Chapter

Therapeutic Properties of *Trichinella spiralis* (Nematoda) in Chronic Degenerative Diseases

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Abstract

Diseases produced by helminth parasites occur frequently in underdeveloped countries where they present a serious public health problem. At the same time, in these regions, a lower rate of autoimmune and allergic diseases has been observed. Due to these observations, some researchers have proposed that some helminths, such as *Trichinella spiralis* or its proteins, have strong anti-inflammatory potential, or have assessed them as modulating agents of the immune response. *T. spiralis* shifts the host immune response from a Th1 profile, characterized by pro-inflammatory cytokines, to a Th2 profile, characterized by the release of different cytokines with anti-inflammatory properties. This parasite has shown high therapeutic potential in a wide variety of disease models. In one of the most promising, the experimental lupus model in mice, the release of anti-inflammatory cytokines IL-4 and IL-10 and delayed onset of the key clinical features of the experimental lupus model for at least 5 months were observed, when previously parasitized. This is the first study to date that focuses on the use of *T. spiralis* as an immunomodulator in lupus disease. In conclusion, further study of the immune response generated by the parasite is necessary to advance the development of new therapies for inflammatory diseases.

Keywords: *Trichinella*, immunomodulation, chronic degenerative diseases, lupus, helminths

1. Introduction

1.1 Helminths and parasitosis

Helminthiases are parasitic diseases caused by helminths, which are colloquially called "parasitic worms". Although a great biodiversity of helminths exists, the most relevant in public and veterinary health are the cestodes (tapeworms), trematodes (flukeworms) and nematodes (roundworms). Helminthiases affect more than 2 billion people worldwide, which can become chronic infections when untreated and

persist for the rest of their host's life [1–3]. The population affected by helminthiases is mostly found in tropical and subtropical areas, where precarious health systems and poor sanitation prevail. This situation contributes to an increase in their prevalence due to global climate change that has caused parasites to undergo evolutionary changes to adapt over time, which in turn generate resistance to antiparasitic treatments [3]. Although the immune system could eliminate the helminth from the host's body, parasites can often evade the immune response and, in the worst-case scenario, the host suffers collateral damage, consequence of the immunopathology caused by the immune attack against helminths, as an attenuated immune response can trigger a tolerance towards the helminth [2].

1.2 T-helper immune response

In vertebrate animals, the immune response is divided in innate- and adaptive immunity. The first one acts in a non-specific but immediate manner, while in the second is antigen-specific and can be classified as cellular (T cells) or humoral (B cells). The adaptive immune response develops antigen-specific immunological memory and drives both inflammation and tissue repair. The cellular immune response play a key role in the development and progression of chronic inflammatory diseases [4]. T cells are divided into several groups, the two most important are the T helper cell and the cytotoxic T cells, both respectively distinguished by CD4+ and CD8+ cell surface markers. The helper (Th) immune response initiates with the interaction of a CD4+ T cell and an antigen-presenting cell. The T-cell receptor, which is in the surface of the CD4 + T cells, bounds with the major histocompatibility complex type II, which is on the cell membrane of an antigen-presenting cell (B cells, dendritic cells, among others). This interaction leads to the differentiation of B cells into plasma cells that produce antibodies. Various cytokines and other co-stimulatory molecules can stimulate the CD4+ T cell population to divide itself into cell subsets, which elicit a different antigen-specific response, as Th1, Th2, Th9, Th17, and Treg [5]. Each subset has specific characteristics and specialized properties. The Th1 response express pro-inflammatory cytokines, as interferon-gamma (IFN-γ), interleukin 2 (IL-2) and tumor necrosis factor beta (TNF-β). Th1 cells respond against intracellular parasites including protozoa, bacteria, viruses, and fungi. Overexpression of Th1 cells can lead to the development of autoimmune diseases such as hypersensitivity, arthritis, and type 1 diabetes. The Th2 express anti-inflammatory cytokines, as IL-4, IL-5 and IL-13; the Th2 is induced as response to helminth parasites, but its over-activation leads to systemic autoimmune inflammatory diseases, such as allergies and atopic dermatitis. Overexpression of Th9 and Th17 cells is also involved in the development of autoimmune and inflammatory disorders. In the case of Treg cells, they regulate the differentiation and proliferation of effector T cells and promote tolerance, thereby limiting the development of autoimmune diseases [5].

1.3 Hygiene hypothesis

In humans, the immune system has adapted to recurrent infections caused by numerous non-pathogenic organisms. Through exposure to environments rich in microorganisms, this adaptation leads to a more effective immune response to invasion by pathogens. However, the elimination of these "microbial allies" from environments in industrialized cities because of advances in medical care, and improvements in hygiene and urbanization, has been associated with a dramatic increase in autoimmune,

allergic, and chronic degenerative conditions of inflammatory origin [6, 7]. In 1989, at the London School of Hygiene and Tropical Medicine, the research group led by Dr. Strachan found an inverse relationship between the number of children in British families, their quality of life, and the rate of hay fever in their children. In other words, the incidence of this disease is higher in families with fewer children and better hygienic conditions; thus, family members have a more limited exposure to the various antigens found in the environment, and this probably leads to a lack of stimulation of the immune system at an early age [6]. However, it was not the first study with these observations; previously, in 1968, a study showed that the Swedish urban population was more susceptible to developing bronchial asthma and chronic bronchitis compared to Swedes living in rural areas [7]. In 1976, another study performed in Canada reported that the prevalence of some atopic diseases, as asthma, eczema, and urticarial was higher in the white community respect to a native community called Metis, which showed an elevated serum IgE level and a higher prevalence of helminthiases, in addition to untreated viral and bacterial diseases [8]. These studies comprise the origin of the so-called "hygiene hypothesis" as we know it today.

