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Trichinella spiralis: inflammation modulator

Jing Ding, Xiaolei Liu, Xue Bai, Yang Wang, Jian Li, Chun Wang, Shicun Li, Mingyuan Liu and Xuelin Wang

Key Laboratory for Zoonoses Research, Ministry of Education, Institute of Zoonoses, College of Veterinary Medicine, Jilin University, OIE Collaborating Center on Foodborne Parasites in Asian-Pacific Region, Changchun 130062, P.R. China

Review Article

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Author for correspondence:
X. Wang, E-mail: xuelin@jlu.edu.cn

Abstract

The hygiene hypothesis posits that the decreased incidence of parasitic infection in developed countries may underlie an increased prevalence of allergic and autoimmune diseases in these countries. As unique inflammation modulator of intracellular parasitism, *Trichinella spiralis*, or its excretory–secretory (ES) product, shows improved responses to allergies, autoimmune diseases, inflammatory bowel disease, type 1 diabetes, rheumatic arthritis and autoimmune encephalomyelitis by exerting immunomodulatory effects on both innate and adaptive immune cells in animal models. Research has shown that *T. spiralis* differs from other helminths in manipulation of the host immune response not only by well-known characteristics of its life cycle, but also by its inflammation modulation pathway. How the parasite achieves inflammation modulation has not been fully elucidated yet. This review will generalize the mechanism and focuses on ES immunomodulatory molecules of *T. spiralis* that may be important for developing new therapeutics for inflammatory disorders.

Introduction

David P. Strachan proposed the 'hygiene hypothesis' in 1989, which theorized that the decreased incidence of bacterial and parasitic infections in humans living in developed countries underlies immune system deregulations and increased development of allergic and autoimmune diseases observed in these populations (Strachan, 1989, 2000; Yazdanbakhsh & Matricardi, 2004; Maizels, 2016; Harnett & Harnett, 2017; Wu et al., 2017; Bach, 2018; Caraballo, 2018). Epidemiological data show that acute or chronic infection by helminths can provide some protection against these diseases (Weinstock *et al.*, 2002; McSorley *et al.*, 2019) in animal models or human beings. Clinical trials indicate that infection with different helminths can provide immunological protection against multiple sclerosis and type 2 diabetes (Tahapary *et al.*, 2015; Charabati *et al.*, 2020). In recent years, mounting evidence suggests that helminths exert strong immunomodulatory effects on their hosts (Maizels *et al.*, 2004; Varyani *et al.*, 2017). As a unique inflammation modulator, *Trichinella spiralis* is known as a zoonotic nematode of intracellular parasitism that infects a wide range of vertebrate hosts, including humans. Humans consume uncooked meat that carries encysted *T. spiralis* larvae in muscle tissue. Larvae moult and mature into adult worms in the small intestine of the host. The female adult worms release newborn larvae after copulation, which travel through the circulatory system to reach skeletal muscle cells (Fabre *et al.*, 2009; Hernandez-Ancheyta *et al.*, 2018). Unlike other intracellular parasites, *T. spiralis* larvae transform muscle cells into encapsulated nurse cells and occupy the host's muscle cells without killing them, making it one of the most successful parasitic symbiotes, ensuring its survival and immune dialogue with the host by releasing excretory–secretory (ES) products (Hernandez-Ancheyta *et al.*, 2018). *Trichinella spiralis* infection ameliorates immune-mediated diseases in animal models, such as allergies, autoimmune disease, inflammatory bowel disease (IBD), type 1 diabetes, rheumatoid arthritis (RA) and autoimmune encephalomyelitis. The reduced disease symptoms are likely to be mediated by *T. spiralis* exerting immune effects on T cells, dendritic cells (DCs), natural killer (NK), mast cells and eosinophils (Saunders *et al.*, 2007; Gruden-Movsesijan *et al.*, 2010; Du *et al.*, 2011; Park *et al.*, 2011; Cho *et al.*, 2012; Kuijk *et al.*, 2012; Aranzamendi *et al.*, 2013a; Sofronic-Milosavljevic *et al.*, 2013; Zhao *et al.*, 2013; Yang *et al.*, 2014; Eissa *et al.*, 2016; Cheng *et al.*, 2018; Sun *et al.*, 2019; Xu *et al.*, 2019a; Xu *et al.*, 2019b) (table 1). Research has shown that *T. spiralis* differs from other helminths in manipulation of the host immune response not only by well-known characteristics of its life cycle, but also by its inflammation modulation pathway. How the parasite achieves inflammation modulation has not been fully elucidated yet. It is clear that the application of live parasite poses a health risk and ethical issues that need to be avoided. *Trichinella spiralis* ES products that enable cross-talk between the parasite and the host have attracted a lot of attention (Wang *et al.*, 2017). Obtained results indicated that this protein complex mixture from ES products and its particular components (either in native or recombinant form) could largely reproduce the effects of infection on the

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Table 1. Immune-mediated diseases ameliorated by *T. spiralis* infection or its product.

Disease	Administration	Immune response cell	Reference
experimental allergic airway inflammation (EAAI)	*T. spiralis* muscle larvae	Treg cell or DCs	Park *et al.* (2011) and Aranzamendi *et al.* (2013a)
experimental autoimmune encephalomyelitis (EAE)	*T. spiralis* muscle larvae; soluble extract from *T. spiralis* muscle larva		Gruden-Movsesijan *et al.* (2010), Sofronic-Milosavljevic *et al.* (2013) and Kuijk *et al.* (2012)
inflammatory bowel disease (IBD)	*T. spiralis* muscle larvae ES products from adults *T. spiralis* (AES)	T and Treg cell	Cho *et al.* (2012), Zhao *et al.* (2013), Cheng *et al.* (2018), Eissa *et al.* (2016), Du *et al.* (2011), Yang *et al.* (2014), Xu *et al.* (2019a) and Xu *et al.* (2019b)
Rheumatoid arthritis (RA)	*T. spiralis* muscle larvae; soluble extract from *T. spiralis* muscle larva		
Type 1 diabetes mellitus (T1DM)	*T. spiralis* muscle larvae	T and NK cell	Saunders *et al.* (2007)
Asthma	ES products from adults *T. spiralis* (AES)	T and Treg cell	Sun *et al.* (2019)

host immune system (by shifting the immune response from pro- to anti-inflammatory type) (Aranzamendi *et al.*, 2013a; Yang *et al.*, 2014; Kobpornchai *et al.*, 2020). Therefore, almost any research performed in the field seeks to explore the molecular mechanisms and to identify molecules important for creation of the anti-inflammatory milieu achieved by this parasite. This review will generalize the mechanism and focuses on the ES immunomodulatory molecules of *T. spiralis* that may be important for developing new therapeutics for inflammatory disorders.

