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Review

Signal Transducers and Activators of Transcription (STAT) Family Members in Helminth Infections

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Abstract

Helminth parasites are a diverse group of multicellular organisms. Despite their heterogeneity, helminths share many common characteristics, such as the modulation of the immune system of their hosts towards a permissive state that favors their development. They induce strong Th2-like responses with high levels of IL-4, IL-5 and IL-13 cytokines, and decreased production of proinflammatory cytokines such as IFN-γ. IL-4, IFN-γ and other cytokines bind with their specific cytokine receptors to trigger an immediate signaling pathway in which different tyrosine kinases (e.g. Janus kinases) are involved. Furthermore, a seven-member family of transcription factors named Signal Transducers and Activators of Transcription (STAT) that initiate the transcriptional activation of different genes are also involved and regulate downstream the JAK/STAT signaling pathway. However, how helminths avoid and modulate immune responses remains unclear; moreover, information concerning STAT-mediated immune regulation during helminth infections is scarce. Here, we review the research on mice deficient in STAT molecules, highlighting the importance of the JAK/STAT signaling pathway in regulating susceptibility and/or resistance in these infections.

Key words: STAT, helminths, *Taenia crassiceps*, alternatively activated macrophages, filariasis, schistosomiasis.

Introduction

Helminth parasites are a very diverse group of multicellular organisms, including both flat worms like *Taenia sp.,* and cylindrical worms like *Ascaris sp*. Although they exhibit different mechanisms of infection and distinct sites at which they lodge in the host, they share some common features such as the immune modulation induced by the host immune system that triggers a permissive state for their development [1]. Helminth parasites possess sophisticated and efficient mechanisms to regulate the immune response to survive inside their hosts. A distinctive characteristic in these infections is that they induce the skewing of the immune response toward a Th2 response with high concentrations of interleukin (IL)-4, IL-13, IL-5 and IgE, IgG1 and IgG4 isotype antibodies that commonly are related to protective effects [2]. On the other hand, a hypo-responsive state is also consistently observed in lymphocytes, mainly by a reduced proliferative response to parasites, non-related antigens, or polyclonal stimuli [3]. In addition to Th2 polarization, the recruitment of immunomodulatory cell populations, such as T regulatory cells (Tregs) and alternatively activated macrophages (AAMΦs), has also been recently described in different helminthic infections, and these cells may be dependent on such signals. The role of this group of cells depends on the

particular type of helminth. For example, in *Nippostrogylus brasiliensis* infection, AAMΦs actively participate in parasite clearance, while in *Taenia crassiceps* infection; AAMΦs favor parasite persistence [4, 5]. Interestingly, experimental infections with *Brugia malayi*, *Schistosoma mansoni* or *Litomosoides sigmodontis* induce an increased and rapid recruitment of $CD4^+CD25^+Foxp3^+$ Tregs [6, 7, 8], which can be beneficial for both, the parasite and the host. It is clear that the strong regulatory mechanisms developed by helminths are necessary either to successfully colonize their hosts or to complete their life cycle while minimizing damage to the host. However, the mechanisms used by helminth parasites to achieve modulation of the host immune response have not been clarified.

During helminth infection, high levels of different types of cytokines are secreted. Cytokines initiate signaling when they bind to their specific receptors, inducing several important conformational changes mainly oligomerization or multimerization of their receptors. These changes are followed by activation of several downstream signaling molecules including those named JAK (Janus Kinases). JAKs signaling molecules are tyrosine kinases, found constitutively associated with each receptor chain [9]. Once the JAKs are activated, they autophosphorylate and transphosphorylate the receptor tyrosine intracellular motifs, which serve as recruiting sites for the SH2 domain of STATs.

Upon activation, the STATs are phosphorylated and dimerize through their SH2 domain to form homo- or heterodimers. These dimers translocate to the nucleus where they bind to the promoter region of genes via specific DNA binding domains and thus bring about the transcription of their respective genes. Here, we discuss the signature cytokines and complex STAT-mediated signaling networks involved in regulating the host response and determining disease outcome during various helminth infections.

The JAK/STAT families

JAK/STAT families are two groups of proteins that, constitute diverse signaling pathways [10] involved in cytokine signaling. STAT family members act as transcriptional factors, activated upstream by JAK proteins. Furthermore, STAT proteins are phosphorylated on tyrosine residues by JAKs. The STAT family is composed of seven proteins (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6), while the JAK family is composed of four proteins (JAK1, JAK2, JAK3 and TyK2). All of these proteins are constitutively present in the cytoplasm without previous stimuli. These two large groups of molecules represent a signaling pathway that can be quickly activated from the cellular membrane to the nucleus.