Several studies have documented the existence of an inverse relationship between the increased incidence of inflammatory and metabolic diseases and a decreased prevalence of parasitic helminthiases, such as filariasis, where helminths mildly immunosuppress the host in a chronic and non-specific manner [9]. This modulation is associated with the development of a particular immune mechanism referred to as Th2 (T helper 2) response. Derived from the hypothesis that helminths have evolved in parallel with their hosts, it is possible to think that helminths can survive and perpetuate their life cycle because they "control" the host's immune response. Helminths can live for prolonged periods by maintaining their hosts as asymptomatic carriers. It is likely that their surface proteins, as well as those secreted, excreted, and shed from the parasite, play a significant role in immunomodulation, which, collaterally, can benefit the host by reducing the consequences of exacerbated inflammatory responses from a Th1 response. This mechanism is a normally occurring part of many autoimmune disorders. Parasitic infections have also been observed to have beneficial effects on clinical outcomes of allergy patients [1, 10, 11]. The relationship between Th1 and Th2 immune response mechanisms can be understood, considering the immune system as a dynamic but regulated entity within a balance between these Th1 and Th2 antagonistic responses. Naturally, there are certain cells, such as T regulatory lymphocytes (Tregs), which upon receiving certain stimuli can suppress competing responses and maintain the system balance [5].

2. Use of helminths in experimental therapies

Data from tropical and subtropical countries have shown that inflammatory and autoimmune diseases are rare, but helminthiases are very abundant there. However, in those regions, the anthelmintic treatment is associated with an increased rate of chronic degenerative diseases [1]. These findings have suggested that helminths or their products may be useful to control inflammatory diseases amending the host immune response from Th1 to Th2. The Th2 response is characterized by the production of anti-inflammatory cytokines (IL-4, IL-5, IL-10, and IL-13), non-specific and parasite-specific IgEs, as well as the mobilization of mast cells, basophils, and eosinophils. During infection, Treg cells release cytokines (Il-10 and TGF-β) that negatively regulate the Th1 cell subset [3, 12].

Helminths secrete enzymes and hormones, along with their debris, these molecules are the excretory and secretory products (ESP). ESP are the main mechanism of immune response evasion due to their high antigenicity and ability to migrate away from the helminth, "distracting" the immune response and ensuring parasite survival [3, 13]. Studies on helminth immunomodulation derived from this observation have raised interest in the use of total extracts, ESP or even, recombinant proteins as immunomodulatory treatment in animal models and human clinical trials. Most of these studies have reported clinical improvement, but do not address the molecular mechanisms involved in the process [1, 12].

A vast variety of parasites and their ESP have been used in studies that seek to find emerging therapies for many diseases, for example the findings on *Trichuris suis* ova (TSO). This approach has shown therapeutic effects in diseases such as rheumatoid arthritis, inflammatory bowel disease, or multiple sclerosis, with phase 1 and 2 clinical studies being carried out. Patients received a controlled treatment of 2500 TSO units every 2 weeks for 12 months and a low clinical efficacy was obtained, with just small variations in the immune response of the patients receiving the parasite [14]. Another trial used the nematodes *Trichuris vulpis* and *Uncinaria stenocephala* as a treatment in a model of atopic dermatitis in dogs, which were infected with larval eggs of *T. vulpis* (two groups with 500, and 2500 eggs respectively) or *U. stenocephala* (three groups with 100, 500, and 2500 eggs respectively). The results showed that all dogs improved their lesions; however, there was no change in the inflammation caused by subcutaneous infiltrates. In a subsequent randomized study with *T. vulpis*, no difference was found between parasitized dogs and those receiving a placebo, and it was concluded that *T. vulpis* did not generate significant changes [15]. There are few examples of parasites used as disease modulators and how parasites or molecules derived from them can induce an anti-inflammatory response through Th2/regulatory responses directly associated with the established helminth response.

3. *Trichinella spiralis*

Trichinella is a nematode genus comprised of 12 species and 3 genotypes. *Trichinella spiralis*, *T. nativa*, *T. murrelli*, *T. britovi*, *T. patagoniensis*, *T. nelsoni* species and T6, T8 and T9 genotypes are distinguished by encapsulation in the host muscle tissues, while *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis* species do not induce capsule formation. The genus *Trichinella* is cosmopolitan and parasitizes more than 150 species of domestic and wild vertebrates, mostly carnivorous mammals. All *Trichinella* species can be transmitted zoonotically, although the one most frequently related to human disease is *T. spiralis*. The parasite load is correlated with the severity of the disease and is the cause host death [13, 16–19].

