This is a repository copy of *CD4CD25 Regulatory Cells Contribute to the Regulation of Colonic Th2 Granulomatous Pathology Caused by Schistosome Infection*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/63952/

Version: Published Version

**Article:**
Turner, Joseph D, Jenkins, Gavin R, Hogg, Karen G et al. (5 more authors) (2011)
CD4CD25 Regulatory Cells Contribute to the Regulation of Colonic Th2 Granulomatous Pathology Caused by Schistosome Infection. PLOS NEGLECTED TROPICAL DISEASES. e1269. pp. 1-11. ISSN 1935-2727

https://doi.org/10.1371/journal.pntd.0001269

---

**Reuse**
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.
CD4\(^+\)CD25\(^+\) Regulatory Cells Contribute to the Regulation of Colonic Th2 Granulomatous Pathology Caused by Schistosome Infection

Joseph D. Turner, Gavin R. Jenkins, Karen G. Hogg, Sarah A. Aynsley, Ross A. Paveley, Peter C. Cook, Mark C. Coles, Adrian P. Mountford*

Centre for Immunology and Infection, Department of Biology, The University of York, York, United Kingdom

**Abstract**

Eggs of the helminth *Schistosoma mansoni* accumulate in the colon following infection and generate Th2-biased inflammatory granulomas which become down-modulated in size as the infection proceeds to chronicity. However, although CD4\(^+\)CD25\(^+\)FoxP3\(^+\) regulatory T cells (T\(_{\text{regs}}\)) are known to suppress Th1-mediated colitis, it is not clear whether they control Th2 –associated pathologies of the large intestine which characterise several helminth infections. Here we used a novel 3D-multiphoton confocal microscopy approach to visualise and quantify changes in the size and composition of colonic granulomas at the acute and chronic phases of *S. mansoni* infection. We observed decreased granuloma size, as well as reductions in the abundance of DsRed\(^+\) T cells and collagen deposition at 14 weeks (chronic) compared to 8 weeks (acute) post-infection. Th2 cytokine production (i.e. IL-4, IL-5) in the colonic tissue and draining mesenteric lymph node (mLN) decreased during the chronic phase of infection, whilst levels of TGF-\(\beta\)1 increased, coincident with reduced mLN proliferative responses, granuloma size and fibrosis. The proportion of CD4\(^+\)CD25\(^+\)FoxP3\(^+\)T\(_{\text{regs}}\) CD4\(^+\) cells in the mLN increased during chronic disease, while within colonic granulomas there was an approximate 4-fold increase. The proportion of CD4\(^+\)CD25\(^+\)FoxP3\(^+\)T\(_{\text{regs}}\) in the mLN that were CD103\(^+\) and CCR5\(^+\) also increased indicating an enhanced potential to home to intestinal sites. CD4\(^+\)CD25\(^+\) cells suppressed antigen-specific Th2 mLN cell proliferation in vitro, while their removal during chronic disease resulted in significantly larger granulomas, partial reversal of Th2 hypo-responsiveness and an increase in the number of eosinophils in colonic granulomas. Finally, transfer of schistosome infection-expanded CD4\(^+\)CD25\(^+\)T\(_{\text{regs}}\) down-modulated the development of colonic granulomas, including collagen deposition. Therefore, CD4\(^+\)CD25\(^+\)FoxP3\(^+\)T\(_{\text{regs}}\) appear to control Th2 colonic granulomas during chronic infection, and are likely to play a role in containing pathology during intestinal schistosomiasis.

---

**Introduction**

Schistosomiasis is an important parasitic helminth disease afflicting more than 200 million people, causing approximately 280 thousand deaths annually, with a further estimated 700 million at risk of infection [1,2]. In the case of *Schistosoma mansoni*, infections are typically chronic (>10 years) and the majority (>90%) give rise to an intestinal form of disease [3] caused by the deposition of parasite eggs in the intestinal mesenteries (mainly of the colon and terminal ileum) and the subsequent development of Th2-associated granulomatous infiltrates rich in macrophages and eosinophils [4]. Such infections lead to diarrhoea, pseudopolyps, microulceration, bleeding and fibrosis [5]. Recent re-appraisal of Disability-Associated Life Years (DALYs) attributable to schistosomiasis, where more subtle disease manifestations such as intestinal schistosomiasis have been included, raises the disease burden caused by this infection as much as 40-fold, putting schistosomiasis on a par with malaria as a global public health problem [6]. Variation in granuloma size in the colon between patients is positively associated with peripheral blood mononuclear cell (PBMC) reactivity to soluble egg antigens (SEA) [7]. Thus, changes in lymphocyte responsiveness appear to be related to the size of granulomas in the intestine and by implication, the severity of pathologies in patients with intestinal disease.

