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Review

Helminth therapy: Advances in the use of parasitic worms against Inflammatory Bowel Diseases and its challenges

M. MARUSZEWSKA-CHERUIYOT*, K. DONSKOW-ŁYSONIEWSKA, M. DOLIGALSKA

Department of Parasitology, Faculty of Biology University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland, E-mail: mmaruszewska@biol.uw.edu.pl

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Summary

Development of modern medicine and better living conditions in the 20th century helped in reducing a number of cases of infectious diseases. During the same time, expansion of autoimmunological disorders was noticed. Among other are Inflammatory Bowel Diseases (IBD) including ulcerative colitis and Crohn’s disease which are chronic and relapsing inflammation of the gastrointestinal tract. Absence of effective treatment in standard therapies effects the search for alternative opportunities. As per hygienic hypothesis increasing number of cases of autoimmune diseases is as a result of reduced exposure to pathogens, especially parasites. Thus, one of the promising remedial acts against IBD and other allergic and autoimmune disorders is “helminth therapy”. Cure with helminths seems to be the most effective therapy of IBD currently proposed. Helminth therapy focuses on advantageous results that have been obtained from the clinical trials, but its mechanisms are still unclear. Explanation of this phenomenon would help to develop new drugs against IBD based on helminth immunomodulatory molecules.

Keywords: helminth therapy; Heligmosomoides polygyrus; Inflammatory Bowel Diseases; ulcerative colitis

Introduction

Helminths have co-evolved with their hosts over millions of years to arrive at a form of mutualism where both the host and the parasite derive some benefit from their relationship. The immunosuppression and immunoregulation induced by helminths is obviously beneficial for the parasite: it prevents the parasite being killed or expelled and improves its fitness; it also inhibits inflammatory reactions and otherwise innocuous antigens, thus benefitting the host by preventing local and peripheral pathologies generated against it (Barthlott et al., 2003). The absence of effective forms of treatment and the unsatisfactory causal effects of conventional therapies for autoimmune diseases has aroused interest in new forms of treatment (Chandrashekara, 2012). The key aspects of research into helminth therapy (HT) and helminth-derived product therapy (HDPT) concern the use of live helminths as treatment, as well as the characterization of the key molecules responsible for immunomodulation. These could be used as drugs to control inflammation and autoimmune diseases. HT currently seems to be the most effective therapy for autoimmune disorders (Wilson & Maizels, 2004); however, live nematode therapy remains undoubtedly controversial, especially as the mechanism of disease prevention and inhibition is unknown (Erb, 2009). In addition, the therapeutic effects of helminths are undoubtedly complex, and for this reason, the use of individual immune-active components isolated from nematode products as potential drugs is not as meaningful as previously believed.

* – corresponding author
Inflammatory Bowel Disease (IBD) Problems

Inflammatory Bowel Disease (IBD), comprising Crohn’s disease (CD) and ulcerative colitis (UC), is a chronic, idiopathic, and relapsing inflammation of the gastrointestinal tract. This disorder is most common in young adults, but can also develop in childhood and old age. The worldwide incidence rate of CD varies between 0.1 – 16/100,000 persons worldwide, while UC is more common and varies greatly between 0.5 – 24.5/100,000 persons, with the prevalence rate of IBD reaching up to 400/100,000 persons. However, the incidence and prevalence of the disorder are probably higher because despite the existing Montreal classification of IBD (Satsangi et al., 2006), precise diagnosis is limited by the lack of gold standard criteria for identification, resulting in inconsistent case ascertainment and disease misclassification (Lakatos, 2006; Molodecky et al., 2012).

The inflammation in UC is characterized by superficial ulcerations, granularity and a distorted vascular pattern. Histological features include an expansion of the lamina propria with inflammatory cells and crypt abscesses. There are usually no fistulae or granulomas: the typical histopathologic features of Crohn’s disease. As a consequence, the symptoms of UC are a progressive loosening of bloody stools, rectal bleeding, diarrhea, tenesmus with cramping abdominal pain and a severe urgency to have a bowel movement up to 20 times a day (Brandtzaeg et al., 1997).