3.1 Life cycle, physiopathology and diagnosis

The adult worm settles in the small intestine, while the larvae inhabits the skeletal muscle, this is the muscle larva (ML) which lives inside of a myocyte surrounded by a collagen capsule. In general, there is one ML per myocyte but, sometimes two or more larvae are found [20]. The enteric phase occurs during the first week after infection and is associated with gastroenteritis, diarrhea, and abdominal pain. The life cycle begins when the host ingests raw or undercooked meat with viable ML. In the stomach, the ML is released from the collagen capsule. In the duodenum, the ML

invade the epithelial columnar cells and molt four times to become adult. At 90 hours after copulation, females deposit the first larval stage, called newborn larva (NBL), which enters the bloodstream. The migration and invasion phase continues during 3 to 7 days and at the end of 30 days post infection, the NBL matured into ML, and the invaded myocyte was repaired, but does not recovered its contractile functions; on the contrary, the glycocalyx hypertrophies, generating a collagen capsule and surrounding itself with a network of new blood capillaries. This new structure is the nurse cell (NC), which allows to the ML remains in hypobiosis for months or years to be transmitted by ingestion to a new host to complete the life cycle. During this last phase, fever, myalgia, and arthralgia are observed; however, individuals with a low parasite load may remain asymptomatic [4]. The diagnostic methods are (1) trichinoscopy, where the ML and the NC are sought by microscopic examination of striated muscle; this technique is used in *post-mortem* studies and food safety protocols. (2) The artificial enzymatic digestion allows isolate ML from meat samples; this method is used in food safety. (3) Antigen detection seeks parasite proteins as biomarkers, mainly in experimental issues (4). Nowadays ELISA and Western blotting are used to determine parasite-specific antibodies in the host serum; this is the gold standard to corroborate clinical suspicion. (5) The molecular diagnosis uses diagnostic probes specific to unique DNA sequences of the parasite for taxonomic purposes [21, 22].

3.2 Immunobiology

ESP from ML and intestinal larval stages as well as from adult helminths play an important role in a successful infection and trigger an early immune response in the host. Many of these proteins are glycosylated and have an N-terminal signal peptide indicating that they are secreted proteins. The high immunogenicity is due to these glycosylations being formed by repetitive chains of oligosaccharides, such as tyvelose and fucose, that confer them modulating properties of the host immune response. Tyvelose is the main antigenic component of ESP and is part of the ML immunodominant antigens [13, 23, 24]. Proteomics and immunoproteomics analyses have shown that some of these proteins are serine proteases, a family of proteolytic enzymes with varied biological functions during a parasite infection. These functions involve host tissue invasion, migration, and proteolysis by helminths. Serine proteases purified from ESP participate in the degradation of host intestinal tissues. They also allow the penetration of a wide range of tissues for acquiring nutrients, and mediate apoptosis-like cell death and phagocytosis, which contributes to a higher parasite-mediated immunosuppression. ESP may play an important regulatory activity by controlling host immune reaction and recognition. In addition to serine proteases, different studies have found other functional proteins involved in the interactions between *T. spiralis* and its host, such as multiple DNase II isoforms that could function as immunomodulators [25].

4. Therapeutic potential of *Trichinella spiralis*

4.1 Therapeutic potential on cancer

In 1970, Weatherly and collaborators [26] conducted one of the first studies where the therapeutic potential of *T. spiralis* was assessed. The authors observed that parasitized mice survived for longer according to the dose of parasites administered, as well

as having a decrease in breast tumor size compared to non-parasitized mice. Since then, many aspects of the inhibitory effect of *T. spiralis* on cancer have been studied and described, both in animal and *in vitro* models with promising results, ranging from the induction of apoptosis in cancer cells to the total or partial inhibition of the growth of some of the tumors studied. The increase in the survival rate of subjects has also been observed *in vivo* in different experimental models, such as in mice that have been parasitized with ML and inoculated with sarcoma 180 tumor cells, where the suppressive effects on cancer development were observed [27–29]. Inhibition of tumor cell growth has also been observed in experimentally infected mice and rats; the inhibition of the development of B16 melanoma, mammary gland cancer, and the number of histiocytomas appears to be directly proportional to the dose of infection [30–34]. The antitumor effects of *T. spiralis* ML have been tested in BALB/c mice with A549 lung cancer, HCT-8 human colorectal carcinoma and C6 glioma [35–37]. In ICR mice, the mouse esophageal carcinoma and mouse ascitic hepatoma (H22) were studied, while in C57BL/6 mice, the hepatoma by Hepa1–6 carcinoma cells were studied [29, 38, 39]. In all experiments, an inhibitory effect of cancer was reported. In addition, studies have been conducted in mouse models of SP2/0 myeloma and colon cancer also immunized with extracts of the parasite and with their ESPs, which immune- modulate the development of both types of cancer [40–42].

ESPs contain some bioactive substances with known antitumor properties, such as the translationally controlled tumor-protein (TCTP) associated with growth, cell cycle regulation and antiapoptotic and immunomodulatory properties. The presence of caveolin-1 (cav-1), an essential protein component of caveolae that acts as a tumor suppressor, has also been described. Other proteins with antitumor properties are some heat shock proteins (HSPS), such as sHSP, HSP60, HSP70, and H3 and H2B histones, involved in fold stability, intracellular arrangement, and proteolytic turnover of many key regulators of growth, differentiation, and survival; they are vital to prevent cell death and maintain homeostasis in *T. spiralis* [20]. The antitumor properties of ESP and ML extracts were also studied in *in vitro* models of esophageal carcinoma, sarcoma 180, chronic myeloid leukemia, hepatomas, lung cancer, B16 melanoma, human cervical carcinoma, and Graffi myeloid tumor. The incubation of cell cultures with ESP or parasite extract showed results that ranged from tumor apoptosis to inhibitory effect on the proliferation of carcinogenic cells [29, 33, 43–47]. These reports are detailed in **Table 1**.

