



## Helminth therapy for autism under gut-brain axis- hypothesis

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### ABSTRACT

Autism is a neurodevelopmental disease included within Autism Syndrome Disorder (ASD) spectrum. ASD has been linked to a series of genes that play a role in immune response function and patients with autism, commonly suffer from immune-related comorbidities. Despite the complex pathophysiology of autism, Gut-brain axis is gaining strength in the understanding of several neurological disorders. In addition, recent publications have shown the correlation between immune dysfunctions, gut microbiota and brain with the behavioral alterations and comorbid symptoms found in autism. Gut-brain axis acts as the “second brain”, in a communication network established between neural, endocrine and the immunological systems. On the other hand, Hygiene Hypothesis suggests that the increase in the incidence of autoimmune diseases in the modern world can be attributed to the decrease of exposure to infectious agents, as parasitic nematodes. Helminths induce modulatory and protective effects against several inflammatory disorders, maintaining gastrointestinal homeostasis and modulating brain functions. Helminthic therapy has been previously performed in diseases such as ulcerative colitis, Crohn’s disease, diabetes, multiple sclerosis, asthma, rheumatoid arthritis, and food allergies. Considering gut-brain axis, Hygiene Hypothesis, and the modulatory effects of helminths I hypothesized that a treatment with *Trichuris suis* soluble products represents a feasible holistic treatment for autism, and the key for the development of novel treatments. Preclinical studies are required to test this hypothesis.

### Introduction

Autism is a neurodevelopmental disorder included within the Autism Spectrum Disorder (ASD). Due to the absence of neurobiological markers, the diagnosis of autism and other ASDs are based on defined core behaviors. Autism is characterized by specific impairments in social interaction, communication skills, restricted interests, and repetitive, and stereotypic behaviors. Autism is often associated to other comorbid symptoms as mental retardation, epilepsy [142], hyperactivity, attention deficits [91,130], sleep (Richdale and Schreck) and gastrointestinal alterations [98,109]. Symptoms generally emerge within the 3 first years of life [17]. Most cases of autism are idiopathic; nevertheless, evidences suggest the contribution of multiple genetic, environmental and immune factors in the pathogenesis of autism [114,119]. Autism does not have a cure, available treatments are directed towards the management of specific symptoms such as repetitive and stereotypic behaviors, irritability, aggressiveness and hyperactivity [28,68,108,131]. Despite of the complex pathophysiology, gut-brain axis is recently gaining strength in the understanding of several disorders. Gut-brain axis represents a bidirectional communication set integrating neural, hormonal and immunological signaling between

brain and gut. Under this axis, brain influences the gastrointestinal tract and vice versa [30,35]. Simultaneously, the Hygiene Hypothesis (HH) suggests that the increase on the incidence of autoimmune diseases in the modern world can be attributed to the decrease of exposure to infectious agents. HH hypothesis proposes that in areas where standards of living include a high degree of sanitation and where there is a limitation in the exposure to infectious agents including helminths, the immune system is not activated by appropriate stimulus. Early parasitic infections induce modulatory and protective effects against several inflammatory disorders, maintaining gastrointestinal (GI) homeostasis and brain functions [153]. Accordingly, helminthic therapy has been developed to treat autoimmune diseases as Chron’s and Bowel disease, resulting on gut microbiome enhancement, restoring of gastrointestinal homeostasis and host aberrant behaviors. Hereby, I maintain the potential therapeutic effects of helminthic therapy with *Trichuris suis* secretory products as a potential holistic treatment autism, under the brain-gut axis.

### Hypothesis

I hypothesize, that the treatment with *Trichuris suis* soluble products

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(TsSPs) represents a feasible treatment for the modulation and improvement of autistic behaviors, overactive pro-inflammatory immune response, and gastrointestinal dysfunctions observed in autism. I base this hypothesis on the immunological dysfunctions observed in autism, the impact of the immune system, and the gastrointestinal tract on the central nervous system (gut-brain axis), and the immunogenic properties associated to *T. suis*.

