Received Date : 16-Mar-2016
Revised Date : 03-Oct-2016
Accepted Date : 07-Oct-2016
Article type : Original Paper

Mucosal and systemic immune modulation by *Trichuris trichiura* in a self-infected individual

Anders Dige[1], Tue Kruse Rasmussen[2,3], Peter Nejsum[4], Rikke Hagemann-Madsen[5], Andrew R.Williams[4], Jørgen Agnholt[1], Jens F Dahlerup[1], Christian L Hvas[1]

[1]Department of Hepatology and Gastroenterology, Aarhus University Hospital, 8000 Aarhus C, Denmark

[2]Department of Biomedicine, Aarhus University, 8000 Aarhus C, Denmark

[3]Department of Rheumatology, Aarhus University Hospital, 8000 Aarhus C, Denmark

[4]Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark

[5]Section for Pathology, Lillebaelt Hospital, 7100 Vejle, Denmark

Running title

Immune modulation from *Trichuris trichiura*

Keywords

Trichiura, trichiuris, helminths, immunology

Corresponding author

Anders Dige, MD PhD

Department of Hepatology and Gastroenterology

Aarhus University Hospital

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pim.12394

Noerrebrogade 44

DK-8000 Aarhus C

Denmark

E-mail: andedige@rm.dk (AD)

Disclosures

None

Abstract

Helminthic therapy of immune-mediated diseases has gained attention in recent years, but we know little of how helminths modulate human immunity. In this study, we investigated how self-infection with *Trichuris (T.) trichiura* in an adult man without intestinal disease affected mucosal and systemic immunity. Colonic mucosal biopsies were obtained at baseline, during *T. trichiura* infection, and after its clearance following mebendazole treatment. Unexpectedly, the volunteer experienced a *Campylobacter* colitis following *T. trichiura* clearance, and this served as a positive infectious control. *T. trichiura* colonisation induced equally increased expressions of T-helper (h)1-, Th2-, Th17-, and Treg- associated cytokines and transcription factors, measured by quantitative polymerase chain reaction. We observed several indicators of modulation of systemic immunity during the *T. trichiura* infection. Plasma eosinophils and anti-*Trichuris* antibodies rose markedly during the inoculation phase, and a shift towards a Th2-dominated T cell response at the expense of the Th1-response was observed in circulating T cells. Taken together, our findings corroborate that helminths modulate regional and systemic human immunity.

Introduction

The potential for helminthic therapy in organ-specific immune-mediated diseases has gained increased attention in recent years[1–4]. Autoimmune and allergic diseases are associated with an inappropriate immunological response towards auto-antigens or allergens. Because helminths escape immunological clearance through the induction of anti-inflammatory immunological responses, a helminthic infection could mediate beneficial immunomodulatory effects to the host during autoimmune and allergic conditions. Studies that have used animal models of inflammatory diseases have indicated helminthic infections induce regulatory dendritic cells, regulatory macrophages, and regulatory T cells[1,5,6]. Furthermore, helminths have been reported to suppress pro-inflammatory T-helper (Th)1-type responses and induce a modified Th2-type response[1,5,7,8].

Our understanding of the immunological effects induced by helminthic infections in humans is primarily based on studies of chronically infected individuals in endemic areas[2]. It has been shown that parasite-specific T cells from chronic helminthic infected individuals are unresponsive[9,10] possibly as a result of infection-induced regulatory FOXP3+ T cells[11,12]. Based on the findings in animal studies, it may be further anticipated that modulation of the adaptive Th-cell responses also occurs in human helminth infections[1,2,4,6,8].

Results from clinical studies that have investigated the effects of helminthic therapy in inflammatory and allergic conditions, such as allergic rhinitis[13], asthma[14], inflammatory bowel disease (IBD)[15,16], celiac disease[17], and multiple sclerosis (MS)[18], have been published, and additional studies are underway[2]. In IBD and MS, the parasite most frequently used is the pig whipworm, *Trichuris (T.) suis*, whereas asthma patients and patients with celiac disease have been infected with the hookworm *Necator americanus*[2].

Few clinical studies have investigated the immunological changes induced by helminths in autoimmune diseases. One study demonstrated helminth-infected MS patients had increased numbers of circulating regulatory T cells and reduced numbers of pro-inflammatory cytokine producing T cells compared with non-infected patients[19]. Indeed, anti-parasitic treatment was associated with a decrease in the numbers of regulatory T cells and an increase in the numbers of pro-inflammatory cytokine producing T cells[20]. In a single self-infected ulcerative colitis patient, the investigators observed increased numbers of mucosal IL-4 and IL-22 producing T cells following *T. trichiura* infection[21]. However, these studies did not enable the discrimination of changes associated with the helminth infection from disease-specific pathology. An increased understanding of how helminths modulate human immunity is warranted and could potentially lead to the development of new treatment strategies.

