Helminths in alternative therapeutics of inflammatory bowel disease

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Inflammatory bowel disease (IBD), which includes Crohn’s disease and ulcerative colitis, is a nonspecific chronic inflammation of the gastrointestinal tract. Despite recent advances in therapeutics and newer management strategies, IBD largely remains untreated. Helminth therapy is a promising alternative therapeutic for IBD that has gained some attention in the last two decades. Helminths have immunomodulatory effects and can alter the gut microbiota. The immunomodulatory effects include a strong Th2 immune response, T-regulatory cell response, and the production of regulatory cytokines. Although concrete evidence regarding the efficacy of helminth therapy in IBD is lacking, clinical studies and studies done in animal models have shown some promise. Most clinical studies have shown that helminth therapy is safe and easily tolerable. Extensive work has been done on the whipworm Trichuris, but other helminths, including Schistosoma, Trichinella, Heligmosomoides, and Ankylostoma, have also been explored for pre-clinical and animal studies. This review article summarizes the potential of helminth therapy as an alternative therapeutic or an adjuvant to the existing therapeutic procedures for IBD treatment. (Intest Res, Published online)

Key Words: Inflammatory bowel disease; Ulcerative colitis; Crohn disease; Helminths; Microbiota

INTRODUCTION

Inflammatory bowel disease (IBD) is a nonspecific chronic relapsing and remitting inflammation of the gastrointestinal tract,¹ which affects about 6.8 million people globally.² IBD includes Crohn’s disease (CD) and ulcerative colitis (UC).³ The patients with CD and UC show some common clinical symptoms, such as abdominal pain, fatigue, continuous or intermittent diarrhea, and rectal bleeding,¹ but show differences in the anatomical distribution of inflammatory lesions.⁵ Traditional IBD treatment includes immunosuppressants, corticosteroids, and aminosalicylates. The traditional treatment is now supplemented with biological therapies, which include anti-tumor necrosis factor (TNF),⁶ anti-integrins,⁷ anti-interleukins (ILs),⁸⁻¹⁶ and Janus kinase (JAK) inhibitors.¹⁷⁻²⁰ Nearly 50% of patients do not respond or respond slowly to traditional and biological therapies,²¹ which necessitates the continued development of new therapeutic options for IBD treatment.

Researchers have been exploring the potential of helminths in various autoimmune disorders, including IBD.²² Intestinal helminthic infections are common in developing countries with high population densities and poor sanitation. Coincidentally, these are also the regions of lower IBD prevalence. Autoimmune and inflammatory diseases are common in developed countries, though incidences of such diseases are also rising in developing countries. According to the "hygiene hypothesis,"
there exists a relationship between an increase in allergic and autoimmune diseases and a lack of exposure to helminth infections.23 The hygiene hypothesis is supported by epidemiological and clinical evidence.24-29

Once considered a rare disease, the frequency of IBD has increased in recent years. According to the “IBD hygiene hypothesis,” modern lifestyle has changed the intestinal microbiota, affecting the development of immune regulatory circuits, predisposing us to IBD.30 Therefore, the loss of exposure to parasitic worms has been hypothesized to increase the risk of IBD,30,31 suggested by the inverse relationship between intestinal helminthic infections and the incidence of IBD.27,28,30,31 Possibly because helminths can modulate host immunity to provide protection against IBD. Helminth infection in childhood can protect against the development of IBD.31 Kabeerdoss et al.32 reported that compared to CD patients, control subjects had higher T cell reactivity to the hookworm (Ancylostoma and Necator americanus) antigen, suggesting that hookworm infection may prevent the development of CD. This review describes the potential of using helminth therapy as an alternative therapeutic option or an adjuvant to the existing therapeutic procedures for IBD treatment. It also describes the relationship between helminths and gut microbiota. Furthermore, the review also describes various helminth species that have been explored for pre-clinical and animal studies.