There is no effective treatment for colitis and therapy is based on encouraging long-term remission with anti-inflammatory medications. The four major classes of medication used today to treat UC are aminosalicylates, steroids, immune modifiers (azathioprine, 6-MP, and methotrexate) and antibiotics administered orally or rectally; however, all have restrictions, including side effects, refractoriness or unresponsiveness. In one-quarter to one-third of patients with UC, medical therapy is not completely successful or complications arise. Complications of UC can include bleeding from deep ulcerations and rupture of the bowel (Leitner & Vogelsang, 2016). Patients are at increased risk of colonie epithelial dysplasia and carcinoma, with an age-specific risk that is at least three times greater than that in the general population. As the risk of developing cancer increases in patients with long-term UC (7 to 10 years) with a rate of approximately 0.5 – 1 % per year, endoscopic surveillance examinations are performed annually and surgery offered for patients with ileal pouch-anal anastomosis. Over the long term, up to 25 % of those with UC will require surgery (Bernstein, 2001).

Although knowledge of UC dates back to the 19th Century, the pathogenic cause remains unknown. Its pathogenesis is believed to be associated with a deregulated proinflammatory response to commensal gut bacteria; it is restricted to the epithelial mucosa of the colon in an even and continuous distribution not related to any intestinal infection. Recent genetic studies have identified about 163 genes which are crucial in the development of IBD; most of them are common between Crohn’s disease and ulcerative colitis (Cleynen et al., 2016). In addition, several environmental risk factors are known to be associated with IBD disease cases including diet, intestinal microbiota composition, medication and vaccination, physical exercise, stress, appendectomy, breastfeeding, air pollution and heavy metals, as well as exposure of vitamin D to UV (Niewiadomski et al., 2016). Smoking has also been proposed to have an influence on the pathogenesis of IBD (Samuelsson, 1976). A sizable proportion of previous research indicates that cigarette increase the chance of developing Crohn’s disease despite protecting against the development of colitis. The mechanism of this phenomenon remains unclear, but can be consequence of changes in the composition of the intestinal microbiota (Biedermann et al., 2013).

There is a high probability that the increase in prevalence of IBD seen in the 20th Century is associated with the industrial revolution in Europe and North America. Regional variation has also been observed, insofar as there is large difference in the numbers of cases of autoimmunological diseases, including IBD, between Western and Eastern countries: Based on a review of data from 1920 – 2008, the highest annual incidence of UC was 24.3/100,000 person-years in Europe and 19.2/100,000 person-years in North America compared to 6.3/100,000 person-years in Asia and the Middle East. The highest reported prevalence of UC was observed in Europe (505/100,000 persons) and North America (248/100,000 persons). It has been found that 60 % of documented studies of UC report an increasing number of incidences (Molodecky et al., 2012). Other reports indicate that children who have moved from countries with a low IBD incidence to countries with a high incidence have the same probability of developing IBD as the children living in the high-incidence regions (Brobert et al., 1992; Li et al., 2011).

Hence, environmental factors appear to play a role in the development of IBD, and differences in lifestyle and medical level are reflected in the results of studies. This trend has been attributed to the Hygienic Hypothesis, a term first used by Strachan (1899). The Hygienic Hypothesis implies that a lack of immune system activation in adulthood occurs as a result of maintaining high cleaning standards and avoiding contact with microorganisms during childhood, a potential consequence of which can be the development of a range of immunological disorders, including IBD and allergic diseases. Contact with pathogens is influenced by many factors, including education level, diet, antibiotics and vaccinations, medical and deistical admission, sharing bedrooms or even having pets (Leong et al., 2016). If permanent contact with bacteria and viruses is maintained, interaction with multicellular parasites such as nematodes or tapeworms can be eradicated thanks to extensive access to antihelminth drugs and adherence to hygiene rules.