In this single-individual study, we aimed to gain insight into the immunomodulatory effects of *T. trichiura* in a self-infected volunteer without intestinal disease using living *T. trichiura* eggs. We investigated both regional and systemic effects by investigating changes in mucosal T-cell-associated cytokine production and circulating T cell phenotypic characteristics. The volunteer unexpectedly acquired a *Campylobacter* infectious colitis after mebendazole-induced clearance of the helminth infection and prior to the follow-up colonoscopy. Therefore, this colonoscopy served as a positive control for mucosal inflammation.

Methods

Study subject and design

The included volunteer was a 38 years old male with no prior history of intestinal diseases but with mild untreated psoriasis. He was 1.92 m tall and maintained a constant body weight of 71 kg throughout the study period. We aimed to induce a moderate *T. trichiura* infection in the volunteer (i.e. 5,000 eggs per gram of faeces according to World Health Organization[22]). We estimated the volunteer´s daily faecal production to 250 grams. Based on a calculation assuming that 80% of the

ingested eggs would hatch and a sex ratio of 1:1 we estimated that a dose of 600 eggs would result in a moderate infection. Because we, based on the lacking fecal egg excretion 9 weeks after the initial egg dose and slight decrease in eosinophils, doubted that the volunteer was infected with *T. trichiura* he voluntarily ingested an additional 3 small doses of eggs. Repeated dosing was used in this second egg ingestion to mimic "natural infection" as we expected that this would result in a higher establishment rate. The volunteer underwent ileo-colonoscopies prior to self-infection with *T. trichiura* eggs, during the established infection, following the clearance of the *T. trichiura*, at which time point the stool cultures were positive for *Campylobacter jejuni*, and following the clearance of this infection. Biopsies at all ileo-colonoscopies were obtained systematically from 4 locations: the terminal ileum, the caecum/ascending colon, the transverse colon and the sigmoid colon. The volunteer was monitored clinically every week for the development of diarrhea, weight loss and anemia. During the study period, blood and faecal samples were obtained every second week. Biochemical parameters (i.e., C-reactive protein, total and differential leukocyte counts, and haemoglobin) were monitored at the times of blood sampling.

Identification and estimation of *T. trichiura* eggs

Intestinal colonisation with adult *T. trichiura* was verified by visual identification of eggs using a dissecting microscope. Eggs in the faeces were identified, and the numbers were estimated using the concentrated McMaster method with an analytical sensitivity of 20 eggs per gram (epg) of faeces as described[23]. Eight expelled worms (see below) were identified as *T. trichiura* by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the Internal Transcribed Spacer 2 region, which has been previously reported[24] and by sequence analysis of 435 base pairs (bp) of the 18S region (rDNA) followed by a BLAST search (GenBank) (data not shown).

Video capsule enteroscopy

The small intestine was visualised by video capsule enteroscopy (PillCam SB, Given Imaging, Yoqneam, Israel) 2 days prior to the ileo-colonoscopies.

Faecal calprotectin

Faecal samples were analysed for faecal calprotectin using a calprotectin ELISA according to the manufacturer's instructions (Bühlmann Laboratories, Schönenbuch, Switzerland).

Reverse transcriptase PCR (RT-PCR) for mucosal cytokines

RT-PCR was performed to quantify the expression levels of IL-4, IL-10, IL-17A, IL-22, TGF-β, IFN- γ, GATA3, FoxP3, and RORC in messenger (m) RNA purified from the intestinal biopsies obtained from the volunteer. In brief, the biopsies were homogenised in TRIzol (Life Technologies), and mRNA was isolated using chloroform and isopropanol. Messenger RNA was pelleted by washing in methanol and diluted in RNase free water. The samples were DNase treated using a DNA-free kit (Ambion, Austin, Texas, United States, cat. no. AM1906) according to the manufacturer's instructions. The concentration and purity of the RNA were determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific), and the RNA was diluted to a concentration less than 50 ng/µL. The samples were stored at -80°C until use. Predesigned primer and probe sets for IL-4, IL-10, IL-17A, IL-22, TGF-β, IFN-γ, GATA3, FoxP3, RORC, GAPDH and ACTB (Life Technologies, cat. no. Hs00174122, Hs00961622, Hs00174383, Hs01574154, Hs00932747, Hs00989291, Hs00231122, Hs01085834, Hs01076122, Hs02758991, and Hs01060665, respectively) labelled with the FAM-BHQ system as a fluorescence/quencher were used. RT-PCR was performed using the TaqMan RNA-to-CT 1-Step Kit (Life Technologies, cat. no. 4392938) on an MX3000 RQ-PCR machine (Stratagene) with the following cycle parameters: 15 minutes at 48°C, 10 minutes at 95°C and 40 cycles of 15 seconds at 95°C and 1 minute at 60°C.