4.2 Therapeutic potential in autoimmune and allergic diseases

Currently, more than 80 autoimmune diseases have been described, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and type I diabetes. Autoimmune diseases affect between 5 and 9% of the world's population and arise from the loss of immune tolerance to self-antigens. Loss of immune tolerance leads to the development of autoreactive T and B cells and the attack of the body's own tissues; for example, an organ-specific attack is presented in rheumatoid arthritis where the target organ is the joints, or it can occur systemically as is the case in SLE [48]. Because *T. spiralis* can induce a Th2-type response in its host to limit the inflammation within the tissue where it is, many studies are focused on the search for new therapies for autoimmune diseases based on these properties. The same happens for allergic diseases, where *T. spiralis* and its ESPs have also shown encouraging results. That is the case of some studies conducted in animal models of allergic asthma, a chronic inflammatory disorder of the respiratory tract with a strong relationship with an exacerbated Th2 response [49].

	Model	Parasite dose	Duration	Effect	Author
In vivo	Sarcoma 180 in (1) HaM/ICR mice, (2) ICR/CD-1 mice & (3) ICR mice	(1) 3000 ML (2) 50–400 ML (3) 400 ML, oral	(1) 28 & 56 days (2) 2–8 & 34 weeks (3) 7 days	(1) Increased survival, no effect on tumor growth (2) Protection in the first weeks (3) Suppression of cancer development	(1) Lubiniecki et al. [27] (2) Molinari and Carric [28] (3) Wang et al. [29]
	B16 melanoma in (1) B6D2F1/J mice (2) & (3) C57Bl/6 mice (4) variant B16-F10 in C57Bl/6 mice	(1) 200 ML, (2) 5 & 20 larvae/g, (3) & (4) different ML doses, oral	(1) 176 days (2) 2 months (3) variable (4) 40 days	(1) & (2) No signs of tumor growth (3) & (4) Suppression of melanoma development	(1) Molinari and Ebersole [30] (2) Pocock and Meerovitch [31] (3) Kang et al. [32] (4) Vasilev et al. [33]
	Malignant fibrous histiocytoma and mammary gland cancer in rats	Prophylactic infection with ML	—	Decreased number of histiocytomas. Suppression of mammary gland cancer	Apanasevich and Britov [34]
	Lung cancer with A549 cells in BALB/c mice	Different doses, attenuated and non-attenuated ML, oral	7 & 11 days	Inhibition of cancer development with attenuated and non-attenuated ML	Gong et al. [35]
	HCT-8 carcinoma in BALB/c mice	Different ML doses, oral	20 days	Decrease in size and weight of tumors	Li et al. [36]
	C6 Glioma in BALB/c mice	Different ML doses, oral	11 days	Parasite load-dependent antitumor effect	Liu et al. [37]
	Esophageal carcinoma in ICR mice	400 ML, oral	7 days	Suppression of cancer development	Wang et al. [29]
	Hepatoma (1) & (2) H22 in ICR mice (3) Hepa1-6 in C57BL/6 mice	(1) 400 ML (2) & (3) different ML doses, oral	(1) 7 days, (2) & (3) variable	Suppression of cancer development	(1) Wang et al. [29] (2) Ding et al. [38] (3) Zhang et al. [39]
	(1) & (2) Myeloma SP2/0 in BALB/c mice	(1) Immunized with crude extract & ESP (2) parasitosis with oral ML	(1) 30 days, (2) 11 days	(1) Induction of protective immunity with antitumor effect (2) Suppression of myeloma development	(1) Gong et al. [40] (2) Deng et al. [41]
	Mouse model of colon cancer induced by 1,2-dimethylhydrazine	Immunized with ESP	—	Enhanced immunomodulation in cancer development	Eissa et al. [42]

	Model	Parasite dose	Duration	Effect	Author
In vitro	Esophageal carcinoma, hepatoma H22, sarcoma 180, human chronic myeloid leukemia (K562)	Incubation with crude extract	—	Inhibition of carcinogenic cell proliferation	Wang et al. [29]
	Human hepatoma (1) & (2) H7402 (3) HepG2	Incubation with (1) & (2) crude extract (3) ESP	24 h	(1) Tumor apoptosis (2) & (3) Inhibition of carcinogenic cell proliferation	(1) Wang et al. [43] (2) Wang et al. [29] (3) Liu et al. [44]
	Lung cancer in (1) NCI-H446 cells (2) SCLC-H446 cells	Incubation with ESP	(1) 24 h, (2) 12, 24 & 48 h	Dose- and time-dependent inhibitory effect on cancer cells.	(1) Chang et al. [45] (2) Luo et al. [46]
	B16 melanoma	Incubation with crude extract	—	Tumor apoptosis	Vasilev et al. [33]
	Graffi myeloid tumor cells, human cervical carcinoma (HeLa)	Biological active substances from Wistar rats parasitized with 1000 ML orally administered	24 h	Inhibitory effect on Graffi myeloid tumor cells, milder growth of HeLa cells.	Tsocheva-Gaytandzhieva et al. [47]

Muscle l(ML), excretory and secretory products (ESP).

Table 1.
Therapeutic effects of T. spiralis *on cancer.*

Among the existent animal models for autoimmune diseases, a model of type 1 diabetes in non-obese mice, when parasitized with ML, showed a decrease in the number of cytotoxic pancreatic cells, which in turn, delayed the disease progression up to 37 weeks [50]. Likewise, a suppressive effect on the disease was observed in parasitized animals with experimental autoimmune encephalomyelitis (EAE) in a study of new treatments for multiple sclerosis; here, there was an increase in the expression of the Th2 profile and a suppression of the disease signs and symptoms [51–53]. In the case of chronic intestinal disease, immunization with recombinant proteins derived from the TsP53, Cystatin-B and paramyosin proteins of *T. spiralis* (rTsP53, Tsp_03420, and rTsPmy, respectively) led to a decrease in the expression of Th1-type cytokines and disease progression [54–56]; in addition, ML parasitosis and ESP immunization also showed anti-inflammatory effects [57–60]. Another animal model used to observe the therapeutic potential of *T. spiralis* is the collagen-induced rheumatoid arthritis model, where parasitosis with ML and immunizations with its extracts and the rTsPmy protein decreased disease progression, inflammation, and histopathological damage in the synovial tissue of the joint cavities [10, 61, 62].

