Review

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Helminthic therapy: improving mucosal barrier function

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The epidemiology of autoimmune diseases and helminth infections led to suggestions that helminths could improve inflammatory conditions, which was then tested using animal models. This has translated to clinical investigations aimed at the safe and controlled reintroduction of helminthic exposure to patients suffering from autoimmune diseases (so-called 'helminthic therapy') in an effort to mitigate the inflammatory response. In this review, we summarize the results of recent clinical trials of helminthic therapy, with particular attention to mechanisms of action. Whereas previous reviews have emphasized immune regulatory mechanisms activated by helminths, we propose that enhancement of mucosal barrier function may have an equally important role in improving conditions of inflammatory bowel diseases.

Rationale for helminthic therapy

Based on the epidemiology of autoimmune diseases, environmental factors such as helminth infection have long been part of the hygiene hypothesis to explain why autoimmunity may be less prevalent in the developing world. Subsequently, helminth infections in animal models have been shown to improve the conditions of certain inflammatory diseases, leading to clinical trials of 'helminthic therapy' (Table 1). Helminths are multicellular metazoan organisms with the potential to cause significant tissue injury as they mature, migrate and feed within the host. Due to effective immune evasion strategies, these parasites can persist in the host for many years. The immune response that optimizes host fitness must be well-adapted for: (i) expelling large multicellular pathogens; (ii) wound healing and tissue repair and (iii) mitigating inflammatory pathology associated with chronic infection. These mechanisms are encompassed within the T_H2 immune response elicited by helminth infection and the activation of regulatory networks that dampen effector T cell responses [1,2]. Elements of the T_H2 immune response, as well as the induction of regulatory T cells, may contribute in varying degrees to the benefits of helminth infection in different autoimmune and inflammatory disease settings. The type 2 immune response triggered by gastrointestinal helminths includes cytokines produced by $CD4^+$ T_H2 cells and innate lymphocytes (e.g. IL-4, IL-5 and IL-13), activation of alternatively activated macrophages and mast cells, increased goblet cell hyperplasia and mucus production, and increased turnover of intestinal epithelial cells [3]. We propose that these alterations to mucosal barrier function in the gut play a protective role against pathology associated with inflammatory bowel diseases (IBD) (especially ulcerative colitis, UC) and may potentially be as mechanistically important as immune regulation in modulating the inflammatory response.

Clinical trials with *Trichuris suis* ova

Therapeutic infection with the pig whipworm *Trichuris suis* was first investigated in 2003 in an exploratory open-label study of seven patients with IBD [4]. *T. suis* ova (TSO) are considered to be ideal agents because they produce self-limited colonization in humans and remain isolated to the gastrointestinal tract. Additionally, ova can be obtained under pathogen-free conditions, can be stored for approximately 2 years and any unexpected long-term colonization can be effectively eradicated with short courses of oral antihelminthic agents. The ova contain the infective J1 larval stage that has developed from a single cell within the egg, which is excreted from the feces of infected animals. The larvae hatch from the eggs in the cecum and will penetrate the mucosa before undergoing several molts to mature into the adult stage that can start shedding eggs in pigs. In humans, the lifecycle is attenuated and the larvae do not fully mature into adults.

Subsequent clinical trials of TSO reported significant improvement in patient responses for both UC and Crohn's disease with essentially no adverse effects [5–8]. In a landmark randomized, placebo-controlled, double-blind study of 54 subjects with moderate to severe UC, subjects in the treatment group ingested 2500 TSO every 2 weeks for a total of 12 weeks [5]. Subjects were evaluated with the Ulcerative Colitis Disease Activity Index (UCDAI) at week 0 and week 12 (which requires inspection of the colonic mucosa by endoscopy) in addition to biweekly assessment with a symptom-based index [9]. After 12 weeks of therapy, 43.3% of the individuals treated with TSO had improved symptoms (defined as a decrease in UCDAI of $\geq$4 points) compared to 16.7% in the placebo group, which was a statistically significant response rate. Furthermore, TSO-treated subjects reported significant improvement in their symptoms (compared with placebo) as early as week 6. This was the first trial to define a subgroup of relatively treatment-refractory patients who responded to helminthic therapy in a controlled setting. There was a