Contribution of immunology to clearing host inflammation during infection with *T. spiralis*

Humans can contract *T. spiralis* by consuming uncooked meat that carries encysted *T. spiralis* larvae in muscle tissue. Larvae are released by gastric fluids and migrate to the small intestine of the host, where they moult and mature into adult worms (intestinal stage). The female adult worms release newborn larvae after copulation, which travel through the circulatory system to reach skeletal muscle cells (muscle stage). *Trichinella spiralis* larvae transform muscle cells into encapsulated nurse cells and establish a niche in muscle tissues (nurse cell formation) (Fabre *et al.*, 2009; Hernandez-Ancheyta *et al.*, 2018). The cross-talk between the host and *T. spiralis* has evolved to maximize the immune modulating efficiency of *T. spiralis*, which regulates the dominant ascending T helper cell type 2 (Th2) immune responses. *Trichinella spiralis* alters the host's immune system to ensure its survival by releasing ES molecules (Ilic *et al.*, 2012; Wang *et al.*, 2017) (fig. 1).

Contribution of immunology to clearing host inflammation in intestinal stage of *T. spiralis* infection

In intestinal stage of *T. spiralis* infection, the B cell compartment is affected in *T. spiralis*-infected individuals and is characterized by an increase in immunoglobulin (Ig)G and IgE in the host (Watanabe, 2014; Moon *et al.*, 2018). Allergen-specific IgG can prevent the binding of the allergen to IgE residing on mast cells and basophils. As a result, increasing allergen-specific IgE leads to increased clinical sensitivity, as mast cells become cross-linked with basophils, leading to the upregulation of IgE receptors (Valenta *et al.*, 2017). *Trichinella spiralis* infection can protect itself from the host by inducing immune cell of host to produce large amounts of polyclonal nonspecific IgE. By saturating the IgE binding sites on the mast cell and basophils, it inhibits the binding of allergen-specific IgE and ameliorates allergic responses (Gurish *et al.*, 2004; Erb, 2007). Cytokines produced by B cells modulate the differentiation of $CD4^+$ T cells towards either Th1 or Th2 cells (Harris *et al.*, 2000). Regulatory B (Breg) cells are required for the polarization of the Th cell response towards Th2 (Ronet *et al.*, 2010) (fig. 1). Breg cells participate in the suppression of experimental autoimmune encephalomyelitis (Wolf *et al.*, 1996). ES molecules that are secreted from *T. spiralis* induce Breg cells (Kobpornchai *et al.*, 2020); however, the molecules that are involved in this induction remain unclear.

As an important pathogen, *T. spiralis*-stimulated DCs can provoke regulatory immune responses associated with toll like receptors (TLRs) (Broide, 2009; Langelaar *et al.*, 2009; Yu *et al.*, 2013; Szkudlapski *et al.*, 2014; Kim *et al.*, 2015; Wojtkowiak-Giera *et al.*, 2018). Pathogens mainly activate DCs via a pattern recognition receptor (PRR) signalling pathways which play a key role in host cell recognition and response to pathogens (Everts *et al.*, 2010). DCs are an important antigen-presenting cell with many types of PRRs expressed on the surface of DCs or in DCs to identify and distinguish different pathogen-related antigens (Carvalho *et al.*, 2009). Among these PRRs, TLRs are the most important members expressed on the surface of DCs or in DCs (Sun *et al.*, 2011). Activation of TLRs leads to the recruitment of adaptor proteins, including TIR domain-containing adapter protein 1 (TIRAP1), TIR domain-containing adaptor protein-inducing interferon (TRIF) and myeloid differentiation primary response protein 88 (MyD88). The recruitment of these proteins leads to the activation of nuclear factor kappa B (NF-κB), adaptor protein 1 (AP1) and interferon regulatory factors (IRFs), which results in the production of proinflammatory cytokines. Maintenance of the TLR-dependent signalling pathway is critical for controlling intestinal inflammation, and deregulation of this pathway contributes to various clinical IBD phenotypes (Sun *et al.*, 2011).

Thirteen mammalian TLR paralogues have now been identified (ten in humans and 12 in mice) (Beiting *et al.*, 2004). TLR1–TLR9 were more studied in the murine disease model correlated with human beings (Yu *et al.*, 2013; Kim *et al.*, 2015; Sun *et al.*, 2015; Ilic *et al.*, 2018; Wojtkowiak-Giera *et al.*, 2018). Different stages of *T. spiralis* infection had different impacts on the expression of TLR1–TLR9 (Yu *et al.*, 2013; Kim *et al.*, 2015).The expression of *TLR4* and *TLR9* significantly increases in the small intestine after *T. spiralis* infects the intestine and releases ES molecules compared with TLR1, TLR2 and TLR5