In United States schoolchildren, the prevalence of hookworm fell from 65 % in 1910 to fewer than 2 % in 1980 (Kappus et al., 1994). The co-evolution of host and parasite resulted in the development of a very complicated mechanism for avoiding the host immunological system, thus increasing the potential for the parasite to
survive and reproduce: Gastrointestinal nematodes cause chronic infection and induce immunosuppression. Such regulation of the immune response by the parasite also offers positive benefits for the host organism: the nematodes control the immunity caused by infection, as well as the responses to various non-nematode antigens (Barthlott et al., 2003). However, a strong inflammation response can result in damage to the infected area (Maizels et al., 2004). Such deprivation of contact with multicellular parasites observed in Western countries resulting from their high level of hygiene can affect the immunological balance by forcing inequalities in the host-parasite arrangement constructed over millions of years. These phenomena result in the creation of new variants of the Hygienic Hypothesis, such as the Lost Friends Theory or the Biome Depletion Theory. With these theories in mind, it seems like the best option in allergies and autimmune disorders treatment is reconstruction of human biome (Bilbo et al., 2011).

Immunological Response in the Intestine

The mucosal membrane of the intestine plays a crucial role in the immunological system. The digestive tract is in constant contact with both commensal and pathogenic microorganisms (Macdonald & Monteleone, 2005). The immune system must therefore be able to control symbiotic bacteria and tolerate them, while being able to eradicate pathogens. During colitis, the gut epithelial barrier is dysfunctional (McGuckin et al., 2009), and the recognition and response to multiple antigens, commensals or nutrients results in local inflammation of the colon. A hypothesis proposed by Shorter et al. (1972) presents that IBD, including colitis, occurs as a result of the establishment of a state of hypersensitivity to the bacterial antigens which are normal components of the intestinal microbiota. It is known that other factors further aggravate epithelial-associated dysfunction, which then develops into chronic inflammation of the gastrointestinal tract. Nonetheless, the intestinal microbiota is crucial for the development of IBD and influences the mucosal immune response during active disease.

In healthy patients, the immune system associated with the mucosal gut develops a tolerance to commensal microorganisms and food antigens. Three types of Antigen-Presenting Cells (APC), viz. dendritic cells (DC), macrophages and B lymphocytes, play a fundamental role in this process (Mann & Li, 2014). DC are able to stimulate primary lymphocyte T cells and differentiate into regulatory T cells (Treg) (Rescigno & Sabatino, 2009), both macrophages and B-cells maintain the survival of Treg, while also secreting interleukin 10 (IL-10) and transforming growth factor β (TGF-β); thus they maintain immune homeostasis and tolerance (Mann & Li, 2014; Hadis et al., 2011).

In colitis, antigens emerge from pathogens, food and commensal bacteria which cause intestinal inflammation as a result of the activity of innate immune cells. DC and macrophages secrete proinflammatory cytokines; tumor necrosis factor α (TNF-α), IL-6 and IL-1β. In the adaptive immune response, T helper type 1 cells (Th1) are activated, resulting in strong production of proinflammato-

Colitis Helminth Therapy in Animal Models

Colitis induction methods

Before helminth therapy can be introduced in IBD patients, it is necessary to understand the mechanism of their immunoregulatory abilities. Elliott and colleagues (2000) propose the hypothesis that exposure to helminths can prevent IBD and highlighted the need to formulate a novel chronic intestinal inflammation model for humans. Since then, a few models of colitis induction have been used in rodents to identify a cure for IBD, the main ones being chemically-inducible models, spontaneous models, genetically-modified models and adoptive transfer models (Witrz & Neurath, 2007). Mucosal immune system dysfunction and display of disease manifestation can be achieved in three ways: through defects in epithelial integrity and permeability, deficiency in innate immune cells or by deficiency in adaptive immune cells. These effects can be achieved chemically using dinitrobenzene or trinitrobenzene sulfonic acid (DNBS/TNBS)-induced colitis (Chassaing et al., 2015), re-

Trematoda

Another issue concerns the variety of species of parasite used in the animal models. Three classes of parasite can be used, namely trematodes, cestodes and nematodes, referred to as helminths. One of the parasite genera belonging to the trematode Schisto-

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Schistosoma mansoni eggs which showed attenuation of intestinal inflammation manifestations. A similar effect has been already demonstrated in TNBS-induced colitis rats after administration of S. mansoni larvae (Morels et al., 2004). In a different study, infection of mice with S. mansoni larvae protected against the manifestation of DSS colitis with macrophage participation (Smith et al., 2007). The influence of S. japonicum eggs demonstrated a preventive outcome on TNBS-induced colitis in mice (Zhao et al., 2009; Xia et al., 2011). In another study, infection with S. mansoni larvae of DSS-induced colitis mice resulted in reduced manifestations and lower levels of Th1 and Th2 cytokines (Bodammer et al., 2011) (Table 1).