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Table 1. Selected clinical studies of helminthic therapy in human disease

Year of publication	Patient population (institution)	Number of subjects	Trial design	Clinical assessment measures	Main observations
2003 [4]	Crohn's disease and ulcerative colitis (University of Iowa)	*Initial phase*: 4 (CD) 3 (UC) *Maintenance phase*: 2 (CD) 2 (UC)	Open-label Phase I pilot *Initial phase*: 2500 TSO PO × 1 Clinical monitoring q2 week × 12 weeks *Maintenance phase*: 2500 TSO q3week × 28 weeks	Safety assessment Remission: CDAI < 150 IBDQ >170 SCCAI < 4	• No adverse events observed • After single dose of TSO in CD: 75% achieved remission, 66% relapse rate by week 12 • After single dose of TSO in UC: 100% achieve remission, 33% relapse rate by week 12 • Maintenance phase: 100% achieved remission with triweekly dosing for > 28 weeks
2005 [8]	Active Crohn's disease (CDAI > 220) (University of Iowa)	29	24-week open-label Phase I study 2500 TSO PO q2weeks	Response: CDAI decrease >100 Remission: CDAI <150	• At week 12, 75.9% responded, 65.5% remitted • At week 24, 79.3% responded, 72.% remitted
2005 [5]	Moderate-severe ulcerative colitis (University of Iowa)	54	12-week randomized double-blind placebo-controlled trial 2500 TSO PO q2weeks	Response: UCDAI < 4 Remission: UCDAI < 2	• At week 12, 43.3% (TSO) vs. 16.7% (PBO) • At week 12, nonsignificant differences in remission rates observed between treatment groups
2006 [32]	Crohn's disease (Townsville Hospital, Australia)	9	45-week open-label POC study 25–50 NA L3i SC at 0 and 27 weeks	Response: CDAI < 150 IBDQ > 170	• CDAI remained unchanged until week 17 • Four week cumulated CDAI scores decreased after 20 weeks (mean 141 *v* 87) • IBDQ improved after 20 weeks (mean 151 *v* 179) • Adverse events included anemia, painful transient enteropathy and peripheral eosinophilia
2009 [30]	AMP-responsive Asthma	32	16-week randomized double-blind placebo-controlled study 10 NA L3i SC	Change in provocation dose of inhaled AMP required to reduced forced expiratory volume in 1 s by 20% (PD_{20}AMP)	• No significant differences in mean airway responsiveness (PD_{20}AMP), asthma control or allergen skin testing were observed
2010 [15]	Treatment-naïve relapsing remitting multiple sclerosis (University of Wisconsin)	5	Phase I baseline vs. treatment study 2500 TSO PO q2weeks × 3 months	Neurological function (MSFC and EDSS) Gadolinium-enhancing lesions on MRI Immunologic assessments (e.g. serum *T. suis* specific IgG_1 and IgA)	• No adverse events observed • No significant change observed in baseline versus treatment neurological functioning • Mean number of new gadolinium-enhancing MRI lesions ($n–Gd^+$) decreased (6.6 at baseline to 2.0 at 3 months). The number of $n–Gd^+$ rose to 5.8 2 months after TSO discontinued.
2010 [10]	Allergic rhinitis (Statens Serum Institut and University of Copenhagen, Denmark)	100	27-week randomized double-blind placebo-controlled trial 2500 TSO PO q2weeks × 25 weeks	Symptom severity score of allergic rhinitis Skin prick testing Immunologic assessments (e.g. serum *T. suis*-specific IgA and IgE, serum grass-specific IgE and total histamine)	• Treatment with TSO caused transient diarrhea peaking at day 41 in 33% of participants (placebo, 2%) • No significant change in symptom scores, total histamine, grass-specific IgE or diameter of wheal reaction in skin prick testing with several allergens observed
2010 [63]	Ulcerative colitis (University of San Francisco)	1	Case study 1500 *Trichuris trichiura* PO ova ad lib for 5 years	Mucus production Mucosal gene expression Flow cytometric analysis of effector T helper cell cytokine production	• Helminth exposure associated with clinical remission and mucosal healing • Helminth exposure associated with increased $IL–17^+IL–22^+$ cells compared to episodes of colitis • Helminth exposure associated with genes involved in carbohydrate and lipid metabolism
2011 [31]	Celiac disease (Princess Alexandra Hospital, Brisbane, Australia)	20	21-week randomized double-blind placebo-controlled study 10 and 5 L3i NA or placebo SC at 0 and 12 weeks. At week 20, subjects underwent a 5 day 16 gram gluten challenge	Duodenal histologic Marsh score Systemic IFN–γ measured by QE65–ELISpot	• No significant differences in duodenal pathology was found between hookworm infected and placebo injected groups • No significant difference in gluten-specific IFN-γ-producing PBMCs was observed following gluten challenge between the study groups • Hookworm-infected subjects experienced an injection site reaction, painful transient enteritis and a modest leukocytosis with eosinophilia

