

Safety and efficacy of helminth treatment in relapsing-remitting multiple sclerosis: Results of the HINT 2 clinical trial

John Fleming, Gianna Hernandez, Leslie Hartman, Jane Maksimovic, Sara Nace, Benjamin Lawler, Todd Risa, Thomas Cook, Rashmi Agni, Mark Reichelderfer, Christopher Luzzio, Loren Rolak, Aaron Field and Zsuzsanna Fabry

Abstract

Background: The hygiene hypothesis suggests that microbial replacement may be therapeutic in allergic and autoimmune diseases. Nevertheless, the results of helminth treatment, including in multiple sclerosis (MS), have been inconclusive.

Objective: To assess safety and brain magnetic resonance imaging (MRI) activity in subjects with relapsing-remitting multiple sclerosis (RRMS) during oral administration of ova from the porcine whipworm, *Trichuris suis* (TSO).

Methods: A total of 16 disease-modifying treatment (DMT) naive RRMS subjects were studied in a baseline versus treatment (BVT) controlled prospective study. MRI scans were performed during 5 months of screening-observation, 10 months of treatment, and 4 months of post-treatment surveillance.

Results: No serious symptoms or adverse events occurred during treatment. For the cohort, there was a trend consistent with a 35% diminution in active lesions when observation MRIs were compared to treatment MRIs ($p=0.08$), and at the level of individuals, 12 of 16 subjects improved during TSO treatment. T regulatory lymphocytes were increased during TSO treatment.

Conclusion: TSO is safe in RRMS subjects. Potentially favorable MRI outcomes and immunoregulatory changes were observed during TSO treatment; however, the magnitude of these effects was modest, and there was considerable variation among the responses of individual subjects.

Keywords: Multiple sclerosis, helminth therapy, hygiene hypothesis, *Trichuris suis*, phase 1 clinical trial, whipworm

Date received: 3 June 2017; revised: 11 August 2017; accepted: 15 September 2017

Introduction

The hygiene hypothesis and variants such as the microbial deprivation, old friend mechanism, and biodiversity hypotheses are based on epidemiological observations which have demonstrated an inverse relationship between the prevalence of allergic-autoimmune diseases and exposure to environmental microbes, such as endemic parasites.^{1,2} Specifically, it has been suggested that as an unanticipated consequence of improved public hygiene, diminished exposure to evolutionarily normal microbiota in industrialized countries may adversely affect immunoregulation and therefore predispose to immunopathology.³⁻⁷ In animal

models and clinical trials, live microbes, purified microbial products, and microbial-based cellular immunotherapeutics have been administered in an effort to ameliorate allergy or autoimmunity.⁸⁻¹³ The effects of anti-helminth deworming programs on allergy, autoimmunity, and immune function have varied in different studies.¹⁴⁻¹⁶

In the case of multiple sclerosis (MS), a report by Leibowitz et al.¹⁷ in 1966 first suggested that high levels of sanitation were associated with the risk of developing this disease, a finding confirmed by subsequent epidemiological surveys.¹⁸ Additionally, an observational

Multiple Sclerosis Journal

1-11

DOI: 10.1177/
1352458517736377

© The Author(s), 2017.
Reprints and permissions:
[http://www.sagepub.co.uk/
journalsPermissions.nav](http://www.sagepub.co.uk/journalsPermissions.nav)

Correspondence to:

J Fleming
Department of Neurology,
University of Wisconsin
School of Medicine and
Public Health, 7th Floor
MFCB, 1685 Highland
Avenue, Madison, WI 53705,
USA.
fleming@neurology.wisc.edu

John Fleming
Christopher Luzzio
Department of Neurology,
University of Wisconsin
School of Medicine and
Public Health, Madison,
WI, USA

Gianna Hernandez
Rashmi Agni
Zsuzsanna Fabry
Department of Pathology
and Laboratory Medicine,
University of Wisconsin
School of Medicine and
Public Health, Madison,
WI, USA

Leslie Hartman
Jane Maksimovic
Sara Nace
Aaron Field
Department of Radiology,
University of Wisconsin
School of Medicine and
Public Health, Madison,
WI, USA

Benjamin Lawler
Loren Rolak
Department of Neurology,
Marshfield Clinic Health
System, Marshfield WI, USA

Todd Risa
Department of Radiology,
Marshfield Clinic Health
System, Marshfield WI, USA

Thomas Cook
Department of Biostatistics
and Medical Informatics,
University of Wisconsin
School of Medicine and
Public Health, Madison, WI, USA

Mark Reichelderfer
Department of Internal
Medicine, University
of Wisconsin School of
Medicine and Public Health,
Madison, WI, USA

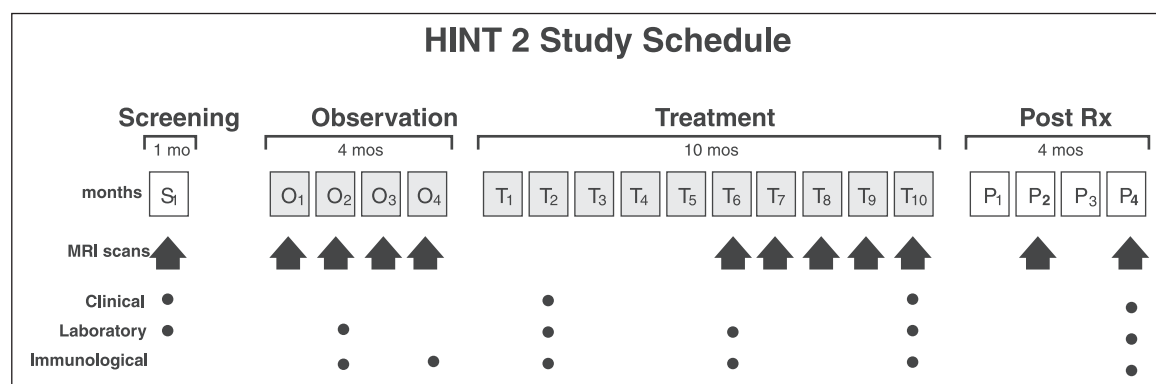


Figure 1. Study design. The phases of the study are shown for intake and screening (S), no-treatment observation (O), TSO treatment (T), and post-treatment evaluation (P) periods, with each month indicated by a numeral in subscript. Monthly brain MRIs are indicated by thick arrows; during the post-treatment phase only one MRI was mandated per protocol (P2 or P4 month). Clinical assessments (examining neurologist, EDSS; technician, MSFC; and treating neurologist, medical evaluation), standard safety laboratory determinations, and research immunological determinations were performed at the time points indicated by respective dots.