JAK protein family

These proteins are called Janus kinases because of the homology of the kinase and pseudokinase domains with Janus, the Roman god of two faces [11]. The first JAK identified was Tyk2 by Krolewski in 1990 [12], using libraries of complementary DNA from human T lymphocytes, while JAK1, JAK2 and JAK3 where identified through conserved motif clonation of the catalytic domain [11, 13, 14]. JAK proteins are molecules structurally composed of seven regions (JH1- JH7). The JH1 domain bears the kinase activity (Fig.1), while JH2 is homologous to JH1 but without the residues required for kinase activity (pseudokinase domain), so this domain is usually associated with regulatory functions [15].

JAK proteins interact with different intracellular domains of cytokine receptors and are present in a variety of cell subtypes. Their participation in mutated cell lines resistant to IFN-α stimuli was observed, and signaling in those cells was restored when different JAK proteins were transfected. Thus it was shown that IFN-α signaling requires JAK1 and Tyk2, while IFN-γ signaling requires JAK1 and JAK2 [16. 17, 18]. After that discovery, the participation of other JAK proteins in cytokine signaling was described (Table 1).

As shown in Table 1, although JAKs participate in multiple signaling pathways, their importance in modulating immune responses is evident. Therefore, their function is mainly in cytokine signaling.

Table 1. JAK Family

	Cytokine or factor
JAK1	IL-2, IL-7, IL-9, IL-15, IL-4, IL-13, IL-6, IL-11, IFN-α, IFN-β, IFN-γ, IL-10, CT-1
JAK2	IL-3, IL-12, IL-13, IL-6, IL-11, IFN-γ, CT-1, Growth hormone, Prolactin, Eritropoyetin
JAK3	IL-2, IL-7, IL-9, IL-15, IL-4
TyK2	IL-6, IL-11, IL-12, IL-13, CT-1, IFN-α, IFN-β, IL-10

STAT family

The STAT proteins are a family of transcription factors composed of 7 members. In 1994, Darnell and colleagues identified the first two members of the family, STAT1 and 2, by purification of factors linked to IFN stimulated genes; the other family members were described subsequently and are STAT3, STAT4, STAT5a, STAT5b and STAT6 [19, 20, 21, 22, 23]. These

proteins act as transcription factors when they form homo- and heterodimers among them. Dimerization is possible once STATs have been phosphorylated at tyrosine residues in their SH2 domain (Fig.1). STATs are the only family of transcription factors that contain SH2 domains for phosphotyrosines that serve mainly for both binding to the activated cytokine receptor and activating STATs through tyrosine phosphorylation [24]. STAT proteins together with JAKs are involved in many cytokine signaling pathways (Table 2).

Table 2. STAT Family

	Cytokine or factor
STAT1	IL-2, IL-6, IL-10, IFN-α, IFN-β, IFN-γ, IL-27
STAT2	IFN-α, IFN-β
STAT3	LIF, IL-10, IL-6, IL-27, Growth hormone
STAT4	IL-12
STAT5a/b	Prolactin, Growth hormone, Thrombopoietin
STAT6	IL-4, IL-13

The Janus kinase/signal transducers and activators for transcription (JAK/STAT) pathway regulate a large plethora of biological processes including cell proliferation, differentiation, cell migration and apoptosis [25]. Ligand binding induces an intracellular activation through the multidimerization of different cytokine receptors units [25], followed by JAKs recruitment to the cytoplasmatic domain of the cytokine receptor. JAK activation occurs when a tyrosine residue in the cytokine receptor is phosphorylated, creating a docking site for cytoplasmic STAT. While STAT proteins are attached to the cytokine receptor, JAK proteins phosphorylate them at a tyrosine residue, detaching the STAT protein from the cytokine receptor so that the STATs form homo- and heterodimers that will translocate to the nucleus and bind DNA sequences to promote gene expression (Fig. 2).

An important characteristic observed in diverse helminthic infections is the participation of some members of the STAT protein family and their associated tyrosine kinases family (JAK kinases) in the regulation of susceptibility and resistance to the infection. Several reports have shown that STAT proteins participate in diverse cytokines signaling pathways, such as IL-4 and IL-13, particularly involving STAT6, responsible for triggering the Th2 immune responses at the transcriptional level, which is a feature of helminth infections.