HELMINTHS AND GUT MICROBIOTA

Helminths and microbiota are “old friends” of humans, as humans have coexisted with both for their entire existence. The human gut microbiota includes approximately 40 trillion microorganisms.35 The composition of the human gut microbiota is affected by a number of factors.37 Several studies have been conducted to know the effects of helminths on gut microbiota. Although further studies are required to reach a consensus, most of these studies have concluded that helminth infection results in a change in the gut microbiota composition,34,36,37 which can affect anti-inflammatory responses.38 The therapeutic effects of helminth therapy may be due to a shift in the gut microbiota, which depends on the type of helminth species. Helminths may affect gut microbiota and induce dysbiosis by interacting directly with the gut microbiota through physical contact, by producing chemical products, or by competing for nutrients.39 Various antimicrobial peptides present in helminth excretory/secretory (ES) products can directly influence gut microbiota.40 Moreover, helminth-derived extracellular vesicles contain antimicrobial peptides and proteins that can affect the gut microbial composition.41

Most studies showing the effect of helminths on gut microbiota are carried out in animals. These studies indicate that helminth infection can alter the gut microbiota composition in animal models.29,42-44 Heligmosomoides polygyrus infection increases Lactobacillaceae/Lactococcus abundance in a susceptible mouse.43,44 Trichuris suis infection alters the abundance of Proteobacteria and Deferrribacteres in porcine colon microbiota47 and increases the abundance of lactobacilli and decreases the diversity of the murine intestinal microbiota.48 Moreover, T. suis infection along with dietary inulin enhances the modifications of the gut microbiota to promote beneficial bacteria such as Prevotella in the porcine gut.49 Helminth infection can alter alpha diversity and microbial richness, and fecal short-chain fatty acid content in mice.50 Trichuris trichiura infection changes gut microbial communities in both mice and humans.51 Recently, Guiver et al.52 reported that helminth infection in mice decreased α-diversity along the gradient of infective doses and increased β-diversity as the infective dose increased. Studies have reported that chronic infections with helminths, particularly Trichuris muris or H. polygyrus, reduce the abundance of Bacteroidetes and increase the abundance of Lactobacillaceae.29,33,36,38,53 Contrary to these studies, Su et al.54 found that H. polygyrus infection increased the abundance of Bacteroidetes and decreased the abundance of Firmicutes and Lactobacillales. A study carried out by Broadhurst et al.55 in a primate model of idiopathic chronic diarrhea reported that T. trichiura was associated with an increase in microbial diversity and increased abundance of Bacteroidetes and Tenericutes.

Studies carried out on humans also reported a change in gut microbiota with helminth infection. A study by Ramanan et al.56 found that the gut microbiota of people living in a region with low helminth infections have a higher abundance of Bacteroides, and those living in a region with high helminth infections have a higher abundance of Faecalibacterium and Prevotella. Another study by Jenkins et al.57 reported that the intestinal microbiota of people with helminth infection had an increased abundance of Verrucomicrobiaceae and Enterobacteriaceae, and those without helminth infection had an increased abundance of Leuconostocaceae. Gordon et al.58 reported that helminth infection increased the richness and diversity of the gut microbiota with an increased abundance of Faecalibacterium. A meta-analysis by Kupritz et al.58 reported that helminth infection can influence the alpha and beta diversity of
the human fecal microbiome. Contrary to these studies, Cooper et al. observed that T. trichiura infection did not alter the human fecal microbiota. Other studies carried out in humans also reported that helminth infection could produce little or no changes in gut microbiota.60,61

Studies have also found a two-way interaction between helminths and microbiota. The eggs of T. muris require direct contact with Escherichia coli to hatch.62 T. muris also requires the presence of Bacteroides thetaiotaomicron for successful infection.61 T. muris limits subsequent infections by other parasites of the same species by altering the host gut microbiota.63

**HELMINTHS AND THE HOST IMMUNE SYSTEM**

Parasitic helminths have evolved with the human immune system and are capable of immunomodulation to promote their own survival.64 The immunomodulatory molecules secreted by parasitic helminths to evade the host immune system can down-regulate cellular responses, reducing the excessive proinflammatory responses to some diseases. The helminths inhabiting the human intestine either attach to the intestinal epithelium or live freely in the intestinal lumen. The larvae of some intestinal helminths live within the epithelial layer of the intestine and therefore may interact directly with the host immune system. Helminths or their products can modulate various innate and adaptive immune cells, including dendritic cells (DC) that support the outgrowth of regulatory T cells (Tregs) and macrophages.65-72

