD25 + T cells by grass pollen immunotherapy. J Allergy Clin Immunol 2003;111:1255-61.
49. Committee for Medicinal Products for Human Use, European Medicines Agency. Guideline on the clinical development of medicinal products for the treatment of allergic rhinoconjunctivitis. 2004. Available at: www.emea.europa.eu/pdfs/human/ewp/245502en.pdf. Accessed September 2009.
50. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. Seoul, Korea, October 2008. Available at: http://www.wma.net/e/policy/b3.htm. September 2009.
51. Dahl R, Stender A, Rak S. Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. Allergy 2006;61: 185-90.
52. Dahl R, Kapp A, Colombo G, de Monchy JG, Rak S, Emminger W, et al. Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006;118:434-40.
53. Kringel H, Roepstorff A. Trichuris suis excretory/secretory antigen-specific antibodies in serum from single-inoculated pigs. Parasite Immunol 2007;29: 327-30.
54. Stahl Skov P, Norn S, Weeke B. A new method for detecting histamine release. Agents Actions 1984;14:414-6.
55. Beer RJ. Morphological descriptions of the egg and larval stages of Trichuris suis Schrank, 1788. Parasitology 1973;67:263-78.
56. Beer RJ. Studies on the biology of the life-cycle of Trichuris suis Schrank, 1788. Parasitology 1973;67:253-62.
57. Summers RW, Elliott DE, Weinstock JV. Why Trichuris suis should prove safe for use in inflammatory bowel diseases. Inflamm Bowel Dis 2005;11:783-4.
58. Weinstock JV, Elliott DE. Helminths and the IBD hygiene hypothesis. Inflamm Bowel Dis 2009;15:128-33.
59. Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. J Allergy Clin Immunol 2009;123:735-46.
60. Mortimer K, Brown A, Feary J, Jagger C, Lewis S, Antoniak M, et al. Dose-ranging study for trials of therapeutic infection with Necator americanus in humans. Am J Trop Med Hyg 2006;75:914-20.
61. Croese J, O'Neil J, Masson J, Cooke S, Melrose W, Pritchard D, et al. A proof of concept study establishing Necator americanus in Crohn's patients and reservoir donors. Gut 2006;55:136-7.
62. Bousquet J, Van CP, Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001;108(suppl 5):S147-334.

TABLE E1. Detailed inclusion and exclusion criteria for a clinical trial of TSO

Inclusion criteria
1. Men, or women not of childbearing potential, age ≥18 and ≤65 years. Women must be postmenopausal or sterilized to have no childbearing potential.
2. Symptoms of grass pollen allergy the last 2 pollen seasons, or more.
3. FEV_1>70% of expected.
4. Scoring all symptoms of allergic rhinitis >50 mm on a 100-mm visual analogue scale (0, not bothersome; 100, worst) during previous pollen season.
5. Specific IgE class ≥2 to allergen extracts of grass (ie, ≥0.7 kUA/L).
6. A positive SPT (≥3 mm) to allergen extracts of grass.
7. Prepared to grant authorized persons access to medical patient records at the trial clinic.
8. Likely to comply with instructions (willing to participate for 24 weeks, visit the clinic, make the diary, and measure daily lung function).
For a volunteer to be included in the trial, all of the criteria listed above must be answered yes.
Exclusion criteria
9. Significant asthma.
10. Use of systemic steroids during the last 2 months.
11. Immune therapy for grass pollen allergy the last 2 years.
12. Planning travel abroad during the trial period (excluding areas with similar grass and birch pollen counts compared with the Copenhagen area).
13. Past or current severe diseases (a history of Crohn disease, ulcerative colitis, multiple sclerosis, active hepatitis B or C, cytomegalovirus, herpes simplex, HIV, other kinds of immune deficiency, and cancer).
14. Antihelminth treatment within the last 2 weeks (eg, Vermox, Janssen Pharm A/S, Birkerød, Denmark).
15. Known or possible hypersensitivity to *Trichuris* species, or compounds made of *Trichuris* species.
16. Past or recent drug abuse.
17. Participation in other clinical trials.
18. Employed at the trial clinic, or the Department of Epidemiology Research at Statens Serum Institut (sponsor's department).
For a volunteer to be excluded from the trial, at least 1 of the criteria listed above must be answered yes.