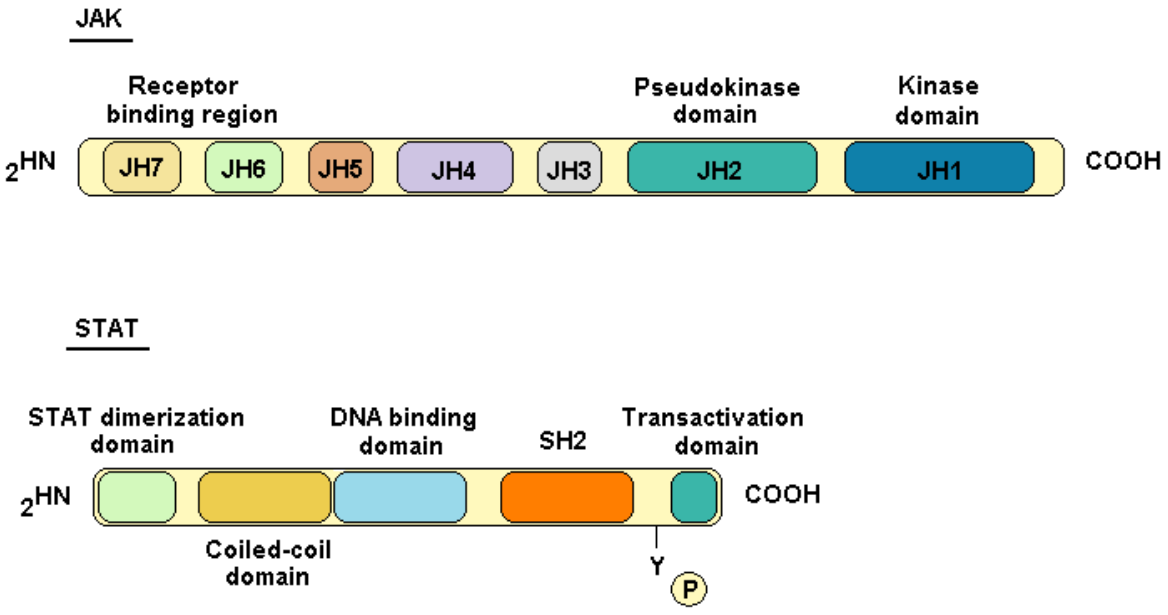

Figure 1. JAK and STAT protein structures. JAK proteins feature 7 domains; they owe their name due to the homology of JH1 and JH2 to the two faced god Janus. The difference between both domains is that JH2 lacks some residues implicated in kinase activity. STAT proteins are transcription factors that feature five conserved domains.

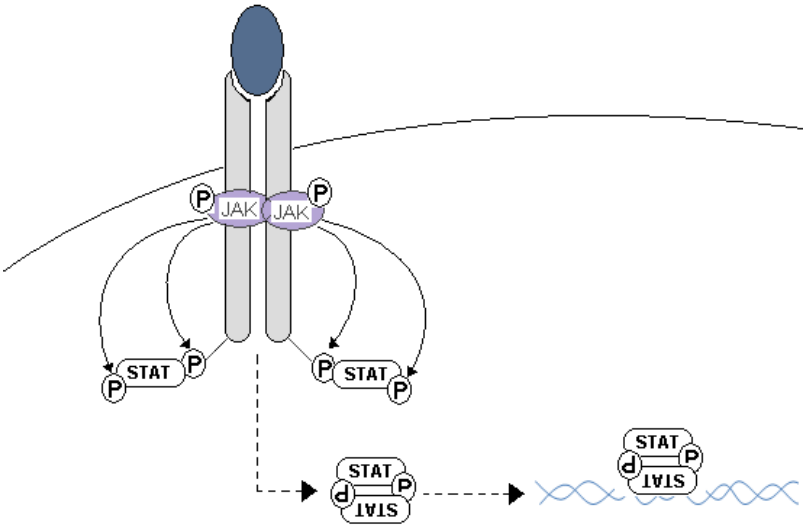

Figure 2. JAK/STAT signaling pathway. Cytokine binds with the target receptor; this causes dimerization of receptor chains and the juxtaposition of JAKs, and their reciprocal phosphorylation. Once activated, JAKs phosphorylate the intra-cytoplasmic regions of the cytokine receptor; creating a docking site for cytoplasmatic STATs that once joined with the receptor are activated by JAK phosphorylation in a tyrosine residue. STATs dimers act as transcriptional factors recognizing specific DNA sequences.

IFN-γ is a cytokine affected by helminth infections. One of the most important functions of this cytokine is classic activation of macrophages, and it is also known that chronic infections with this kind of parasite preferentially induce an alternative state of activation in this cell lineage. It has been shown that AAMΦs are induced by stimulation with IL-4, and they do not have the faculty to adequately respond to IFN-γ [26, 27]. This may be the key to the relationship between helminth infections and the JAK/STAT proteins because is STAT1 precisely the transcription factor involved in the IFN-γ signaling pathway.