In the case of allergic diseases, the use of ESPs from *T. spiralis* has also shown promising results in animal models of allergic asthma, a chronic inflammatory respiratory disorder triggered by an exacerbated Th2 response. In some cases, ML and its soluble extracts were used in mice with airway inflammation; as a result, improvements were observed in the disease progression, observed by the reduction of the levels of infiltrated eosinophils and ovalbumin-specific IgE, the decrease in IL-4, and the increase in IL-10 and TGF-β [49, 63]. This modulation was also observed in a mouse model of sepsis-induced acute lung injury where immunization with ESP from the parasite increased survival by 50% and reduced inflammation by a decreased production of pro-inflammatory cytokines [64]. These studies on autoimmune and allergic diseases are mentioned in **Table 2**.

4.3 Therapeutic potential of T. spiralis and other nematodes on experimental lupus mice models

SLE is a chronic autoimmune disease that can affect all organs and tissues of the body due to a set of alterations in the innate and adaptive immune system, such as inefficient removal of apoptotic bodies, generation of autoantibodies that activate the complement cascade, and deposition of immune complexes in tissues that triggers an uncontrolled inflammatory process [65]. Renal, dermatological, and cardiovascular symptoms may occur as clinical features. It has an estimated worldwide prevalence between 6.5 and 178.0 per 100,000 people while its incidence varies from 0.3 to 23.7 cases per 100,000 people per year. The disease occurs in the young population, mainly females (9:1 ratio) [66]. SLE is a social and public health problem because 10–25% of patients who develop SLE die within 10 years of diagnosis. Currently, many of these patients die due to the uncontrolled inflammatory activity associated to the disease or because of the immunosuppressive treatment to which they are subjected [67]. Although the etiology of the disease is not completely known, its clinical heterogeneity suggests that different subsets of immune cells play a vital role in its pathogenesis, especially autoreactive B cells and autoantibody-producing plasma cells. Likewise, T cells play a fundamental role in the progression of the disease due to the loss of the delicate balance between Th1 and Th2 responses [68]. Another pivotal part in this disease is the increased levels of a variety of pro-inflammatory cytokines, such as type I (IFN-α, IFN-β, IFN-κ), type II (IFN-γ) and

	Disease	Mouse model	Parasite dose	Effect	Author
Autoimmune diseases	Type 1 diabetes mellitus	Non-obese diabetic mice	Parasitosis with ML orally administered	Infection protected animals from disease for <37 weeks. Mediated by increased CD4+ T cell numbers and decreased CD8+ and NK T cell numbers in the pancreas.	Saunders et al. [50]
	Multiple sclerosis	Experimental autoimmune encephalomyelitis (EAE)	(1) & (2) Parasitosis with ML orally administered (2) Transfer of T cells from parasitized rats to rats with EAE (3) Immunized with ESP	(1) Parasitosis decreased disease in a dose-dependent manner. (2) Infection maintained a Th2 profile immunity after EAE treatment. T cell transfer protected from disease. (3) ESP caused significant suppression of clinical signs in EAE.	(1) Gruden-Movsesijan et al. [51] (2) Gruden-Movsesijan et al. [52] (3) Kuijk et al. [53]
	Inflammatory bowel disease	Colon damage by dinitrobenzene sulfonate or trinitrobenzene sulfonate	Immunized with recombinant proteins (1) 53 kDa protein rTsP53, (2) Cystatin-B (Tsp_03420) & (3) paramyosin (rTsPmy) of *T. spiralis* (4), (5) & (6) parasitosis with ML orally administered (7) Immunized with ESP of adult worms	(1) RTsP53 significantly improved the disease progression and decreased Th1-type cytokines. (2) A better Th1 / Th2 response balance was observed, and it improved the disease outcome. (3) & (4) *T. spiralis* infection and use of rTsPmy inhibited colitis and increased regulatory cytokines and Treg cells. (5) & (6) Colon damage was reduced through an increased Th2 response. (7) ESP immunization showed anti-inflammatory therapeutic effects.	(1) Du et al. [54] (2) Xu et al. [55] (3) Hao et al. [56] (4) Cho et al. [57] (5) Zhao et al. [58] (6) Xu et al. [59] (7) Yang et al. [60]
	Rheumatoid arthritis	Collagen-induced arthritis	(1) Immunized with soluble ML extract (2) Parasitosis with ML orally administered (3) Immunized with recombinant paramyosin protein (rTsPmy)	(1) Decreased clinical disease progression. (2) Inhibition of Th1/Th17 pro-inflammatory responses and polarization to Th2/Treg. (3) Decreased inflammation, histopathological damage and complement deposition in joints.	(1) Eissa et al. [61] (2) Cheng et al. [10] (3) Chen et al. [62]
Allergic diseases	Asthma	Allergy-induced airway inflammation model in BALB/c mice	(1) Parasitosis with ML orally administered (2) Immunized with adult parasite extract.	(1) Improved lung function and decreased inflammation through the increase of regulatory cytokines IL-10 and TGF-β in parasitized mice. (2) Reduced allergic airway inflammation by an IL-4-mediated upregulation of IL-10 and TGF-β, which in turn stimulated a Th2/Treg response.	(1) Park et al. [63] (2) Sun et al. [49]
	Acute lung injury	Experimental lung injury induced by sepsis	Immunized with ESP	Improved survival of mice by 50% and decreased pro-inflammatory cytokine production, which reduced inflammation and lung tissue injury.	Li et al. [64]

Muscle larvae (ML), excretory and secretory products (ESP).