Abbreviations: CD, Crohn's disease; UC, ulcerative colitis; TSO, *Trichuris suis* ova; UCDAI, ulcerative colitis disease activity index; IBDQ, inflammatory bowel disease questionnaire; SCCAI, Simple Clinical Colitis Activity Index; MSFC, multiple sclerosis functional composite; EDSS, expanded disability status scale.

trend towards improved response in those subjects with extensive colonic involvement and shorter duration of disease activity. A 24-week open-label study of TSO in 29 patients with active Crohn's disease showed an even more robust response rate (79.3%), with an impressive remission rate of 72.4% with no adverse events reported [8]. Currently, larger Phase II dose-escalation trials of TSO in Crohn's disease are ongoing in Europe (Dr. Falk Pharma, GmbH; NCT01279577) and the United States (Coronado Biosciences and OvaMed GmbH; NCT01434693). We are currently recruiting moderate to severe UC patients to conduct an exploratory mechanistic trial of TSO in order to better characterize the mucosal immune response at New York University School of Medicine (NCT01433471).

TSO therapy is also being evaluated in extra-intestinal diseases such as multiple sclerosis [10,11]. A strong body of epidemiological and experimental evidence suggests that parasitic infection may be protective for multiple sclerosis [12]. In an uncontrolled prospective double-cohort study of 12 patients with relapsing–remitting multiple sclerosis (RRMS) who presented with infection with different organisms (*Hymenolepis nana*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides sterocolaris* and *Enterobius vermicularis*), helminth-infected patients had a significantly lower number of exacerbations and fewer magnetic resonance imaging changes compared with uninfected patients [12]. Furthermore, increased regulatory cytokine production (e.g. IL-10 and TGF-β) and $CD4^+CD25^+FoxP3^+$ T cell clones were noted to be significantly enriched in the infected cohort [12]. Additional studies by this group demonstrated that helminth infections induced regulatory B cells capable of suppressing the immune response through IL-10 production [13]. Interestingly, when some of these patients were ultimately treated with antihelminthics for worsening parasite-associated symptoms, a flare in clinical and radiologic MS activity occurred that was accompanied by an increase in IFN-γ and IL-12 producing cells and a decline in IL-10, TGF-β and regulatory T cells [14].