JAK/STAT in helminth infections

Large amount of information has demonstrated that the JAK/STAT pathway is involved in helminth infections, suggesting that family members of these families might participate in mediating susceptibility or resistance to different helminth infections.

Helminth parasites are a heterogeneous group with common characteristics, such as host modulation of the immune response to generate a permissive environment that favors infection and induction of the development and polarization of the immune response towards a Th2 dominant profile. It is known that development of Th2 immune responses is IL-4-dependent, while the Th1 immune responses depend mainly on IL-12 and IFN-γ [28]. These important cytokines exert their biological effects signaling through the JAK/STAT pathway. However, the mechanisms involved in helminth immune modulation remain unclear, but there is evidence that indicates the importance of the presence or absence of some JAK/STAT proteins to modify the resistance or susceptibility to these parasite infections

STAT1 and helminths

STAT1 is an important player in IFN-γ signaling. It has been demonstrated that IFN-γ is a very important cytokine in the development and regulation of Th1-type immune response and inflammatory responses. Whereas IFN-γ plays a critical role in protecting animals against viral, bacterial, and protozoan pathogens, its role on many helminthic infections is practically unknown. Moreover, it was previously thought that Th1-mediated immune responses did not participate as effector responses against helminth infections. However, several new evidences show that immune response against these parasites is a very complex event. Effectors' mechanisms against helminths are not exclusively limited to Th2-type responses, but Th1-type responses may participate and develop effector mechanisms depending on diverse factors such as the type of helminth infection, site where the helminth is established (intestinal lumen, liver, muscle, lung or brain), and the life cycle stage of the parasite (eggs, larvae or adult).

In contrast to other STAT family proteins, very few reports exist regarding how STAT1 participates in helminth infections. Schistosomiasis is a parasitic disease caused by trematode flatworms of the genus *Schistosoma*, and in particular *Schistosoma mansoni*, affecting both human and cattle. The immune response against this parasite is initiated by soluble egg antigens (SEA) derived by laying eggs in the lung and liver. During the development of the infection, a significant decrease of Th1-type cytokine (IL-12, IFN-γ and TNF-α) expression has been observed followed by a clear augmentation in the production of regulatory and Th2-type cytokine (TGF-β, IL-10, IL-4) ex-

pression in splenocytes, protecting host-pathology and control etiology of sickness [29].

Due to the fact that STAT1 is not only involved in the Th1 response but also in regulating cytokine signaling (i.e., IL-10), Goh and colleagues [30] analyzed the effect of SEA in activating different signaling pathways that could induce Th2 immune responses. Interestingly, among the molecules activated by SEA, this group observed that p38 and Erk 1/2 signaling pathways were activated but STAT1 was not.

In murine schistosomiasis, IFN-γ and the Th1 response can protect against severe fibrosis by preventing AAMΦ activation and thereby limiting the fibrosis by enhancing effects of the Th2 response [31]. However, in humans Mwatha and colleagues [32] reported Th1-type immune responses in individuals with severe hepatosplenomegaly, whereas individuals infected but with less-severe hepatosplenomegaly mounted Th2-type responses. Thus, STATs controlling Th1 or Th2 responses may be involved in regulating the balance between pathology and elimination of this helminth infection.

In contrast to the dearth of knowledge regarding participation of STAT1 in helminth infections, there is evidence of its importance in protozoan infections. When C57BL/6 mice that mount a strong Th1 response against *Leishmania major* infection lack STAT1, they became highly susceptible to this infection [33]. In some helminth infections such as filariasis, cysticercosis and echinococcosis, the early immune response is Th1-mediated, which sometimes correlates with rapid control of the infection or maintenance of low parasite burdens. However, this response is mainly transient, and it rapidly shifts towards Th2 immune responses that may favor the establishment of the helminth. Keeping in mind that STAT1 is key molecule in Th1-type immune response development, this suggests that a rigorous study on STAT1 participation in helminth infections is clearly missing in the literature of the immunoparasitology field, therefore we cannot rule out a possible role for STAT1 in regulating control or pathology of helminth infections.

A role for STAT4 during helminth infections

The transcription factor STAT4 participates in the IL-12 signaling pathway. IL-12 functions as the main physiological inducer of IFN-γ by activated T cells and promotes Th1-type $CD4^+$T cell differentiation and therefore may play an important role in response to helminth infections. Thus, STAT4 and STAT1 both induce and sustain a Th1 immune response, and as described below, this response has been implicated in resistance against some helminths among them *Taenia crassiceps* infection [34, 35, 36].