TABLE E2. Daily scoring of rescue medication adapted from Dahl et al* and used for calculating of medication scores among 96 subjects in a randomized clinical trial of TSO, Denmark, 2008

Step	Active agent of the rescue medication, trade name (recommended dose)	Score/dose	Maximum/d (guidance)
	Allergic rhinitis		
1	Antihistamine tablets (desloratidine), Aerius (5 mg once daily)	6 per tablet	6
1	Antihistamine eye drops (levocabastine), Livostine (0,5 mg/mL; 1 drop in each eye twice daily)	2 per drop	8
2	Budesonide nasal spray, Rhinocort (as much as 32 μg; 2 sprays per nostril twice daily)	1 per spray	8
3	Prednisone tablets (as much as 50 mg once daily)	1.6 per 5 mg	16
	Total		38
	Asthma		
A	Inhaled ß$_2$-adrenergic-agonists, Airomir (100 μg per inhal.; 1–2 inhal. twice daily)	2 per inhal.	8
B	Inhaled budesonide, Pulairmax/Symbicort mite (200 μg per inhal.; 1–2 inhal. twice daily)	2 per inhal.	8
C	Prednisone tablets (as much as 50 mg once daily)	1.6 per 5 mg	16
	Total		32

inhal., Inhalation.

*Dahl R, Stender A, Rak S. Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. Allergy 2006;61:185-90.

TABLE E3. Correspondence* between diary records and use of rescue medication that subjects with grass pollen–induced allergic rhinitis (N = 96) received from the trial clinic during a randomized clinical trial of TSO, Denmark, 2008

	N	Percentage
Tablets with antihistamine		
Returned all† containers after use	61	64
Recorded and used ≥10 tablets	46	48
Mean correspondence (Q1, Q3)	46	93 (83, 100)
Nasal sprays with budesonide		
Returned all spray containers after use	57	59
Recorded and used ≥10 sprays	51	53
Mean correspondence (Q1, Q3)	51	85 (65, 98)
Eye drops with antihistamine		
Returned all eye drop containers after use	57	59
Recorded and used ≥10 eye drops	45	47
Mean correspondence (Q1, Q3)	45	109 (66, 99)
Inhalations with ß$_2$-adrenergic agonists		
Returned all the inhalators after use	30	31
Recorded and used ≥10 inhalations	21	22
Mean correspondence (Q1, Q3)	21	107 (73, 136)
Inhalations with budesonide		
Returned all the inhalators after use	10	10
Recorded and used ≥10 inhalations	11	11
Mean correspondence (Q1, Q3)	11	51 (33, 58)

*The mean correspondence percentage was calculated as the number of doses recorded in the diary divided by the number of used doses. The number of used doses was calculated as the weight of a new container minus the weight of the returned container, divided by the weight of 1 dose. However, for inhalations with budesonide, the number of used doses was calculated as the number of doses in a new container (200) minus the number on the returned inhalator's display of remaining doses.
†Included all subjects who returned all containers excluding tablet containers, because tablet containers were not counted on return—only tablets.

Correspondence

Looking into the future of *Trichuris suis* therapy

To the Editor:

We were recently intrigued by the findings of a clinical trial using *Trichuris suis* ova as treatment for patients with allergic rhinitis.[1] *T suis* ova are prime candidates for the clinical therapy of allergic and inflammatory diseases in the growing field of helminth immunomodulation, having previously been successfully applied in patients with Crohn disease.[2] A plethora of epidemiologic and laboratory studies have brought the beneficial effects of helminth infections into focus in recent years. Loss of helminth infections (including *Trichuris trichiura*) through deworming of children living in endemic regions resulted in a consequent increase in skin reactivity to house dust mite allergens.[3,4] Similarly, we were able to show that helminth infection abrogated clinical symptoms and key immunologic parameters in a murine disease model of airway hyperreactivity.[5]

During the trial performed by Bager et al,[1] 100 patients presenting with grass pollen–induced allergic rhinitis symptoms received doses of 2,500 live *T suis* ova every 3 weeks. However, no clinical parameters, such as grass-specific IgE levels, were significantly altered. This led to the conclusion that *T suis* ova therapy might not be applicable in human subjects to treat allergic rhinitis or other allergic airway diseases.