### Immune system and autism

Brain-immune system connection warrants the correct development of the brain but also participates on neuroinflammatory processes, contributing to neurodevelopmental disorders such as autism [9,128,133]. The balance between health and disease within the context of the brain-immune system is still not well defined, but it has been reported that immune system regulates the behavior, and cognitive functions in ASD individuals [69]. Atypical immune responses have been detected in the brain, cerebral spinal fluid and serum of autistic patients. Among them, a marked astroglial and microglial activation, an imbalance of the Th1/Th2 immune response and an increased on pro-inflammatory cytokine profiles as IFN- $\gamma$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$  and chemokines as MCP-1 [94,143]. In autism, Th2 response activation has been related to language, visual and motor skills, IL-4 has been linked to learning and memory, and IFN- $\gamma$  has been linked to social and spatial learning and memory [52,61]. Abnormalities in peripheral immune response were also noted in plasma of children, involving dysfunction of immune cells and abnormal level of immunoglobulins and cytokines [15,137]. Autism immune dysfunctions are commonly associated to an altered genetic expression but have been also described *in utero* due to maternal infections or maternal anti-brain antibodies, and postnatally associated to inflammatory processes or seric anti-brain autoantibodies [105].

#### Immune genes linked to autism

Autism is strongly associated with genetic expression. So far more than 200 genes have been suspected to be risk genes for autism, affecting brain development or to immune system pathways [68,120]. These genes most often increase predisposition to develop autism. Autism predisposition implies single or multiple genes mutations and variations, as well as different modes of inheritance in autistic patients and their families [59,60]. Probable gene candidates have been mapped in several chromosomes [3], some of them directly linked to genes associated to brain development and immunity, while other genes are involved in multiple biological processes [25]. Two genes linked to the immune component of autism are in chromosome 6 (6p21.3), the major histocompatibility complex (HLA0-DRB1) and the complement component C4B. HLA0-DRB1 is highly associated with language impairment in autism [110,146]. C4B plasmatic level is low in autistic patients [111] affecting the classic immune pathway, the complement system. Another gene linked to autism and immune regulation is MET. MET “C” variant (7q31) encodes for a receptor tyrosine kinase that is a negative regulator of cortical development and of the immune responsiveness to gastrointestinal events [24,58]. Hypomorphic mutations of MET induce abnormal migrations of interneuron progenitors from the ganglionic eminence to the cortex with the result of a reduced number of cortical interneurons [24,25,60]. In addition, a correlation between the gene for the macrophage inhibitory factor (MIF) gene and the severity of autism has been described [64]. Further research will probably unravel many more immune genes related to the immune-based ASD etiology. Despite the high heritability, only a 10–20% of autistic cases can be strictly correlated to genetic alterations or mutations.