For all experiments, the individual samples were run in duplicate with a maximum acceptable inter-replicate difference of 1 cycle. The fold change was calculated using the ΔCt method and expressed relative to the geometric means of control housekeeping genes (GAPDH and ACTB).

Peripheral blood mononuclear cell isolation and flow cytometric analysis of intracellular cytokines and FOXP3 expression in circulating T cells

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque (GE Healthcare Bio-Sciences, Uppsala, Sweden) and stored at -140°C. Prior to staining, the PBMCs were thawed and washed twice with phosphate-buffered-saline (PBS) with 20% heat-inactivated pooled human AB serum. Staining for intracellular cytokines in the PBMCs was performed as previously described[25]. In brief, the PBMCs were surface-stained with anti-CD45RO FITC (BD, cat.no. 555492), anti-CD3 PerCP (BD, cat.no. 345766), anti-CD8-PE-Cy7 (BD, cat.no. 557746), and anti-CD4 APC-Cy7 (BD, cat.no. 557871) prior to fixation, permeabilisation and staining with anti-IL17A Alexa-647 (eBiosciences, cat.no. 51-7179-42), anti-IL-4 PE (eBiosciences, cat.no. 12-7049-42), anti-IL22 PE (R&D, cat. no. IC7821P) and anti-IFN-γ APC (eBiosciences, cat. no. 17-7319-82). In parallel, the PBMCs were surface-stained with anti-CD3 PerCP (BD, cat.no. 345766), anti-CD25 PE-Cy7 (BD, cat.no. 557741), and anti-CD4 APC-Cy7 (BD, cat.no. 557871) prior to staining for intracellular FOXP3 FITC as previously described[26]. After staining, the cells were fixed in 250 µl PBS with 1% formaldehyde. Flow cytometry was performed within 24 hours using a FACS Canto analyser (BD Biosciences), and 10^5 events in the forward-side scatter lymphogate were recorded. The combination of forward-scatter-height and forward-scatter-amplitude was used to exclude events without single cell appearances. The gating for IL-17A, IL-22, γ-interferon and IL-4 positive events was based on isotype controls. The

gating of FOXP3 positive events was based on a biological control as previously described, which used the CD4+CD3+ T cells with the lowest CD25 expression (lowest quartile) as "non-FOXP3" expressing cells as previously recommended[27]. All flow cytometry data were analysed using FACS Diva 5.1 software (BD Biosciences).

Microscopic examination of intestinal biopsies

Forceps biopsies, two per bowel segment, were placed on Millipore paper and routinely prepared. All biopsies were fixed for approximately 24 hours in 10% neutral buffered formalin; biopsies were then paraffin embedded and cut in 3 µm thin serial sections, with 3 sections from each block. Each slide that contained 3 serial sections was stained with haematoxylin and eosin for evaluation of eosinophil infiltration. Ileac biopsies were also stained with Alcian Blue-PAS for the visualisation of mucin droplets.

Measurement of anti-*Trichuris* antibodies in serum

Anti-*Trichuris* antibodies was measured using ELISA. *Trichuris suis* excretory/secretory (ES) material was produced by *in vitro* culture of adult worms collected from the caecum and colon of experimentally infected pigs, essentially as described[28], and protein content of ES products was measured by the BCA assay (Pierce). ELISA plates (Nunc Maxisorb) were coated overnight at 4°C with 5 µg/mL of ES products in carbonate/bicarbonate buffer (pH 9.6). Plates were blocked with 1% BSA in PBS, before the addition of sera and then detection antibodies: goat anti-human IgA-HRP conjugate (Sigma-Aldrich) or mouse anti-human IgG_1 (Sigma Aldrich; clone 8c/6-39) followed by goat anti-mouse IgG-HRP conjugate (Abd Serotec). All incubation steps were for 1 hour at 37°C and plates were washed 5 times between incubations with PBS containing 0.02% Tween 20. Plates were developed with TMB substrate and the reaction stopped with 0.2M H_2SO_4. Plates were read at 450 nm with a Multiskan FC plate reader (ThermoFisher). Titres were calculated as the inverse of the dilution which gave an OD value at least 3 standard deviations above the mean of the negative control (pooled naïve serum).