In 2011, the first prospective use of TSO in five subjects with treatment-naïve relapsing–remitting multiple sclerosis (RRMS) was published in the helminth induced immunomodulator therapy (HINT)-1 study [15]. The mean number of new gadolinium-enhancing lesions in treated individuals decreased from 6.6 at baseline to 2.0 at the end of 12 weeks of TSO treatment. Lesion incidence increased to a mean of 5.8, 2 months after the completion of the treatment phase, indicating that any protective effects were transient. TSO treatment was associated with relative increases in T_H2 cytokines without significant decreases in T_H1 cytokines. Acute phase reactants such as high-sensitivity C-reactive protein (hs-CRP) rose during the first 2 months of TSO administration and fell during and after the last 4 weeks of ova exposure. Anti-*T. suis* IgG1 antibodies showed a durable response, whereas *T. suis*-specific IgA returned to baseline after treatment and IgE was undetectable during the study period. Peripheral $CD4^+CD25^+Foxp3^+$ cells modestly increased in only two of the five subjects under study.

As of January 2012, additional clinical trials of TSO in adult autism (NCT01040221), peanut and tree nut allergy (NCT01070498) and multiple sclerosis (NCT01413243) are ongoing.

Safety concerns of TSO

TSO has been extensively studied in IBD patients on concomitant prednisone, thiopurines and other immunosuppressants, suggesting relative safety even in immunocompromised hosts [16]. In a 2010 randomized double-blind, placebo-controlled investigation of 100 subjects with allergic rhinitis (the largest clinical trial of a helminthic agent in a human study population to date), treatment with TSO showed no significant effect on symptom scores or subclinical measures of allergic reactivity compared with placebo [10]. However, this was the first clinical trial to detail treatment-emergent symptoms such as diarrhea, excessive flatulence and upper abdominal pain in the majority of TSO-treated subjects [17–21]. These events peaked 30–50 days after the first treatment with TSO, were generally transient (median duration of 2 days) and could be related to the expulsion of *T. suis* larvae from the gut [21]. These symptoms were not observed in earlier studies of TSO in IBD patients, perhaps because they were occurring in the context of already moderate to severe gastrointestinal pathology in the study cohorts. Concerns have been raised about aberrant migration of *T. suis* in its non-natural host to other organs and tissues [19] but this has not occurred in any of the subjects studied in any trial of TSO to date. Secondary bacterial infection, specifically *Campylobacter jejuni*, could also be a concern [22,23]. A case of life-threatening campylobacterjejunosis leading to toxic megacolon and acute renal failure associated with concomitant *T. trichiura* infection has been reported [20] but this has also never been observed with TSO-treated subjects.

Clinical trials with *Necator americanus*

The only other helminth studied in clinical trials thus far is the hookworm *N. americanus* but this has been less successful than its porcine whipworm counterpart. Hookworm infection is highly prevalent in impoverished regions of the world [24] and can also upregulate immunoregulatory molecules such as IL-10, TGF-β and metalloproteases [25–28]. However, hookworm infections can be far from benign, with the most common hookworm-related injuries being a pruritic maculopapular pruritic skin eruption, gastrointestinal symptoms such as diarrhea, increased flatulence, abdominal pain, cough, dyspnea, malaise and iron deficiency anemia secondary to chronic blood loss [25].

Due to the requisite extra-intestinal phase of its lifecycle and the fact that humans are the natural host, there is probably a much narrower therapeutic window between achieving effective immune modulation and causing unacceptable adverse events for *N. americanus*. Dose-ranging studies of therapeutic infection of *N. americanus* in humans have shown that doses higher than 10 larvae correlate with more frequent adverse events than low-dose inocula [25,29]. This is a very small number compared with the 2500 TSO being used in Phase II trials. In a 2009 randomized double-blind investigation, 32 subjects with asthma were randomized to receive 10 larvae or placebo for 16 weeks, with the primary outcome being a change in the

provocation dose of inhaled AMP required to reduce the forced expiratory volume in 1 s by 20% (PD_{20}AMP) from baseline to week 16. Although the absolute mean PD_{20}AMP increased more in the hookworm group, the differences between the treated groups were nonsignificant [30]. Furthermore, gastrointestinal side effects such as abdominal pain, loss of appetite and nausea were significantly higher in the *N. americanus* treated group than in the placebo group.