CD4+ T cells are major determinants in the generation of the host’s protective immune response against helminths. CD4+ T cells are divided into different sub-populations, such as Th1 and Th2, based on the type of cytokines produced. Th1 cells produce cytokines that contribute to inflammation and perpetuate autoimmune response, such as IL-2, IL-12, and interferon gamma (IFN-γ), while Th2 cells produce cytokines that have anti-inflammatory response, such as IL-10. The host immune responses to helminths include a strong Th2 immune response, Treg response, and the production of regulatory cytokines, such as IL-10.73-81 The specific Th2 response and Th2 cytokines induced by helminths82,85 can attenuate Th1-driven inflammatory responses in the host.82,85,86 Helminths are also capable of inducing immune Tregs that express markers such as Foxp3 and CD25.87-90 Such Tregs include type 1 Tr (Tr1) cells that secrete high levels of IL-10.88,90 The induction of Th2 and Tr1 can explain the beneficial effects of helminth therapy in IBD patients.84 The Th2 response elicited by helminth infection also induces alternatively activated macrophages,91,94 which can negatively regulate Th1 response.95,96

**HELMINTHS IN IBD THERAPEUTICS**

Several helminths, including Trichuris, Schistosoma, Trichinel- la, Heligmosomoides, and Ancylostoma, have been considered potential candidates for developing alternative or adjuvant therapy for IBD (Fig. 1). Trichuris is a genus of parasitic roundworms in the family Trichuridae. Trichuris is commonly referred to as whipworm. The important species of Trichuris are T. suis, T. muris, and T. trichiura. The natural host of T. suis is the pig, but it can transiently colonize humans without causing disease.97 T. trichiura is known as the human whipworm because humans are its natural hosts. The natural host of T. muris is the mouse. Schistosoma is a genus of parasitic flatworms, commonly known as blood flukes, belonging to the class Trematoda. Schistosoma mansoni is the leading cause of schistosomiasis in humans. S. mansoni and Schistosoma japonicum are potential candidates for IBD treatment, and considerable research has been carried out on these 2 species. S. japonicum has a wide host range and infects many species of mammals. Trichinella spiralis is a parasitic roundworm that causes trichinosis in humans. It is transmitted to humans by consuming undercooked or raw meat. T. spiralis completes its life cycle in a single host, during which it releases many proteins that help it to evade the host immune response.98-100 Another species, Trichi- nella papuae, has also been considered for treating IBD.101 H. polygyrus is a roundworm commonly found in the intestine of rodents. Ancylostoma is a genus of roundworms commonly referred to as hookworms. Only a few studies have explored the potential of Ancylostoma in IBD treatment.102,103

Clinical trials have been carried out for only a few, including Trichuris and Schistosoma (Table 1). Most of the studies on the efficacy of helminth therapy in IBD are restricted to animal models. In a recent meta-analysis, Shields and Cooper concluded that it is difficult to reach any conclusion regarding the efficacy of helminth therapy. Nevertheless, helminth therapy is safe and tolerable. Large clinical cohort studies must be carried out to establish the efficacy of helminth therapy in IBD.

**1. Human Clinical Trials**

Clinical trials have been carried out using Trichuris, protein derived from Schistosoma, and excretory products derived from H. polygyrus. Several studies have been conducted to evaluate the effectiveness of T. suis for IBD treatment.27,20,65,107-110 T. suis
was the organism of choice for early clinical trials in humans. In one of the first small open-label trials, 7 patients were enrolled, 4 with CD, and 3 with UC. Each patient who received a single dose of 2,500 viable ova demonstrated improvement in clinical symptoms. Another study by the same group enrolled 29 patients with CD and found that after 12 weeks of *T. suis* ova (TSO) therapy, 66% of patients showed remission. A similar study carried out in patients with UC also reported that patients receiving 2,500 viable *T. suis* eggs showed a significant improvement over placebo. Human clinical trials...
have revealed that TSO therapy can reduce the disease severity in some patients with UC and CD, is well tolerated and not associated with short- or long-term side effects.\textsuperscript{85,107,111-113} In a case study, Broadhurst et al.\textsuperscript{114} reported that patients with severe refractory UC achieved clinical remission when infected with \textit{T. trichiura}. They also showed that \textit{Trichuris} infection escalated mucosal expression of IL-4 and IL-22.