Ethical statement

The volunteer is a researcher within parasitology with main focus on *T. trichiura* and *T. suis*. Before the study, he signed a declaration of informed consent.

The Central Denmark Region Committees on Health Research Ethics confirmed that the study, according to the Danish legislation, did not need a formal notification to the Ethics Committee. The study was registered at www.Clinicaltrials.gov (ClinicalTrials no. NCT02399683). Infection of pigs for *T. suis* material was approved and conducted according to the guidelines of the Danish Experimental Animal Inspectorate (Licence number 2015−15−0201−00760).

Results

Clinical course of self-infection with *T. trichiura*

Colonisation with *T. trichiura* following self-infection was monitored by faecal egg counts. Because no eggs were observed during faecal microscopy 9 weeks following the first inoculation with 600 *T. trichiura* eggs, the volunteer ingested an additional 650 eggs in the following 4 weeks (350 eggs in week 9, 200 eggs in week 10, and 100 eggs in week 12) . Four weeks after this second inoculation, a faecal egg count of 100 epg was observed, and this count increased rapidly to 7,620 epg 3 weeks later (Figure 1A). Following a colonoscopy during which the presence of adult worms was restricted to the caecum/ascending colon and the transverse colon, the volunteer received mebendazole, after which the egg numbers decreased rapidly to 300 eggs at one week post-treatment. Because low drug efficacy has been reported for *T. trichiura*[29], a dose regimen of 100 mg mebendazole twice daily for 5 consecutive days was used, which was an effective treatment. Two weeks following the mebendazole treatment, the faecal samples were negative and remained negative for the remainder of the study period.

Serum IgA and IgG_1 anti-*Trichuris* antibody levels were below detection level before the *T. trichiura* infection and rose markedly at the time for established the *T. trichiura* infection. Antibody levels dropped after the infection but remained both just above the detection level for the rest of the study period (Figure 1B).

Capsule enteroscopy of the small intestine was normal with no visible worms in any investigation.

Circulating eosinophils began to increase 4 weeks following the first inoculation. They continued to increase during colonisation, but they began to decrease when the eggs became apparent in the stool samples and normalised after the mebendazole treatment (Figure 2). Microscopic examination of the ileal and colonic biopsies obtained during the *T. trichiura* infection all revealed a lightly to moderately inflamed mucosa. The inflammatory cells represented both acute and chronic inflammation, but were dominated by eosinophils that were predominantly localised superficially in the lamina propria. The infiltration was much more pronounced in the regions colonized with *T.trichiura*, i.e., the caecum/ascending and transverse colon, with cryptitis and a slight disruption of single crypts (Figure 3). No eosinophils was observed in the biopsies obtained before the *T.trichiura* infection. Faecal calprotectin levels remained normal during the *T. trichiura* infection (Figure 2). The volunteer did not experience intestinal symptoms during the *T. trichiura* infection.

One week prior to the scheduled follow-up ileo-colonoscopy, the volunteer developed abdominal discomfort. A colonoscopy revealed redness, oedema and small ulcers in the entire colon and terminal ileum. Examination of the intestinal biopsies revealed signs of acute and chronic inflammation in all investigated colonic regions and the terminal ileum, but no eosinophil infiltration.

Faecal cultures proved positive for *Campylobacter* species. During this *Campylobacter* colitis, we observed a marked increase in the faecal calprotectin to 830 mg/kg (Figure 2). The symptoms and faecal calprotectin levels normalised spontaneously during the following week. At the ileo-colonoscopy 4 weeks later, the colon and terminal ileum were normal.

T. trichiura induces a regional mucosal cytokine response

We used quantitative PCR (qPCR) analysis of intestinal mucosal biopsies to investigate the mucosal T cell responses to the *T. trichiura* infection. We aimed to elucidate whether the *T. trichiura* infection was associated with a subset-specific T cell response by examining representative genes from the different T cell subsets, i.e., the Th-1-, Th2-, Th17- and Treg cells, in the qPCR analysis (Table 1). We determined that *T. trichiura* induced a pronounced increase in the expression of all investigated T cell subset-representative genes (Figure 4). The increased expression was restricted to the colonic regions colonised with *T. trichiura* during the colonoscopy, i.e., the caecum/ascending and transverse colon (Figure 4). The Th1, Th2, Th17, and Treg subset-representative genes were all similarly increased with corresponding increases in the subset-representative transcription factors and their respective cytokines (Figure 4).

