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Regulatory T Cells Limit Induction of Protective Immunity and Promote Immune Pathology following Intestinal Helminth Infection

Deepali V. Sawant,*1 David M. Gravano,*1,2 Peter Vogel,† Paul Giacomin,‡,3 David Artis,‡ and Dario A. A. Vignali*

Foxp3+ regulatory T cells (Tregs) have a well-characterized role in limiting autoimmunity and dampening deleterious immune responses. However, a potential consequence of the immunosuppressive function of Tregs can be the limitation of protective immunity to infectious pathogens. Parasitic infections are a potent stimulus for the generation of Treg responses, which may be beneficial to both the parasite and the host by promoting persistence of infection and limiting immune-mediated pathology, respectively. In this study, we explore the functional role of Tregs post–low-dose infection with the intestinal helminth parasite Trichuris muris, which yields a chronic infection because of inefficient induction of Th2 responses. Early Treg depletion postinfection resulted in expedited worm clearance, and was associated with reduced Th1-mediated inflammation of the intestinal environment. Interestingly, this protective immunity was lost, and worm burden enhanced if Tregs were depleted later once the infection was established. Early and late Treg depletion resulted in enhanced Th2 and reduced Th1 cytokine and humoral responses. Blockade of the Th2 cytokine IL-4 resulted in a moderate increase in Th1 but had no effect on worm burden. Our findings suggest that Tregs preferentially limit Th2 cell expansion, which can impact infections where clear immune polarity has not been established. Thus, the impact of Treg depletion is context and time dependent, and can be beneficial to the host in situations where Th1 responses should be limited in favor of Th2 responses. 


Intestinal helminth parasites are large, multicellular organisms that can establish long-term chronic infections that last for years. Resistance to infection is typically mediated by a Th2 response that is characterized by the production of cytokines such as IL-4, IL-5, and IL-13 (1). It has been suggested that a key part of the survival strategy of helminth is immune evasion through induction of immune suppression via multiple mechanisms (2). The role of Foxp3+ regulatory T cells (Tregs) during parasitic infection has generated considerable interest because Treg populations expand post–parasite infection and exposure to parasite excretory/secretory (E/S) Ags (3–9), leading to the hypothesis that helminths directly drive Treg induction to avoid immune-mediated parasite expulsion (10). Helminth-mediated Treg induction may also have beneficial effects for the host, such as reduced immunopathology (11, 12).

Studies that investigated the functional role of Tregs in helminth immunity and inflammation in the intestine have used various methods of Treg depletion, including Ab-mediated depletion and a genetic model to specifically ablate Tregs by administration of diphtheria toxin (DT) (11, 12). However, the approaches used led to incomplete or transient Treg ablation, leading to somewhat conflicting results regarding the benefits of Tregs in helminth infections and their influence on immunopathology. Ab-mediated depletion studies with the filarial parasite Litomosoides sigmodontis have shown that Treg depletion can expedite parasite clearance and amplify Th2 cytokine production (13–15). These studies used high doses of parasite infection that promote robust Th2 responses, and thus the effect of Treg depletion on the polarization of the immune response is less clear. Recently, it has been shown that Treg depletion can skew the Th polarization preferentially toward the Th2 lineage, increasing the ratio of IL-4/IFN-γ-producing cells (16). A Th2 cell response predominates because of a preferential ability of Tregs to control Th2 cell expansion through the induction of apoptosis. This result was obtained independent of a disease setting; thus, the physiological and functional ramifications of a Treg depletion–mediated shift in Th cell responses in a disease setting are currently unknown.