Despite the potential of TSO therapy, many studies have found contradictory results. In a Cochrane review, Garg et al.\textsuperscript{108} concluded that there was insufficient evidence regarding the efficacy and safety of TSO therapy to treat IBD patients. Schölmerich et al.\textsuperscript{109} reported that TSO therapy did not show any clinical efficacy against active CD. A meta-analysis by Huang et al.\textsuperscript{110} concluded that TSO therapy showed no statistical benefit for IBD patients.

A protein derived from \textit{Schistosoma}, P28 glutathione-S-transferase (P28GST), combines the activities of antioxidant glutathione S-transferase and prostaglandin D synthase.\textsuperscript{115,116} P28GST was first developed as a vaccine against schistosomiasis and demonstrated safety and tolerability, both in adults and children.\textsuperscript{117,118} In a pilot Phase 2a study, Capron et al.\textsuperscript{119} evaluated the safety of P28GST in patients with mild CD. They found a decrease in baseline Crohn's Disease Activity Index and blood calprotectin levels, suggesting that P28GST can be used as a safe therapeutic option for CD. Cook et al.\textsuperscript{120} reported that a secretory product of \textit{H. polygyrus}, Hp-TGF-\textbeta mimic (Hp-TGM), could induce Foxp3 expression in a more efficient manner than transforming growth factor beta (TGF-\textbeta). Moreover, Tregs induced by Hp-TGM had superior suppressive function than Tregs induced by TGF-\textbeta. Croese et al.\textsuperscript{121} reported that hookworm \textit{Necator americanus} could improve disease activity in 9 patients with CD after 20 weeks of administration.

\section*{2. Studies Carried on Animal Models}

While there are only a few studies on humans that suggest potential of helminths in IBD therapeutics, many studies have been carried out in animals, particularly mouse model of IBD. These studies have explored the potential of whole organisms, eggs/ova, antigens, soluble and secreted proteins, metabolites, ES, and recombinant products from different helminths.

\subsection*{1) Whole Organisms}

Experiments in animal models have been carried out using both adult worms and larvae from various helminth species. The use of whole organisms has shown some potential in animal models and can be replicated in large-scale clinical trials. In one of the early studies, Readon et al.\textsuperscript{122} showed that the tapeworm \textit{Hymenolepis diminuta} infection could ameliorate dextran sodium sulfate (DSS)-induced colitis. Studies have found that \textit{S. mansoni} could attenuate intestinal inflammation in trinitrobenzene sulfonic acid (TNBS)-induced colitis.\textsuperscript{123} DSS-induced colitis,\textsuperscript{124,125} and \textit{Toxoplasma gondii}-induced ileitis.\textsuperscript{126} Studies have found that \textit{S. japonicum} cercariae could attenuate the inflammatory response in a DSS-induced IBD mouse model by promoting IL-10, repressing IFN-\gamma production and expression, and enhancing the Treg subset population.\textsuperscript{127,128}

Khan et al.\textsuperscript{129} reported that \textit{T. spiralis} infection could protect mice from dinitrobenzene sulfonic acid (DNBS)-induced colitis. Subsequent studies found that \textit{Trichinella} can induce a strong Th2/Treg response, induce the production of immunoregulatory cytokines,\textsuperscript{109,130,131} and recruit Tregs.\textsuperscript{132} Studies with \textit{T. spiralis} have also found that it could ameliorate inflammation in a mouse model of acetic acid-induced colitis.\textsuperscript{133} DSS-induced colitis,\textsuperscript{134,135} \textit{Citrobacter rodentium}-induced colitis,\textsuperscript{136} and TNBS-induced colitis\textsuperscript{137} by recruiting Foxp3-expressing Tregs, immunomodulating Tregs to produce anti-inflammatory cytokines, inhibiting proinflammatory cytokines and PD-1 upregulation and M2 macrophage polarization.\textsuperscript{135,140}

\textit{H. polygyrus} improves colitis by affecting mucosal Th1 cytokine production, suppressing IL-17 production from lamina propria mononuclear cells and mesenteric lymph node cells and augmenting IL-4 and IL-10 production.\textsuperscript{141,142} \textit{H. polygyrus} secretes a substance that mimics the mechanism by which TGF-\textbeta promotes the expression of Foxp3 in CD4+ T cells.\textsuperscript{143} Foxp3+ Tregs maintain immune homeostasis in the intestine, and any defects therein are linked to IBD. This is evident from the fact that mice with IL-10- Foxp3-expressing T cells develop spontaneous colitis.\textsuperscript{144} \textit{H. polygyrus} cannot prevent colitis in transgenic mice harboring T cells that do respond to TGF-\textbeta.\textsuperscript{145}