The expression of most investigated T cell subset-representative genes was down regulated during the *Campylobacter* infection in the colonic regions in which the *T. trichiura* had been resident, although the expression remained increased compared with the baseline. The expression of IL-21 and T-bet increased further during the *Campylobacter* colitis, although the increase in T-bet was only modest (Figure 4). In the colonic regions where *T. trichiura* was absent (i.e., the ileum and descending colon), we observed increased expression of IL-17A, IL-21, IFN-γ, IL-10 and TGF-β during the *Campylobacter* infection compared with baseline levels. The expression of the remaining investigated T cell subset-representative genes was unchanged during the *Campylobacter* infection in the regions in which *T. trichiura* had not been resident (Figure 4). The levels of the investigated T cell subset-representative genes returned to baseline at the 4-week follow-up after the patient recovered from the *Campylobacter* colitis (Figure 4).

T. trichiura modulates systemic immunity

To investigate whether the *T. trichiura* colonisation-induced modulation of the mucosal T cell response was also reflected in systemic immunity, the intracellular cytokine production in circulating T cells was analysed (Figure 5). Because the main production of the selected cytokines is identified in activated memory T cells, CD45RO was used to enrich for these cells. We observed a more than two-fold increase in the frequency of circulating IL-4 producing CD4+CD45RO+ T cells and more than a 30% increase (from 2.8% to 3.7%) in the numbers of IL-22 producing CD4+CD45RO+ T cells during the *T. trichiura* infection compared with the baseline levels (Figure 5). The number of IFN-γ-producing CD4+CD45RO+ T cells decreased with 33 % during the infection (from 10.6% to 7.1 %). There were no clear changes in the frequencies of IL-17A-producing CD4+CD45RO+ T cells (range

0.2-0.6%) (Figure 5) or CD4+FOXP3+ Tregs (range 3.8-4.2%) (data not shown). The frequencies of IL-4-, IL-22-, and γ-interferon-producing CD4+CD45RO+ T cells decreased during and following the *Campylobacter* colitis (Figure 5).

Discussion

This study describes the successful self-infection with *T. trichiura* in a volunteer without intestinal disease, with concomitant monitoring of the mucosal and systemic T cell-specific immune responses. Our study is unique in that the unexpected *Campylobacter* colitis, which was present at the follow-up colonoscopy, provided a positive *in vivo* control for intestinal inflammation. The main findings included a marked regional increase in mucosal gene expression of all investigated T cell-subtype specific cytokines and a modulation of systemic immunity demonstrated by eosinophilia, the formation of anti-Trichuris antibodies and change in the circulating CD4+CD45RO+ T cell cytokine production.

The present study is the first to investigate changes in intestinal T cell cytokine production in an intestinally healthy individual during *T. trichiura* infection. A similar study from a single ulcerative colitis patient who was self-infected with *T. trichiura* has been published[21]. However, because the study was conducted in a patient with active ulcerative colitis, it was not possible to clarify whether the immunological observations reflected helminth-induced modulation of immunity or coincidental changes in disease activity. Previous studies of immunological changes during helminthic infection have primarily described chronic infections in parts of the world where helminthic infections are endemic[2]. These observations may not be applicable to patients in industrialised countries with no or very limited previous exposure to helminths. Indeed, the absence of exposure to helminths and other classes of microorganisms forms the basis for the hygiene hypothesis, which links the increase in autoimmune disease incidences to the decreased exposure to helminths[30].

Colonisation with *T. trichiura* was demonstrated 13 weeks after the initial egg ingestion. Because the precise pre-patency period of *T. trichiura* is unknown[31] and eosinophil levels began to fall, a second inoculation was performed at week 9. We find it unlikely that worms could have grown to maturity within 4 weeks (the second dose) and believe that the colonisation originated from the primary inoculation.

Based on the changes in the mucosal expression of T cell representative genes, we determined that infection with *T. trichiura* was associated with increased mucosal T cell activation. This increased T cell activation was paralleled by a mild to moderate inflammatory response dominated by eosinophil granulocytes, which was most pronounced in the regions colonised by *T. trichiura*. The increased activation of mucosal T cells was not mirrored by an increase in faecal calprotectin, which is a sensitive marker of intestinal inflammation[32]. The increased T cell activation was restricted to colonic regions where the *T. trichiura* was resident, i.e., the caecum/ascending and transverse colon. Interestingly, the mucosal T cell response to *T. trichiura* was not limited to a specific T cell subset because we observed a similarly increased expression profile of all investigated

T-cell subtype specific genes. Based on findings from animal models and studies in MS patients, one would have expected a more polarised Th2-like or regulatory T cell response[5,8,19,20]. In an ulcerative colitis patient who was self-infected with *T. trichiura*, an intestinal T cell response dominated by IL-4 and IL-22 producing T cells was observed[21]. In these studies, however, both disease-related and helminth-induced changes could have caused the described changes.