Trichuris muris is a well-characterized murine helminth infection model that is closely related to the human whipworm Trichuris trichiura (17). During T. muris infection, the polarization of the Th cell response is critical to the infection outcome because a Th2-cell dominated response confers resistance, whereas a Th1 cell–dominated response confers susceptibility to chronic infection (18, 19). Although most mouse strains are resistant to high doses of T. muris infection, mice given a low dose of T. muris exhibit...
a chronic infection because of a more Th1 polarized response (20, 21). Low-dose infection models may more closely mirror the human infection patterns (21); thus, determining the role of Tregs in this context is critical. In this study, we explore whether Treg deletion during a low-dose *T. muris* infection is beneficial to the host, via induction of a more robust Th2 cell response and expedited worm clearance, or more detrimental, because of immune hyperactivation and increased Th1 or Th2 cytokine-mediated pathology. Our results demonstrate that Tregs preferentially block effector Th2 responses during *T. muris* infection; thus, Treg ablation protects the host from worm-driven intestinal pathology. Our study also identifies that the outcome of Treg depletion is temporal and, in this model, confers beneficial effects to the host (reduced worm burden and histopathology) only when Tregs are targeted early during the onset of infection, whereas Treg depletion later in infection can enhance parasite burden and immune pathology.

### Materials and Methods

#### Mice and parasites

Foxp3*ΔT*GFp mice (100% C57BL/6 based on 96-marker single nucleotide polymorphism analysis) were provided by A.Y. Rudensky (Sloan-Kettering Institute) (22). C57BL/6 mice were purchased from The Jackson laboratory (Bar Harbor, ME). Mice were 8–10 wk of age at the time of infection. *T. muris* embryonated eggs, and E/S Ags were generated as previously described (23). For the low-dose infection, embryonated eggs were individually counted using a dissecting microscope and aliquoted. Mice were infected by oral gavage with 30 embryonated eggs in a volume of 200 μl (low-dose infection). All experiments were performed in American Association for the Accreditation of Laboratory Animal Care–accredited, specific pathogen-free, murine norovirus free, and Helicobacter-free facilities at St. Jude Animal Resource Center in accordance with federal, state, and institutional guidelines, and all protocols were approved by the St. Jude Animal Care and Use Committee.

#### Experimental design

For all experiments, mice were infected with 30 embryonated *T. muris* eggs via oral gavage on day 0, followed by Treg depletion (early versus late) by five i.p. injections of 10 μg/kg DT (Sigma-Aldrich, St. Louis, MO) in 200 μl sterile PBS or sterile PBS alone, and harvested on day 35. For the early DT experiments, mice were administered DT or PBS on days 0, 2, 4, 6, and 8, whereas for the late DT experiments, DT or PBS was administered on days 9, 11, 13, 15, and 17. Optimal dosage of DT was determined by in vivo titration to ensure maximal Treg depletion in Foxp3*ΔT*GFp mice with apparent toxicity in C57BL/6 mice. For experiments involving IL-4 neutralization, mice received three i.p. injections of 0.5 mg anti–IL-4–PE (clone 11B11; Biolegend), anti–IFN-γ–PE/Cy7 (clone XMG1.2; Biolegend), anti–IL-17A PerCP/Cy5.5 (clone TC11-18H10.1; Biolegend), and anti–IL-13 eFluor660 (clone eBio13A; eBioscience) using Cytofix/Cytoperm kit (BD Biosciences, San Jose, CA) following the manufacturer’s instructions.

#### Cytokine analysis

Draining (mesenteric) (DLNs) and non-draining (axillary and brachial) lymph nodes (NLDNs) were removed and mashed over 70-μm cell strainers. For intracellular cytokine staining, cells were stained at 5 × 10^6 cells/ml in DMEM with PMA/Ionomycin (100 and 500 ng/ml, respectively; Sigma-Aldrich) or 5 μg/ml *Trichuris* E/S Ag for 12–16 h in the presence of monensin (1:1000; eBioscience).

