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Treating Inflammatory Bowel Disease: from Helminths to Ova

Tomer Bashi MD[1], Miri Blank PhD[1] and Yehuda Shoenfeld MD FRCP (Hon.) MaACR[1,2]

[1]Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer, Israel
[2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

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During the last century, the western lifestyle had led to a decrease in the infectious burden. On the other hand, there is a high rate of autoinflammatory disorders expressed by a higher prevalence of autoimmune and autoinflammatory diseases and allergies. Strachan, who first proposed "The Hygiene Theory" while following more than 17,000 British children born in 1958, noticed an inverse correlation between hay fever and the number of older siblings. This hypothesis states that limited exposure to microorganisms such as helminths and microbes in childhood will eventually lead to an off-balanced immune system [1].

The eradication of helminths was shown to increase atopic skin sensitization in Venezuela, Gabon, and Vietnam. Moreover, the disappearance of malaria due to mosquito-eradication programs was linked to the increase of multiple sclerosis in Sardinia with respect to the high genetic susceptibility of human leukocyte antigen (HLA) DR3 on the island.

Helminths aim to survive in the host and therefore try to induce a tolerance scenario. Yet, it is important to keep in mind that immunomodulation is affected by several key elements such as the helminth's species and the host's immune system response. In most cases helminths will induce tolerance, but in some scenarios they may cause inflammatory responses.

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the digestive tract and manifests primarily as Crohn's disease (CD) and ulcerative colitis (UC). CD may involve inflammation in any part of the gastrointestinal tract (from mouth to anus), while UC is confined to the large intestine (the colon and rectum) [2]. In the late 1990s, Weinstock [3] raised the "inflammatory bowel disease hygiene hypothesis," based on the increasing prevalence of IBDs which was in reverse correlation to the prevalence of helminths in the United States. He proposed that exposure to helminths might protect against IBDs [3]. Further proof was given by a study carried out in sub-Saharan Africa where helminth intestinal infestation is frequent. There was a low incidence and prevalence of IBD that could not be explained by genetic factors, since the incidence of IBDs in the black populations of the USA and Britain was approaching that of the Caucasian population [3].

Helminths, their ova and their antigens have been used in many studies that have attempted to treat IBDs in murine models as well as in humans [3-8] [Table 1]. One model of experimental colitis is interleukin-10 (IL-10) knockout mice. IL-10 is an important immunoregulatory cytokine that down-modulates macrophage activation, thus IL-$10^{-/-}$ deficient mice develop spontaneous chronic colitis. Colonization of *Heligmosomoides polygyrus* in IL-10 knockout mice with piroxicam-induced colitis was shown to suppress established inflammation and to decrease lymphocytic infiltration. Moreover, in vitro analysis of lamina propria mononuclear cells (LMPC) showed that the cells from mice bearing *H. polygyrus* did not release interferon-gamma (IFNγ) or IL-12p40, unlike control mice LMPC [6]. Rag-deficient mice (mice born without mature T and B cells) injected with IL-$10^{-/-}$ T cells fed with ovalbumin displayed intense inflammation of the small bowel and colon.

Table 1. Treating inflammatory bowel disease with helminths and their derivatives

Helminth	Model	Treatment type	Proposed mechanism	Ref
Heligmosomoides polygyrus	Piroxicam-induced colitis in IL-10 knockout mice	Colonization	Reduces IL-17 production and IFNγ, IL-12 release	[6]
	Rag-deficient mice injected with IL-$10^{-/-}$ T cells fed with ovalbumin	Colonization	Suppression of IL-17, IFNγ and induction of IL-10 modulates intestinal dendritic cell function to act as regulatory agents	[5,6]
Schistosoma mansoni	TNBS-induced colitis in mice	Ova	Reduced IFNγ and IL-4 levels	[8]
	TNBS-induced colitis in mice	*S. mansoni*-homogenized proteins	Decreased inflammation and MPO activity	[8]
Trichinella spiralis	DNBS-induced colitis in mice	*T. spiralis* antigens from frozen skeletal muscle larvae	Down-regulation of MPO, IL-1β and iNOS. Up-regulation of IL-13 and TGFβ	[7]
Trichuris suis	IBD patients	Ova	Not tested	[3]