Studies have indicated that \textit{H. polygyrus} infection can alleviate IBD by reducing the production of IFN-\gamma and IL-17,\textsuperscript{146} inducing Foxp3+ Tregs, mucosal production of IL-10,\textsuperscript{147,148} elevating Th2 cytokine expression, increasing mast cell infiltration,\textsuperscript{149} and by down-modulating Smad7 expression in intestinal CD4+ T cells.\textsuperscript{150} \textit{H. polygyrus} larvae can also reduce inflammation in a DSS-induced colitis model.\textsuperscript{151} Blum et al.\textsuperscript{152} found that \textit{H. polygyrus} bakerii modulated intestinal DC function in Rag IBD murine models reconstituted with IL-10+ T cells. Moreover, \textit{H. polygyrus} exacerbates \textit{C. rodentium}-induced colitis in murine models, probably due to an influx of alternatively activated macrophages or increased IL-10 production by DC caused by \textit{H. poly-
girus infection, which impedes host protection against C. rodentium infection.

2) Eggs/Ova

Leonardi et al.\textsuperscript{155} reported that TSO therapy could significantly reduce the disease activity in a rabbit model of DSS-induced colitis. However, TSO exacerbated colitis in immunosuppressed rabbits. Exposure to schistosome eggs protects a mouse model of TNBS-induced\textsuperscript{156} and DSS-induced colitis\textsuperscript{157} by inhibiting Th1-type inflammation dependent on the IL-4 signaling pathway.\textsuperscript{158} S. mansoni egg antigen can induce Foxp3\textsuperscript{+} Tregs and Th2 cytokines and has modulatory effects on a DSS-induced colitis mouse model.\textsuperscript{159} Extracellular vesicles from DC treated with S. japonicum soluble egg antigen can ameliorate DSS-induced colitis in mice.\textsuperscript{159} Recently, Hou et al.\textsuperscript{160} reported that pre-exposure to S. japonicum eggs could alleviate colitis in the TNBS-induced colitis model, which was related to increased Treg immune response, decreased Th17 immune response, and reconstruction of Treg/Th17 balance. Another study by Zhu et al.\textsuperscript{161} reported that Schistosoma soluble egg antigen could decrease colonic inflammation and the disease activity index score in mice with DSS-induced IBD. This study also reported changes in the intestinal microflora with Schistosoma soluble egg antigen intervention.

3) Antigens, and Soluble and Secreted Proteins

T. spiralis antigens can reduce the severity of colitis in a mouse model of DNSS-induced colitis, as evident from a down-regulation of IL-1β production, myeloperoxidase activity, and inducible nitric oxide synthase and an upregulation of IL-13 and TGF-β production.\textsuperscript{162} In a meta-analysis, Li et al.\textsuperscript{163} concluded that Trichinella and its derived antigens are effective in alleviating IBD in mouse models. S. mansoni soluble proteins can ameliorate TNBS-induced colitis in mice by increasing the expression of regulatory cytokines and suppressing the expression of proinflammatory cytokines.\textsuperscript{163} Protein P28GST from schistosomes can reduce intestinal inflammation in experimental colitis by down-regulating the Th1/Th17 inflammatory response.\textsuperscript{135,164} Foligné et al.\textsuperscript{165} reported that P28GST administration could modulate the diversity and composition of mouse fecal microbiota and significantly reduce colitis in a mouse model. They found that P28GST increased the immunoregulatory response through Th2/Treg cells and M2 macrophages. It also reduces the local expression of proinflammatory mediators, such as TNF-α, and enhances the expression of anti-inflammatory cytokines, such as IL-5, IL-13, and IL-10.\textsuperscript{167,168} Studies have found that a secreted protein of S. japonicum, rSj16, shows protective effects on DSS-induced colitis by inhibiting the peroxisome proliferator-activated receptor (PPAR)-alpha signaling pathway\textsuperscript{166} and reducing apoptosis.\textsuperscript{167}