In order to investigate the phenomenon of Th2-associated colonic inflammation and possible mechanisms underlying its regulation, we utilized a murine model of infection with *S. mansoni* which provides a well accepted permissive experimental host. In the murine model, myeloid antigen presenting cells, including dendritic cells [8,9], and basophils [10], are primed to induce potent anti-egg Th2 CD4\(^+\) lymphocyte responses. Th2 activation appears necessary to protect the host from lethal hepatic and intestinal damage during acute infection [11] and to keep Th1 inflammatory immunopathology in check [12]. However, survival to the chronic stage of infection, representative of human disease, is dependent on modulation of the Th2 granulomatous response in order to subvert IL-4/IL-13-driven morbidity [13]. ‘Naturally occurring’ T\(_{\text{regs}}\) bearing the IL-2 receptor \(\alpha\) chain molecule...
Author Summary

Schistosomiasis is an important parasitic helminth disease affecting more than 200 million people worldwide. Infections are typically chronic and in the case of Schistosoma mansoni and S. japonicum the majority give rise to an intestinal form of disease caused by the deposition of parasite eggs in the colon and terminal ileum. The eggs cause Th2-associated inflammatory immune granulomas to form, which as the disease develops, are down-regulated by cells of the immune system. However, the mechanisms which underpin the down-regulation of granulomas in the large intestine are not known. In order to investigate the phenomenon of Th2-associated colonic inflammation, we utilized a murine model of infection with S. mansoni and compared immune responses at the acute and chronic phases of infection. We show that a type of regulatory T helper lymphocyte (CD4+CD25+FoxP3+Tregs) contributes to regulation of colonic inflammation. These cells modulate anti-egg Th2 responses within the mesenteric lymph nodes and granulomatous pro-fibrotic Th2 responses within the colon. Our study highlights the importance of CD4+CD25+FoxP3+Tregs as a source of regulatory pressure on granuloma formation in the colon and by implication humans with chronic intestinal schistosomiasis.

Materials and Methods

Ethics statement

All experiments were carried out in accordance with UK Animal’s Scientific Procedures Act 1986 and with the approval of The University of York Ethics Committee.

Experimental infection and parasitological readout

C57BL/6 (B.6) and hCD2-VaDsRed-B.6 mice were maintained within the University of York under specific pathogen-free conditions. hCD2-DSRed-B6 mice, were a gift of D. Kioussis and A. Patel (National Institute for Medical Research, London) and express fluorescent DSRed T cells (>90% CD3+) to facilitate in situ detection of T cells by multiphoton microscopy (see below). Eight to ten-week female mice were infected percutaneously via the abdomen with 25 S. mansoni cercariae, and infections allowed to mature for either 8 or 14 weeks representing the acute and chronic phases of infection respectively. Adoptive transfer recipients were infected with 100 cercariae. Egg burdens in the 5 cm of colon proximal to the cecum were enumerated following digestion in 4% KOH. Eggs in faecal material were enumerated following dispersion in PBS, filtration through 100 μm pore mesh, and concentration. Colonic granulomas were isolated as previously described [16]. Volumes were calculated by measuring the longest and widest points and extrapolating volume using standard formulae for sphere or cylinder, depending on individual granuloma shape.

Histology and confocal microscopy

Colonic tissue were fixed in 4% formaldehyde and embedded in wax. Transverse cross-sections (5 μm) were stained with H&E, or haematoxylin and Van Geison (Department of Veterinary Pathology, University of Liverpool). Digital photomicrographs were analysed using AxioVision software (Zeiss).