More recently, a 2011 randomized double-blind clinical trial of *N. americanus* larvae or placebo in 20 human leukocyte antigen (HLA)–DQ2 positive patients with well-controlled clinically inactive celiac disease was completed in Australia [31]. After subcutaneous inoculations of 10 and 5 stage three larvae at 0 and 12 weeks (or placebo mixed with Tabasco sauce in order to simulate the expected hookworm-associated pruritic skin eruption that follows inoculation), subjects underwent a 5-day gluten challenge followed by esophagogastroduodenoscopy with duodenal biopsies for determination of histopathologic Marsh score (a widely used scoring system which grades duodenal inflammation and villous atrophy). No significant difference was observed in pathologic grade or systemic inflammatory immune response determined by gluten-specific IFN-γ producing peripheral blood mononuclear cells (PBMCs) after gluten challenge between the hookworm-infected and placebo-injected groups, although the adverse reactions were more similar than previously reported between the two groups [32]. Subsequent characterization of the circulating and mucosal immune response to *N. americanus* in these subjects showed a strong mucosal T_H2 response as well as increased IL-22 expression accompanied by declines in IFN-γ and IL-17A secretion from cultured duodenal pinch biopsies [33,34]. The authors' comment that the relatively low inoculation dose of hookworm (15 worms) used in the trial may have been insufficient to induce an immunosuppressive phenotype in this patient population.

Regulatory mechanisms in helminthic therapy

In recent years, immune regulation has been the major mechanism proposed to explain the potential beneficial effects of helminths [35]. Because these mechanisms have been reviewed in detail recently [35], in this review we provide just a brief summary of studies addressing the function of immunoregulatory cell populations during helminth infection.

Regulatory T cells (Tregs) clearly expand during a wide range of helminth infections [1,2,36]. The use of depleting (anti-CD25) and neutralizing antibodies (anti-CTLA–4 and anti-GITR) can enhance lymphocyte proliferation and cytokine responses. In some but not all cases this will promote parasite clearance but may also lead to inflammatory pathology [2]. Recently, the use of a mouse strain engineered for the inducible deletion of FoxP3 expressing Tregs (depletion of regulatory T cell, DEREG) has established a role for this subset in regulating effector responses that mediate parasite killing [37] and inflammatory pathology [38]. Thus, helminth-elicited Treg populations appear to benefit both parasite and host. However, functional studies of Treg populations in mouse models of helminthic therapy have shown considerable heterogeneity in outcomes. Suppression of allergic airway disease by infection with *Heligmosomoides polygyrus* or with *Schistosoma mansoni* eggs was dependent on Tregs [39,40]. However, neither the depletion of $CD25^+$ cells nor TGF-β neutralization affected the suppression of airway inflammation mediated by *Litomosoides sigmodontis* [41]. In contrast to the asthma model, *H. polygyrus*-mediated inhibition of diabetes was not reversed by CD25 depletion or IL-10 neutralization [42]. The antidiabetic effect of schistosomal egg antigens (SEA) was transferable by adoptive transfer of unfractionated but not CD25-depleted splenocytes from SEA-exposed mice [43]. By contrast, although the suppression of diabetes by *L. sigmodontis* infection did not appear to be dependent on $CD25^+$, $FoxP3^+$ Tregs or IL-10 signaling, neutralization of TGF-β reversed the therapeutic effect [44]. TGF-β was also shown to be critical in *Fasciola hepaticus*-mediated protection against experimental autoimmune encephalomyelitis, a model of multiple sclerosis [45].

Therefore, distinct Treg subsets induced by the same helminth infection may mediate protection against different inflammatory diseases (e.g. TGF-β is essential for *L. sigmodontis*-mediated suppression of diabetes but not allergic airway disease). Finally, for some models of helminthic therapy (e.g. chemically-induced colitis) a role for helminth-elicited Tregs remains to be demonstrated.