study in patients with relapsing-remitting multiple sclerosis (RRMS) demonstrated dramatic clinical, immunological, and neuroimaging improvements in patients who had been naturally infected with human helminth parasites in the field.^{19,20} Exploratory 1a clinical trials of helminth therapy in MS have been inconclusive, likely because of small sample size and other study design limitations.^{21–23} In order to assess the effects of helminth therapy in MS, live ova of the porcine whipworm, *Trichuris suis* (TSO), were administered orally to 16 disease-modifying treatment (DMT) naïve RRMS subjects for 10 months in the phase 1b HINT 2 (Helminth-Induced Immunomodulation Therapy) clinical trial. HINT 2 is the first prospective hygiene hypothesis-based clinical trial in MS or any neurological disease which has employed contrast-enhanced magnetic resonance imaging (MRI) endpoints as recommended for exploratory trials in MS,²⁴ subjects with early disease and without confounding DMT exposure, baseline versus treatment (BVT) control design, and treatment of sufficient duration to assess the possibility of late adverse events (AEs) or benefits.

Materials and methods

Study design

A BVT design was employed, in which each subject served as their own longitudinal control. BVT study design has been preferred for most phase 1 investigations of putative MS treatments, as statistical power in BVT design is achieved with fewer subjects than parallel groups (PG) design.^{24,25} HINT 2 was divided into periods for screening (1 month), observation without treatment (4 months), TSO treatment (10 months), and

post-treatment observation (4 months) (Figure 1). Specifically, the 10-month treatment period was chosen in order to assess any possible AE or treatment effect only apparent during chronic TSO administration. The primary outcome measures were safety, as determined by the incidence of AE, and change in MS activity, as judged by the total number of gadolinium-enhancing (GdE) lesions observed on masked readings for (a) five brain MRIs obtained during the screening-observation phases of the trial compared to (b) five brain MRI scans obtained during the last months of treatment (Figure 1: MRI scans for months S₁, O₁, O₂, O₃, O₄ vs months T₆, T₇, T₈, T₉, T₁₀). The study was conducted at the Marshfield Clinic (Marshfield, WI) and the University of Wisconsin Hospitals and Clinics (Madison, WI).

T. suis ova production, testing, and administration

TSO was purchased from Ovamed, Hamburg, Germany (lot number 9009102901) where it was certified at Good Manufacturing Practice (GMP) pharmaceutical standards of purity and sterility. Details of post-production storage, testing for adventitious agents, and ova stability are presented in Supplementary Material. Based on previous studies,^{21,26–28} GMP TSO was administered to subjects at a dose of 2500 viable ova orally every 2 weeks.

Subjects and ethical statement

Subjects were recruited if they met the criteria of the McDonald Committee for RRMS,²⁹ had a confirmed diagnosis within 3 years of study entry, had never received DMT prior to the study and were willing to

forego DMT during the study, and demonstrated an active brain MRI with at least one GdE typical of MS. Detailed eligibility criteria are provided in Supplementary Material. Additionally, healthy subjects, demographically matched as closely as possible to HINT 2 MS subjects, donated control blood samples for immunoassays. All subjects provided written informed consent. HINT 2 was reviewed and approved by the University of Wisconsin, Madison, Health Sciences IRB (2012-0484-CR005), conducted under Investigational New Drug numbers 13585 and 14174 of the US Food and Drug Administration, and registered with ClinicalTrials.gov (NCT00645749).

Clinical, laboratory, and safety assessments

At the indicated time points (Figure 1), an Expanded Disability Status Scale³⁰ (EDSS) assessment was performed by an examining neurologist who was blinded throughout the study to all other clinical information, and a Multiple Sclerosis Functional Composite (MSFC) evaluation³¹ was performed by a certified technician; subsequently, these assessments were reviewed and an unmasked interim history and physical examination were performed by a treating neurologist. These assessments were also performed at unscheduled clinic visits for AEs or possible relapse. Routine laboratory assessments, including a complete blood count, liver and renal function tests, stool ova and parasite determination, and pregnancy tests in female subjects were performed at the University of Wisconsin Hospital and Clinics clinical laboratory per the schedule indicated in Figure 1. Enzyme-linked immunosorbent assays (ELISA) specific for antibodies to *T. suis* and flow cytometric assays for cell phenotype were performed as previously described.²¹ Colonoscopies with mucosal biopsies were performed in selected subjects. In all cases, the primary indication for colonoscopy was routine colorectal cancer screening; additionally, secondary informed consent was obtained for two to five research biopsies of colonic mucosa. Mucosal samples were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. Safety was assessed by application of Food and Drug Administration (FDA)–Center for Biologics Evaluation and Research (CBER) criteria, modified as indicated in Supplementary Material and graded as follows: Grade 1, mild; Grade 2, moderate; Grade 3, severe; Grade 4, potentially life-threatening; and Grade 5, death.

MRI assessments

Brain MRIs were performed with and without intravenous contrast (double-dose gadolinium, 0.2 mmol/kg body weight) on a 3-Tesla scanner (GE Healthcare,

Waukesha, WI, at the University of Wisconsin site and a Siemens Healthineers, Malvern, PA, at the Marshfield Clinic site) at the time points indicated in Figure 1. For masked readings of each subject's MRI, all identifying information was removed and replaced by a code number, after which the scans were presented in random (not chronological) order to three board-certified neuroradiology fellows who counted the number of GdE. Following the initial reading, the coded images were reviewed by the masked attending neuroradiologist, who resolved any discrepancies or errors during the first reading. Subsequently, the MRI data were locked, and the images were reassembled in chronological order for tabulation of GdE. Details of the MRI sequences for HINT 2 are provided in Supplementary Material.