However, the trial might have failed to yield positive therapeutic results because of problems with the study design. Although a dose of 2,500 ova every 21 days might be sufficient for therapeutic effects when directly delivered to diseased tissue, such as the colons of patients with Crohn disease, it might be insufficient to effectively alter systemic responses, considering the lack of colonization and rapid clearance of *T suis* ova.

Furthermore, the 96 patients who completed the trial received only 3 doses before the onset of the pollen season. It is currently unknown by which mechanisms *T suis* ova mediate their therapeutic effects or how many repeat exposures are necessary. Moreover, timing of helminth exposure is clearly important because early infections with *T trichiura* in human subjects directly correlated with reduced skin test reactivity to 7 common allergens.[6] Thus a study in which all patients received a consistent and high number of doses before allergen exposure might prove more efficacious.

One attractive possibility to exploit the therapeutic effects of helminth treatment is to isolate the potent immunomodulatory components produced by these worms, and we recently reported the striking effects of the filarial worm–derived protein Av17 in suppressing airway hyperresponsiveness in a murine model.[7] Interestingly, our laboratory now has evidence suggesting excretory/secretory products of *T suis* larvae are also highly effective in preventing the onset of airway hyperreactivity in the mouse. In such a system administration of *T suis* excretory/secretory products before antigenic airway challenge resulted in a complete ablation of clinical symptoms, cell recruitment, and cytokine production (unpublished data).

Thus despite the findings of Bager et al,[1] there is considerable hope for the future of *T suis* therapy, and the characterization and production of recombinant *T suis* products might be an effective way to bring this tool to the clinic.

Matthew R. Hepworth, PhD[a]*
Eckard Hamelmann, MD[b,c]*
Richard Lucius, PhD[a]
Susanne Hartmann, PhD[a]

From [a]the Department of Molecular Parasitology, Humboldt University, Berlin, Germany; [b]the Department of Paediatrics, Allergy Center, Charité Hospital, Berlin, Germany; and [c]University Children's Hospital, Ruhr-University Bochum, Bochum, Germany. E-mail: Susanne.Hartmann@hu-berlin.de.

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REFERENCES

1. Bager P, Arnved J, Rønborg S, Wohlfahrt J, Poulsen LK, Westergaard T, et al. *Trichuris suis* ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. J Allergy Clin Immunol 2009; [Epub ahead of print].
2. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy in Crohn's disease. Gut 2005;54:87-90.
3. van den Biggelaar AH, Rodrigues LC, van Ree R, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004;189:892-900.
4. Flohr C, Tuyen LN, Quinnell RJ, Lewis S, Minh TT, Campbell J, et al. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin Exp Allergy 2009; [Epub ahead of print].
5. Hartmann S, Schnoeller C, Dahten A, Avagyan A, Rausch S, Lendner M, et al. Gastrointestinal nematode infection interferes with experimental allergic airway inflammation but not atopic dermatitis. Clin Exp Allergy 2009;39:1585-96.
6. Rodrigues LC, Newcombe PJ, Cunha SS, Alcantara-Neves NM, Genser B, Cruz AA, et al. Early infection with *Trichuris trichiura* and allergen skin test reactivity in later childhood. Clin Exp Allergy 2008;38:1769-77.
7. Schnoeller C, Rausch S, Pillai S, Avagyan A, Wittig BM, Loddenkemper C, et al. A helminth immunomodulator reduces allergic and inflammatory responses by induction of IL-10-producing macrophages. J Immunol 2008;180:4265-72.

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*These authors contributed equally to this manuscript.