A systemic response towards *T. trichiura* was suggested already during the inoculation phase where circulating eosinophil numbers increased as the infection was established. Following the clearance of *T. trichiura*, the plasma eosinophils normalised. Changes in the circulating eosinophils could therefore represent a sensitive tool to monitor patients in future trials with *T. trichiura*. Another indication of systemic immunomodulation by *T. trichiura* was our finding of altered cytokine production in circulating T cells. The infection was associated with increased IL-4 and IL-22 productions. However, we also observed a decreased IFN-γ production in circulating T cells. The increased IL-4 and decreased IFN-γ production in circulating T cells suggest a selection of the Th2 response on the expense of the Th1 response in *T. trichiura* infected individuals. An increase in circulating Th2 cells from *T. trichiura* infection has also been reported in a single-patient report on self-infection in UC[21]. Our finding of decreased circulating Th1 cells following *T. trichiura* colonisation supports the principle of treating autoimmune related diseases with *T. trichiura* because these diseases are associated with the abnormal activation of Th1 cells. We did not observe an increase in the numbers of circulating FOXP3+ Tregs during *T. trichiura* infection, which has been previously reported in chronically helminthic infected MS patients[19].

The volunteer developed a *Campylobacter* colitis just after *T.trichiura* had been eradicated. Although we consider this an unexpected complication it may not be coincidential. Studies have shown that *T. suis* infections in pigs are associated with an increased abundancy of *Campylobacter* in the colon microflora, but also that co-infected animals more frequently develop diarrhea and that this is more severe in animals only infected with either *Campylobacter* or *T. suis*[33,34]. Furthermore, an *in vitro* study reported that secretory products from *T. suis* damaged epithelial cells, although it did not increase the cells susceptibility to *Campylobacter* infection[35]. Based on these findings, it has been speculated that *Trichuris* infections may modulate the course of human *Campylobacter* infections[36]. To our knowledge, only one case of concomitant *T. trichiura* and *Campylobacter* has been reported in humans. This individual presented with a very severe and complicated *Campylobacter/Trichuris* colitis[37]. Our observation highlights that *Campylobacter* and *Trichuris* infections may be associated in humans. We recommend that future studies applying *Trichuris* infections to humans to investigate patients' *Campylobacter* status, and any present *Campylobacter* should be considered eradicated before individuals are infected with *Trichuris*. Furthermore, our study indicates that any sign of intestinal inflammation, i.e., clinical symptoms or an increase in faecal calprotectin in a helminth-infected individuals, should lead to the suspicion of bacterial superinfection.

This study has clear limitations, the most prominent of which is that we only examined one study subject. To what extent the more specific immunological changes we observed in the included individual reflects the general response of human immunity during a *T. trichiura* infection is of course speculative and should be confirmed in larger studies. However, taking the

longitudinal study design in to consideration, our findings regarding changes of mucosal and systemic immunity, although only observed in one individual, demonstrate that immunity is modulated by a *T. trichiura* infection. Our investigation focused on adaptive immunity in the form of mucosal and circulating CD4+ T cells. The modulation of mucosal and systemic immunity during helminthic infection may include non-T cell responses, which have been demonstrated in animal studies[1,7]. Because we performed qPCR analysis on forcep biopsies, the increased cytokine expression associated with the *T. trichiura* infection may also reflect changes in other immune and epithelial cells. However, this does not alter our conclusion that these changes were not restricted to the response of a specific T cell subtype.

In conclusion, the present study demonstrates that *T. trichiura* infection induces a regional mucosal cytokine response that represents a general activation of all Th-subsets. Furthermore, the infection modulated systemic immunity via the induction of a Th2-like dominated response at the expense of a Th1-like response in circulating mononuclear cells. Taken together, our findings support that helminths modulate both regional and systemic human immunity. Immune modulation induced by helminths may play a role in the future treatment of autoimmune diseases.