TNBS = 2,4,6-trinitrobenzene sulfonic acid, DNBS = dinitrobenzenesulphonic acid, IBD = inflammatory bowel disease, IL = interleukin, IFNγ = interferon-gamma, TGFβ = transforming growth factor-beta, MPO = myeloperoxidase

When infected with *H. polygyrus*, gut inflammation was abrogated, with suppression of IL-17 and IFNγ and induction of IL-10 [6]. It was demonstrated that direct interaction with innate immune system cells by *H. polygyrus* can inhibit colitis, without direct interactions with T or B cells [5]. Moreover, *H. polygyrus* induced tolerogenic dendritic cells in the intestinal of infected Rag-deficient mice [5].

Another model of experimental colitis is trinitrobenzene sulfonic acid (TNBS)-induced colitis, characterized by ulcer formation, infiltration of the lamina propria with chronic inflammatory cells, and transmural lymphocytic inflammation [8]. *Schistosoma mansoni* ova exposure attenuated TNBS-induced colitis and protected BALB/c mice from lethal inflammation. IFNγ levels were reduced while IL-4 and IL-10 mRNA levels were enhanced due to production by αCD3-stimulated spleen and mesenteric lymph node cells [8]. Furthermore, treatment with *S. mansoni*-derived proteins during TNBS-induced colitis in mice showed a significant decrease in macroscopic inflammation score as well as a decrease in colonic inflammation and myeloperoxidase (MPO) activity. The effect resulted in reduction of gastrointestinal motility [8].

Treatment with *Trichinella spiralis* frozen skeletal muscle larvae prior to dinitrobenzene sulphonic acid (DNBS)-induced colitis in C57BL/6 mice significantly reduced the severity of colitis. MPO activity was down-regulated, as well as IL-1β production and iNOS expression. IL-13 and tumor growth factor-beta (TGFβ) production in colon was up-regulated [7].

Moreover, Pineda et al. [9] found a compound, ES-62, secreted from the rodent nematode *Acanthocheilonema viteae* which has immunoregulatory capabilities. ES-62 is surrounded by a moiety of phosphorylcholine (PC) attached to the core by glycans. The immunomodulatory effect of ES-62 was attributed to PC [9]. Recently we successfully employed helminth PC-based conjugates to treat colitis in a mice model [10].

Furthermore, the use of toll-like receptor (TLR)-signaling antagonists and TLR-negative regulator agonists from helminths or helminth products should be considered as treatment for IBD. TLR signaling may contribute to destructive host responses and chronic inflammation, while helminths may play an important role in down-regulation of gene activation to control overwhelming inflammation and pro-inflammatory cytokine production [9].

Murine studies have led to human therapy trials with helminth ova. A preliminary study conducted in the early 2000s indicated that *Trichuris suis* (pig whipworm) seemed to be safe and possibly effective in the treatment of inflammatory bowel disease. *T. suis* met the safety requirements. It can colonize humans but only for a short time. A single dose of *Trichuris suis* ova (TSO) (containing up to 7500 ova) was well tolerated and did not result in short- or long-term treatment-related side effects [3].

Summers and co-authors [3] studied seven IBD patients. In an initial treatment and observation period, a single dose of 2500 live TSO was given orally and the patients were followed for 12 weeks. Six of them achieved remission. The benefit was temporary in some patients with a single dose, but it could be prolonged with maintenance therapy every 3 weeks. In a later TSO study involving 29 patients with active CD who ingested 2500 live TSO every 3 weeks for 24 weeks, disease activity was monitored. The results were impressive: at week 24, 23 patients responded and 21/29 remitted. Furthermore, in a randomized, double-blind placebo-controlled trial, 54 patients with active UC received 2500 TSO or placebo orally at 2 week intervals for 12 weeks. The results demonstrated improvement according to the intent-to-treat principle in 13 of 30 patients with ova treatment as compared to 4 of 24 patients given placebo. Improvement was also found to be significant by week 6 [3].

Correspondence:
Dr. Y. Shoenfeld
Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer 52621, Israel
Fax: 972-35352855
email: shoenfel@post.tau.ac.il

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"We adore chaos because we love to produce order"

M.C. Escher (1898-1972), Dutch graphic artist known for his often mathematically inspired woodcuts, lithographs and mezzotints. These feature impossible constructions, explorations of infinity and architecture