For multiphoton imaging, proximal colon segments were mounted within 10 mm depression slides, and granulomas imaged from the serosal surface to egg mid-point using a 510 NLO laser-scanning microscope (LSM, Zeiss) with multi-photon laser (Coherent) tuned to 872 nm. 3D projections of ‘half-granulomas’ were rendered from 2 stacks using Velocity 4 software (Improvision). Quantification of Ds-Red+ lymphocytes, granuloma and collagen volumes were performed using “ROI” and “RGB” measurement tools within Velocity.

For immunofluorescent staining, frozen tissues were cryosectioned at 8 μm intervals, fixed with 10% methanol, permeabilised with 0.5% saponin (Sigma), and blocked with 5% rabbit serum / 1% FCS. Sections were labelled with anti-CD4 AF488 and anti-FoxP3 AF647 (both eBioscience) and fluorescence captured using the 510 NLO LSM. Settings for acute and chronic fluorescence images are matched both with respect to laser scanning settings at the time of image capture and post-image digital enhancement. Baseline laser scanning settings were undertaken on isotype controls and resultant negative control images contain undetectable fluorescent signal.

Anti-CD25 mAb treatment

Three doses of anti-CD25 mAb (50 μg; clone PC61, a gift from F. Powrie, University of Oxford), or purified rat IgG2a, were delivered intraperitoneally to infected mice at 9, 11, and 13 weeks.

Tregs cell purification, adoptive transfer and in vitro culture

Tregs from the mLN were purified by depletion of non-CD4+ cells following isolation of CD25+ cells using antibodies conjugated to magnetic beads (Miltenyi Biotec). For adoptive transfer, 2.5×10^6 CD4+CD25+Tregs (>90% purity) were injected via the lateral tail vein. Total mLN cells (2×10^6/ml), sorted CD4+CD25+ effector cells (1×10^6/ml), and CD4+CD25+Tregs (0.5×10^6/ml) from infected mice cultured in complete RPMI-1640 medium (containing 10% FCS, 50 μg/ml penicillin/streptomycin), in combination with naive mLN CD4+CD25- cells (0.1×10^6/ml) as a source of APC. Cells were stimulated with plate-bound anti-CD3 mAb (1 μg; Becton Dickinson), or SEA (50 μg/ml) [16]. Cells were cultured for 72 h and supernatants retained for cytokine analysis. Proliferation was measured from 72 to 96 h by ^3H-thymidine incorporation and scintillation counting.

Cytokine and collagen quantifications

ELISAs were used to quantify IL-4, IL-5 and IFNγ [17], while IL-10 and IL-13 were measured by Cytoset (Invitrogen) or DuoSet (R&D Systems) kits respectively. A TGFβ-sensitive, mink lung epithelial cell bio-assay (MLEC transfected with firefly luciferase; gift from Daniel Rilkin, NY Medical Center) was used to determine levels of bio-active TGFβ1 [18]. As the bio-assay was not compatible with tissue extracts, a TGFβ1 ELISA (R&D Systems) was employed. In order to determine cytokine levels in the colon, frozen tissues were first homogenised in proprietary...
Quantitative Real Time PCR

Total colonic mRNA was used to generate cDNA using Superscript III DNA polymerase (Invitrogen) and foxp3 transcript analysed by qRT-PCR (ABI PRISM 7000; Applied Biosystems) using Taqman probes (Sigma-Aldrich). The relative expression of foxp3 was normalised to values obtained for β-actin. Primer pairs and probes were: foxp3 5’-GGAGCTTCAGTGTCATGGA; 5’-CAGAGCCTGACTGTAGTGA; 5’-CAGACGCCTGACTGTAGTGA; 5’-CAATTCCCGCCCTGTTT and 3’-GAGCAGCCTGCCTGGAGG. For intracellular staining of FoxP3, cells were fixed in 1% formalin, re-suspended in permeabilisation buffer (Becton Dickinson) prior to staining with FoxP3-PE, CD3-FITC (14.4), CD25-FITC (7D4), anti-CTLA-4-FITC, or anti-CCR5-biotin (eBioscience). For staining with anti-CD25-APC (PC-61), anti-CD103-PE (all eBioscience), anti-CD11c-PE, anti-CD11b-FITC, and anti-CD11c-AMCA, cells were fixed in 1% formalin and permeabilised with BD Perm/Wash buffer. Cells were sequentially detected with streptavidin-PE-Cy7 (eBioscience). For intracellular staining of FoxP3, cells were fixed in 1% formalin, re-suspended in permeabilisation buffer (Becton Dickinson) prior to labelling with anti-FoxP3-PE or -AF647 (eBioscience). Cells were analysed using a Cyan flow cytometer with Summit software (Beckman Coulter).