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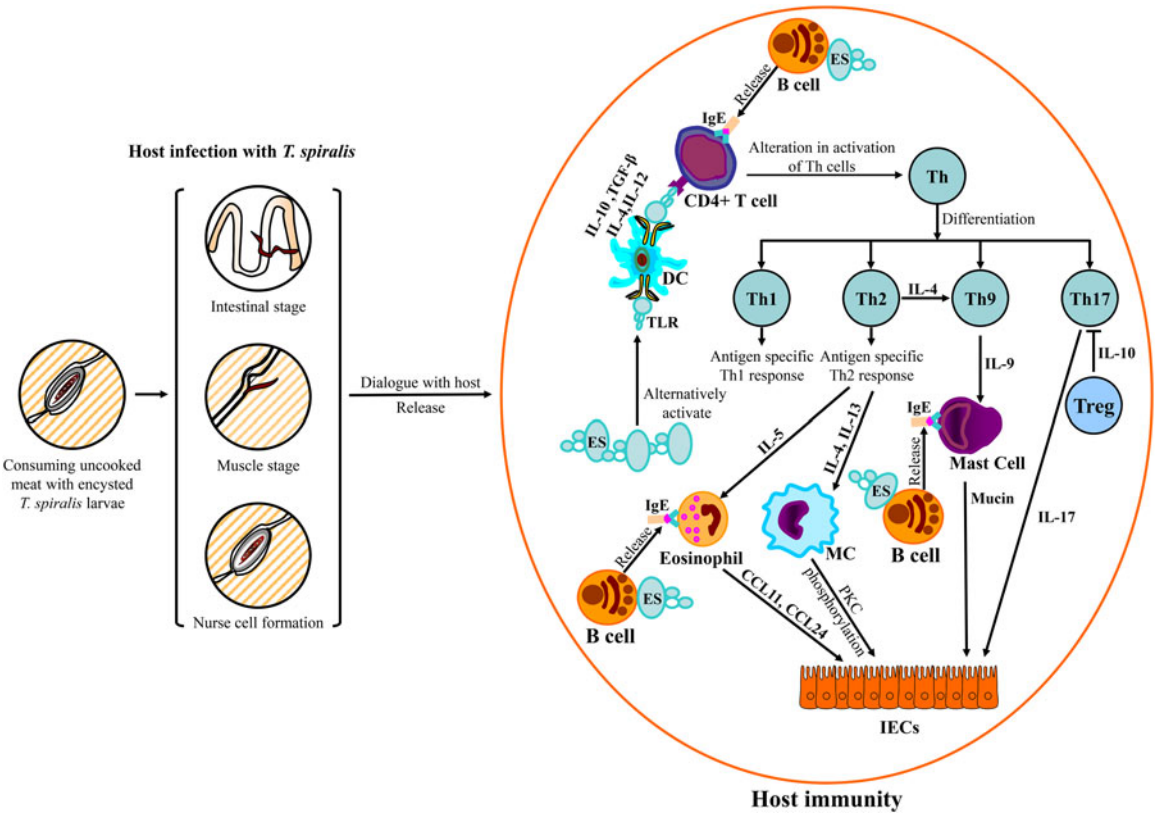


Fig. 1. Host–*Trichinella spiralis* interactions. *Trichinella spiralis* infection alters and activates host Th cells and leads to an antigen-specific Th1 response and dominant elevation of the Th2 response in the host.

(Kim *et al*., 2015). Activated TLR9 triggers intracellular signalling, leading to the activation of macrophages, DCs and B cells and the production of cytokines, chemokines and immunoglobulins (Broide, 2009; Szkudlapski *et al*., 2014). In the adult worm stage of infection, *TLR4* expression is upregulated, and the MyD88-dependent signalling pathway is activated (Yu *et al*., 2013; Wojtkowiak-Giera *et al*., 2018). The muscle larvae then inhibit TLR4- and TRIF-dependent signalling pathways by releasing ES molecules (Yu *et al*., 2013). TLR4 and TLR9 have different adaptor proteins. TLR4 binds to MyD88/TIRAP and TRIF, while TLR9 binds only to MyD88 (Chen *et al*., 2016a). The expression of *TRIF* is the most important change in Th2-related cytokine gene expression in the host (Xu *et al*., 2004; Piras & Selvarajoo, 2014) (fig. 2). Therefore, TLR4 may be more closely associated with the Th2 response in individuals who are infected with *T. spiralis* compared with those of other TLRs (Ilic *et al*., 2018; Wojtkowiak-Giera *et al*., 2018).

DCs could produce interleukin (IL)-12, which influences the development of Th1 or IL-4 and influences the development of Th2 type of immune response in response to pathogen associated molecular patterns (PAMPS) and damage-associated molecular pattern molecules (Qian & Cao, 2018). ES molecules also stimulate DCs, leading to significantly increased IL-10 and transforming growth factor beta (TGF-β) (Sofronic-Milosavljevic *et al*., 2013) (fig. 1). *Trichinella spiralis*-stimulated DCs provoke regulatory immune responses, thus resulting in the regulatory DC phenotype (Langelaar *et al*., 2009). The regulation of DCs occurs in a generalized manner irrespective of their Th1 or Th2 phenotypic affiliation (Mucida & Cheroutre, 2010). Gruden-Movsesijan *et al*. (2010) successfully induced an immune tolerogenic, Th2 response using *T. spiralis*-sensitized DCs in rats and humans (Ilic *et al*., 2018). This is a promising tool to avoid the application of live parasites to treat various autoimmune and allergic diseases.

In the early stages of *T. spiralis* infection, the host also experiences a T helper cell type 1 (Th1) response to protect the host from the parasite, as characterized by increased interferon gamma (IFN-γ) and IL-12 levels in the spleen and serum of the host (Aranzamendi *et al*., 2012; Kang *et al*., 2012; Ding *et al*., 2017). The Th1-type immune response is triggered by transcriptional upregulation and increased secretion of proinflammatory cytokines. Increased circulation of cytokines increases immunity by recruiting immune cells to the intestine and increasing the restoration of intestinal epithelial cells (IECs) that are invaded by *T. spiralis* (Ming *et al*., 2016). Once a *T. spiralis* adult worm occupies a niche in the enterocytes of the jejunum, a well-characterized type 2 cytokine response (IL-4, IL-5, IL-9, IL-10 and IL-13) orchestrated by $CD4^+$ T cells is elicited in the mesenteric lymph nodes. This cell is subsequently recruited to the gut mucosa and clears inflammation in the host gut tissue (Helmby & Grencis, 2003; Sofronic-Milosavljevic *et al*., 2015). *Trichinella spiralis* infection elicits a Th2 response in the host that is characterized by eosinophilia, high serum IgE levels and high serum levels of IL-4, IL-5 IL-9, IL-10 and IL-13 (Finkelman *et al*., 2004; Sofronic-Milosavljevic *et al*., 2015). Eotaxin is a fundamental regulator of physiological eosinophil trafficking during healthy and inflamed states (Conroy & Williams, 2001). Eotaxin-1 (CCL11) and eotaxin-2 (CCL24) are important in intestinal tissue eosinophilia in individuals who are infected with *T. spiralis* (Bruschi *et al*., 2008). The inhibition of eosinophil apoptosis during the later stages of *T. spiralis* infection is related to the presence of IL-5