Statistical assessments

A primary outcome measure of HINT 2 was the mean number of total GdE when observation and treatment MRIs for the study cohort were assessed in a Poisson regression model with a robust variance estimator. Eosinophilia, humoral responses to *T. suis*, and T regulatory enumerations were analyzed by one-way analysis of variance (ANOVA; GraphPad PRISM version 6.0). EDSS and MSFC values were evaluated by unpaired *t*-test (GraphPad PRISM version 6.0). The safety analysis was descriptive rather than inferential, with AEs listed by symptom or disease and frequency without formal statistical analysis for significance. In this phase 1b investigation, all study analyses included all subjects, regardless of adherence to the study protocol.

Results

The demographic characteristics of our subjects are indicated in Table 1. In general, HINT 2 subjects were relatively young, and their RRMS was of short duration and mild disability.

Safety

No serious AE (grade 3 and above) were noted during this study. Similar to prior TSO clinical trials, including the HINT 1 MS study,²¹ the phase 1a MS study of Voldsgaard *et al.*,²³ and the allergic rhinitis study of Bager *et al.*,²⁸ no significant, treatment-related AE were noted in any system, except for the gastrointestinal system, where grade 1 or 2 AE occurred in 9 of the 16 subjects (Table 2). Grade 1 gastrointestinal AE, such as diarrhea, mild abdominal pain, and flatulence, occurred in six subjects, with onset at approximately 30–40 days after the first TSO dose, followed by spontaneous remission in several days. We hypothesize that

Table 1. Demographics of TSO MS subjects.

Study number ^a	Sex	Age (years)	Symptom duration (months) ^b	RRMS diagnosis (months) ^c	Number of clinical relapses in prior year ^d	CSF ^e	EDSS ^f at entry	Prior corticosteroids ^g	MRI GdE at entry ^h
110	F	19	48	4	1	L+	1.5	IV: 7 months	7
111	F	31	7	2	2	NT	1.5	None	12
112	F	38	18	2	1	L+	1.5	IV: 12 months	1
115	M	25	4	1	2	L+	1.5	Oral: 2 months	3
117	M	38	168	6	1	L+	3.5	None	1
118	F	23	26	11	1	H–	1.0	None	6
120	F	32	60	4	0	L+	1.0	None	7
122	F	46	72	4	1	NT	1.0	None	3
125	F	37	18	1	0	L+	1.5	None	2
210	F	33	5	5	2	L+	2	Oral: 1 month	1
212	M	32	23	17	1	NT	2.5	None	1
215	M	33	2	0	1	NT	0	None	5
216	F	25	2	0	1	L+	1.5	Oral: 1 month	1
217	M	31	16	9	2	L+	0	Oral: 3 months	1
218	M	21	3	2	1	NT	0	IV: 2 months	2
219	M	41	15	0	0	NT	1	None	2
Group ⁱ	F 9/16	32±8	30±42	4±5	1±0.6	L+9 NT 6 H–1	1.3±0.9	No steroids: 9; IV steroids: 3; oral steroids: 4	3.4±3.0

TSO: *Trichuris suis*; MS: multiple sclerosis; RRMS: relapsing-remitting multiple sclerosis; CSF: cerebrospinal fluid; EDSS: Expanded Disability Status Scale; MRI: magnetic resonance imaging.

All 16 subjects met the 2010 McDonald criteria for RRMS; when subject histories were reviewed from the first relevant symptom to study entry, five RRMS subjects were subclassified as clinically isolated syndromes on the basis of a single documented typical attack (study numbers 125, 215, 216, 218, and 219), and 11 RRMS subjects were subclassified as clinically definite MS on the basis of two or more documented typical attacks.

^aSubject studies numbers in 100's are from University of Wisconsin site, and numbers in the 200's from Marshfield Clinic site. Gaps in series are due to screen failures.

^bSymptom duration: time in months from (a) first relevant historical symptom recalled by subject to (b) study entry.

^cRRMS diagnosis: time in months from (a) explicit diagnosis of RRMS (McDonald criteria) by a neurologist to (b) study entry; 0 months indicates that the subject was diagnosed at study entry.

^dRelapses based on clear a historical event or physician report during year before study entry.

^eCerebrospinal fluid (CSF) analysis: L+, positive laboratory assessment (documented CSF-specific oligoclonal bands and/or increased IgG index); H– negative CSF by history but without verified laboratory report; and NT, not tested.

^fExpanded Disability Status Scale (EDSS) at study entry.

^gOral and/or intravenous corticosteroids for MS relapse or any indication in the year prior to study entry, indicated by months prior to study entry.

^hGdE (gadolinium-enhancing lesions) on brain MRI at entry performed with 3T scanner with double-dose gadolinium contrast (0.2 mmol/kg body weight).

ⁱMean and standard deviation or summary data for cohort.

these symptoms may represent a robust innate immune response during the first encounter with maturing *T. suis* larvae. Subject number 212 experienced moderate diarrhea (grade 2) with spontaneous resolution in 7 days. Two exceptional subjects, numbers 110 and 111, had grade 2 gastrointestinal symptoms which persisted for several weeks and required a clinic visit for assessment and symptomatic treatment, followed by complete remission of symptoms. In no subject did gastrointestinal symptoms interfere with school or work activities.

Clinically indicated colonoscopies were performed on one healthy control subject, two MS control subjects not receiving TSO, and two HINT 2 MS subjects (study numbers 122 and 125) at month T₁₀ of their TSO treatment. As expected for patients undergoing routine cancer screening, the colonoscopy cohort (mean age 44.6 years, 4/5 female subjects) was older than the general HINT 2 cohort. Apart from benign polyps, no histological abnormalities were observed on hematoxylin–eosin-stained colonic mucosal biopsies in any subject, including those treated with TSO

Table 2. Adverse events, MS relapses, and missing MRI scans.