Cestoda
One of the cestode class, Hymenolepis diminuta is also successfully used a model of colitis parasite therapy. Preventive and curative treatment of tapeworm larvae resulted in normalization of colonic ion transport in DSS-induced colitis mice. No differences in histological or cytokine level were observed (Reardon et al., 2001). Hunter et al. (2005) demonstrated a reduction of DNBS-induced colitis symptoms and higher levels of IL-10 and IL-4 in a mouse model. The same team showed increased levels of Th2 and Treg response interleukins in a colitis model induced by oxazalone (Hunter et al., 2007). Elsewhere, infection with H. diminuta larvae of DNBS-induced colitis mice resulted in higher levels of Th2 and Treg, and a lower level of Th1 cytokines (Melon et al., 2010) (Table 1).

Nematoda
The most promising group of intestinal parasite seems to be the nematodes. Trichinella spiralis infection was found to protect mice from developing DNBS-induced colitis (Khan et al. 2002). Another study a decade later reported attenuation of DSS-induced colitis by Trichinella papuae larvae (Adisakwattana et al., 2013). Elsewhere, Trichuris trichiura eggs restored mucosal barrier functions and reduced overall bacterial attachment to the intestinal mucosa in idiopathic chronic diarrhea in macaque monkeys (Broadhurst et al., 2012). The majority of investigations about mechanisms of helminth therapy in human IBD is focused on the gastrointestinal nematode Heligmosomoides polygyrus. This parasite of mice, with a simple and short life cycle, is an excellent model of human infection with Necator americanus (Monroy & Enríquez, 1992). Both nematodes have been phylogenetically placed in the order Strongylida (Gouy et al., 2011). Another advantage of using H. polygyrus is that its laboratory breeding procedure is uncomplicated. Different tribes of mice react differently to H. polygyrus infection, which enables the investigation of the influence of genetic conditioning to the host immunological response. The inflammatory response is reduced during H. polygyrus infection, thus demonstrating the suitability of the nematode model in IBD suppression process. To date there have been numerous reports demonstrating that H. polygyrus infection is an effective therapy for colitis. Elliot et al. (2004) first demonstrated that H. polygyrus larvae can treat colitis in IL-10−/− mice, and later demonstrated suppression of mucosal IL-17 production in the same model (Elliott et al., 2008). Infection by H. polygyrus larvae in IL-10−/− mice with T cell transfer colitis effects induced CD8+ regulatory cells (Metwali et al., 2006). Promising results have been achieved on the same model, showing that DC plays a crucial role in the regulatory immune response in colitis (Hang et al., 2010; Blum et al., 2012). Studies on TNBS-induced colitis mice revealed attenuation of the disease with mast cell infiltration following infection by H. polygyrus larvae (Selawan et al., 2007; Sutton et al., 2008). Our own previous studies found infection with H. polygyrus larvae to have a curative effect on DSS-induced colitis with macrophage infiltration and decreased levels of MOR1, POMC and β-endorphin observed in the colon (Donskow-Lysiniewska et al., 2012). Administration of the same larvae to antigen-driven colitis mice also resulted in protection from disease with the induction of Foxp3+ Treg cells (Leung et al., 2012).

A treatment effect is not achieved in every model of intestinal inflammation. Investigations in mice with Citrobacter rodentium-induced colitis infected with H. polygyrus larvae found that DC activation and IL-10 production impaired the host response to C. rodentium (Chen et al., 2005; Chen et al., 2006). Similarly no curative effect was observed in mice with TGF-βRII DN colitis caused by blocking the effect of TGF-β on T cells; the findings showed that TGF-β signaling to T cells can play an essential role in the regulatory abilities of helminths (Ince et al., 2009).