#### Ab ELISAs

For *T. muris* Ag-specific IgG1 and IgG2a ELISAs, microtiter plates were coated with 5 μg/ml *T. muris* E/S Ag. Plates were then blocked in 1% BSA. Serially diluted serum samples were incubated at room temperature starting with an initial serum dilution of 1/10 in PBS + 1% BSA. Ag-specific IgG1 and IgG2a were detected with biotinylated anti-mouse IgG1 and IgG2a (clones A85-1 and R19-15, respectively; BD Biosciences) followed by incubation with streptavidin-HRP and developed with TMB substrate. The reaction was terminated with IN H2SO4, and OD450 was determined with a spectrophotometer. Total serum IgE was determined using the Mouse IgE ELISA MAX kit (Biolegend) following the manufacturer’s instructions.

#### Statistical analysis

All results are expressed as the mean ± SEM. Statistical analysis was performed using GraphPad Prism software using unpaired Student t test or two-way ANOVA analysis. The p values were categorized into the following levels: *.p < 0.05, **p < 0.01, ***p < 0.001.

### Results

#### Early Treg depletion postinfection expedites *T. muris* clearance

During a low-dose infection, the ability of a highly resistant strain (such as BALB/K) versus a less resistant strain (C57BL/6) to clear *T. muris* infection can be clearly delineated at 35 d postinfection (18). Thus, we chose this time point to analyze the ability of Treg-depleted mice to clear *T. muris* infection. Foxp3*ΔT*GFp mice can sustain near-complete Treg depletion for up to 2 wk, at which point significant mortality is seen because of the ensuing autoimmunity (22). Therefore, our experimental design involved DT administration for a maximum of 10 d to avoid increased mortality associated with long-term treatments. To gain a comprehensive assessment of the role of Tregs during immunity to this intestinal helminth parasite, we used two approaches for Treg depletion: early DT involved DT administration during the initial stages of infection (days 0–8), whereas late DT involved Treg depletion once the infection was established (days 9–17; Fig. 1A). To validate that Tregs could effectively be depleted after DT administration, we infected separate cohorts of mice with a low (30) dose of *T. muris* eggs, performed early versus late DT treatments, and analyzed the mice 1 wk after the last DT treatment. We observed a near-complete absence of Foxp3^+^ cells within the CD4^+^ T cell fraction from both the gut-draining mesenteric lymph node (MLN) and the non-gut-draining axillary and brachial lymph nodes (Fig. 1B). However, by day 35, Treg showed a robust recovery with both DT treatment approaches (Fig. 1B, 1C). Interestingly, early Treg depletion resulted in a modest but significantly increased ability to clear *T. muris* infection by day 35, whereas late Treg depletion reduced worm clearance (Fig. 1D). These data reveal a differential role for Tregs at different time points during infection on worm burden.

#### Differential *T. muris*–mediated immunopathology after early versus late Treg depletion

We next assessed the impact of Treg depletion on *Trichuris* infection–induced intestinal inflammation. Because low-dose *T. muris* infection in C57BL/6 mice results in Th1 cytokine-mediated chronic infection and intestinal inflammation, we hypothesized...
that T<sub>reg</sub> depletion in this model would exacerbate tissue pathology consistent with previous studies using different models of helminth infection (11, 12). Surprisingly, histological analysis of intestinal sections revealed that total inflammation scores were significantly lower in the cecum of <i>T. muris</i>–infected mice treated with early DT compared with control mice (Fig. 2A–C). Although the numbers, distribution, and composition of inflammatory cell infiltrates in the cecal mucosa were similar in both treatment groups (+DT, −DT), higher overall inflammation severity scores were assigned to the untreated mice (−DT) largely because of the much greater extent and severity of submucosal edema (Fig. 2B). In contrast with the effects with early DT, the late DT treatments in <i>T. muris</i>–infected mice increased intestinal histopathology (Fig. 2A–C). The most notable difference between the mice receiving late DT and not treated with DT (−DT) was the lower number of intraepithelial globule leukocytes (also known as mucosal mast cells) and lymphocytes in the mucosa of late DT–treated mice compared with untreated mice (data not shown). Overall, the histopathology results were concordant with the worm burden data for early versus late DT treatments (Fig. 1D), suggesting a differential effect of T<sub>reg</sub> on parasite burden and intestinal inflammation during the early versus late stages of infection.