IL-12 signaling leads to activation of STAT4 by JAK2 and Tyk2 [37, 38]. STAT4 dimers translocate into the nucleus and bind DNA sequences at the IFN-γ activation site (GAS). In T cells and NK cells, the main action of IL-12 is the induction of IFN-γ [39]. By this pathway, STAT4 mediates most of the pro-inflammatory activities of IL-12 and is critical for Th1 differentiation and directing the cell-mediated immune response [40, 41]. Rodriguez-Sosa and colleagues evaluated the role of STAT4 in experimental cysticercosis [34]. Surprisingly they found an unexpected participation of STAT4 in mediating resistance against this helminth. In this study, the authors observed that while wild type (WT) C57BL/6 mice were highly resistant to *T. crassiceps* infection, this group found that C57BL/6 mice lacking STAT4 ($STAT4^{-/-}$) were highly susceptible to *T. crassiceps* infection. It is worth noting that in cysticercosis caused by *T. crassiceps*, there are sex-associated differences in susceptibility, where males are more resistant, and females show more susceptibility to this infection [42]. Although C57BL/6 is a resistant strain of mouse, these differences in susceptibility became evident in $STAT4^{-/-}$ mice, where both males and females became highly susceptible, but females still presented a greater parasite burden. In addition to the loss of resistance to this infection in $STAT4^{-/-}$ mice, the Th1 immune response was also critically affected; they found a decrease in IgG2a specific antibodies, while there was an increase in IgG1 specific antibodies titers. The absence of STAT4 in this infection also affected splenocyte proliferation to specific antigens where they found an important decrease that was more evident as infection progressed. As expected, the absence of STAT4 decreased the levels of IFN-γ from CD4+ enriched cells, whereas the levels of IL-13 and IL-10 were significantly increased.

Another case in which STAT4 appears to be involved is in the effect of vaccination on experimental schistosomiasis. Wynn et al [43] demonstrated that IL-12 enhances vaccine-induced immunity to schistosomes by augmenting both humoral and cell-mediated immune responses, and because IL-12 biological activity is STAT4-dependent, cysticercosis caused by *T. crassiceps* is not the only helminth infection related to STAT4 and protection. Interestingly, this protein has been also involved in anti-filarial responses. Those parasites are typical of tropical environments and are capable of avoiding immune response to facilitate their permanence in their host [44, 45]. Like other nematodes, filarias induce a Th2 response in their host [46]. Recent studies trying to un-

cover how filarias avoid immune response have demonstrated that these parasites are capable of ontogenic transformations of surface epitope expression [47, 48]. Besides surface antigens, Muthian and colleagues [35] demonstrated another immune evasion mechanism related to secreted lipids from the filarial parasite *Setaria digitata*, named, secreted filarial lipids (SFL). This group observed that proliferation of T cells induced by myelin antigens was decreased when co-stimulated with SFL, as well as IL-12 and IFN-γ production. Given that IFN-γ production is related to STAT4 activation, they analyzed the effect of SFL on STAT4 phosphorylation in IL-12-stimulated T cells; they observed that SFL decreased STAT4 activation in a dose-dependent manner. This same phenomenon was observed in the proteins responsible for STAT4 phosphorylation in response to IL-12, JAK2 and TyK2. Thus, the infection with *S. digitata* inhibits Th1 response by secreting lipid antigens that inhibit STAT4 activation favoring polarization toward a Th2 immune response. This suggests an important immune evasion mechanism where helminth-derived products drive host immune response toward a Th2 profile that, in some infections, works as an effector mechanism to remove parasites, but in other cases works as an evasion mechanism that favors establishment of the infection. Surprisingly, STAT4 has been recently implicated in Th2 immune response development; Syrbe et al [36] observed that although they employ *Nippostrongylus brasiliensis,* an infection model that triggers Th2 immune response, when mice lacking STAT4 were infected there was a decrease in the number of cells producing IL-4 . Thereby it is possible that STAT4 participates in Th2 immune response development, at least during *N. brasiliensis* infection.