		Study periods		
		Screening- observation (5 months)	TSO treatment (10 months)	Post-treatment (4 months)
Adverse events				
	Gastrointestinal ^a	0	9 No AE, <i>n</i> = 7 Grade 1, <i>n</i> = 6 Grade 2, <i>n</i> = 3 Grade 3 and above, <i>n</i> = none	0
	Non- gastrointestinal ^b			
	Migraine headache	1	7	1
	Pharyngitis	1	1	0
	Sinusitis	0	1	0
	Bronchitis	0	1	0
	Flank pain	0	1	0
	Back pain	0	1	0
	Alopecia	0	1	0
	Wisdom tooth	0	1	0
	Syncope	1	0	0
MS Relapses ^c		2	2	3
Missing MRIs ^d		5/80	6/80	0/16

MS: multiple sclerosis; MRI: magnetic resonance imaging; TSO: *Trichuris suis*; EDSS: Expanded Disability Status Scale.

^aAll gastrointestinal symptoms were considered TSO treatment-related. The duration of symptoms was 1 week or less in seven subjects. In subject 110, grade 2 symptoms were intermittent for 30 days; and in subject 111, grade 2 symptoms were intermittent or recurrent for 75 days.

^bNon-gastrointestinal symptoms were all grade 1, transient, and were considered not clearly related to TSO treatment.

^cA relapse was defined as new symptoms noted by the subject, in the absence of fever, infection, or other factor contributing to a pseudoattack, in the presence of objective signs on neurological examination corresponding to the new symptoms, with a duration of 24 hours or greater, and an increase in EDSS of at least one point in the total scale or two points in the relevant functional system subscale. Relapses by subject number and study month of relapse were: observation (subjects 118, O4; 216, O4), treatment (subjects 212, T1; 118, T8), and post-treatment (subjects 115, P1; 218, P2; 125, P4).

^dMissing MRI scans: in total, per protocol, 176 brain MRIs with contrast were planned. In the table, the number of missing scans is shown in the numerator and the planned scans in the denominator for each study period. The most common reasons for a missed scan were failure to obtain IV access, subject transportation, and MRI machine non-operative. In HINT 2 all study data, including MRI scans, were included for all subjects, regardless of adherence to protocol.

(Figure 2). Prior to the investigations, it was not known whether ingestion of the polyethylene glycol (PEG) colonic preparation solution would dislodge *T. suis* larvae from the colonic epithelium; for this reason and in order to maintain any putative therapeutic TSO effect at month T₁₀, the colonoscopies on the two MS-TSO subjects were delayed until approximately 3–4 weeks after the last day of ova administration during month T₁₀. In colonoscopies performed on the two subjects who had received TSO, robust, live larvae were observed in subject 122 (Figure 2) but not in subject 125; it is not clear whether the difference in larval detection was due to PEG preparation, colonoscopy timing, or other factors. No AE beyond transient,

expected discomfort and sedation were encountered during or following colonoscopies.

No neurological AE were noted during clinical assessments and MRI over-reads in real time, apart from typical MS symptoms, signs, and lesions. There were seven relapses of MS during the study: two relapses occurred before TSO treatment, two during TSO treatment, and three after TSO treatment (Table 2); one subject required corticosteroid treatment during their relapse. After relapse, all subjects regained baseline function. Apart from transient and reversible alterations in the masked assessments during relapses noted above, there was no significant change in clinical disability during

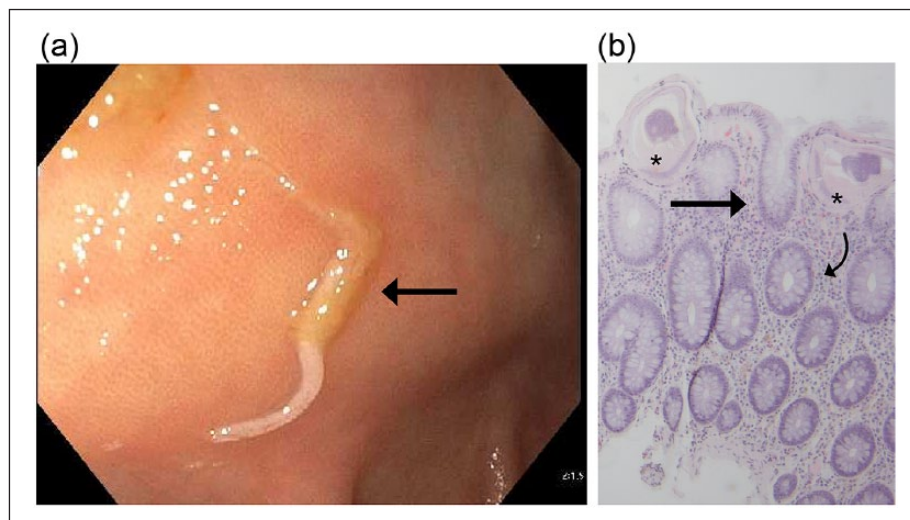


Figure 2. Colonoscopy. Representative images are from subject 122 at month T₁₀ of TSO treatment. (a) Colonic mucosa with live, active *T. suis* larva with its anterior end embedded in superficial colonic epithelium (arrow) and posterior end free in lumen of colon. (b) Colonic biopsy demonstrating *T. suis* larvae in cross section (stars), normal-appearing colonic crypt (straight arrow), and normal-appearing lamina propria (curved arrow) ($\times 100$). The anterior end of the worm has burrowed into the superficial epithelium near the crest of the colonic crypt and is covered by a thin layer consisting of the apical surfaces of epithelial cells.³² These photographs have not been published previously but were taken from the same subject reported on previously,⁸ with permission (Wiley).

the clinical trial; for example, the EDSS mean and standard deviation was 1.3 ± 0.9 at study entry (month S₁) and 1.1 ± 1.3 at the last treatment visit (month T₁₀) (non-significant difference, *t*-test). The corresponding MSFC values were 0.52 ± 0.34 at month S₁ and 0.86 ± 0.28 at month T₁₀ (*p*-value 0.007, *t*-test). These results likely reflect the demographics of our study participants, who had declined conventional DMT, were relatively young, and whose MS was non-disabling and of short duration.