Dendritic cells (DCs) and macrophages (MΦs) are also important immune regulatory cells during helminth infection [2]. Balb/c mice infected with male worms of *S. mansoni* are protected from DSS-induced colitis through a macrophage dependent pathway and not through IL-10, TGF-β or Tregs [46]. Extracts from the tapeworm *Hymenolepis diminuta* can reduce colitis, perhaps because it suppresses macrophage activation [47]. Intestinal DCs of *H. polygyrus*-infected mice are also diminished in their ability to activate T cells [48] and may be protective in an $IL\text{-}10^{-/-}$ T cell transfer model of colitis. Because intestinal DCs and MΦs are important in regulating mucosal homeostasis [49], it is not surprising that changes in their phenotype may occur during helminthic therapy. In addition, because the immunoregulatory, alternatively activated macrophages (or M2 cells) are induced by helminths to repair tissue damage [50] and have been shown to suppress colitis [51], they may play a critical role in helminthic therapy for IBD.

Enhancement of the mucosal barrier in helminthic therapy for IBD

The immune response to intestinal helminth infection drives a potent physiologic response with the dual aims of parasite expulsion and mucosal healing [3]. We propose that these T_H2-associated host responses that drive resistance against intestinal helminths may also underlie the protective effects against IBD (Figure 1).

Recently, the mucosal response of an individual who self-infected with *T. trichiura* to treat his own symptoms of UC was longitudinally characterized [52]. He was able to put his disease into remission twice by self-infection. Although we originally speculated that regulatory mechanisms might play an important role in his situation, a detailed analysis of mucosal pinch biopsies collected during colonoscopy suggested that the effect of T_H2 cytokines