Statistical analyses

Significant differences between two experimental groups were determined by unpaired Student’s t test, and between three or more groups by 1-way ANOVA with Tukey post-hoc tests using Prism software (GraphPad). Because colonic egg counts were skewed, analysis was undertaken after Log10 transformation. All data are representative of a minimum of two independent experiments. Significance is indicated ***P<0.001, **P<0.01, *P<0.05. Significance values are shown on the figures with line connectors between the appropriate groups. Where statistical significance was not achieved (P>0.05), figures are intentionally left blank.

Results

Colonic granuloma size and anti-egg Th2 responses decline with chronicity of infection

Egg deposition and anti-egg granulomatous responses in proximal colon were examined over a time-course of infection in B6 mice. The numbers of eggs increased during infection from 292±1.8 (day 42) to 2339±863.5 (day 98) (Fig. 1A). Mean areas of isolated colonic granulomas declined from the acute to chronic time point visualised by H&E staining (Fig. 1B & C), supporting previous observations [16,19]. Estimates of volumes of granulomas declined from the acute to chronic phase of infection, from 29.2±0.6% to 18.8±0.7% was revealed by enumeration of double positive versus single positive cells in anti-CD4 / anti-FoxP3 immunostained cryosections of colonic tissue (Fig. 3D & E). This profound (>10 fold) proportional elevation in FoxP3+ cells compared with total number of T lymphocytes in gut tissue was corroborated by qRT-PCR of FoxP3 transcript normalised to CD3 transcript (Fig. 3F). Thus, during enteric S. mansoni infection, relative and absolute expansion in the numbers of CD4+FoxP3+ cells occurs preferentially within gut-associated lymphoid tissue (GALT). Furthermore, relative increases of CD4+FoxP3+ cells within colonic granulomas are apparent during chronic disease.

Increased numbers of CD4+FoxP3+Treg cells occur in the mLN and colonic granulomas as chronicity proceeds

The proportion of CD4+FoxP3+Treg in the mLN as a proportion of total CD4+ cells, as determined by flow cytometry of cell suspensions, increased from 13.1±0.2% in naive mice to 16.0±0.4% during acute infection (P<0.001), and increased further to 20.6±0.2% during chronic infection (P<0.001; Fig. 3A). Absolute numbers of both mLN CD4+ effector and CD4+FoxP3+Treg cells increased during acute disease from naive levels, and remained significantly elevated during chronic infection (Fig. 3A). The increase was confirmed by enumeration of FoxP3+ Treg in stained sections of mLN from naive mice and those with acute and chronic infection (Fig. 3B & C). In contrast, absolute numbers or proportions of CD4+FoxP3+Treg in the spleens did not expand (12.8±0.6% cf. 13.9±0.8% cf. 14.8±2.3%, Fig. 3A).
CD103, the αE molecule of the αEβ7 mucosal integrin involved in homing of T cells to intestinal sites [20], increased on mLN CD4+FoxP3+Tregs at the chronic stage compared to naive mice (31.0 ± 2.3% cf. 62.4 ± 3.1%, P < 0.001, Fig. 3G). CCR5 is also involved in T cell homing to intestinal inflammatory sites [21], and significantly, the proportion of CCR5+CD4+FoxP3+Tregs in the
mLN increased markedly (9.6 ± 2.3% vs. 19.3 ± 2.6%; Fig. 3G). Thus, increases in the number of mLN CD4+FoxP3+ Tregs expressing CD103 and CCR5 suggests these cells have enhanced potential to be recruited and retained within the colonic infection site during chronic infection.