Table 2.
Therapeutic effects of T. spiralis *on autoimmune and allergic diseases.*

type III (IFN-λ1, IFN -λ2, IFN -λ3 and IFN -λ4) interferons, tumor necrosis factor α (TNF-α), interleukins (IL)-1, IL-2, IL-6, IL-10, IL-12, IL-16, IL-17, IL-23, and others. Due to their correlation with the disease, they have been proposed as therapeutic candidates since there is a lack of effective treatments [69]. Thus, the study of new therapies based on immunomodulation that can ameliorate the symptoms and severity of the disease and improve the quality of life of patients has become highly relevant. To date, the few published reports focus on the therapeutic potential of the nematode *Acanthocheilonema viteae* in SLE, based on the use of a dominant ESP protein called ES-62, a widely tested glycoprotein with therapeutic effects in inflammatory diseases such as arthritis and asthma. This glycoprotein, administered in the MRL/Lpr lupus model, induced a decrease in the production of antinuclear autoantibodies, reduced aortic atherosclerotic lesions, and diminished fibrosis by up to 60%. These results have encouraged the use of drug-like small molecule analogs (SMAs) based on the active phosphorylcholine found on the N-glycans of ES-62, with similar outcomes to those obtained by the original protein, setting up a novel approach to control atherosclerosis in SLE [70, 71].

Phosphorylcholine effect on the intestinal microbiome was also studied in mice from the MRL/Lpr lupus model that received a synthetic conjugate called TPC (Tuftsin-Phosphorylcholine); this is made up of a tetrapeptide with immunostimulatory effects called Tuftsin, part of an IgG molecule, and phosphorylcholine. The mice treated with TPC had significant changes in the intestinal microbiome, such as the increase of the populations of beneficial bacteria of the genera *Turicibacter, Bifidobacterium, Mogibacteriaceae, Clostridiaceae, Adlercreutzia, Allobaculum* and *Anaeroplasma* and the reduction of pro-inflammatory bacteria, like the genus *Akkermansia*. Furthermore, TPC treatment was related to a significant decrease in proteinuria levels and an improvement in the disease progression [72].

Due to the immunomodulatory properties shown above, it is important that further studies be carried out, focused on other parasites or their derivatives with a potential therapeutic effect in lupus disease, like *T. spiralis*.

In 2004, Baeza et al. developed a murine model of experimental lupus that shares strikingly similar characteristics to the human disease, such as the presence of anti-histone, anti-nuclear and anti-coagulant antibodies, as well as anti-cardiolipin and anti non-bilayer phospholipid arrangements (NPA). NPA are three-dimensional structures in the cell membrane, different from the canonical bilayer, formed by the polar fractions of the phospholipids; this rearrangement causes the generation of auto antibodies. The lupus mice present glomerulonephritis, splenomegaly, arthritis-like joint lesions, alopecia and facial lesions resembling human malar erythema. IgG anti-NPA antibodies are found in lupus model mice and in some human patients with anti-phospholipid antibody syndrome [73–75].

The influence of *T. spiralis* infection have been studied in the experimental lupus murine model to find out whether the parasite had a therapeutic effect on the progression and outcome of this inflammatory disease. One of our experiments consisted in study mice were orally infected with 100 ML and at day 30 *post* infection induce lupus by intrasplenic administration of 100 μL of liposomes incubated with the promazine to trigger the formation of non bilayer phospholipid arrangement or NPA [76, 77]. The NPA administration was weekly by intraperitoneal until the end of the experiment. Blood samples were taken every 30 days for 6 months to determine the presence of pro- (IL-1α, IL-17a, IFN-γ and TNF-α) and anti-inflammatory cytokines (IL-4 and IL-10) by flow cytometry. In addition, body weight, clinical lesions and, antibodies to the ML were evaluated by ELISA [22].

The levels of IFN-γ and IL-1α did not show significant differences between experimental groups. **Figure 1** shows that first month *post-infection*, low levels of IL-4 and IL-10 were observed in mice with lupus and lupus infected mice respect to infected. However, unexpected at fifth moth *post-infection*, the lupus infected mice group increased the IL-4 levels (**Figure 1a**); in accordance with data published in different experimental models of inflammatory diseases for arthritis, colitis and airway inflammations [2], is possible that a modulation towards an anti-inflammatory-type response by IL-4 along with the induction of Tregs initiated by IL-10 (**Figure 1b**) were observed. In our observations, levels of IL-17a were higher in the lupus infected mice respected to lupus mice (**Figure 1c**), which contrasts with data shown by Cheng and collaborators, who found that the decrease in this cytokine did not produce any effect in an arthritis model [10]. IL-17a is commonly related to an inflammatory response, but also participate in tissue regeneration [78]. The overexpression of TNF-α in the infected mice and lupus infected mice (**Figure 1d**) is in accordance with results reported by Kim and Moudgil in 2008, using an arthritis model developed by the administration of heat-killed *M. tuberculosis* in rats and subsequently administered with TNF-α and IFN-γ. Authors observed that high levels of TNF-α had a protective effect against arthritis progression [79]. Our data shows absence of arthritis-like lesions in lupus infected mice in concordance with data reported by Cheng and collaborators in 2018, where a therapeutic effect of *T. spiralis*