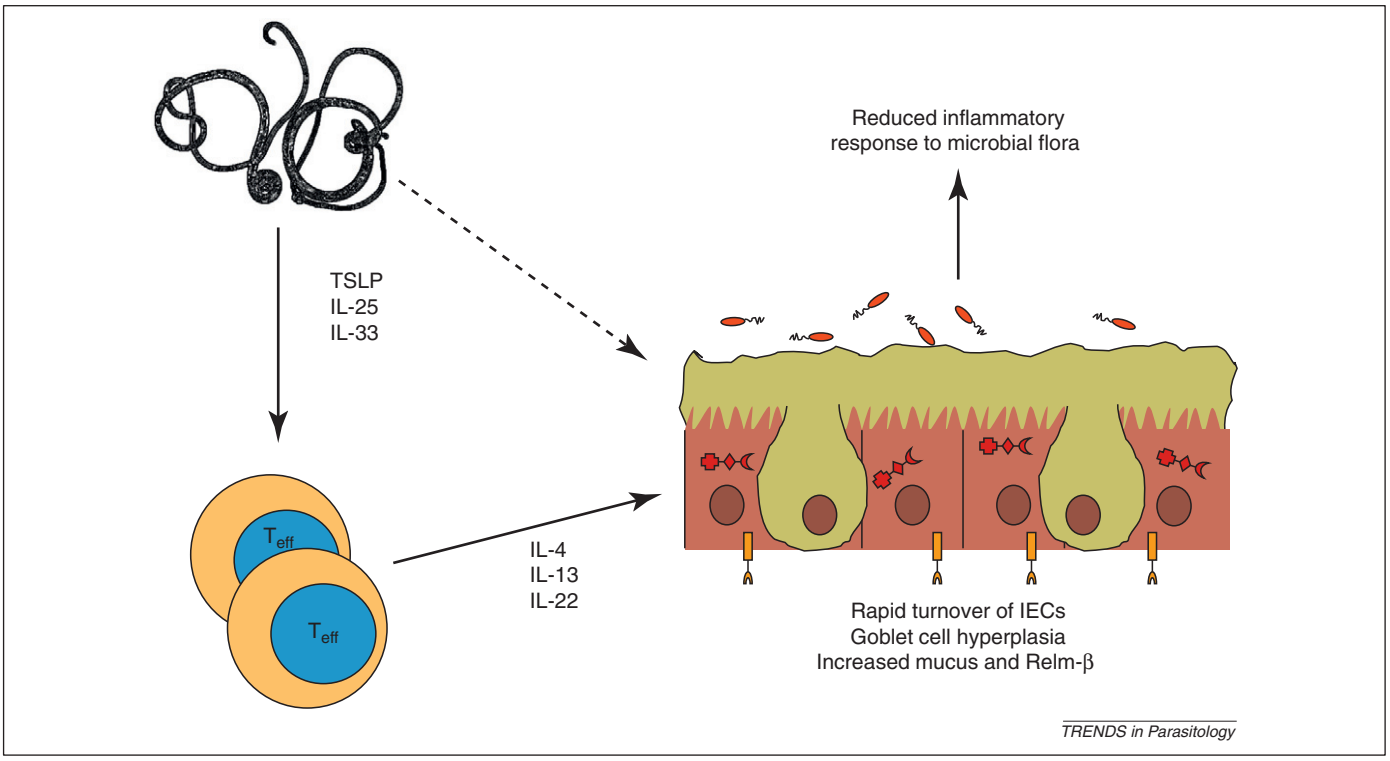

Figure 1. A mechanistic model for improved mucosal barrier function from helminthic therapy with *Trichuris* worms. Interactions between the parasites and intestinal epithelial cells leads to the production of cytokines such as TSLP, IL-25, IL-33 and other yet unidentified factors that can induce the differentiation of naïve T cells into effector cells that produce T_H2 cytokines such as IL-4 and IL-13, as well as IL-22. These cytokines can then increase the turnover of intestinal epithelial cells (IECs) as well as induce goblet cell differentiation, maturation and hyperplasia. Goblet cells increase production of mucus and molecules such as Relm-b, which improves the physical barrier separating the IECs from gut microbiota in the lumen of the colon. This could lead to a reduced inflammatory response to luminal bacteria and improve the conditions of individuals with ulcerative colitis.

and IL-22 on mucosal barrier function may play an even greater role in symptomatic improvement. T_H2 cytokines and IL-22 have profound effects on colonic epithelial cell function [53–55], including the stimulation of goblet cell and Paneth cell differentiation with their attendant mucus production, antimicrobial peptide expression and the activation of antiapoptotic pathways. Furthermore, accessory cells recruited and activated by type 2 cytokines, most notably alternatively-activated macrophages, can promote mucosal healing [56]. Additionally, IL-13 and IL-22 can increase the proliferation and turnover of IECs, which serves the dual function of parasite expulsion and mucosal healing [3,53]. Taken together, these functions enhance the epithelial barrier against luminal antigens and they have demonstrated protective effects in murine models of colitis [56–58]. Mucus hypersecretion, a ubiquitous feature of the host response to intestinal worms, may therefore be implicated in the protective effect of helminth infection in the setting of IBD by reinforcing the mucosal barrier. Although triggered to expel intestinal helminths, this response could also protect against exposure to luminal bacteria in patients with IBD.

Intestinal mucus is a carbohydrate-rich gel, approximately 1 mm thick, charged with the formidable task of separating the intestinal epithelium from $\sim 10^{13}$ commensal bacteria. The scaffolding of the mucus gel is primarily composed of mucins, high molecular weight glycoproteins bearing O-linked oligosaccharides that are commonly decorated with chemical moieties such as sulfate and acetyl groups. Of the 19 mucins identified in humans, Muc2 is the most important mucin secreted in the intestine [59]. Muc2 forms two distinct layers following secretion by goblet cells. The loosely packed outer layer is the main bulk of the mucus gel and harbors a large number of bacteria. Conversely, the thin inner layer is composed of tightly packed lamellar sheets that are normally impermeable to bacteria [60]. Below the Muc2 layers, transmembrane mucins (e.g. Muc3) cover the apical surface of enterocytes. A lipid fraction largely composed of amphipathic phospholipids contributes to the viscosity and hydrophobicity of the mucus gel [61]. Phosphatidylcholine (PC) and lyso-PC are the most abundant phospholipids in colonic mucus [62].