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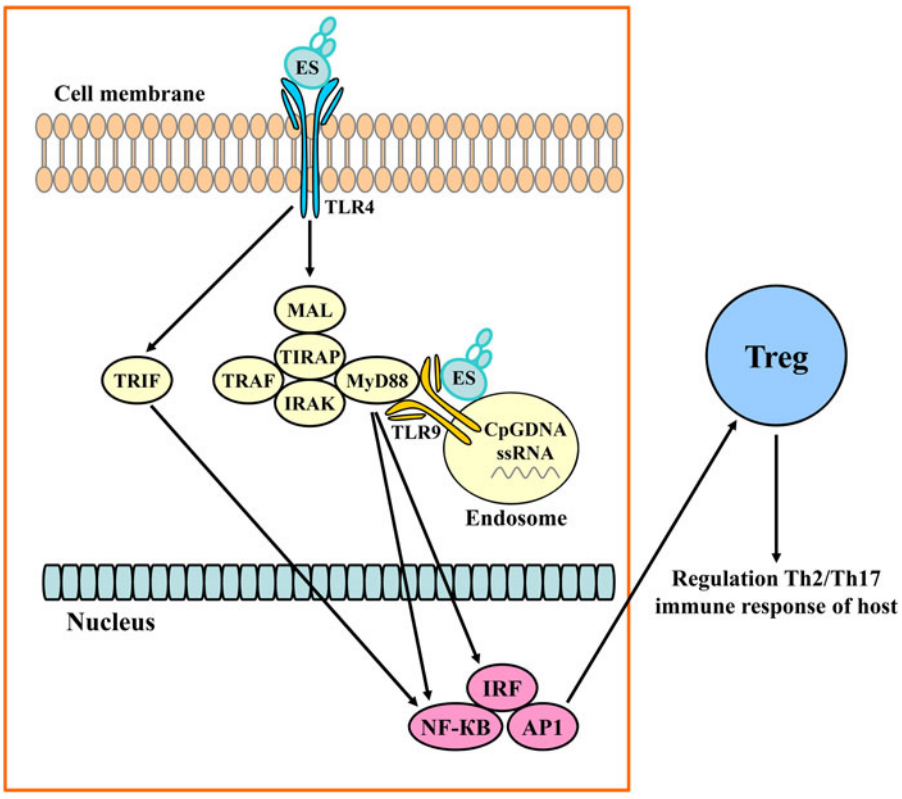


Fig. 2. ES-induced signalling changes in extracellular TLR4 and TLR9. ES molecules that are secreted from *T. spiralis* selectively activate TLR4 and TLR9. Activated TLR4 induces signals through the activation of the MyD88 adaptor protein and intermediate MyD88 adaptor-like (MAL)/TIRAP, TNF receptor associate factor (TRAF), and interleukin-1 receptor associated kinase (IRAK) signal adaptor protein molecules. Activated TLR9 induces signals through activation of the MyD88 adaptor protein. TLR4- and TLR9-mediated signals induce downstream activation of nuclear factor kappa B (NF-κB), adaptor protein 1 (AP1) and IRF to regulate Th2/Th17 immune responses in Treg cells.

(Danilowicz-Luebert *et al.*, 2011). Elevated levels of Th2 cytokines activate mast cells to release mucin and induce goblet cell hyperplasia, induce peritoneal macrophages to become activated M2 macrophages and enhance protein kinase C phosphorylation during the intestinal phase of *T. spiralis* (Kamal *et al.*, 2001; Knight *et al.*, 2004; Dzik *et al.*, 2010). ES products from different stages of *T. spiralis* alter macrophage phenotypes by inhibiting the production of proinflammatory cytokines, such as tumour necrosis factor alpha (TNF-α), IL-1β, IL-6 and IL-12, which then ameliorate the effects of inflammatory diseases (Akiho *et al.*, 2002). *Trichinella spiralis* ES proteins ameliorate intestinal injury by promoting IRF4 and M2-related metabolic regulation via STAT6-dependent transformation of classically activated M1 macrophages to alternatively activated M2 macrophages. M2 macrophages have anti-inflammatory properties, and, thus, ES products may have potential as an immunotherapeutic against inflammatory disorders by inducing M2 macrophages (Han *et al.*, 2019; Kang *et al.*, 2019). The immune response to *T. spiralis* is a powerful way to reduce inflammation of the host intestine. *Trichinella spiralis* infection does not trigger apoptotic events and results in significantly increased expression of *IL-1β, IL-8, ENA-78*, and *MCP-2* in IECs (Stadnyk *et al.*, 2000; Milcheva *et al.*, 2013) (fig. 3). *Trichinella spiralis* retunes the immune cell repertoire of its host, which further explains why helminths are involved in the immunoregulation of the host and can be applied to treat excessive inflammatory responses (Saunders *et al.*, 2007; Du *et al.*, 2011; Gruden-Movsesijan *et al.*, 2011; Park *et al.*, 2011; Cho *et al.*, 2012; Kuijk *et al.*, 2012; Aranzamendi *et al.*, 2013a; Sofronic-Milosavljevic *et al.*, 2013; Zhao *et al.*, 2013; Yang *et al.*, 2014; Eissa *et al.*, 2016; Cheng *et al.*, 2018; Inclan-Rico & Siracusa, 2018; Xu *et al.*, 2019a) (fig. 4). Recent studies have shown that Th2-related innate immune responses are regulated by epithelial cells and innate helper T cells, and triggering this response leads to intestinal mucosal restoration (Yang *et al.*, 2014; Xu *et al.*, 2019a). *Trichinella spiralis* infection activates the response in intestinal tuft cells, which are epithelial chemosensory cells found in mucosal tissues and release the alarmin IL-25 and type 2 innate lymphoid cells (ILC2s) that expand upon *Trichinella* infection and provide the early signals that drive type 2 immunity in the gut (Luo *et al.*, 2019).