Correspondence

Trichuris suis might be effective in treating allergic rhinitis

To the Editor:

We compliment Bager et al[1] for performing the first randomized, double-blind controlled trial of *Trichuris suis* ova in the treatment of allergic rhinitis. They concluded that the agent had no therapeutic effect on grass pollen–induced disease. Our studies demonstrated that *T suis* exposure reduced disease activity in patients with immune-mediated ulcerative colitis and Crohn disease.[2,3] Mechanistic studies in animals demonstrated that helminth exposure reduced hyperactive airway disease through induction of regulatory T cells.[4,5] In the study by Bager et al,[1] *T suis* ova treatment produced definite biologic effects, including increased eosinophil counts and *T suis*–specific IgE, IgG4, and IgA levels. These observations established that *T suis* had a significant effect on the human immune system. However, several features of the study design and conduct possibly caused the investigators to miss a treatment benefit.

The most important problem with the study was late administration of *T suis* ova with respect to the tree allergy season because it takes time for the helminths to induce clinically significant disease modulation. Subjects were enrolled from March 10 through May 15, and the allergic rhinitis season was from May 28 through July 27, with the peak beginning June 9. Distribution of enrollment dates is not described in detail with respect to the allergy season, but by season peak, some subjects had received only 2 treatments administered 21 days apart, and 23% of subjects were treated for only 3 to 5 weeks. Thus many subjects were treated too late for therapeutically apparent effects by season peak. In our studies clinical effects were not apparent until 6 weeks.[2] *Post hoc* analysis of those patients treated for 6 to 15 weeks increases type 2 error.

Subject characteristics were similar except that severity of allergic rhinitis was greater in the *T suis* ova–treated group (moderate-severe, 94%; mild, 6%) than in the placebo-treated group (moderate-severe, 82%; mild, 18%). Differences in severity might have skewed the results against the *T suis* ova–treated subjects, particularly when combined with incomplete *T suis* exposure at peak season.

Although the primary change in symptom score demonstrated no difference between *T suis* ova– and placebo-treated subjects, other parameters were statistically significant or showed a definite trend in favor of *T suis* ova. Medications used were 31 tablets (29% days) with *T suis* ova versus 45 tablets (35%) with placebo ($P = .04$), and mean daily medication scores were less with *T suis* ova (4) than with placebo (5.1, $P = .11$). There was little comment about these results and no discussion that higher use of antihistamines might have reduced the symptom score in the placebo-treated subjects.

Thus it is premature to conclude that *T suis* ova had no therapeutic effect for treatment of grass pollen–induced allergic rhinitis.

Bager et al[1] report that some *T suis* ova–treated subjects had transient diarrhea around 30 to 50 days of treatment. However, diarrhea was not statistically different than in the placebo group. In our studies we used authenticated tools to measure stool patterns and saw improvement in diarrhea.[2] In the study by Bager et al,[1] subjects did not spontaneously report diarrhea, and the severity and duration was not described. Therefore it is difficult to determine whether this description is clinically significant.

Robert W. Summers, MD[a]
David E. Elliott, MD, PhD[a]
Joel V. Weinstock, MD[b]

From [a]Department of Internal Medicine, the University of Iowa Carver College of Medicine, Iowa City, Iowa, and [b]Department of Internal Medicine, Tufts–New England Medical Center, Boston, Mass. E-mail: david-elliott@uiowa.edu.

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REFERENCES

1. Bager P, Arnved J, Rønborg S, Wohlfahrt J, Poulsen LK, Westergaard T, et al. *Trichuris suis* ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. J Allergy Clin Immunol 2009 [Epub ahead of print].
2. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology 2005;128:825-32.
3. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy in Crohn's disease. Gut 2005;54:87-90.
4. Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med 2005;202:1199-212.
5. Kitagaki K, Businga TR, Racila D, Elliott DE, Weinstock JV, Kline JN. Intestinal helminths protect in a murine model of asthma. J Immunol 2006;177:1628-35.

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Correspondence

Reply

To the Editor:

We thank the authors for their comment on our study of *Trichuris suis* ova for the treatment of allergic rhinitis.[1,2] The study was a randomized, double-blind, placebo-controlled clinical trial in which 96 subjects with grass pollen allergy received 8 treatments with 2,500 *T suis* ova or placebo.[3] We observed no beneficial clinical effect on their pollen allergy, as well as no subclinical effect after 6 months (168 days).