All studies clearly show that various species of intestinal parasites have curative and protective effects in animal models. The reports also give an insight into the mechanisms of IBD treatment in humans with helminths. However, these achievements are closely dependent on the method of inducing intestinal inflammation, as well as the choice of parasite species (Table 1).

Therapeutical Potential of Intestinal Helminths
The most common genera of nematodes distributed in human digestive tract are Ascaris, Trichuris (whipworm), Necator and Ancylostoma (hookworms). Two species of which, Trichuris suis and Necator americanus, have been investigated in clinical examinations of UC and CD patients. Although T. suis is a natural parasite in the caecum and colon of pigs, it can also infect other hosts, including humans; however, the worms can only survive in the human digestive tract for a few weeks (Helmy, 2015). There is discrepancy in host species and deficiency in inflammatory response for the parasite marked T. suis for the most promising nematode for human IBD therapy. The initial results of small clinical studies of IBD treatment with T. suis were published in 2003 by Summers et al. A group of UC and CD patients received a single oral dose of 2500 live T. suis eggs, and were then monitored every 14 days for 12 weeks. A second group of patients received the same administered dosage every 21 days for 28 weeks. After
| Author                  | Model of colitis induction | Parasite class / species                  | Main outcomes                                                                 |
|-------------------------|----------------------------|-------------------------------------------|-------------------------------------------------------------------------------|
| Eliot et al., 2003      | TNBS                       | Trematoda / Schistosoma mansoni           | Th1 response reduction, Th2 and Treg response induction                       |
| Morels et al., 2004     | TNBS                       | Trematoda / S. mansoni                    | Th2 response induction                                                         |
| Smith et al., 2007      | DSS                        | Trematoda / S. mansoni                    | Macrophage participation                                                       |
| Zhao et al., 2009       | TNBS                       | Trematoda / S. japonicum                  | Th1 response reduction                                                         |
| Xia et al., 2011        | TNBS                       | Trematoda / S. japonicum                  | Lower intestinal bacterial translocation frequency                             |
| Bodammer et al., 2011   | DSS                        | Trematoda / S. mansoni                    | Th1 and Th2 response reduction                                                 |
| Reardon et al., 2001    | DSS                        | Cestoda / Hymenolepis diminuta            | No changes in response noticed                                                 |
| Hunter et al., 2005     | DNBS                       | Cestoda / H. diminuta                     | Th2 and Treg response induction                                                |
| Hunter et al., 2007     | Oxazolone                  | Cestoda / H. diminuta                     | Th2 and Treg response induction                                                |
| Melon et al., 2010      | DNBS                       | Cestoda / H. diminuta                     | Th2 and Treg response induction                                                |
| Broadhurst et al., 2012 | Idiopathic chronic diarrhea in macaques monkeys | Nematoda / Trichuris trichiura | Mucosal barrier functions restored and overall bacterial attachment to the intestinal mucosa reduced |
| Khan et al., 2002       | DNBS                       | Nematoda / Trichinella spiralis           | Th2 response induction                                                         |
| Adisakwattana et al., 2013 | DSS                       | Nematoda / T. papuae                     | Th2 response induction and Treg response changes                               |
| Elliott et al., 2004    | IL-10−/−                   | Nematoda / Heligmosomoides polygyrscopy   | Th1 response reduction and Treg response induction                             |
| Chen et al., 2005       | Citrobacter rodentium      | Nematoda / H. polygyrscopy               | STAT 6-mediated mechanism                                                      |
| Chen et al., 2006       | Citrobacter rodentium      | Nematoda / H. polygyrscopy               | CD11c+ dendritic cells activation and IL-10 production                         |
| Metwali et al., 2006    | IL10−/− T cell transfer    | Nematoda / H. polygyrscopy               | CD8+ regulatory cells induction                                                |
| Setiawan et al., 2007   | TNBS                       | Nematoda / H. polygyrscopy               | Th1 response reduction and Treg response induction                             |
| Elliott et al., 2008    | IL-10−/− mice              | Nematoda / H. polygyrscopy               | Suppression of mucosal IL-17 production mast cells infiltration                |
| Sutton et al., 2008     | TNBS                       | Nematoda / H. polygyrscopy               | A role of TGF-β signaling to T cells in regulatory response                    |
| Ince et al., 2009       | TGF-βRII DN                | Nematoda / H. polygyrscopy               | A role of dendritic cells in regulatory immune response                        |
| Hang et al., 2010       | IL10−/− T cell transfer    | Nematoda / H. polygyrscopy               | Induction of tolerogenic dendritic cells                                       |
| Blum et al., 2012       | IL10−/− T cell transfer    | Nematoda / H. polygyrscopy               | Macrophage infiltration and MOR1, POMC, β-endorphin increased levels           |
| Donskow-Lysoniewska et al., 2012 | DSS                       | Nematoda / H. polygyrscopy               | Induction of Foxp3+ Treg cells                                                |
| Leung et al., 2012      | Antigen driven             | Nematoda / H. polygyrscopy               |                                                                           |