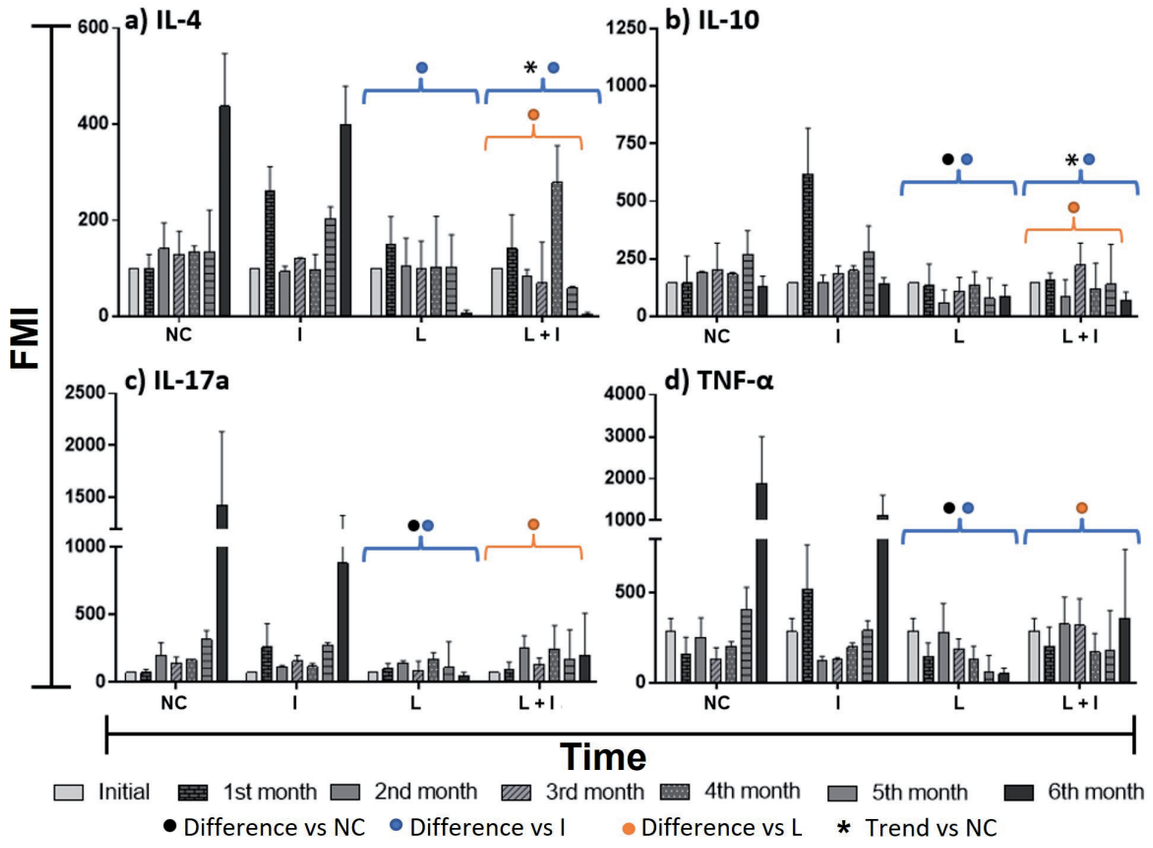

Figure 1.
FMI levels of intracellular cytokines in peripheral blood of the experimental mice. Bar graphs show the levels of cytokines IL-4, IL-10, IL-17a, and TNF-α. Untreated (NC, negative control), infected (I), lupus (L), and lupus infected (L + I) mice. Circles indicate significant differences, asterisks trends between study groups, and square brackets the time during which the statistical difference is valid. Orange color stands for 5 months and blue color for 6 months of study. To determine the differences ($p < 0.05$), a two-way ANOVA for independent samples was performed. FMI stands for fluorescence mean intensity.