4) Metabolites, ES, and Recombinant Products

Metabolites from H. polygyrus can lower disease activity in a mouse model of DSS-induced colitis by decreasing LPS-induced TNF and increasing IL-10 release by bone marrow DC.\textsuperscript{168} The ES products from T. spiralis suppress macrophage-mediated inflammatory responses, reduce proinflammatory cytokine production, stimulate the differentiation of host Treg cells by activating DC, decrease the expression of IFN-γ and nuclear factor (NF), increase the expression of IL-4 and IL-10, change the ratio of M1/M2 phenotypic macrophages in the spleen, reduce TNF-α secretion, inducing IL-10 expression.\textsuperscript{169-175} T. spiralis-derived protein paramyosin ameliorates the progression of colitis by inducing Tregs, reducing proinflammatory responses, and enhancing regulatory cytokine production in a mouse model of DSS-induced colitis.\textsuperscript{176} Various T. spiralis-derived recombinant products have also been explored for IBD treatment including a recombinant protein from T. spiralis, rTsP53,\textsuperscript{177} recombinant serine protease,\textsuperscript{178} and recombinant serine protease-like protein (rTs-ADSp-7).\textsuperscript{179}

H. polygyrus ES products and recombinant protein, TGF mimic (TGM), showed some benefit in DSS-induced and TNBA-induced colitis, with a reduction in inflammatory cytokines.\textsuperscript{180,181} Studies have also found anti-inflammatory properties of low-molecular-weight metabolites derived from somatic extracts and ES products of Ancylostoma caninum\textsuperscript{184,185} and Ancylostoma ceylanicum.\textsuperscript{163} The recombinant macrophage migration inhibitor-like factor from the herring worm Anisakis simplex third-stage larva has shown to ameliorate DSS-induced colitis in a mouse model.\textsuperscript{182}

RAMIFICATIONS OF HELMINTH THERAPY

The promising results shown by helminth therapy suggest that the therapy can be used as an alternative or adjuvant therapy to treat various autoimmune or allergic diseases.\textsuperscript{186} Nevertheless, most helminths have pathogenic potential in humans, which makes their direct use in treatment unethical. Clinical trials have also shown that helminth therapy is not free of side effects (Table 1). Long-term infection by hookworm can result in iron deficiency anemia.\textsuperscript{184} Certain worms may increase mucus secretion and gastrointestinal motility, resulting in diarrhea.
and abdominal cramps. Furthermore, colonization by specific helminths may intensify the inflammatory response to an existing enteric bacterial infection. Helminths can also suppress host innate or adaptive immune responses to other parasites, predisposing patients to various infections. Although many clinical trials in IBD patients have revealed no side effects of *T. suis*, Kradin et al. reported that TSO therapy for pediatric CD caused infection in one patient. Similarly, acute exposure to *N. americanus* can cause diarrhea, vomiting, and abdominal pain. Several studies have found that the use of the crude extract, purified, or recombinant molecules/proteins derived from helminths can produce similar anti-inflammatory effects as whole organisms. Therefore, in place of living helminths, their extracts or recombinant molecules can be used for IBD treatment.

**CONCLUSIONS AND FUTURE PERSPECTIVES**

Helminths and microbiota are two “old friends” of humans. Helminths play an important immunoregulatory role in mucosal immunity and in maintaining a healthy gut ecosystem. There is sufficient evidence to conclude that helminth infections are immunomodulatory and regulate the host’s immune response. Studies have confirmed the safety and tolerability of helminth therapy. Various parameters need to be standardized to increase the efficacy and success rate of helminth therapy. These parameters include the helminth species to be used, the optimal dose and frequency of administration, and the route and mode of administration. A detailed study of host genetic factors, environment, diet, and the exact mechanism of action will surely help in designing better helminth therapy for IBD. In place of whole live helminths, proteins, and ES products can also be used for IBD treatment. The commercial production of such products can be done by expressing them in a suitable host, such as bacteria or yeast. The use of helminth-derived recombinant anti-inflammatory molecules can rule out the fear and concerns associated with the use of live helminths. Nevertheless, large clinical cohort studies must be carried out to know the safety and antigenicity of such recombinant products.

**ADDITIONAL INFORMATION**

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