The majority of Schistosome-expanded CD4+CD25+ mLN cells are FoxP3+ and suppress antigen-specific CD4+ proliferative responses in vitro

More than 75% of CD4+CD25+ mLN cells co-expressed FoxP3, regardless of the stage of infection confirming that the majority of CD4+CD25+ cells can be classified as CD4+FoxP3+Tregs (Fig. 4A). In addition, while CD4+CD25+ effector cells from mice with an acute infection exhibited a strong proliferative response in vitro to SEA, CD4+CD25+Tregs, taken at the chronic stage of infection did not proliferate (Fig. 4B). Moreover, following depletion of CD4+CD25+Tregs or after their re-addition in a 1:2 Treg / effector T cell ratio, we observed that while the Treg conferred a significant degree of suppression on the anti-SEA response, chronic CD4+CD25+ effector T cells remained hyporesponsive, or anergic, compared with their acute-stage counterparts (Fig. 4D). Taken together, these in vitro assays provide evidence that schistosome-expanded CD4+CD25+Tregs suppress the pre-dominant Th2 anti-SEA response. However, compared with Tregs from naive mice, they are not enhanced in their ability to suppress antigen-specific CD4+ proliferation.

Anti-CD25mAb treatment impairs the regulation of colonic granuloma size

Depletion of CD25+ cells is a common technique to experimentally induce Treg deficiency [14,22,23,24,25,26]. Whilst not all CD25+ cells are FoxP3+ Tregs (75%–80% in our infection...
Regulation of Th2-Biassed Granulomas in the Colon

A

B

C

D

E

F

G

www.plosntds.org 6 August 2011 | Volume 5 | Issue 8 | e1269
model) and FoxP3+ Treg populations can be rapidly induced following CD25 depletion during infection [27], a single antibody treatment with anti-CD25 clone PC61 can significantly reduce FoxP3+ cells by 70% [28], with reduced FoxP3+ cells persisting in the face of ensuing inflammation for two weeks [27]. We therefore treated 9 week schistosome infected mice with a regimen of PC61 once per two weeks for six weeks and assessed CD25+ depletion and effects on intestinal granuloma parameters one week (+14 weeks infected) following the last PC61 antibody treatment.

Anti-CD25 mAb treatment of infected mice effectively depleted CD25+ cells in the mLN, and reduced the proportion of CTLA-4+ lymphocytes (Fig. 5A). Anti-CD25-treated mice also retained significantly larger granulomas in chronic colonic tissues compared to their isotype control cohorts (P<0.01; Fig. 5B). Eosinophils adjacent to schistosome eggs were significantly more numerous in anti-CD25 treated mice, while the numbers of large mononuclear cells remained stable (Fig. 5C & D). This suggests that reductions in CD25+ cells, the majority of which are FoxP3+ Tregs, lifted suppression of eosinophil recruitment, or their retention within colonic granulomas. It was also co-incident with increased antigen-specific mLN cell proliferation and IL-4 production at the chronic phase of infection (Fig. 5E). However, in CD25+-depleted mice, colonic granulomas remained, on average, significantly smaller in area compared to those at the acute stage of infection (43,397±5,477 μm² compared with 107,128±12,062 μm², P<0.01). Thus, in vivo depletion of CD25+ lymphocytes partially, yet significantly, reverses the down-modulation of Th2 granulomatous pathology in the colon during chronic S. mansoni infection.

Transfer of infection-expanded CD4+CD25+ Tregs modulates acute colonic granuloma development

Purified CD4+CD25+ mLN cells (2.5×10⁶, >90% purity; Fig. 6A) from mice at the chronic stage of infection were administered to hCD2-VaDαR6-B.6 mice co-incubated with the onset of egg deposition. Four weeks later (+9 weeks post-infection),...
the number of eggs in tissues or excreted from CD25+ cell recipients were not significantly different compared with controls (Table S1). This showed that immune cell transfers did not significantly affect adult worm development, fecundity, or egg transmission. Recipients of CD4+CD25+ cells had significantly smaller granuloma area and collagen deposits but Ds-Red+ T cell numbers within the colonic granulomas (open arrow) in isotype mAb treated (left) and anti-CD25 treated (right) infected mice. Scale bar = 200 μm; egg denoted ‘Sm’. D) Numbers of eosinophils (left) and large mononuclear cells enumerated from the sections above; Bars are mean cell counts / group (n = 4) with 3–4 fields of view / mouse. E) Antigen-specific mLN responses in anti-CD25 mAb treated mice; data are means of cpm 3H-thymidine incorporation and IL-4 secretion pg/ml (n = 3).