The Role of STAT6 in resistance to helminth infections

It is generally recognized that IL-4 and IL-13-producing $CD4^+$ Th2 cells and eosinophils play an important role in inhibiting and killing helminth parasites and impeding helminth establishment and growth, as signature molecules associated with Th2-type immune responses are the predominant cytokines present either in circulation or at the site of infection during helminth parasitic infections [2]. These infections can be related to STAT6 because this transcription factor is a key molecule involved in IL-4 and IL-13 cytokine signaling [49]. The IL-4R and IL-13R share a common receptor chain involved in signal transduction. Both IL-4 and IL-13 are activators of STAT6, which mediates most biological activities of these cytokines. $STAT6^{-/-}$ mice display impaired Th2 differentiation and lose responsiveness to IL-4 and IL-13, but these animals are capable to maintain normal responses to other cytokine signals. The importance of IL-4, IL-13 and STAT6 in mediating resistance during helminth infection was clearly demonstrated in experimental models of infection with the gastrointestinal parasite *Trichinella spiralis*. It has been observed that while IFN-γ deficiency accelerates infection clearance, mice with IL-4Rα deficiency did not achieve worm expulsion, nor did $STAT6^{-/-}$ mice. The $STAT6^{-/-}$ mice also displayed an increase in parasite burden and significant changes in cytokine profile production, evidenced by a decrease in IL-4 and IL-13 and increase in IFN-γ production eleven days post infection. Moreover, it was observed that STAT6 was participating through mast cell-dependent mechanisms [50]. Thus, STAT6 and Th2 immune response are important in *T. spiralis* clearance.

Besides mast cell participation in *T. spiralis* expulsion, other important cell population that has been implicated in this process is the goblet cells, whose participation is STAT6-dependent, suggested by Khan and colleagues in 2001 [51]. This scientific group worked with $STAT6^{-/-}$ mice and observed that these mice lost the resistance to infection comparing with WT mice, as demonstrated by higher parasite burden. Furthermore, it was also observed that the production of IL-4 and IL-13 was decreased 14 days post infection associated with a significant decrease in goblet cell numbers. In addition, the decrease in goblet cell production is important in the context that *T. spiralis* infection is characterized by an induced goblet cell hyperplasia necessary for worm expulsion [52, 53].

T. spiralis clearance has also been shown to be related to intestinal muscle hypercontractility. Mice strains that show a quick clearance of this worm also show greater gut muscle hypercontractility [54]. This hypercontractility depends on both $CD4^+$ and $MHC\text{-}II^+$ T cells [55], although mechanisms involved in these events remain unclear. Thus, trying to determine possible mechanisms, in 2001 Khan and colleagues [56] analyzed the participation of IL-4 and STAT6 in hypercontractility development and its relationship with parasite expulsion. They observed that *T. spiralis* infection induced an increase in IL-4 and IL-6 cytokine production in mesenteric lymph node and spleen cell culture supernatants stimulated with ConA. However, this increase was not observed in $STAT6^{-/-}$ mice. Together with these results, they found a marked attenuation of carbachol-induced muscle contraction in $STAT6^{-/-}$ mice but only a slight decrease in muscle hypercontractility in $IL\text{-}4^{-/-}$ mice. Parasite burden was delayed in $STAT6^{-/-}$ mice, but was almost normal in mice lacking IL-4. Thus, the

absence of STAT6 is important in gut hypercontractility that can be related to the inefficiency of parasite expulsion. However, this seems to be an IL-4 independent event, so perhaps other cytokines such as IL-13 or IL-9 may be responsible for *T. spiralis* gastrointestinal infection clearance.

However, *T. spiralis* infection is not strictly restricted to the gastrointestinal site, but the larval stage of this parasite rapidly can get into the blood stream and disseminate in the host. In order to investigate the influence of Th2 cytokines during muscle infection with *T. spiralis*, Beiting et al [57] infected STAT6$^{-/-}$ mice. These mice were in a BALB/c background, which would be expected to mount a strong Th2 response. Tissue muscle recovered from BALB/c mice at 18 dpi contained numerous mature larvae and fully developed nurse cells surrounded by focal infiltrates. In contrast, nurse cells from STAT6$^{-/-}$-infected mice had markedly reduced inflammatory infiltrates around infected cells and reduced numbers of lymph node cells. When stimulated with larval antigens, lymph node cells from BALB/c mice produced significantly more IL-4, IL-5, and IL-13, but less IFN-γ, compared with their STAT6$^{-/-}$ counterparts, whereas IL-10 concentrations were comparable in BALB/c and STAT6$^{-/-}$ mice. Interestingly, despite impairment of Th2 responses and inflammation in STAT6$^{-/-}$ mice, larval burdens were similar to those in BALB/c mice. These data highlights a more important role for STAT6 in eliminating gastrointestinal worms than in tissue-dwelling helminths.