Table 1. Summary of helminth therapy with live parasites in animal models in IBD.
accurate monitoring, no side effects were noticed and a further repeated dosage resulted in the improvement of all medicated patients (Summers et al., 2003). Similarly, in a second clinical trial, a repeated dose of 2500 viable T. suis eggs was given to 29 of CD patients every 21 days for 24 weeks, and no adverse reaction was observed. After 24 weeks of therapy, 79.3 % of patients responded to treatment and 72.4 % were in remission, as evaluated based on Crohn’s disease activity index (Summers et al., 2005a). A similar examination was conducted on 59 UC patients. A dose of 2500 T. suis eggs or placebo was given every two weeks for 12 weeks: It was found that 42.3 % of patients who received T. suis and 16.7 % of those who received placebo responded, but only 10 % of the first group and 4.2 % of the second displayed remission, with no side effects to induction. The outcome was calculated based on UC disease activity index (Summers et al., 2005b).

Almost a decade after helminths were first demonstrated to have promising effects in IBD therapy in humans, Sandborn et al. (2013) demonstrated novel findings concerning the safety and tolerance of various doses of T. suis in clinical trials with patients with CD. The patients received one dose of 500, 2500, 7500 T. suis eggs or placebo. They were then evaluated for 14 days, and then by telephone interview one, three and six months after receiving the dose. All doses, including the 7500-egg dose, was very well tolerated without any short or long-term adverse reactions (Sandorn et al., 2013). In the meantime, N. americanus has been proposed as an alternative for T. suis and studies have been carried out to investigate tolerance to infection and a number of other practical topics. Humans can be infected with N. americanus larvae third-stage (L3) by skin contact with contaminated soil. The adult worms are situated in small intestine of the host and can survive for five years, although expulsion of the parasite is possible with anthelmintic medicines. It is important to note that one consequence of infection is anemia caused by the helminth feeding on blood. In the experiment, CD patients were inoculated with a single or a repeated dose of 25 – 50 infective larvae (L3). Despite a promising remission effect, the presence of the worms yielded a mild itch, painful transient enteropathy and blood eosinophilia (Croese et al., 2006).

The outcomes of these studies clearly show the great potential of helminths in IBD therapy, and further study is needed in this area. While just one study has examined N. americanus, and side effects were observed, the parasite may still serve as a promising alternative for T. suis, especially since N. americanus is very well tolerated in CD therapy (Daveson et al., 2011; Croese et al., 2015). Live T. suis and the haematophagous hookworm N. americanus have been suggested as effective treatments for IBD, and three clinical trials have been initiated: NCT01413243 (Correale, 2014; Ruyssers et al., 2008). Nonetheless, a greater understanding of the mechanism by which inflammation is suppressed in the intestine by helminths is essential for further progress in clinical practice (Table 2).