Contribution of immunology to clearing host inflammation in the muscle stage of *T. spiralis* infection

In the muscle stage of *T. spiralis* infection, the Th2 cytokines IL-4, IL-10 and IL-13 are upregulated (Akiho *et al.*, 2002). The muscle stage also induces a Th9 response by producing high levels of IL-9, which mediates the immune response by increasing the levels of circulating IgE antibodies (Beiting et al., 2007; Ilic et al., 2011, 2012). The research indicates that IL-9 is a potent Th2 adjuvant and has an important role in the induction of a Th2-type response. During *T. spiralis* infection of muscle cells, the expression of the chemokine receptors *CXCR3*, *CCR4*, *CCR5*, *CCR9* and *CCR10* correlates with increased Treg cell recruitment to infected muscle cells (Ahn *et al.*, 2016) (fig. 3). *Trichinella spiralis* infection upregulates *TLR9* expression, which then stimulates DCs to produce IL-10 and promote the induction of Treg cells (Kim *et al.*, 2015). In addition, *T. spiralis* regulates the host's Th2 and Th17 immune responses in mice by releasing ES molecules into the small intestine and muscle tissue. Hence, TLR9 may be closely associated with Treg cell-mediated immune responses in individuals who are infected with *T. spiralis* (Aranzamendi *et al.*, 2013b; Kim *et al.*, 2015).

Long-term *T. spiralis* infection in muscle tissue induces immunomodulation and is mainly characterized by a Th2 response in which it is generally beneficial to the host to repair or prevent tissue

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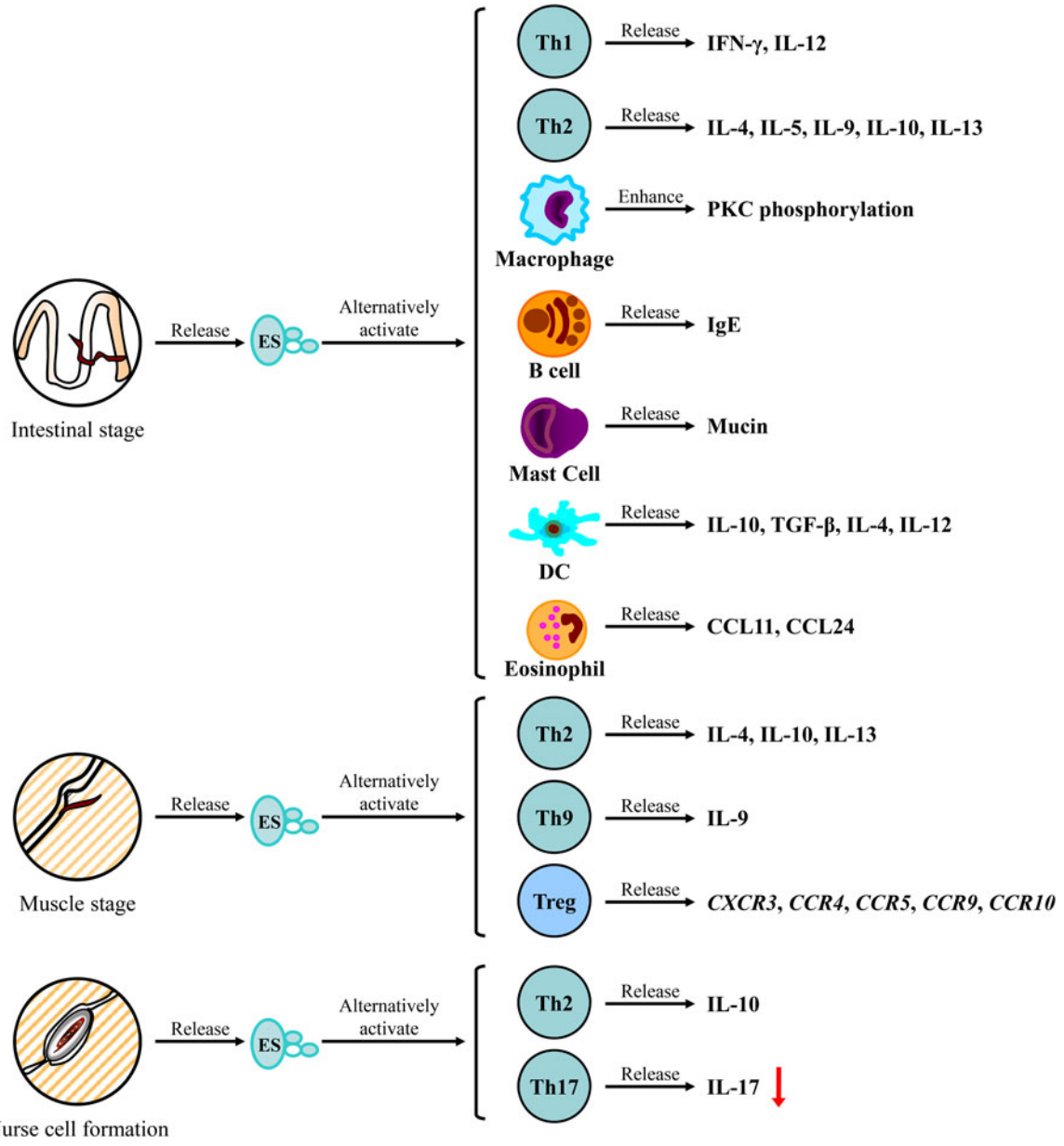


Fig. 3. The immune response in different *T. spiralis* infection stages. *Trichinella spiralis* infection in the intestine, muscle and nurse cell formation stages leads to the secretion of special excretory–secretory (ES) antigens that activate Th1, Th2, Th9, Th17, and Treg cells, DCs, B, macrophages and mast cells, depending on the stage. Activated cells produce or release cytokines to alter the immune regulation of the host and ensure the survival of *T. spiralis* through its different life stages.

damage that results from inflammation (Fabre *et al.*, 2009). The parasite orchestrates a long-lasting molecular cross-talk with the host through muscle larvae excretory-secretory products (ES L1). Those products can successfully modulate parasite-specific immune responses as well as responses to unrelated antigens (either self or non-self in origin), providing an anti-inflammatory milieu and maintaining homeostasis (Sofronic-Milosavljevic *et al.*, 2015).