**FIGURE 1.** Early T<sub>reg</sub> depletion accelerates clearance of low-dose <i>T. muris</i> infection. (A) Schematic of the early (left) versus late (right) DT treatment regimens for depletion of T<sub>reg</sub> after low-dose <i>T. muris</i> infection. Mice were infected via oral gavage with 30 embryonated <i>T. muris</i> eggs on day 0 (D0), DT was administered five times every other day using one of the two approaches (early DT: D0–D8 or late DT: D9–D17), and mice were analyzed at D35. (B) Representative flow cytometric plots depicting Foxp3<sup>+</sup> T<sub>reg</sub> depletion and recovery after treatment with DT (+DT) or PBS (−DT). MLN and NDLN cells were isolated from infected Foxp3<sup>DTR-GFP</sup> mice and surface stained for CD4 and intracellular Foxp3. Cells were gated on CD4<sup>+</sup> T cells and analyzed for percentage of GFP<sup>+</sup>Foxp3<sup>+</sup> T<sub>reg</sub> 1 wk after the last DT treatment (left) and at the time of analysis, D35 (right). (C) T<sub>reg</sub> recovery assessed by the percentages of GFP<sup>+</sup>Foxp3<sup>+</sup> T<sub>reg</sub> at D35 for early DT (left) and late DT (right) treatment approaches. Data averaged from 2 independent experiments (n = 8–10 mice/group). (D) Total worm burden at day 35 from the cecum of mice infected with low-dose <i>T. muris</i> as in (A) and treated with early and late DT approaches. Each data point on the scatter plots represents an individual mouse with mean indicated. Data averaged from 4 independent experiments for early DT approach (n = 20–22 mice/group) and 3 independent experiments for late DT approach (n = 12–15 mice/group). **p < 0.01, ***p < 0.001 (unpaired Student t test).

**FIGURE 2.** T<sub>reg</sub> depletion during <i>T. muris</i> infection leads to induction of potent Th2 responses

Because tissue pathology and host susceptibility are associated with the balance between a protective Th2 cytokine response and a detrimental Th1 cytokine response, we assessed alterations in the polarity of the immune response after T<sub>reg</sub> depletion in low-dose <i>T. muris</i>–infected mice. It was recently reported that T<sub>reg</sub> depletion results in a preferential expansion of Th2 cells over Th1 cells (16). We hypothesized that this skewed response after T<sub>reg</sub> depletion could impact disease outcome post–low-dose <i>T. muris</i> infection. T<sub>reg</sub> cells are known to curtail excessive Th1, Th2, and Th17 effector responses to maintain immune homeostasis (24, 25). Consistent with published reports, there was a significant expansion of both IFN-γ and IL-4–producing CD4<sup>+</sup> T cells in the gut-draining and non–gut-draining axillary and brachial lymph nodes after T<sub>reg</sub> depletion (Fig. 3A–D). The overall fold induction of IFN-γ<sup>+</sup> CD4<sup>+</sup> T cells (as assessed by the ratio of +DT/−DT) was higher in the DLNs (∼6.3 fold for early DT and ∼8.3 fold for late DT) relative to the NDLNs, consistent with the increased Th1 polarization in the gut post–low-dose <i>T. muris</i> infection (Fig. 3A, 3B). The percentages of IL-4–producing CD4<sup>+</sup> T cells were strikingly high in the NDLNs, compared with DLNs for both the DT treatment approaches (Fig. 3C, 3D). This could be attributed to the
counterregulation to Th2 induction mediated by the increased Th1 responses at the site of infection (DLN), whereas Th2 responses are unrestrained in the NDLNs after Treg depletion. Importantly, however, the fold induction of IL-4+ CD4+ T cells (ratio of +DT/−DT) is higher (∼10- to 20-fold in DLNs and ∼36-fold in NDLNs) relative to the fold induction of IFN-γ+ CD4+ T cells (∼10-fold at both DLNs and NDLNs) with the two DT treatments (Fig. 3B, 3D). IL-17+ CD4 T cells were also increased after early and late Treg depletion (Supplemental Fig. 1A, 1B), although the overall induction was lower than that of IFN-γ– and IL-4–producing CD4+ T cells. These results highlight the preferential induction of potent Th2 responses after Treg depletion that can affect the disease outcome in scenarios where clear immune polarity is not established. Thus, in the low-dose T. muris infection setting, Tregs limit Th2 effector responses far more strongly than Th1 responses, thereby inadvertently restraining protective antihelminthic immunity. Importantly, although comparable Th2 responses were induced after both early versus late Treg depletion, these responses have a favorable outcome to the host (reduced worm burden and parasite-driven intestinal pathology) only when unleashed during the initial stages of infection, but fail to have an effect once the infection is well established.