References

1. Elliott DE, Weinstock JV. Helminth-host immunological interactions: prevention and control of immune-mediated diseases. Ann N Y Acad Sci 2012; 1247: 83-96.
2. Khan AR, Fallon PG. Helminth therapies: translating the unknown unknowns to known knowns. Int J Parasitol 2013; 43: 293-299.
3. Weinstock JV. Autoimmunity: The worm returns. Nature 2012; 491: 183-185.
4. Wolff MJ, Broadhurst MJ, Loke P. Helminthic therapy: improving mucosal barrier function. Trends Parasitol 2012; 28: 187-194.
5. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 2003; 3: 733-744.
6. McSorley HJ, Hewitson JP, Maizels RM. Immunomodulation by helminth parasites: defining mechanisms and mediators. Int J Parasitol 2013; 43: 301-310.
7. Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. Nat Rev Immunol 2011; 11: 375-388.
8. Taylor MD, van der Werf N, Maizels RM. T cells in helminth infection: the regulators and the regulated. Trends Immunol 2012; 33: 181-189.
9. Grogan JL, Kremsner PG, Deelder AM et al.. Antigen-specific proliferation and interferon-gamma and interleukin-5 production are down-regulated during Schistosoma haematobium infection. J Infect Dis 1998; 177: 1433-1437.

10. King CL, Mahanty S, Kumaraswami Vet al.. Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. J Clin Invest 1993; 92: 1667-1673.

11. Metenou S, Dembele B, Konate S et al.. At homeostasis filarial infections have expanded adaptive T regulatory but not classical Th2 cells. J Immunol 2010; 184: 5375-5382.

12. Nausch N, Midzi N, Mduluza T et al.. Regulatory and activated T cells in human Schistosoma haematobium infections. PLoS One 2011; 6: e16860.

13. 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.

14. Feary JR, Venn AJ, Mortimer K et al.. Experimental hookworm infection: a randomized placebo-controlled trial in asthma. Clin Exp Allergy 2010; 40: 299-306.

15. Summers RW, Elliott DE, Urban JF et al.. Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology 2005; 128: 825-832.

16. Summers RW, Elliott DE, Urban JF et al.. Trichuris suis therapy in Crohn's disease. Gut 2005; 54: 87-90.

17. Daveson AJ, Jones DM, Gaze S et al.. Effect of hookworm infection on wheat challenge in celiac disease--a randomised double-blinded placebo controlled trial. PLoS One 2011; 6: e17366.

18. 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.

19. Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol 2007; 61: 97-108.

20. Correale J, Farez MF. The impact of parasite infections on the course of multiple sclerosis. J Neuroimmunol 2011; 233: 6-11.

21. Broadhurst MJ, Leung JM, Kashyap V et al.. IL-22+ CD4+ T cells are associated with therapeutic trichuris trichiura infection in an ulcerative colitis patient. Sci Transl Med 2010; 2: 60ra88.

22. World Health Organization 2012 Soil-transmitted helminthiases: eliminating soil-transmitted helminthiasis as a public health problem in children: progress report 2001-2010 and strategic plan 2011-2020.

23. Food and Agriculture Organization of the United Nations: Epidemiology, diagnosis and control of helmith parasites of swine.

24. Nissen S, Al-Jubury A, Hansen TV et al.. Genetic analysis of Trichuris suis and Trichuris trichiura recovered from humans and pigs in a sympatric setting in Uganda. Vet Parasitol 2012; 188: 68-77.

25. Dige A, Stoy S, Rasmussen TK et al.. Increased levels of circulating Th17 cells in quiescent versus active Crohn's disease. J Crohns Colitis 2013; 7: 248-255.

26. Dige A, Hvas CL, Deleuran B et al.. Scand J Gastroenterol 2011; 46: 1206-1214.

27. Law JP, Hirschkorn DF, Owen RE et al.. The importance of Foxp3 antibody and fixation/permeabilization buffer combinations in identifying CD4+CD25+Foxp3+ regulatory T cells. Cytometry A 2009; 75: 1040-1050.

28. Kringel H, Roepstorff A. Trichuris suis excretory/secretory antigen-specific antibodies in serum from single-inoculated pigs. Parasite Immunol 2007; 29: 327-330.

29. Olsen A, Namwanje H, Nejsum P et al.. Albendazole and mebendazole have low efficacy against Trichuristrichiura in school-age children in Kabale District, Uganda. Trans R Soc Trop Med Hyg 2009; 103: 443-446.