The comments by Hepworth et al mainly concern the future of the use of parasite therapy in preventing the development of allergic disease (eg, the treatment of nonallergic children). Our study did not address this issue because all subjects had allergy. We encourage studies on this hypothesis despite our negative result.

However, any such study should perhaps consider why only allergen-specific immunotherapy can be effective in curing allergies to pollen, dust mites, and food.[4-6] For parasites to be prime candidates for any similar effect, they clearly need to use an equally effective immunologic mechanism. We believe it is an important message from our study that *T suis* ova therapy is not allergen specific and was ineffective against grass allergy.

Hepworth et al reference the dichotomy observed in studies of schoolchildren that skin reactions to allergens developed after deworming, whereas in other studies the risk of such reactions were reduced in children who had infections with, for example, *Trichuris trichiura*.[7-10] A possible reason for these observations could be that parasite antigens were cross-reacting with allergens[11] and thereby either induced skin reactions or tolerance to allergens. This view would be compatible with the mechanisms underlying allergen-specific immunotherapy.[12,13] For example, controlled dosing with allergen extracts is important for efficacy, and with higher doses, there is a concern for allergic reactions.[12]

Hepworth et al write that subjects in our trial received only 3 doses before the onset of the pollen season (May 28). To be correct, 3 to 5 doses were received by 77% of the subjects before the date of the peak of the grass pollen season (June 9), and efficacy was equally absent in this group. To comply with a minimum of 4 to 6 weeks lag to a disease response as reported by Dr. Summers et al, *all* subjects received 2 to 5 doses at least 4 weeks before June 9 (90%, ≥6 weeks). This compliance was identical by treatment group despite that subjects on *T suis* had an unexpected 3 to 19-fold higher rate of episodes with moderate to severe diarrhea, abdominal pain, and flatulence (median duration, 2 days) compared with placebo.

Hepworth et al also write that a dose of 2,500 *T suis* ova every 21 days might be insufficient to effectively alter systemic responses considering the lack of colonization and rapid clearance of *T suis*. However, we observed an altered systemic *T suis* IgG response in more than 92% of subjects. In addition, Table I demonstrates that subjects who did not clear *T suis* by means of diarrhea or subjects who responded in the upper quartile of *T suis* IgG titers (>29.9 milligram antibody per liter serum) showed no reduction in their skin reactions to allergens or grass IgE titers after 6 months. A few insignificant changes in the size of skin reactions were likely an after effect of higher pollen and more variable indoor allergen exposure during the summer months.

TABLE I. Unchanged allergic reactivity after 6 months in high responders to 8 treatments with 2,500 *T suis* ova in a clinical trial, Denmark, 2008

	High responders to *T suis* ova treatment					
	No diarrhea, entire trial (n = 14)*			High *T suis* antibody level on day 168 (n = 24)†		
	n	Day 1 mean	Day 168 mean	n	Day 1 mean	Day 168 mean
Skin reactions to 10 allergens‡ (mm)						
Grass	14	9.0	10.3	24	8.3	10.1
Birch	5	6.4	6.2	10	6.1	7.8
House dust mite *(Dermatophagoides pteronyssinus)*	4	6.6	9.0	8	7.1	6.1
House dust mite *(Dermatophagoides farinae)*	4	6.4	9.0	6	5.6	5.7
Dog	11	4.4	4.2	20	4.1	4.1
Cat	7	3.6	5.3	12	5.4	8.1
Horse	1	4.0	0.0	4	3.6	1.8
Mugwort	3	6.5	5.5	4	6.0	5.6
Mold (*Alternaria alternata*)	1	7.5	4.0	3	3.3	3.3
Mold (*Cladosporium herbarum*)	0	–	–	1	0.0	7.0
No. of reactions of 0 mm§	14	6.6	6.9	24	6.5	6.6
Grass IgE (kUA/L)	14	11.4	19.7	24	13.3	21.2

All differences in means between day 1 and day 168 were insignificant by means of the *t* test.