Treg depletion alters T. muris–specific Th1/Th2 cell polarization and humoral responses

To determine whether T. muris–specific immune parameters were altered after Treg depletion in low-dose T. muris–infected mice, we next assessed the percentages of Ag-specific IFN-γ and IL-4+ CD4 T cells (Fig. 4). The overall Th1/Th2 trend in the DLNs and NDLNs after both early versus late Treg depletion was comparable with the effects observed after polyclonal stimulation (Fig. 3); however, the magnitude of response was lower as anticipated. Consistent with the induction of chronic Th1 response post–low-dose T. muris infection, Ag-specific IFN-γ+ CD4+ T cells were significantly elevated in the MLNs (DLNs, site of infection) after Treg depletion, compared with the NDLNs (Fig. 4A). Although there was a trend toward increased percentage of Ag-specific IL-4+ CD4+ T cells after DT treatment, the effects did not reach significance (Fig. 4B), thus further corroborating that only the Th1 induction in this model was T. muris specific, whereas the potent Th2 responses noted after polyclonal stimulation (Fig. 3C, 3D) were attributed primarily to Treg ablation.

Parasite-specific serum IgG1 and IgG2a titers correlated with Th2 IL-4–dependent IgG1 class switching and Th1 IFN-γ–dependent IgG2a class switching, respectively (26). Whereas low-dose T. muris infection of non-DT–treated (−DT) Foxp3DTR-GFP mice resulted in robust Ag-specific IgG2a titers, parasite-specific IgG2a was nearly undetectable in both early and late Treg-depleted mice (Fig. 5A), consistent with potent induction of Th2 responses and a shift away from Th1 with Treg depletion. Ag-specific IgG1 titers, however, did not show a significant alteration after Treg depletion at either time point (Fig. 5B). Elevation in total serum IgE is another characteristic response to helminth infection and is associated with...
a Th2 cytokine response (27). Treg depletion causes a substantial increase in total IgE (Fig. 5C), consistent with Tregs preferentially curbing Th2 responses. Again, comparable humoral responses are induced with both the early and late DT treatment approaches, reinforcing the observation that the enhanced Th2 cytokine and humoral responses after Treg depletion can mediate effective parasite clearance only during the onset of infection, but not once it becomes established. Indeed, enhanced Th2 responses during the latter stages of T. muris infection appear to negatively impact worm burden and inflammation.