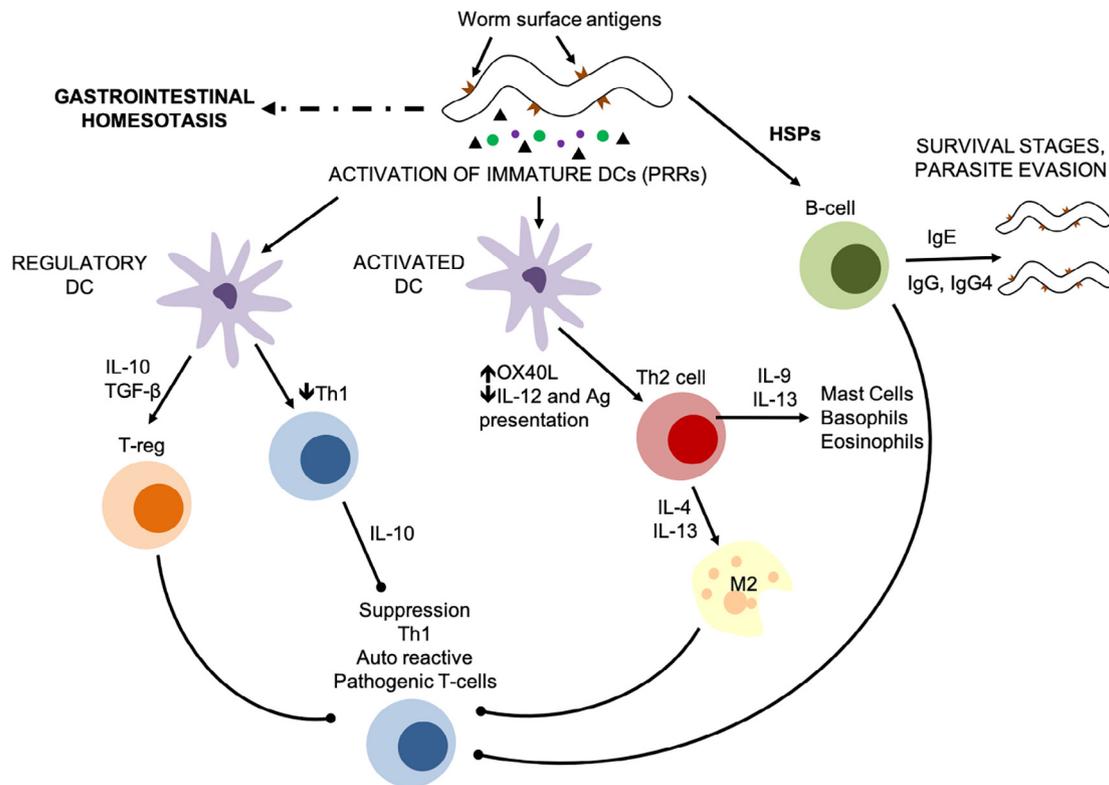
#### Th1/Th2 imbalance in autism

Variations on immune cells and cytokines levels and their receptors

have a deep impact on the maintenance of neural tissues, cognitive functions and emotional processes [16]. Some of the autistic phenotypes present a chronic Th1 pro-inflammatory cytokine response and a reduced Th2 anti-inflammatory response [94]. This imbalance in the Th1/Th2 immune response leads to an increase in the number and state of activation of microglial and astroglial cells, and an increase of pro-inflammatory cytokines (IL-6, IL-1 $\beta$ , IL-8 and IL-12p40) levels [16,116,142,147–149]. Studies in postmortem brain and cerebrospinal fluid from ASD individuals revealed high levels of transforming growth factor  $\beta$  (TGF- $\beta$ ) [142]. Contrary, lower levels of TGF- $\beta$  were observed in peripheral blood in adults [113] and children with severe autistic behavior scores compared to control patients [12]. The levels of plasmatic leptin, a pro-inflammatory hormone/cytokine, have been also found elevated in early-onset autistic children, compared to regressive autistic and control cases [14]. MCP-1, RANTES, and Eotaxin chemokines were found in high levels in plasma in association with impaired behaviors in autistic children [13]. Contrary, adhesion molecules as sPECAM-1 and sP-selectin were significantly reduced in plasma of autistic children. Furthermore, low levels of sPECAM-1 seem to be negatively correlated with autistic stereotypy and abnormal brain functions [115]. The evaluation peripheric plasma samples, confirmed this trend in adult autistic males [137]. Activated microglia and astroglial, and interferon  $\gamma$  (INF- $\gamma$ ), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF- $\alpha$ ), MIF, and platelet-derived growth factor (PDGF) [13,64,84] have also been described as markers of neuroinflammatory processes in autism [18,33,142]. In addition, Natural Killer T- cells have been largely implicated to autism presenting less responsive to cytokines [48,132]. In the same way, the evaluation of plasma revealed the presence of anti-brain auto-antibodies correlated with aberrant behaviors such as decreased cognitive and adaptive functions [63]. Autoantibodies react against neural progenitor cells (NPCs) inducing alterations on CNS and neural death [100]. In addition, a significant decreased in plasmatic IgG and IgM levels have been associated with severe autistic behaviors [72].

#### Gastrointestinal dysfunctions and gut microbiome in autism

Children with autism commonly suffer from immune-related comorbidities such as recurrent ear infection, upper respiratory infections, adverse reactions to multiple medications, longer duration of illnesses, GI inflammation [82], and a higher prevalence of inflammatory bowel disease [23,31,87]. GI affections in autistic patients are associated with more severe behavioral dysfunctions [109]. Common comorbid symptoms include abdominal pain, gaseousness, diarrhea or constipation [106,109]. Some patients suffer alterations on the permeability of the gastrointestinal tract (“leaky gut”) [36,40]. This particular condition enables, bacterial neuroactive molecules and GI antigens, to cross gut mucosa reaching the bloodstream affecting brain functions [92]. In addition, altered gut microbiota and metabolic dysfunctions are commonly observed in ASD patients [21,40,71] and autistic animal models [41]. Alteration in gut microbiota ecology (dysbiosis) [21,40], species richness [4,39,53,54], and abundances are suspected to be directly or indirectly associated to ASD symptoms, by influencing the immune system, particular metabolic pathways, abnormal behaviors [77], and the severity of the disease [38]. Clinical studies with children supplemented with probiotics, resulted in improvements in ASD symptoms [32,92,138]. Gut microbiota acts as a “second brain”, in a bidirectional interaction with the CNS [21]. Behavior is modulated by bacterial products [30,35]. Bacterial neuromodulatory metabolites can reach the brain, via the enteric nervous system by the vagus nerve (VN) [92], through neuroendocrine system by the hypothalamic-pituitary-adrenal (HPA) axis, or by the activation of the mucosal immune cells [30,92]. Mucosal immune cells release pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, IFN- $\gamma$ , TNF- $\alpha$ ), would cross the blood-brain barrier (BBB), reaching the CNS [13,94]. The evaluation of immunoglobulins revealed a general increase of isotopic IgG4 antibodies and allergen-specific IgE in the gut