Contribution of immunology to clearing host inflammation in nurse cells formation of *T. spiralis* infection

After nurse cells form, the anti-inflammatory cytokine IL-10 remains upregulated, while the inflammatory pathology mediator IL-17 is downregulated (fig. 3), which balances the T cell response and limits inflammation in the host (Beiting *et al.*, 2004; Elliott & Weinstock, 2012). The ES antigens released by *T. spiralis* during each of its developmental stages induce different mediators to increase the populations of Treg cells in the host (Ilic *et al.*, 2012). *Trichinella spiralis* leverages this immunomodulation to prevent its eradication by an excessive immune response, as excessive inflammatory responses by the host may lead to organ damage (Kahl *et al.*, 2018). Treg cells control Th2-dominated diseases, such as allergies (McKay, 2009). The induction of Treg cells by *T. spiralis* is important in remodelling the immune response of the host (Gruden-Movsesijan *et al.*, 2011; Park *et al.*, 2011; Aranzamendi *et al.*, 2013a). The immune response suppresses the differentiation of Th17 cells through the interaction of Foxp3 and RORγt, which are transcription factors that are expressed in Treg and Th17 cells, respectively (Elliott & Weinstock, 2012; Fasching *et al.*, 2017). Th17 cells, which express

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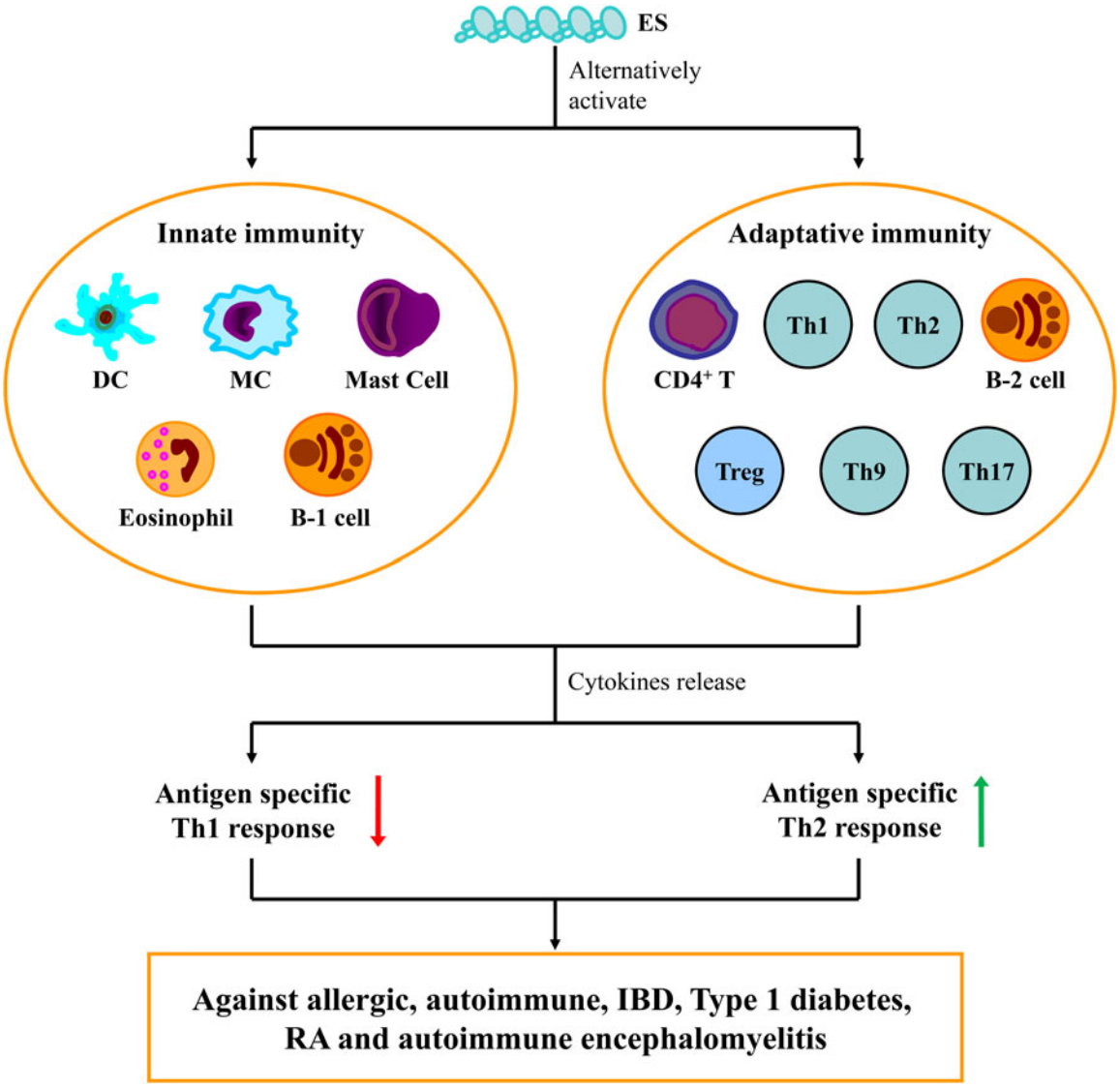


Fig. 4. *Trichinella spiralis*-induced immune cells involved in remodelling the immune response of the host. *T. spiralis* ES antigens from all stages induce a network of innate immune cells (DCs, macrophages, mast cells, eosinophils and B-1 cells) and adaptative immune cells ($CD4^+$ T, Th2, Treg, Th9, Th17 and B-2 cells) to release cytokines which leads to an antigen-specific Th1 response and dominant elevation of the Th2 response in the host. Immunomodulation protects against excessive inflammatory responses in allergies, autoimmune diseases, IBD, type 1 diabetes, RA and autoimmune encephalomyelitis.

high levels of IL-17, participate in the progression of many autoimmune diseases (Bedoya *et al.*, 2013). *Trichinella spiralis* inhibits the formation of Th17 cells, thus downregulating the expression of IL-17 in its host (Ahn *et al.*, 2016; Steel *et al.*, 2019).

Trichinella spiralis infection is characterized by a Th2 response (Saunders *et al.*, 2007). ES products released by the different life stages of *T. spiralis* are also involved in the Th2 response (Liu *et al.*, 2014; Cvetkovic *et al.*, 2016; Guo *et al.*, 2016). A study showed the Th2 response can influences the development of autoimmune responses (Cho *et al.*, 2012), so that *T. spiralis* or its products can be applied in controlling the levels of inflammation in infected tissues.