### Challenges of Helminth Therapy

During HT, the amelioration of symptoms was only seen when the helminth infection was present; removal of the parasites resulted in the remission of IBD pathology and the inhibition of immunomodulatory response (Fleming et al., 2011). Furthermore, many patients feel uneasy about receiving live worms for therapy. In addition, aside from the ethical concerns, there are many practical considerations that may reduce the efficacy of this approach. Nematode L4 larvae invade tissues, and even small numbers of hookworms can induce gastrointestinal or other tissue pain in the early stages of infection; they can also exhibit aberrant migration in the human host and influence the physiology of their respective niches. Live parasite infections result in the induction of danger signals and pro-inflammatory stimuli, thus leading to inflammation. Furthermore, in addition to the desired helminth immunomodulators, the host is exposed to the full spectrum of helminth-derived products including potent antigens, inflammatory stimuli and potentially disease-causing allergens. It is important to note that only

### Table 2. Summary of clinical trials of helminth therapy with live parasites in IBD.

| Author          | Scheme of trial                                                                 | Results                                                                 |
|-----------------|--------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Summers et al., 2003 | Single or repeated dose of 2500 live eggs of T. suis administered every 3 weeks for 28 weeks to 3 UC and 4 CD patients. | Remission noticed on every patient administered with repeated dose. |
| Summers et al., 2005a | Repeated dose of 2500 live eggs of T. suis administered every 3 weeks for 24 weeks to 29 CD patients. | No side effects. |
| Summers et al., 2005b | Repeated dose of 2500 live eggs of T. suis administered every 2 weeks for 12 weeks to 54 UC patients. | No side effects. |
| Sandom et al., 2013 | Single dose of 500, 2500 or 7500 live eggs of T. suis administered to 36 CD patients. | Every dose very well tolerated. Quantity of dose has no influence on gastrointestinal tract response. |
| Croese et al, 2006 | Single or repeated dose of 25-50 L3 larvae of N. americanus administered to 9 CD patients. | Side effects: itching, enteropathy, eosinophilia. Condition of majority of patient’s improved. |

Abbreviations: UC – ulcerative colitis, CD- Crohn Disease
the minimum number of larvae was used in the trial for safety reasons, and so potential clinical benefits may have been lost. In addition, as early infection is characterized by obvious symptoms that will reveal to patients whether they are in a placebo or treatment group, it is very difficult to conduct trials by incorporating proper placebo controls. Furthermore, helminths can influence drug efficacy by modulating the host immune response, and colonization may worsen other pathogenic infections in immunocompromised hosts (Correale, 2014). Treatment with living nematodes therefore has clear disadvantages, and in order to survive for a long time in an adverse and aggressive environment, the nematodes may modify host-cell homeostasis and increase susceptibility to oncogenic transformation by secreting several soluble factors that interact with host cells (Packham & Stevenson, 2005; Donskow et al., 2011; Donskow-Lysoniewska et al., 2013b). The factors secreted by helminths could be involved in neoplasia promotion and progression. Schistosoma haematobium, Spiromera mansonoides, Taenia taeniaformis, and T. solium, all have significant tumor-promoting activity (Herrera & Ostrosky-Wegman, 2001). Excretory-secretory (ES) products from the small intestine nematodes Trichostrongylus vitrinus, T. colubriformis, Cooperia curtei, Nematodirus battus and the abomasal nematode Teladorsagia circumcincta have all been shown to produce over-proliferation in normal intestinal epithelial cells and/or cell lines (Huby et al., 1995). Additionally, our study indicated that living nematode therapy of colitis, the changes in the small intestinal milieu promote intestinal nematode larval adaptation and improve worm growth. The plasticity of the nematode proteome is a consequence of evolutionary adaptation which benefits the host by inhibiting inflammatory disease and also the parasite by increasing its survival (Donskow-Lysoniewska et al., 2013a) (Table 3).