Another helminth parasite infection in which STAT6 appears to be involved is the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. The third-stage larvae of this parasite infect mice through the skin, migrate into the lungs, are coughed up, ingested, travel to gut lumen were mature adults reside, produce eggs that are excreted in the feces, and are themselves expelled approximately 10 days after the initiation of infection [58]. In 1988, Katona and colleagues [59] described that even when Th2 responses are necessary for parasite expulsion, IL-4 is not, while treatment of infected mice with IL-12 blocks worm expulsion [60]. Although those results suggest that the primary mechanism of host protection against *N. brasiliensis* might be production of a Th2 immune response, it seems to be IL-4-independent but may be related to other molecules involved in Th2 polarization, like IL-4 cytokine receptor IL-4Rα or STAT6.

Urban and colleagues [4] analyzed whether *N. brasiliensis* expulsion was affected in mice lacking IL-4Rα or STAT6. They found that both deficient animals failed to expel *N. brasiliensis* and presented elevated egg burdens, whereas WT mice did not. These results were confirmed with the administration of anti-IL-4Rα to WT mice, which lost resistance evidenced by the presence of egg and parasite burden. They also found antibody isotype changes, as WT mice produced IgG1 whereas IgG2a, characteristic in Th1 responses, was not detected. On the other hand, STAT6$^{-/-}$ mice produced greater levels of both IgG1 and IgG2a antibodies during chronic infection. The fact that IL-4 is not the only cytokine that uses IL-4Rα for signaling suggests analysis of the participation of IL-13, which also signals through IL-4Rα, might be appropriate. Administration of soluble IL-13Rα2-Fc, a fusion protein that neutralizes IL-13, in WT mice infected with *N. brasiliensis*, demonstrated that these mice became susceptible to infection. So, protection against *N. brasiliensis* depends mainly on STAT6 but not on IL-4, and STAT6 might be activated through IL-13 signaling, a cytokine related to Th2 immune response in addition to IL-4. This exhibits the importance of STAT6 in protection against gastrointestinal helminth infections.

Another enteric helminth related to this transcription factor is the cestode *Hymenolepis diminuta*. In 2003, McKay and Khan [61] described the importance of STAT6 in this infection by analyzing the participation of IL-4, IL-13 and STAT6 in worm expulsion. They found that adult worms were harbored in the gut only in STAT6$^{-/-}$ mice, and that both IL-4 and IL-13 deficient mice were able to expel *H. diminuta*. Another important finding was the reduced goblet cell numbers found in STAT6$^{-/-}$ mice that was related to susceptibility to infection.

Brugia malayi is a human-infecting tissue-dwelling nematode that induces a Th2 response that also seems to be beneficial in rodent hosts. In the mouse model of infection, immunocompetent mice are able to clear the parasites [62]. In 2001, Spencer and colleagues described that IL-4 is required for clearance of *Brugia* from BALB/c [63] and C57BL/6 mice strains. This was evidenced in IL-4 and STAT6$^{-/-}$ mice, which still harbored live worms 6 weeks post-infection in BALB/c mice and two and four weeks post-infection in C57BL/6 mice, contrary to WT mice in both strains. Therefore, IL-4 favors parasite clearance via the STAT6 signaling pathway in filariasis.

Another helminth infection is murine cysticercosis caused by the helminth parasite *Taenia crassiceps*, which induces a strong Th2-like response. Interestingly, *T. crassiceps*-infected mice develop a transient Th1-like response during the first weeks of infection, but later they develop a dominant Th2 response in chronic phases of infection (>4 weeks) that is associated with an increase in parasite loads [64]. Trying to

confirm that development of Th2 immune response correlates with susceptibility to this infection, Rodriguez-Sosa and colleagues [65] analyzed the role of a Th2-type response induced via STAT6-mediated signaling in BALB/c mice during *T. crassiceps* infection. They found that even when BALB/c mice were susceptible to infection and carried approximately 400 intraperitoneal metacestodes at 12 weeks after infection, STAT6$^{-/-}$ mice resolved the infection as early as 4 weeks post-infection. STAT6$^{-/-}$ mice, together with resistance to infection, developed a dominant Th1-type response with high levels of anti-*T. crassiceps*-specific IgG2a antibodies, and lower levels of specific IgG1 and total IgE antibodies compared to WT mice; they also produced higher levels of IFN-γ and lower levels of IL-4 and IL-13 in the course of infection. Consistent with this, macrophages from STAT6$^{-/-}$ *T. crassiceps*-infected mice displayed an inflammatory phenotype with high IL-12 and NO production compared to macrophages from WT mice which displayed an alternative activation phenotype, with inhibited IL-12 production and NO and high expression of Arginase-1, PD-L2 and IL-4Rα [66]. Together, these results show that murine cysticercosis requires a Th1 immune response development to clear infection, while Th2 immune response mediated by STAT6 is related to susceptibility to this extra-intestinal infection.