Histochemical studies have demonstrated that the mucus gel is abnormal in both quantity and quality in a large fraction of UC patients [63]. Muc2 abundance is lower in rectal mucus samples from UC patients [64] and displays altered glycosylation [65] and reduced sulfation [66]. A causal role for altered expression and post-translational processing of mucins in the pathogenesis of colitis is supported in several mouse models. Genetic deficiency [67,68] or terminal misfolding [69] of Muc2 precipitates severe, spontaneous colitis in mice. Impaired glycosylation of mucins due to specific glycosyltransferase deficiencies also increases susceptibility to colitis [70,71]. More recently, abnormalities in phospholipid species have also been described in UC patients, with a significant decrease in PC [62,72,73]. Intriguingly, clinical trials in which the phospholipid content of mucus in UC patients was restored to

that of healthy individuals by oral intake of delayed-release PC have shown promising results [72–74].

Helminth infection is also associated with qualitative changes in mucus composition, including increased sulfation of mucins [75] and stimulation of bulk mucus production via goblet cell hyperplasia and increased mucin expression [3]. Muc5ac is a mucin that is specifically induced by helminth infection and is important for the expulsion of these parasites [76]. The increased production of resistin-like molecule (RELM)-beta by goblets cells after helminth infection may also play a critical role in reinforcing mucosal barrier function [77,78]. T_H2 cytokines can induce intestinal epithelial cells (IECs) to differentiate into goblet cells producing RELM-beta, which in turn plays a critical role in the expulsion of worms that live in the gut lumen [77,78]. Interestingly, RELM-beta deficient mice are more sensitive to DSS-induced colitis [79] and delivering RELM-beta to the colon can improve TNBS-induced colitis [80]. RELM-beta also regulates expression of antibacterial peptides like REG3 beta/gamma [79], which may be associated with alteration in the gut microbiota [81–83].

These changes in the quality, quantity and antibacterial peptide composition of mucus during helminth infection is likely to have a major impact on the gut microbial environment. *H. polygyrus* infection has been shown to have major effects on the microbiota of mice, especially increasing the abundance of the Lactobacillaceae family [84]. Indeed, successful colonization of the colon with *T. muris* is dependent on the gut microbiota [85]. Further studies may reveal the intricate relationship between helminth infection, gut microbiota and the protection against IBD. It is conceivable that some of the protective effects of helminth infection may be attributable to indirect effects downstream of alterations in gut microbiota rather than direct effects of helminth infection.

Concluding remarks

Although the therapeutic window for *N. americanus* may be too narrow for clinical use, TSO could potentially become the first live parasite that is used as a therapeutic agent. Phase II clinical trials for Crohn's disease are in progress and should be completed in 2012. Although there has been an extensive body of work on the role that regulatory cells and cytokines may play during helminthic therapy, we propose that more direct effects of helminths on mucosal barrier function may play an equally important role in inflammatory diseases of the intestinal tract. We propose a model whereby the immune response that is triggered to expel gastrointestinal parasites, which includes increased mucus production, changes to the composition of mucus secreted by goblet cells and increased epithelial cell turnover, may have a beneficial effect in restoring mucosal barrier function during inflammatory bowel disease and reducing inflammation driven by gut bacteria. To test this model, a clinical trial is being conducted that focuses on elucidating the mechanism of action of TSO, rather than evaluating clinical efficacy (NCT01433471) (P. Loke, unpublished). The further study of host protective mechanisms activated during intestinal helminth infection may identify novel pathways that can bolster mucosal barrier functions without the risks of immunosuppression associated with current treatments for severe IBD.

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