Laboratory and immunological assessments

At the indicated laboratory time points (Figure 1), no abnormality was noted in any subject during assessment of complete blood count, liver and renal function, and pregnancy screening, except for increased blood eosinophils and positive *T. suis* serology during TSO treatment (Figure 3). All subjects had evidence of *T. suis* infection as manifest by at least a twofold increase in blood eosinophils relative to baseline and/or specific antibody for *T. suis*. Interestingly, the peak in blood eosinophils was most prominent during the initial stages of TSO treatment, corresponding to the period in which gastrointestinal symptoms were most common, while the peak of antibody response to *T. suis* occurred at month T₁₀ of treatment. Ova or parasites were not detected in any stool sample. An

increase in peripheral blood lymphocytes with T regulatory phenotype (FOXP3⁺, CD25⁺, CD127⁻, CD4⁺) occurred during treatment and extended into the post-treatment observation period (Figure 4).

MRI assessments

For the entire cohort, the mean number of GdE per MRI scan during the observation period was 3.25; during the treatment period, the mean number of GdE per MRI scan was 2.10. The relative reduction in the group MRI activity when the observation period was compared to the treatment period was 35%; however, this trend failed to meet statistical significance for the study primary endpoint (*p*-value 0.08). Additionally, since in the BVT design of HINT 2 each subject served as his or her control, we also analyzed the MRI changes for each subject for BVT. As expected, there was wide variability in MRI activity at the level of individuals (Figure 5). Although the magnitude of change, expressed as the observation-treatment difference in mean number of GdE per MRI scan, was small in many subjects, 12 of 16 subjects experienced an absolute diminution of GdE during TSO treatment.

Discussion

During the HINT 2 clinical trial, live ova from the porcine whipworm, *T. suis*, were administered to 16

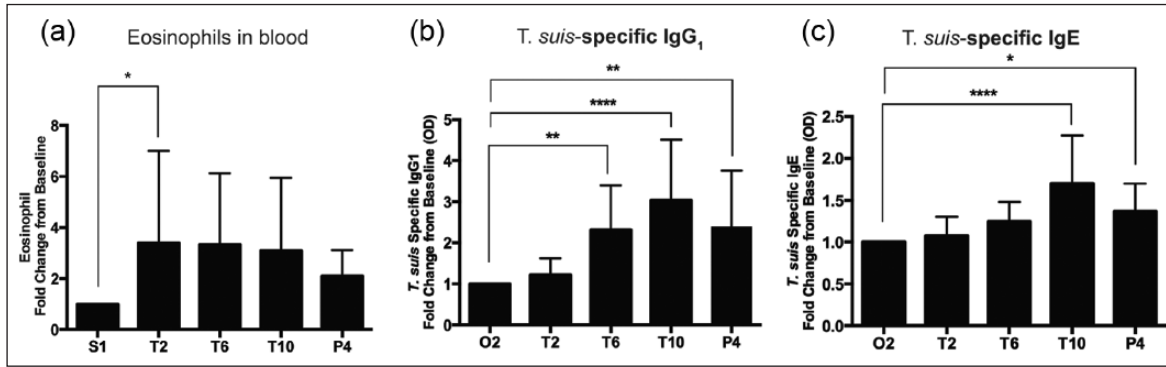


Figure 3. Blood eosinophils and humoral responses to *T. suis*. (a) Fold change in blood eosinophils expressed relative to subjects' pretreatment baseline determination. At entry, all HINT 2 subjects had normal absolute blood eosinophil determinations (30–500 cells/ μ L); during treatment, five subjects experienced absolute eosinophilia (range 600–1230 eosinophils per μ L of blood). (b) *Trichuris suis*-specific serum IgG₁ expressed as fold change relative to baseline determination in optical density units (OD, 465 nm). (c) *Trichuris suis*-specific serum IgE expressed as fold change relative to baseline determination in optical density units (OD, 465 nm). The baseline optical density unit determinations for IgG₁ and IgE ELISA assays in HINT 2 subjects were indistinguishable from those of control healthy blood donors and thus represent the presumed non-specific background of the assay. The data are represented by mean (histogram height) and one standard deviation (bar) and were analyzed by ordinary one-way ANOVA with values at month S₁ as the base for comparison, using GraphPad PRISM version 6.0. Data are not significant unless otherwise stated; significance values are indicated as * p -value ≤ 0.05 , ** p -value ≤ 0.01 , *** p -value ≤ 0.001 , and **** p -value ≤ 0.0001 .