**Th2 responses unleashed after Treg depletion contribute to antihelminthic immunity**

To provide further mechanistic insight, we assessed whether the increased Th2 responses, specifically IL-4, after early Treg depletion contributed to enhanced worm clearance and reduced intestinal pathology. Mice received three injections (1.5 mg/mouse total), on days 2, 5, and 8, of either IL-4 neutralizing Ab (11B11) or isotype control (IgG1) during the early DT treatment regimen (days 0–8), and worm burden and inflammation parameters were assessed at day 35 postinfection (Fig. 6A). Treg recovery was not affected by the Ab treatments, because percentages of Foxp3+ Tregs were either comparable or augmented after DT treatment at both the DLNs and the NDLNs (Supplemental Fig. 2A). Although we observed a trend toward reduced worm burden in the DT treatment groups (+DT+IgG1 and +DT+anti–IL-4) compared with untreated groups (−DT+IgG1 and −DT+anti–IL-4), IL-4 neutralization did not alter worm burden (Fig. 6B). Analysis of the Th1/Th2 cytokine responses demonstrated a significant reduction in the percentage of IL-4+ CD4+ T cells and a reciprocal increase in the
percentage of IFN-γ+ CD4+ T cells after IL-4 neutralization in the DT treatment groups (+DT+IgG1 and +DT+anti–IL-4; Fig. 6C, 6D). Ab treatments did not lead to any major alterations in the Th1/Th2 response in the non-DT groups (−DT+IgG1 and −DT+anti–IL-4). T. muris–specific IgG1 and total IgE trended toward a moderate decrease after IL-4 neutralization; however, we did not observe any increase in T. muris–specific IgG2a (Supplemental Fig. 2B). Interestingly, although we did not see an increase in the worm burden after IL-4 neutralization, histological examination revealed significant increase in the overall inflammation severity and increased edema score in mice receiving the IL-4 blocking Ab (Fig. 6E, 6F). Early Treg depletion resulted in reduced overall intestinal inflammation in the DT groups as expected; however, IL-4 neutralization in the DT groups did not exacerbate the pathology. Thus, although IL-4 blockade led to increased Th1 polarization and concomitantly increased immune pathology, it did not reverse the protective effects of Treg depletion. This could be because of incomplete neutralization of IL-4 (IL-4+ CD4+ T cells after DT treatment were still observed in the DLNs and NDLNs) and/or compensatory effects driven by other Th2 cytokines (e.g., IL-13), which was also increased after Treg depletion (Supplemental Fig. 2C). It is also possible that T cell hyperactivation caused by Treg depletion, rather than increased IL-4, is sufficient to reduce worm burden. Studies to further dissect these processes are warranted.

Discussion

In this study, we sought to address the functional role of Treg in immune polarization in a physiological setting where the balance of polarity is critical in disease susceptibility versus resistance (18, 2909).
**FIGURE 6.** T<sub>reg</sub>-depletion–mediated Th2 induction limits *T. muris*-driven intestinal pathology. (A) Schematic of IL-4 neutralization in low-dose *T. muris*-infected mice after T<sub>reg</sub> depletion by the early DT approach. Infected mice received anti–IL-4 (11B11) neutralizing Ab or isotype (IgG1) control on days 2 (D2), D5, and D8, alongside the early DT treatments (D0–D8) and were analyzed at D35. Thus, the four treatment groups included −DT+IgG1, −DT+anti–IL-4, +DT+IgG1, and +DT+ anti–IL-4. (B) Total worm burden from ceca of *T. muris*-infected mice as treated in (A). Worms counted at D35 for the four treatment groups. (C) Representative flow plots gated on CD4<sup>+</sup> T cells stained for the Th1 cytokine, IFN-γ. DLN and NDLN cells were isolated from *T. muris*-infected mice treated with IL-4 neutralizing Ab or IgG1 isotype alongside early T<sub>reg</sub> depletion and analyzed at D35. Cells stimulated with PMA and Ionomycin for 12–16 h in the presence of monensin. Data represented only the two DT treatment groups (+DT+IgG1 and +DT+ anti–IL-4). (D) Representative flow plots gated on CD4<sup>+</sup> T cells stained for the Th2 cytokine, IL-4. DLN and NDLN cells were isolated and treated as in (C). (E) Inflammation and edema scores as assessed by H&E staining of D35 cecal sections for the four treatment groups as treated in (A). (F) Representative H&E-stained cecal sections for the four treatment groups as treated in (A). Original magnification ×20. Each data point on scatter plots represents an individual mouse with mean indicated. Data represent two independent experiments (n = 5–8 mice/group). *p < 0.05, **p < 0.01, ***p < 0.001 (unpaired Student t test).
20). Therefore, we chose to use the T. muris low-dose infection model. T. muris is a natural coevolved pathogen of mice that closely parallels T. trichura infection in humans (21). Low-dose infection results in a mixed Th1/Th2 response in C57BL/6 mice associated with chronic parasitic infection and intestinal pathology.