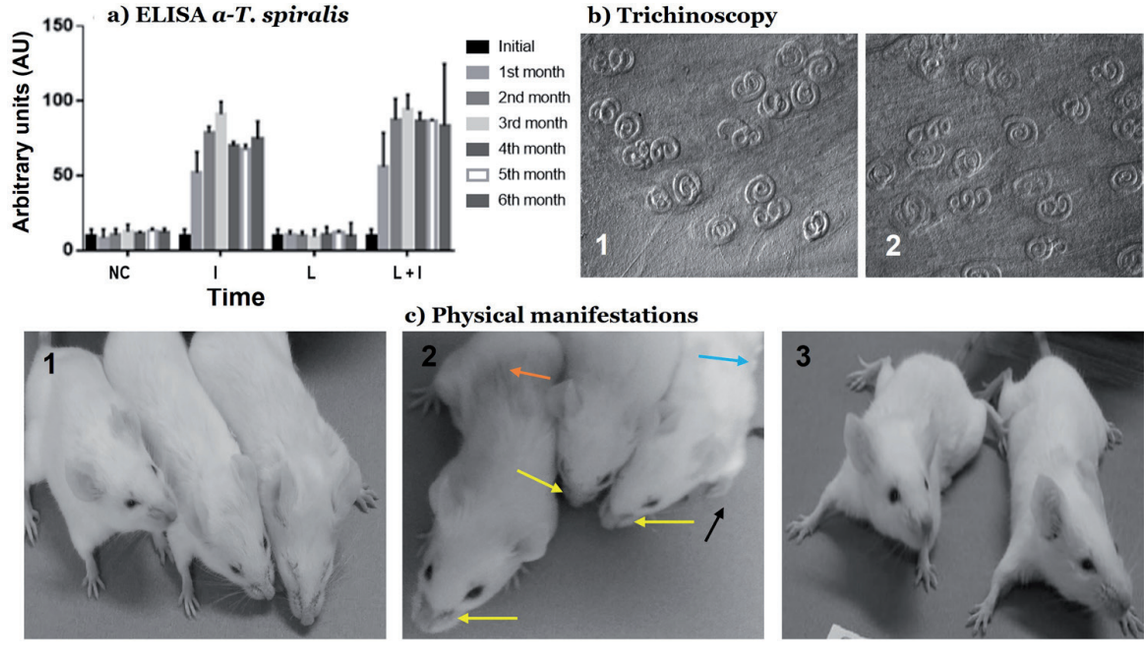

Figure 2.
Physical and clinical characteristics of the mice studied. (a) Bar graphs show serum levels of antibodies specific for T. spiralis. Untreated (NC, negative control), infected (I), lupus (L) and lupus infected (L+I) mice. Circles indicate significant differences between groups. To determine differences ($p < 0.05$), a two-way ANOVA for independent samples was performed. (b) Trichinoscopies of mouse diaphragms from the P (b1) and P+L (b2) groups analyzed by optic microscopy. (c) Photos show some of the lesions presented by mice at the end of the study, indicated by colored arrows; black arrows show joint lesions, yellow arrows facial lesions, orange arrows alopecia spots, and blue arrows piloerection. (c1) infected (I), (c2) lupus (L), and (c3) lupus infected (L+I) mice.

in a collagen-induced arthritis mouse model was observed by promoting tolerance, suppressing inflammatory T-cell activity and reducing tissue damage [10]. IgG serum antibodies to *T. spiralis* was similar between infected and lupus infected mice (**Figure 2a**). Trichinoscopy of all infected mice showed similar parasite loads in diaphragms (**Figure 2b**). Clinical lesions were only observed in lupus mice (**Figure 2c**). At the beginning of the third month, half of the L group had developed alopecia and facial lesions, and more than half of these mice showed arthritis-like articular lesions.

In conclusion, data suggest a *T. spiralis* a protective effect during 5 months through the production of anti-inflammatory cytokines; this effect can delay or reduce the appearance of some of the lupus-related signs. It is imperative to continue the research to gather more data on the mechanisms of immunomodulation triggered by *T. spiralis* to look out for future lupus therapies.

5. Risks of treatment with helminths or helminth products

Nowadays, there are number for alternative therapies to autoimmune disorders, including the use helminth infection; however, these "treatment" is neither attractive nor etic because the use of live worms. Indeed, in the experimental approach, there are many unanswered questions such as appropriate dosing regimens and optimal timing of treatment, in addition to how host genetics, diet, and environment influence disease progression [80, 81]. Because helminth-enabled immunomodulation can extend to other unknown effects on the immune

response to other pathogens or vaccines, these interactions can induce immune downregulation and may lead to predispositions to other types of infections, such as those caused by *Mycobacterium tuberculosis* or malaria [1]. Even though some helminths reduce the risk of developing adenocarcinoma associated with *Helicobacter pylori*, others increase the development of several types of cancer, such as trematodes of genus *Opisthorchis*. In this case, it is important to consider that although all vermiform organisms are considered helminths, there are notable metabolic differences between them; in flatworms (e.g., *Opisthorchis*), the parasite–host contact is carried out through their tegument and involves a whole range of surface proteins. On the other hand, nemathelminths (e.g., *Trichinella*) contact its host through the cuticle that surrounds the parasite, mainly made up of chitin, and turning the ESP into the main antigens recognized by the immune response [1].

In the case of *T. spiralis*, there are some concerns about its use as a therapeutic reagent, the most important being the possible induction of an antibody response, which may reduce the efficacy of its ESP. Even though many of the reports do not use adjuvants or the administrations are intraperitoneally given for short time periods, limiting the response against the parasite proteins, the efficacy could be negatively affected if they are used repeatedly or for prolonged time periods due to the probable production of neutralizing antibodies. Another problem that has arisen from this kind of treatment is the complex composition of the ESP itself, which may lead to the occurrence of side effects or immunological interference, if ESP are used in their entirety. This complexity in composition also represents a problem for scaling their production, limiting ESP clinical use; thus, the characterization of each component that has immunoregulatory properties is of upmost importance [64].

6. Conclusion

Although helminths are different, both biologically and morphologically, most have developed similar strategies to evade innate and adaptive host immune responses, allowing them to establish prolonged parasitism. Among these strategies, the capacity of immunosuppression or immunomodulation stands out, turning helminths into a focus of attention for the study of new therapeutic strategies that allow improving the quality of life during chronic degenerative diseases. The study of *Trichinella spiralis* has shown to have immunomodulatory potential in experimental models of cancer, allergy and autoimmune diseases. In the case of experimental murine lupus, infection with *Trichinella* delayed the presence of signs of disease for 5 months and increased the levels of the cytokines IL-10 and IL-4. To our knowledge, this is the only work reporting the therapeutic effects of *T. spiralis* in an experimental mouse model of lupus.

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Conflict of interest

The authors declare no conflict of interest.

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