*Number of doses: total, 8 (n = 12), 7 (n = 1), and 5 (n = 1); ≥4 weeks before grass peak, 5 (n = 1), 4 (n = 5), 3 (n = 5), and 2 (n = 3). Information on diarrhea was obtained from subject diaries (diarrhea, n = 33; severe diarrhea, n = 11). Two subjects with no diarrhea were excluded because they did not attend the clinical visit for tests at day 168.

†Number of doses: total, 8 (n = 20) and 7 (n = 4); ≥4 weeks before grass peak, 5 (n = 2), 4 (n = 7), 3 (n = 10), and 2 (n = 5). Mean level of *T suis* IgG: day 76 (Quartile 1, day 64; Quartile 3, day 85), 23.8 mgA/L (Quartile 1, 14.1 mgA/L; Quartile 3, 30.1 mgA/L; 6 subjects >29.9 mgA/L); day 168, 50.3 mgA/L (Quartile 1, 33.8 mgA/L; Quartile 3, 57.9 mgA/L; all 24 subjects >29.9 mgA/L).

‡The mean millimeter value was calculated for subjects (n) who had a wheal size of greater than 0 mm on day 1 or day 168.

§Excluding reactions to grass (ie, all ≥3 mm).

Finally, our demonstration of an absence of an effect of *T suis* on measures of allergic reactivity in skin, blood, and airways even after 6 months is not compatible with the insignificant effect on self-reported medication usage noted by Dr Summers et al.

In conclusion, an even closer look at our data suggests that *T suis* will not show sufficient efficacy in treatment of subjects who have already had an allergic disease.

Peter Bager, PhD[a]
Jan Wohlfahrt, PhD[a]
Bjarne Kristensen, MSc[b]
Lars K. Poulsen, PhD[c]
Mads Melbye, DMSc[a]

From [a]Statens Serum Institut, Department of Epidemiology Research, Copenhagen, Denmark; Phadia ApS, Allerød, Denmark; and [c]the Allergy Clinic, National University Hospital, Copenhagen, Denmark. E-mail: pbg@ssi.dk

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REFERENCES

1. Summers RW, Elliot DE, Weinstock JV. *Trichuris suis* may be effective in treating allergic rhinitis. J Allergy Clin Immunol 2010; ■■:■■.
2. Hepworth MR, Hamelmann E, Lucius R, Hartmann S. Looking into the future of *Trichuris suis* therapy. J Allergy Clin Immunol 2009 ■■■:■■■.
3. Bager P, Arnved J, Rønborg S, Wohlfahrt J, Poulsen LK, Westergaard T, et al. *Trichuris suis* ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. J Allergy Clin Immunol 2009 [Epub ahead of print].
4. Penagos M, Passalacqua G, Compalati E, Baena-Cagnani CE, Orozco S, Pedroza A, et al. Metaanalysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. Chest 2008;133: 599-609.
5. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005;60:4-12.
6. Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. Allergy 2007;62:1261-9.
7. van den Biggelaar AH, Rodrigues LC, van RR, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004;189:892-900.
8. Flohr C, Tuyen LN, Quinnell RJ, Lewis S, Minh TT, Campbell J, et al. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin Exp Allergy 2009 [Epub ahead of print].
9. Rodrigues LC, Newcombe PJ, Cunha SS, Alcantara-Neves NM, Genser B, Cruz AA, et al. Early infection with *Trichuris trichiura* and allergen skin test reactivity in later childhood. Clin Exp Allergy 2008;38:1769-77.
10. Flohr C, Quinnell RJ, Britton J. Do helminth parasites protect against atopy and allergic disease? Clin Exp Allergy 2009;39:20-32.
11. Sereda MJ, Hartmann S, Lucius R. Helminths and allergy: the example of tropomyosin. Trends Parasitol 2008;24:272-8.
12. Nelson HS. Allergen immunotherapy: where is it now? J Allergy Clin Immunol 2007;119:769-79.
13. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. J Allergy Clin Immunol 2008;122:298-304.

doi:10.1016/j.jaci.2009.11.040