Application of *T. spiralis* or its products to modulate autoimmune diseases

Trichinella spiralis infection is characterized by a Th2 response, and this Th2 response is critical in controlling the levels of inflammation in infected tissues (Saunders *et al.*, 2007). *Trichinella spiralis* modulates the host immune system and influences the development of autoimmune responses (Cho *et al.*, 2012).

Application of *T. spiralis* or its products to modulate IBD

Trichinella spiralis infection has therapeutic potential in IBD with Th1-driven intestinal inflammation (Zhao *et al.*, 2013). *Trichinella spiralis* infection inhibits epithelial and crypt cell destruction and sustains mast cell stabilization, which prevents mucosal mast cell hyperplasia and exacerbates motor responses against intestinal inflammation (Serna *et al.*, 2006). ES products from adult *T. spiralis* (AES) alleviate IBD with acute inflammatory colonic disease in mice induced by dextran sodium sulphate, which was mediated by inhibition of Th1/Th17 proinflammatory cytokines and an increase in the production of anti-inflammatory Th2/Treg regulatory cytokines in the lymphocytes of colon, mesenteric lymph node and spleen of mice treated with AES (Yang *et al.*, 2014; Kang *et al.*, 2019). Motomura *et al.* (2009) showed

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that *T. spiralis* antigens activate Th2 and Treg cells, leading to the production of IL-13 and TGF-β, respectively, subsequently reducing Th1-mediated inflammatory responses. Therefore, *T. spiralis* and its associated antigens may have bidirectional immunomodulatory effects, and *T. spiralis* antigens may provide a novel strategy to retune the immune cell repertoire and ameliorate colonic inflammation by expanding the Treg cell population and activating the Th2 response.

Application of *T. spiralis* or its products to modulate experimental allergic airway inflammation (EAAI)

Infection with *T. spiralis* confers protection against EAAI (Kang *et al.*, 2014). *Trichinella spiralis* has an immunosuppressive effect through the inhibition of the Th1-related cytokine IFN-γ and the Th17-related cytokine IL-17, promotion of the Th2-related cytokines IL-4, IL-5 and IL-13, release of the Treg-related cytokines IL-10 and TGF-β, induction of regulatory cells and increase in allergen-specific IgE and eosinophils (Conroy & Williams, 2001; Watanabe, 2014; Moon *et al.*, 2018)

Application of *T. spiralis* or its products to modulate experimental autoimmune encephalomyelitis (EAE)

Trichinella spiralis infection is accompanied by increased production of IL-4 and IL-10, which lead to reduced levels of IFN-γ and IL-17 in EAE immune response in mice (Kuijk *et al.*, 2012; Sofronic-Milosavljevic *et al.*, 2013). The decreased production of IFN-γ and IL-17 correlates with the alleviation of EAE symptoms. In addition, the amelioration of EAE symptoms is also attributed to the upregulation of Treg cells, which is a common immune response in individuals who are infected with *T. spiralis* (Beiting *et al.*, 2004; Gruden-Movsesijan *et al.*, 2010; Kuijk *et al.*, 2012).

Application of *T. spiralis* or its products to modulate type 1 diabetes mellitus

Type 1 diabetes mellitus is a Th1 inflammatory disease. Helminth infections induce a Th2 immune response by altering the production of IL-4 (Hubner *et al.*, 2012). *Trichinella spiralis* infection reduces the development of diabetes by inducing the Th2 response, which in turn reduces the Th1-mediated destruction of beta cells (Saunders *et al.*, 2007; Wang *et al.*, 2017).

Application of *T. spiralis* or its products to modulate rheumatoid arthritis

Th17 cells play an important role in the pathogenesis of RA (Kotake *et al.*, 2017). Th17 cell activity is dampened by a variety of regulatory T cells that express the transcription factor Foxp3 (Tahmasebinia & Pourgholaminejad, 2017). Treg cells suppress effector T cells either directly via cell-to-cell contact or via the secretion of TGF-β and IL-10 (Li *et al.*, 1998). *Trichinella spiralis* antigens have anti-arthritic potential by upregulating $Foxp3^+$ Treg cells, which leads to reduced production of IL-17 and increased production of IL-10 (Eissa *et al.*, 2016; Cheng *et al.*, 2018).

Application of *T. spiralis* or its products in therapy of tumour model

Trichinella spiralis muscle larva ES proteins inhibit the proliferation of human H446 small cell lung cancer cells and induce apoptosis by activating mitochondrial apoptosis pathways (Luo *et al.*, 2017). The A200711 protein of *T. spiralis* induces apoptosis in H7402 hepatocellular carcinoma cells (Wang *et al.*, 2013). *Trichinella spiralis* infection reduces B16-F10 melanoma growth and small cell lung cancer metastasis through changes in cytokine profiles of the individuals, including CXCL9, CXCL10 and CXCL13 in mice (Kang *et al.*, 2013). The mechanism is still unknown.

Therapy using live *T. spiralis* may be difficult and unethical to patients with autoimmune diseases. Instead, molecules that are secreted by *T. spiralis* may provide an alternative strategy by generating antigen-specific immunoregulation (Abdoli, 2019; Maizels, 2020).

Molecules released by *T. spiralis*

Trichinella spiralis can survive for long periods in the host, and this is mediated by the parasite's ability to modulate the host immune response through the secretion of immunomodulatory molecules (Yang *et al.*, 2013; Liu *et al.*, 2014; Sun *et al.*, 2015; Tang *et al.*, 2015; Cvetkovic *et al.*, 2016; Guo *et al.*, 2016) (table 2). ES products released by the different life stages of *T. spiralis* are involved in acute inflammatory responses and modulate molecular cross-talk between the parasite and host (Sun *et al.*, 2011; Kang *et al.*, 2019).

It is important to identify and characterize *T. spiralis* immunomodulatory molecules using genomic, proteomic and molecular methods, as they may provide a promising strategy to control and prevent parasite infection, as well as serve as novel immunomodulatory therapeutic drugs for immunopathological diseases (Bai *et al.*, 2012; Cvetkovic *et al.*, 2014).