Past studies have evaluated the role of Treg in intestinal helminth infection through Trg depletion strategies. However, these studies have had limitations in the evaluation of the effect of Treg on the polarization of the immune response. First, the Treg depletion strategies using the CD25-depleting Ab PC61 or DEREg mice have not allowed for complete or sustained Foxp3+ Treg depletion, respectively (11, 12). These shortcomings of previous Treg depletion studies have made interpretation of results difficult. Second, these models have used high doses of helminth infection that drive a strong Th2 polarization and would typically not reflect most natural human parasite infections. This high antigenic load has likely masked the subtle change in immune polarization, which, as we show, has functional ramifications in the low-dose T. muris model.

Because low-dose T. muris infection results in elevated Th1-mediated pathology relative to high-dose infection, it was unclear to us whether Treg depletion would amplify that pathology or reduce it through the promotion of a productive Th2 response. Our data would support the interpretation that through a shift in immune polarization toward Th2, the detrimental Th1 pathology is limited after Treg ablation in this model. Despite the Treg depletion-mediated shift toward a Th2 response to T. muris low-dose infection, the parasite was not completely cleared at 35 d postinfection, whereas strongly Th2-inducing high-dose infections mediate parasite clearance within 21 d. This indicates that despite the elevated Th2 response, resistance to infection was incomplete.

Physiologically, a “trickle infection,” in which low levels of parasitic infectious agents are ingested over time, may be more relevant to human disease than the high-dose infection models that are often used when studying parasitic infection. Studies of trickle infection with T. muris have shown that initially a Th1 response predominates in normally susceptible mice (21). However, as the antigenic load increases with increased infection, a threshold is reached that results in the establishment of a Th2 response that confers resistance. Our data would suggest that during low-dose infections and trickle infections, it may be clinically relevant to target immune regulation to direct the polarity of the immune response more strongly toward Th2 and expedite worm clearance. New efforts to understand how to maximize Th2 responsiveness are warranted because parasites have proved to be highly resistant to sterilizing immunity.

It is intriguing that the Th2 response induced after Treg depletion is insufficient for complete parasite expulsion. It is possible that the strength and/or “quality” of the Th2 response is insufficient. Alternatively, it is possible that other regulatory mechanisms, such as suppressive cytokines or inhibitory receptors, may compensate for the absence of Treg. Further studies will be required to address these possibilities in more detail. Another intriguing finding is the differential impact of Treg on parasite burden and immunopathology. Although the reduced worm burden caused by early Treg depletion could be mediated by increased Th2 response, it is unclear why late Treg depletion causes increased worm burden and immunopathology. Although it is possible these two events are linked, further studies will be required to dissect this relationship in more detail.

Our data may also provide a new interpretation on why parasites have developed strategies to expand Treg early during the course of infection. As has recently been demonstrated, Heligmosomoides polygyrus E/S products alone can directly induce Treg generation via the presence of a molecule that mimics the bioactivity of TGF-β (10). Instead of a broad and nonspecific dampening of immunity, parasites may drive Treg to specifically limit Th2 immunity to prolong their survival.

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Disclosures

The authors have no financial conflicts of interest.

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