30. Rook GA. Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. Immunology 2009; 126: 3-11.

31. Bundy DA, Cooper ES. Trichuris and trichuriasis in humans. Adv Parasitol 1989; 28: 107-173.

32. Sherwood RA. Faecal markers of gastrointestinal inflammation. J Clin Pathol 2012; 65: 981-985.

33. Mansfield LS, Gauthier DT, Abner SR et al.. Enhancement of disease and pathology by synergy of Trichuris suis and Campylobacter jejuni in the colon of immunologically naive swine. Am J Trop Med Hyg 2003; 68: 70-80.

34. Wu S, Li RW, Li W et al.. Worm burden-dependent disruption of the porcine colon microbiota by Trichuris suis infection. PLoS One 2012; 7: e35470.

35. Abner SR, Hill DE, Turner JR et al.. Response of intestinal epithelial cells to Trichuris suis excretory-secretory products and the influence on Campylobacter jejuni invasion under in vitro conditions. J Parasitol 2002; 88: 738-745.

36. Stephenson LS, Latham MC, Ottesen EA. Malnutrition and parasitic helminth infections. Parasitology 2000; 121 Suppl: S23-S38.

37. Shin JL, Gardiner GW, Deitel W et al.. Does whipworm increase the pathogenicity of Campylobacter jejuni? A clinical correlate of an experimental observation. Can J Gastroenterol 2004; 18: 175-177.

Tables

Table 1

Th1	Tbet, IFN-γ
Th2	GATA3, IL-4
Th17	RORC, IL-17A, IL-21, IL-22
Treg	IL-10, FoxP3, TGF-β

Table and figure legends

Table 1. T-cell subtype specific genes analysed by reverse transcriptase polymerase chain reaction (RT-PCR).

Figure 1. Egg excretion and anti-*Trichuris trichiura* antibodies during the *T. trichiura* self-infection. The volunteer ingested *T. trichiura* eggs twice (week 0 and repeated ingestion at week 9-13). Faecal egg excretion was observed from week 13 and increased until week 16 where *T. trichiura* presence was demonstrated during colonoscopy. Egg numbers dropped rapidly after the volunteer received mebendazole. No eggs were detected 2 weeks after mebendazole treatment (A). The levels of anti-*Trichuris* IgA and IgG_1 were below the detection limit (titer 50) before the infection and rose markedly after infection. Both IgA and IgG_1 levels dropped after mebendazole treatment but remained above the detection limit (B). *: titer < detection limit.

Figure 2. The course of the *T. trichiura* self-infection. Levels of plasma eosinophils (open circles) increased after inoculation followed by a drop and eventually normalisation following mebendazole treatment. Faecal calprotectin levels (closed circles) remained normal during *T. trichiura* infection, but rose rapidly during the accidental *Campylobacter* colitis.

Figure 3. Regional *T. trichiura* colonisation in the caecum/ascending and transverese colon was demonstrated (red arrows) during colonoscopy at week 16 post first-infection and did not trigger macroscopically signs of inflammation (A). Histological examination of ileac and colonic biopsies obtained during the *T.trichiura* infection revealed slightly to moderate inflammatory changes representing both acute and chronic inflammation. The infiltratory cells were dominated by eosinophil granulocytes predominantly localised superficially in the lamina propriae. The inflammation was most pronounced in the caecum/ascending and transverse colon, where a slight destruction of single crypts was observed (B).

Figure 4. Regionally increased expression of T cell subtype specific genes. During the *T. trichiura* infection there was a marked increase expression of all investigated T cell subtype specific genes in the colonised regions (caecum/ascending (red) and transverse colon (yellow)) (A) while no changes were observed in the non-colonised regions of the intestine (ileum (dark blue) and descending colon (light blue)) (B). The regional increased expression of T cell subtype specific genes was higher during the *T. trichiura* infection than during the following *Campylobacter* enteritis.

Figure 5. Modulation of circulating CD4+ T cells cytokine production by *T. trichiura*. Representative flow plots. Lymphocytes were gated upon their forward and side scatter appearance (A). Cytokine production was evaluated in CD4+CD4RO+ T cells following 4 hours stimulation with PMA, Ionomycin and Brefeldin A using flow cytometry (in the example interleukin-4 and γ-interferon). Cytokine-positive events were gated based on isotype controls (B). *Trichuris trichiura* induced increased frequencies of circulating IL-4- and IL-22 - producing CD4+CD45RO+ T cells and decreased frequency of interferon-γ -producing CD4+CD45RO+ T cells. Frequencies of IL-4 and IL-22-producing CD4+CD45RO+ T cells dropped during and after the *Campylobacter* colitis. No changes in the frequency of IL-17A - producing CD4+CD45RO+ T cells was observed (C).