L1 products (*T. spiralis* 53 kDa glycoprotein Tsp53) found in *T. spiralis* induce the proliferation of naïve T cells and their polarization towards Th2 and anti-inflammatory responses. Tsp53 induces the production of IL-4 and IL-10 in T cells (Du *et al.*, 2011; Cvetkovic *et al.*, 2016; Nascimento Santos *et al.*, 2017); therefore, Tsp53 is an important anti-inflammatory molecule. Recombinant Tsp53 (rTsP53) downregulates the proinflammatory cytokines TNF-α, IL-6, IL-12 and iNOS and elevates production of the regulatory factors IL-10 and TGF-β1 by inducing the transition of macrophages from M1 to M2 (Chen *et al.*, 2016b). rTsP53 has anti-inflammatory properties in mice by rescuing rTsP53-treated mice from 2,4,6-trinitrobenzene sulphonic acid-induced IBD (Du *et al.*, 2011). Thus, rTsP53 has therapeutic potential for inflammatory disorders. *Trichinella spiralis* cystatin-like protein (Ts-CLP) induces the production of IL-10. IL-10 inhibits TNF-α production, which is a key feature in IBD pathogenesis; therefore, parasitic cystatins may be potential candidates for IBD therapy (Liu *et al.*, 2014; Tang *et al.*, 2015). *Trichinella spiralis* paramyosin (Ts-Pmy) modulates the host immune system by stimulating DCs to differentiate $CD4^+$ T cells into regulatory T cells (Sun *et al.*, 2015; Guo *et al.*, 2016). Ts87, a novel Ts87 gene from the cDNA library of adult *T. spiralis*, balances the Th1/Th2 response and downregulates IL-17 expression (Yang *et al.*, 2013). Further research is required on how Ts-Pmy and Ts87 may be applied to treat inflammatory diseases. As new identified molecular from *T. spiralis*, *T. spiralis* calreticulin (Ts-CRT) can bind to host complement C1q, which not only reduces C1q-mediated activation of classical complement pathway but also inhibits the C1q-induced non-complement activation of macrophages (Zhao *et al.*, 2017). *Trichinella spiralis* novel cystatin (TsCstN) elicits an anti-inflammatory property by suppressing proinflammatory cytokines and interfering with the antigen presentation process through depletion of MHC class II

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Table 2. Molecules released by *T. spiralis*.

Protein	Function	Molecular weight	GenBank accession number	Reference
T. spiralis 53 kDa glycoprotein (Tsp53)	Caused partial maturation of DCs, regulated Th1/Th2 balance	53 kDa	U25127	Cvetkovic *et al.* (2016)
T. spiralis cystatin-like protein (Ts-CLP)	Induced a Th1/Th2-mixed type of immune response and decreased STAT6 transcription	~46 kDa	EU263325.1	Tang *et al.* (2015) and Liu *et al.* (2014)
T. spiralis Paramyosin （Ts-Pmy）	Differentiation of regulatory T cells, interfering with complement activation	102 kDa	EF429310.1	Sun *et al.* (2015) and Guo *et al.* (2016)
Ts87 glycoprotein	Stimulated Th1/Th2 systemic immune response	38 kDa	AF399957	Yang *et al.* (2013)
T. spiralis calreticulin (Ts-CRT)	reducing C1q-mediated activation of classical complement pathway and inhibiting the C1q-induced non-complement activation of macrophages	45.5 kDa	XM_003371331.1	Zhao *et al.* (2017)
T. spiralis novel cystatin (TsCstN)	suppressing proinflammatory cytokines and interfering with the antigen presentation process through depletion of MHC class II expression	13.2 kDa	XP_003369399.1	Kobpornchai *et al.* (2020)
T. spiralis glutathione-S-transferase (TsGST)	as an immune-modulator participates in regulating maturation and function of dendritic cells (DCs) and induces the Th2-type immune response.	30.8 kDa	XM_003373603	Jin *et al.* (2019) and Yang *et al.* (2020a)

expression (Kobpornchai *et al.*, 2020). As an immune-modulator, *T. spiralis* glutathione-S-transferase (TsGST) participates in regulating maturation and function of DCs and induces the Th2-type immune response of its host (Jin *et al.*, 2019; Yang *et al.*, 2020a). The three molecules from *T. spiralis* might be a potential molecular target for antiinflammation.

In addition to the above-mentioned protein molecules, it has recently been discovered that extracellular vesicles (EVs) also have immunomodulatory effects. EVs are small membrane-bound structures secreted by helminths which mediate immune evasion during parasite infection (Yang *et al.*, 2020b). EVs produced by *T. spiralis* muscle larvae can carry immunomodulatory proteins and have the capacity independently to induce regulatory responses (Kosanović *et al.*, 2019).

In the *in vivo* application of *T. spiralis* immunomodulatory molecules, the induction of Th2 and regulatory cytokines is important for creating anti-inflammatory responses. The recombinant antigens with high-quality *T. spiralis* glycans which affect the anti-inflammatory environment and can interfere with the development of inflammatory diseases will instead of native antigen from ES of adult *T. spiralis* or muscle larvae and make is a generalized and cheap therapeutics for inflammatory disorders. A better understanding of the functions of *T. spiralis* immunomodulatory molecules will be important for developing new therapeutics for inflammatory disorders, including allergies and autoimmune diseases (Cvetkovic *et al.*, 2014; Kahl *et al.*, 2018).

Prospective applications of *T. spiralis*-derived compounds

The relationship between humans and *T. spiralis* can be considered mutualistic rather than typical parasitism (Szkudlapski *et al.*, 2014). Live helminth therapy is encouraged to maximize the immune efficiency of helminths; however, it may be difficult and unethical to suggest helminth therapy to patients. Instead, generating antigen-specific immunoregulation through molecules that are secreted by *T. spiralis* may provide an alternative strategy (Sofronic-Milosavljevic *et al.*, 2015; Smallwood *et al.*, 2017; Abdoli, 2019; Maizels, 2020). For example, rTsP53, Ts87, Ts-CLP and Ts-Pmy can serve as prospective immunotherapeutic for inflammatory disorders. These *T. spiralis*-derived compounds would form an ideal basis for therapeutic application in the treatment of autoimmune diseases, including allergies, Crohn's colitis, ulcerative colitis, type 1 diabetes and autoimmune encephalomyelitis.

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