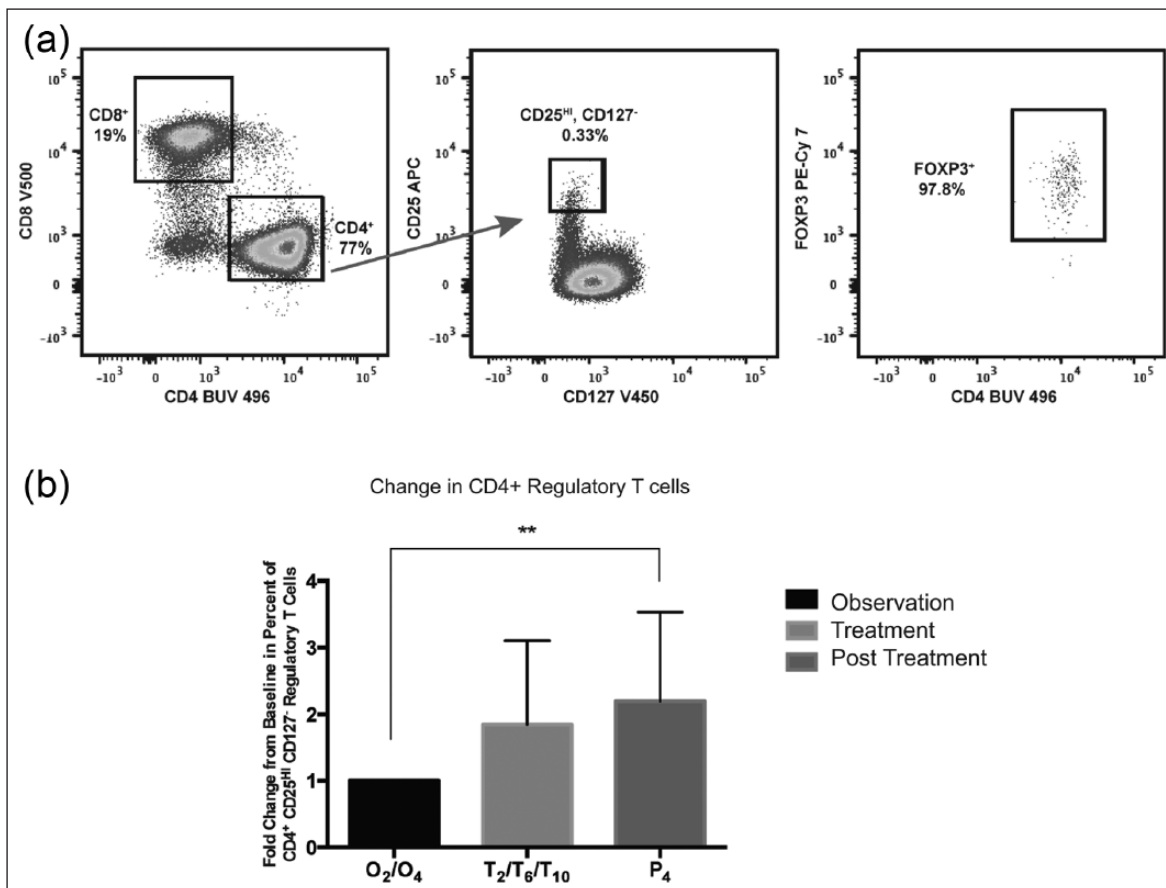


Figure 4. Blood T regulatory cells. (a) Gating strategy during flow cytometry for FOXP3⁺, CD25⁺, CD127⁻, CD4⁺ T cells. (b) The change in cells with a T regulatory phenotype, relative to baseline determinations. Antibodies employed are listed in Supplementary Material. Data and p -values are analyzed and presented as in Figure 3.

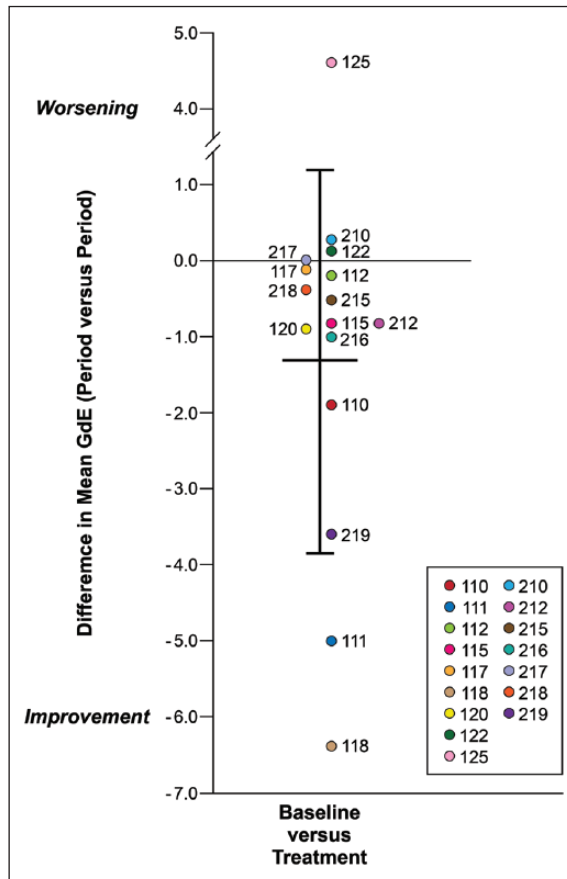


Figure 5. Change in brain MRI activity for individual subjects between baseline and treatment study periods. For each subject, the mean number of GdE on masked readings of double-dose gadolinium-enhanced brain MRI were determined for their (1) baseline period (the set of five scans for months S₁, O₁, O₂, O₃, O₄) and (2) treatment period (the set of five scans for months T₆, T₇, T₈, T₉, T₁₀). Subsequently, the difference between mean GdE at baseline and mean GdE during treatment (BVT difference) was determined for each subject. Determinations for each individual are indicated by a dot and are expressed such that a positive BVT difference indicates worsening (i.e. more GdE during treatment) and a negative BVT difference value indicates improvement (i.e. less GdE during treatment). Each dot represents the data from 10 MRI scans for an individual, as designated by color and subject number in the key.

RRMS subjects in order to assess the safety of this agent and its effects on clinical and MRI outcomes. Following oral administration of TSO, larvae hatched and established a transient infection of the superficial colonic mucosa in these subjects. This is consistent with prior experimental studies of whipworms in animals and humans.^{32–34} Since *T. suis* is not a human pathogen and does not establish a persistent colonization or produce progeny worms in the human host, it was necessary to administer ova

every 2 weeks during the treatment period in order to maintain continuous larval whipworm infection of subjects' colonic mucosa.

There were three principal findings in our study. First, *T. suis* larvae were able to efficiently colonize the colonic mucosa, as evidenced by serum antibody responses to *T. suis* and/or increased eosinophil counts in all subjects. Second, administration of TSO was safe in RRMS subjects, as evidenced by the absence of serious clinical AE, stable neurological function, lack of laboratory evidence of systemic toxicity, and normal colonic histology. In terms of tolerability, transient gastrointestinal symptoms occurred in 9 of the 16 subjects, usually shortly after treatment initiation; however, these symptoms were not of sufficient intensity or duration to interfere with activities of daily living such as school or work. Third, serial neuroimaging studies of the cohort demonstrated a trend for a 35% relative decrease (p -value=0.08) in the mean number of GdE per scan when MRI results before and during TSO treatment were compared. Additionally, since HINT 2 was based on a BVT design, in which each subject served as his or her own control, the MRI data were also analyzed at the level of each individual's experience, that is, the difference in MRI activity for a given subject when baseline assessments were compared to treatment assessments. This analysis showed diminution or stability in mean GdE in 12 of 16 subjects during treatment. Currently, it is not known whether the observed individual differences in MRI activity were due to subject-specific responses to TSO, variability in individual MS natural histories, or chance.