As previously described, while STAT6-mediated Th2 immune response is not always related to helminth resistance, it may be dependent on several factors, such as the site of infection, type of parasite and chronicity of the infection, among others. Thus, recently Mishra et al. [67] analyzed the development of immunopathology in a murine model of neurocysticercosis caused by *Mesocestoides corti* in the absence of STAT6. They found that WT BALB/c mice, as seen with *T. crassiceps* infection [26, 68, 69, 70], induced expression of markers associated with alternatively activated macrophages such as Ym1, Fizz1, Arg-1, MR1 and MGL 1/2 in the brains of infected mice. Interestingly, STAT6$^{-/-}$ mice showed a down-regulation in the expression of AAMΦ associated molecules compared with WT mice. As reported previously by Rodriguez-Sosa et al [71], STAT6 deficient mice with cysticercosis displayed also increased iNOS expression. However, these mice displayed enhanced susceptibility to *M. corti* and greater neuropathology (abnormal vestibular function, tilted head, walking in circles, and morbidity). Thus, it appears that in this model STAT6 may participate by controlling excessive pathology (inflammation) through the development of AAMΦs, which have been proven to have suppressive activity mainly mediated by the Death-ligand pathway [66]. Furthermore, AAMΦs may participate by healing central nervous system damage during neurocysticercosis, as one the newest functions associated with AAMΦs during helminth infections [72]. Interestingly, the development of AAMΦs and the expression of PD-L2 in such cells have been recently confirmed to be STAT6-dependent [73], suggesting that an anti-inflammatory and healing processes during helminth infections may be also associated with STAT6 function.

Table 3. STATs in helminth infections

	Parasite	Effect	References
STAT1	*Schistosoma mansoni*	Soluble egg antigens (SEA) do not induce STAT1 phosphorylation in macrophages but induce ERK and p38 activation.	[30]
STAT4	*Taenia crassiceps*	Genetically resistant C57BL/6 mice became susceptible when lacking STAT4 gene.	[34]
	Setaria digitata	Secreted filarial lipids (SFL) down-regulate Th1 immune responses by decreasing STAT4 phosphorylation in a dose-dependent manner.	[35]
	Nippostrongylus brasiliensis	Decrease in frequency of IL-4 producing cells in infected STAT4$^{-/-}$mice.	[36]
STAT6	*Nippostrongylus brasiliensis*	IL4-Rα and STAT6 are necessaries for worm expulsion; IL-13 is decisive for clearance.	[4]
	Trichinella spiralis	Absence of STAT6 is related to higher parasite burden and lower Th2 cytokines.	[50]
		STAT6 and Th2 cytokines seems to be necessary for goblet cell hyperplasia.	[51]
		Gut muscle hyper-contractility necessary for worm expulsion depends on STAT6 signaling.	[56]
	Hymenolepis diminuta	Resistance is mediated by STAT6 but do not depend on IL-4 or IL-13 alone. The absence of STAT6 down regulates goblet cells.	[61]
	Brugia malayi	IL-4 and STAT6 are required for clearance from BALB/c and C57BL/6 mice.	[63]
	Taenia crassiceps	STAT6 deficient mice were resistant to infection with an intense Th1 response.	[65]
	Mesocestoides corti	In murine neurocysticercosis STAT6 deficient mice succumbed faster given an intense inflammatory response	[67]

Conclusion

The pathology and resolution of different helminth infections is dependent to a large extent on the infecting species, the model used, and the site of infection. STATs molecules do have a role in all cases either by initiating the development of a Th1 response as in schistosomiasis and cysticercosis or development of Th2 response in case of gastrointestinal infections. The major cytokines in both cases are IL-12, IFN-γ, IL-10, IL-4, IL-13 the effects of which are mediated by specific STATs. Thus, even when STAT6 participates in the expulsion of some gut parasites, a STAT4 meditated Th1 immune response seems to be necessary to control extra-intestinal larvae stages. Hence, both of these STATs proteins are necessary in trigger immune responses against helminth infections but play different role. The fact that helminth-derived antigens have been demonstrated to inhibit at least STAT4 activation, it must be enough motivation to try new helminth antigens to study STAT modulation in different target cells, this will help to clarify how helminths modulate the immune response.

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Conflict of Interests

The authors have declared that no conflict of interest exists.

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