doi:10.1371/journal.pntd.0001269.g005

Discussion

Our data provides both in vitro and in vivo evidence that intestinal-associated CD4+CD25+FoxP3+ Treg cells expand during chronic, schistosome-induced colitic inflammation. They mediate significant levels of antigen-specific Th2 suppression in vivo including reduced IL-4 production, eosinophil recruitment, collagen production, and an overall reduction in the size of egg-induced granulomas in the large intestine. Thus, our data demonstrates experimentally, that CD4+CD25+ Treg cells, which expand within the mLN of chronically-infected mice, exert a suppressive effect on the development of acute-phase Th2 inflammation.
heightened bioactive TGFβ1 from mLN cells and within the colon at the chronic phase of infection, we speculate that TGFβ1 might have a role in the induction of gut-homing CD4⁺FoxP3⁺Tregs from naive precursors. Both proportions and absolute numbers of CD4⁺FoxP3⁺Tregs significantly increased within the mLN during acute infection, when anti-egg Th2 responses are at their peak. Although inhibition of Treg induction by Th2 differentiation programs has been reported [33], our data would suggest that Th2 differentiation is insufficient to block outgrowth of a regulatory T cell phenotype during acute intestinal schistosomiasis.

CD4⁺FoxP3⁺Tregs within the mLN of mice with a chronic infection expressed elevated levels of CD103 and CCR5, both of which are associated with homing to mucosal tissues during inflammation [20,21]. However, infection status did not alter the in vitro suppressive ability on a per-cell basis of CD4⁺FoxP3⁺Tregs, significantly increased within the mLN during acute infection, when anti-egg Th2 responses are at their peak. Although inhibition of Treg induction by Th2 differentiation programs has been reported [33], our data would suggest that Th2 differentiation is insufficient to block outgrowth of a regulatory T cell phenotype during acute intestinal schistosomiasis.

CD4⁺FoxP3⁺Tregs, within the mLN of mice with a chronic infection expressed elevated levels of CD103 and CCR5, both of which are associated with homing to mucosal tissues during inflammation [20,21]. However, infection status did not alter the in vitro suppressive ability on a per-cell basis of CD4⁺FoxP3⁺Tregs from naive mice share a common mechanism of Th2-effector cell suppression. Therefore, it is likely that the increased regulatory activity of CD4⁺FoxP3⁺Tregs in vivo during chronic infection is a product of either increased numbers trafficking to the site of egg deposition, or greater survival/retention in colonic granulomas. As recipients of schistosome-expanded CD4⁺CD25⁺Tregs displayed selective suppression of Th2 activity at the enteric infection site versus the mLN, this may reflect a biased homing of Tregs in the colon that provides increased regulatory pressure on the local granulomatous response (i.e., collagen synthesis and eosinophil recruitment). Taken with the observation that ablation of CD4⁺CD25⁺ cells in vivo significantly restores the anti-egg IL-4 and proliferative mLN cell response, these data suggest that intestinal-associated, schistosome infection-expanded FoxP3⁺ Tregs exert layers of Th2 suppression both within gut-draining lymph nodes and within the colonic infection site.

Our data are consistent with recent findings that CD4⁺FoxP3⁺Tregs constitute a partial component of the modulation of granuloma development in the liver [14,24,34]. We show that depletion of CD4⁺CD25⁺ cells in vivo does not fully reverse the Th2 hypo-responsiveness, nor do chronic enteric granulomas in treated mice fully recover the florid cellularity of the acute phase. The use of transgenic mice deficient for foxp3 [35] could help...
further investigation of this phenomenon. The development of other regulatory cells such as alternatively-activated macrophages [36], IL-10-secreting non-FoxP3 cells [14], and regulatory B cells [37], may explain the partial role of CD4+FoxP3+Treg in our system. T cell intrinsic anergy, for instance mediated by GRAIL signalling [38], could also account for the remaining hypo-responsiveness after depletion of CD4+CD25+ cells. However, because the micro-environment of the intestines is favourable towards the expansion of CD4+FoxP3+Treg, it is possible that CD4+FoxP3+Treg-mediated suppression of enteric granulomas is more apparent compared with their hepatic counterparts. This could explain why colonic granulomas modulate more rapidly versus hepatic granulomas after the acute stage, and why hepatic granulomas retain a greater size and cellularity than enteric granulomas during chronic disease [19].