HINT 2 did not demonstrate the very dramatic clinical and MRI improvements in RRMS activity found during an observational study of pathogenic human helminth infections of MS patients in the field;¹⁹ nor did HINT 2 confirm results of an exploratory phase 1a clinical trial of TSO treatment in RRMS which showed no signals of clinical or immunological benefit.²³ The disparities between HINT 2 and previous studies may reflect differences in study design between observational investigations and BVT-controlled clinical trials, numbers or types of subjects, confounding effects of concurrent DMT in some studies, duration of helminth infection, MRI methodology, different biologies of porcine and human helminths, differential epigenetic programming in subjects raised in regions with and without endemic parasitism,³⁵ and other factors.^{13,14,36}

There are several limitations to our study. First, the demographic features of the HINT 2 subjects with early RRMS may have limited our ability to detect

substantial changes in clinical or MRI outcomes after TSO treatment. Second, while the critically important considerations of the dose, frequency, and duration of TSO treatment were based on the best animal and human experimental data available at the time of study design reviewed in Fleming and Weinstock,⁸ Summers *et al.*,²⁶ and Bundy and Cooper,³⁴ we cannot be certain that the conditions of TSO administration were in fact optimal. In several recent clinical trials for inflammatory bowel disease, for example, TSO has been administered at single or repeated doses of 7500 ova without serious AE.^{8,37} Third, it is possible that our MRI results represent regression to the mean. Nevertheless, we believe that regression to the mean during HINT 2 is unlikely since findings of the Miller Taskforce²⁴ and experience with subjects receiving placebo during similar RRMS exploratory trials at our centers suggest that regression to the mean is likely to be minimal or absent when the observation run in period is at least 4–6 months. Additionally, limited post-treatment MRI scans (1–2 studies per subject) showed either no change or an increase in GdE in 9 of 16 subjects (data not shown), suggestive of either stability or mild rebound after TSO cessation in many subjects, rather than continued regression toward a hypothetical natural mean. Fourth, the primary outcome measure, reduction in GdE for baseline versus treatment for the cohort ($p=0.08$), did not reach the conventional standard for statistical significance ($p\leq 0.05$). However, consideration of the totality of our findings nonetheless suggests that there is a genuine but modest trend supporting positive effects of TSO; these findings include the preponderance of favorable MRI responses at the level of individual subjects (Figure 5), an increase in peripheral blood lymphocytes with a T regulatory phenotype (Figure 4(b), $p\leq 0.0001$), evidence of a modified Th2 immune response, and favorable changes in stool microbiota (manuscript in preparation).

In conclusion, the results of the HINT 2 clinical trial, in contrast to earlier, smaller exploratory studies, suggest that TSO treatment may be associated with a trend for protective neuroimaging and immunological outcomes, although these effects were of modest degree and varied considerably among individual subjects. On the other hand, the findings of HINT 2 also indicate that a dramatic improvement of MS, as reported in prior observational studies of natural helminth infection in the field, is highly unlikely, at least for TSO at the doses currently employed. Certainly, we agree with McKay that every “parasite survives at some cost to the health and/or nutritional status of the host”;³⁸ for this reason, treatment of

humans with helminths should not be performed outside of regulated clinical trials. In this regard, recent studies with purified and recombinant molecules derived from helminths have demonstrated profound immunoregulatory effects in animal models *in vivo* and in human T cells *in vitro*.^{9,11} Future research with helminth-derived therapeutics may hold considerable promise.

Acknowledgements

The authors gratefully acknowledge the assistance of Andrea Maser, Namita Azad, Courtney Maxcy, Christopher Roginski, and Pamela Mundt for clinical coordination of the study; Amy Rettammel and Tina Graber for regulatory assistance; Jennifer Becker and Carol Dizack for illustration preparation; Dr David Elliott for assays of TSO embryonation and viability; Dr Derek Hei, Lisa Byrne, and colleagues at Waisman Biomanufacturing Center for direction and assistance with post-production testing of TSO; Dr Freddy Caldera, Dr Ben Hall, and Dr Melissa Harris for colonoscopy procedures; Drs Paul Rutecki (Chair, Neurology), Daniel Muller (Immunology), Lindel Gentry (Neuroradiology), Michael Lucey (Gastroenterology), Andrew Urban (Infectious Diseases), Patricia O’Looney (National Multiple Sclerosis Society), and Bruce Bebo (National Multiple Sclerosis Society) for serving on the DSMB; Drs Joseph Urban and Delores Hill for providing *T. suis* ESP antigen for ELISA testing, and Drs Michael Carrithers and Simon Oh for neurological examinations; Dr Howard Rowley for over-reads of MRI scans; Kelli Hellibrand for patient coordination during MRI scans; Barbara Dziuk for accounting assistance; and Lisa Ortiz for secretarial assistance. The advice and support of Drs Robert Summers, Joel Weinstock, and David Elliott at the University of Iowa and Dr Stephen Reingold at the time of study design are most appreciated. ClinicalTrials.gov: NCT00645749.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The HINT 2 study was supported by National Multiple Sclerosis Society (research grant RG 36113A/1 to J.F.), NIH (grant number 1 R56AI091462-01 to Z.F.), and intramural funds from the Neurology Departments of the University of Wisconsin and Marshfield Clinic.