Other Perspectives

Even though the mechanism of disease prevention is unknown, HT seems to be the most effective therapy of IBD currently proposed. As HT has its disadvantages, an important aim of HDPT research is to characterize the key molecules responsible for immunomodulation for use as drugs to control inflammation and autoimmune diseases such as IBD. For this reason, a number of international studies have attempted to identify the immune-active components of helminths. Some filarial nematode proteins such as cystatin (AvCystation) have been shown to prevent asthma and colitis by induction of IL-10 production by macrophages in animal models of the disease (Schnoeller et al., 2008). A filarial-derived phosphocholine product (ES-62) of Acanthocheilonema vitae modulates dendritic cell and macrophage activity in a toll-like receptor 4 (TLR-4) dependent manner and attenuates the symptoms of collagen-induced arthritis (CIA), aryl hydrocarbon receptor (AHR) knockout, and DSS-induced colitis (Goodridge et al., 2005). The recombinant 53kDa protein from T. spiralis prevents experimental colitis in mice and upregulates Th2 and regulatory cytokines while downregulating some Th1 cytokines (Du et al., 2011).

However, studies of the potential therapeutic use of single immune-active components isolated from nematode products is not as meaningful as previously suggested. The live nematodes express and secrete copious quantities of antigens into host tissues with different immunomodulatory properties, and the immunomodulatory effects, presumably intended for self-protection, must be multiple and complex. These mixtures of proteins, peptides, glycanes and lipids might help the worm to survive in a number of ways, minimizing inflammatory processes or interfering with them, and selectively skewing the phenotype of the immune response generated (Mulyenna et al., 2009).

The protective immune responses to native antigens have been difficult to replicate based on recombinant antigens produced in most popular artificial expression systems, such as bacteria and yeast, as these usually have an incorrect conformation and the lack post-translational modifications of the recombinant molecule. Increasingly, post-translational modifications such as those including phosphocholine (PC) and various glycans are being recognized as the active components of many immunomodulatory components of helminths (ICHs), particularly in interactions with the host (Prasanphanich et al., 2013; Hokke et al., 2007). Furthermore, the use of bare single-defined immunomodulatory products as therapeutics is doomed to failure as such products can be neutralized and rendered ineffective by the host immune response. In addition, the use of helminth excretory secretory (ES) products does not solve the problem. These represent up to 30% of the proteome of an organism, and proteomic studies have found them to

| CONS                                      | PROS                                      |
|-------------------------------------------|-------------------------------------------|
| Exposure to full spectrum of helminth products | Strong immunogenic properties of live parasite |
| Tissue invasion by helminths               | Better than any therapy currently available |
| Ethical aspect                             | An introduction to more extensive research using molecules with immunomodulatory properties |
| Symptoms re-emergence after parasites removal | Less expensive method                     |
| Tumor promoting activity                   |                                           |
| Better adaptation of worms in colitis environment |                                   |
| Difficulty in proper placebo controls use  |                                           |
| Proinflammatory activity of live worms     |                                           |

Table 3: Cons and pros of using helminth therapy with live parasites.
be highly distinct from somatic extracts (McSorley et al., 2013). However, the range of secretory products is wide and varied, comprising a complex mixture of many different substances with particular biological functions which are secreted from cells or glands, as well as various unnecessary metabolic products released from the body. Hence, it is difficult to determine the precise application of ES products from parasitic helminths: the analysis of the smaller molecules among ES products can be confounded by protein breakdown products and media components used for in vitro culture of nematodes, including amino acids with immunomodulatory properties in their own right. In addition, due to the low concentrations of protein caused by high dilutions of cultivation media, ES can often be contaminated by normally non-secreted proteins following nematode cell lysis and death (Smith et al., 2009).

Therefore, the somatic extract might be extremely useful in the development of intervention strategies for inflammatory reactions, especially since the immunomodulatory potency of helminths appears to be largely achieved by their surface glycoproteins (Erb, 2009). As the immune regulation induced during parasitic infection is complex and cannot be generated by single recombinant factors, and therapy with live nematodes could produce a severe infection, it appears essential to devise other modes of treatment following nematode cell lysis and death (Smith et al., 2009).

EFFECTIVENESS

| Live parasites | ES products | Single parasite compounds | Synthesized proteins based on parasite compounds |
|----------------|-------------|---------------------------|-----------------------------------------------|

SAFETY

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