During experimental helminth infections, CD4+CD25+Treg-mediated suppression of Th2 effector responses confer a permissive state by stifling effective Th2-mediated worm attrition [23,25,26]. In the case of gut-helminths, this potentially operates by regulating Th2 cytokine signalling on smooth muscle contractility and epithelial cell turnover [39,40]. Paradoxically, during S. mansoni infections, while Th2 granulomatous responses protect and aid survival of the host [11,41], intact Th2 responses are also essential to propagate the parasite’s life cycle, as egg transmission from the gut is impaired in the absence of Th2 cells [42,43,44], IL-4 [41], or IL-4R+ signalling [45]. Nevertheless, transfer of CD4+CD25+ cells to acute stage recipients does not impinge on egg transmission in spite of a modulatory effect on IL-4 in the colon. In fact, during the chronic stage of infection, where Th2 inflammation in the colon becomes modulated, egg excretion rates are unaffected (our unpublished observations). How intestinal schistosome parasites mediate sustained, chronic egg transmission in the face of marked Th2 hypo-responsiveness remains to be identified.

Enteric helminth infections, or products derived from helminths, are gaining prominence as potential therapies to reverse the effects of inflammatory bowel disease [46]. Some ameliorating capacities may be attributed to antagonism of Th1 processes by induction of IL-4-secreting cells within intestinal tissues rather than by induction of Treg. Indeed, most experimental studies demonstrating the modulatory action of helminth infection used trinitrobenzene sulphate as a haptenizing agent to drive Th1 colitis reminiscent of Crohn’s Disease [46]. Initiation of Th2/NKT colitis by oxazolone in conjunction with Th2-promoting helminth infection exacerbates pathology [47], further supporting that the mechanism of helminth suppression of colitis is based on Th2/Th1 antagonism. Thus, from a clinical perspective, helminth-based therapies might be considered inappropriate for ulcerative colitis or other intestinal disorders with Th2 aetiology. However, our data demonstrates that helminth infection-expanded FoxP3+ Treg clearly regulate coincident pro-fibrotic Th2 processes in the colon. Indeed, distinct molecules released by schistosome eggs deliver triggers that polarize naive CD4+ T cells towards Th2, or a regulatory phenotype [9,8,10,40]. Potentially, the release of somatic molecules with regulatory potential from degrading eggs that fail to breach colonic tissues could favour Treg expansion during chronic disease in the context of a TGFβ-enriched microenvironment. Exploitation of such regulatory molecules may be of benefit in the treatment of intestinal schistosomiasis or other Th2-based intestinal disorders via the expansion of Treg with bystander potential.

Supporting Information

Video S1 Acute colonic granuloma shown in Fig 1F. (MOV)
Video S2 Chronic colonic granuloma shown in Fig 1F. (MOV)
Video S3 Control recipient, colonic granuloma shown in Fig. 6B. (MOV)
Video S4 CD4+CD25+ recipient, colonic granuloma shown in Fig. 6B. (MOV)

Table S1 Acute-stage schistosome egg tissue burdens and excretion following adoptive transfer of chronic infection-expanded CD4+CD25+ cells. (DOC)

Acknowledgments

The authors wish to thank Dr Jane Dalton and Dr Marika Kullberg for critical appraisal of the manuscript and Dr Patricia Coulson for technical expertise.

Author Contributions

Conceived and designed the experiments: APM GRJ KGH JDT. Performed the experiments: APM GRJ KGH PCC SA RAP JDT. Analyzed the data: APM JDT KGH GRJ. Contributed reagents/materials/analysis tools: MC. Wrote the paper: JDT APM.