References

1. Rook GA. Hygiene hypothesis and autoimmune diseases. *Clin Rev Allergy Immunol* 2012; 42: 5–15.
2. Stiemsma LT, Reynolds LA, Turvey SE, et al. The hygiene hypothesis: Current perspectives and future therapies. *Immunotargets Ther* 2015; 4: 143–157.
3. Maizels RM. Parasitic helminth infections and the control of human allergic and autoimmune disorders. *Clin Microbiol Infect* 2016; 22: 481–486.
4. Weinstock JV. Do we need worms to promote immune health? *Clin Rev Allergy Immunol* 2015; 49: 227–231.
5. Wammes LJ, Mpairwe H, Elliott AM, et al. Helminth therapy or elimination: Epidemiological, immunological, and clinical considerations. *Lancet Infect Dis* 2014; 14: 1150–1162.
6. Fumagalli M, Pozzoli U, Cagliani R, et al. Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions. *J Exp Med* 2009; 206: 1395–1408.
7. Maizels RM, Balic A, Gomez-Escobar N, et al. Helminth parasites—Masters of regulation. *Immunol Rev* 2004; 201: 89–116.
8. Fleming JO and Weinstock JV. Clinical trials of helminth therapy in autoimmune diseases: Rationale and findings. *Parasite Immunol* 2015; 37: 277–292.
9. Lopes F, Matisz C, Reyes JL, et al. Helminth regulation of immunity: A three-pronged approach to treat colitis. *Inflamm Bowel Dis* 2016; 22: 2499–2512.
10. Matisz CE, Faz-Lopez B, Thomson E, et al. Suppression of colitis by adoptive transfer of helminth antigen-treated dendritic cells requires interleukin-4 receptor- α signaling. *Sci Rep* 2017; 7: 40631.
11. Navarro S, Pickering DA, Ferreira IB, et al. Hookworm recombinant protein promotes regulatory T cell responses that suppress experimental asthma. *Sci Transl Med* 2016; 8: 362ra143.
12. Pineda MA, Eason RJ, Harnett MM, et al. From the worm to the pill, the parasitic worm product ES-62 raises new horizons in the treatment of rheumatoid arthritis. *Lupus* 2015; 24: 400–411.
13. Smallwood TB, Giacomini PR, Loukas A, et al. Helminth immunomodulation in autoimmune disease. *Front Immunol* 2017; 8: 453.
14. Briggs N, Weatherhead J, Sastry KJ, et al. The hygiene hypothesis and its inconvenient truths about helminth infections. *PloS Negl Trop Dis* 2016; 10: e0004944.
15. Wammes LJ, Hamid F, Wiria AE, et al. Community deworming alleviates geohelminth-induced immune hyporesponsiveness. *Proc Natl Acad Sci U S A* 2016; 113: 12526–12531.
16. Tahapary DL, de Ruiter K, Martin I, et al. Effect of anthelmintic treatment on insulin resistance: A cluster-randomized placebo-controlled trial in Indonesia. *Clin Infect Dis*. Epub ahead of print 3 May 2017. DOI: 10.1093/cid/cix416.
17. Leibowitz U, Antonovsky A, Medalie JM, et al. Epidemiological study of multiple sclerosis in Israel. II. Multiple sclerosis and level of sanitation. *J Neurol Neurosurg Psychiatry* 1966; 29: 60–68.
18. Fleming JO and Cook TD. Multiple sclerosis and the hygiene hypothesis. *Neurology* 2006; 67: 2085–2086.
19. Correale J and Farez MF. Association between parasite infection and immune responses in multiple sclerosis. *Ann Neurol* 2007; 61: 97–108.
20. Correale J and Farez MF. The impact of parasite infections on the course of multiple sclerosis. *J Neuroimmunol* 2011; 233: 6–11.
21. Fleming JO, Isaak A, Lee JE, et al. Probiotic helminth administration in relapsing-remitting multiple sclerosis: A phase 1 study. *Mult Scler* 2011; 17: 743–754.
22. Benzel F, Erdur H, Kohler S, et al. Immune monitoring of *Trichuris suis* egg therapy in multiple sclerosis patients. *J Helminthol* 2012; 86: 339–347.
23. Voldsgaard A, Bager P, Garde E, et al. *Trichuris suis* ova therapy in relapsing multiple sclerosis is safe but without signals of beneficial effect. *Mult Scler* 2015; 21: 1723–1729.
24. Miller DH, Albert PS, Barkhof F, et al. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. *US National MS Society Task Force*. *Ann Neurol* 1996; 39: 6–16.
25. Sormani MP, Miller DH, Comi G, et al. Clinical trials of multiple sclerosis monitored with enhanced MRI: New sample size calculations based on large data sets. *J Neurol Neurosurg Psychiatry* 2001; 70: 494–499.
26. Summers RW, Elliott DE, Qadir K, et al. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. *Am J Gastroenterol* 2003; 98: 2034–2041.
27. Summers RW, Elliott DE, Urban JF, et al. *Trichuris suis* therapy for active ulcerative colitis: A randomized controlled trial. *Gastroenterology* 2005; 128: 825–832.
28. Bager P, Arnved J, Ronborg S, et al. *Trichuris suis* ova therapy for allergic rhinitis: A randomized, double-blind, placebo-controlled clinical trial. *J Allergy Clin Immunol* 2010; 125: 123–130.e1–3.
29. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010

- revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292–302.
30. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology* 1983; 33: 1444–1452.
 31. Cutter GR, Baier ML, Rudick RA, et al. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. *Brain* 1999; 122(Pt 5): 871–882.
 32. Tilney LG, Connelly PS, Guild GM, et al. Adaptation of a nematode parasite to living within the mammalian epithelium. *J Exp Zool A Comp Exp Biol* 2005; 303: 927–945.
 33. Elliott DE, Summers RW and Weinstock JV. Helminths and the modulation of mucosal inflammation. *Curr Opin Gastroenterol* 2005; 21: 51–58.
 34. Bundy DA and Cooper ES. Trichuris and trichuriasis in humans. *Adv Parasitol* 1989; 28: 107–173.
 35. Rook GA, Lowry CA and Raison CL. Hygiene and other early childhood influences on the subsequent function of the immune system. *Brain Res* 2015; 1617: 47–62.
 36. Evans H and Mitre E. Worms as therapeutic agents for allergy and asthma: Understanding why benefits in animal studies have not translated into clinical success. *J Allergy Clin Immunol* 2015; 135: 343–353.
 37. Sandborn WJ, Elliott DE, Weinstock J, et al. Randomised clinical trial: The safety and tolerability of *Trichuris suis* ova in patients with Crohn's disease. *Aliment Pharmacol Ther* 2013; 38: 255–263.
 38. McKay DM. The beneficial helminth parasite? *Parasitology* 2006; 132: 1–12.

Visit SAGE journals online
[journals.sagepub.com/
 home/msj](http://journals.sagepub.com/home/msj)

 SAGE journals