**Fig. 1.** Immunoregulatory patterns of helminths and helminth-derivate products. Helminths ligands tight specific PPRs triggering the activation of T-reg. Activated DCs, expressing OX40L prime the differentiation of Naïve hosts T-helpers cells into Th2 cells, increasing an anti-inflammatory cytokine pool IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, and TGF- $\beta$ . IL-4 and IL-13 help in the activation of M2. IL-9 and IL-13 stimulate the maturation of the effector cells: Mast cells, Basophils, Eosinophils. B-cells secrete helminth specific antibodies IgE, IgG4 and IgE. HSPs (Helminths soluble products), PPRs (Pattern recognition receptors), DCs (Dendritic cells), T-reg (Regulatory T- cells), M2 (Alternative Activate macrophages), B-cell (B-lymphocytes cells).

mucosa of autistic patients [34,48,82,83].

### Helminths and immunomodulation

Parasitic helminths coexist within our organism for millions of years, adapted to live in the gastrointestinal tract, blood, lungs and other tissues of a multitude of animal species. Years of coexistence, have resulted in a complex system of interactions and interdependence between host immune system and parasites [151]. Helminthic infection affects millions of people, causing direct or indirect deleterious effects on health [75,141]. Generally, helminths induce low rates of mortality but high morbidity [27,76]. Despite their negative effects, populations affected by endemic helminthic infections present with a low incidence of autoimmune diseases [20,126,152].

#### The hygiene hypothesis

An intensive anti-parasitic regimen in developed countries has been hypothesized to be a factor responsible for the increase in the prevalence of immune-mediated diseases [145,151]. The positive effects of helminths in immune disorders can be explained with the Hygiene Hypothesis (HH). The HH states that helminthic infections exert modulatory and protective effects against several inflammatory disorders. This hypothesis proposes that in areas where standards of living include a high degree of sanitation and where there is a limitation in the exposure to infectious agents including helminths, the immune system is not activated by suitable stimuli [66], contributing to an immune dysregulation and a higher prevalence of immune pathological diseases. Accordingly, autoimmune diseases are more common in industrialized than in developing areas [112]. This hypothesis is supported by epidemiological studies demonstrating a decrease in the levels of Th1-

related autoimmune diseases and allergen Th2-related diseases as asthma and allergic rhinitis in people parasitized by helminths, including infections by species like *Ascaris lumbricoides* and *Necator americanus* [10,37,51]. Clinical data also support the idea that deworming induces an increase of allergic skin reactions [47,123]. Consequently, diseases as ulcerative colitis, Crohn disease, type-1 Diabetes, multiple sclerosis, asthma, rheumatoid arthritis, and food allergies are increased in regions where sanitation degree has improved [57,151]. Epidemiological studies revealed a possible positive effect of parasites as hookworms and schistosome in the treatment of autoimmune diseases. Several animal models have revealed how helminths can protect from these diseases.

#### Helminths and mechanisms of regulation of the immune system

Despite the wide variability of helminths, most host-parasite relationships present with similar patterns of immune modulation. Helminthic infection induces a modified Type 2 immunity that allows counteracting the allergy Type 2 and the autoreactive Th1 immune responses [51]. Helminth modified Th2 response involves the activation of CD4+ Th2 cells, the recruitment of eosinophils, basophils and mast cells, and a Th2 cytokine profile [97,141,144]. This prevents from an excessive inflammatory response, ensures parasite survival, and elicits chronic infections of limited pathogenicity [11,65,107]. During host-parasite infestation, helminths go through different life stages, interacting with the host's immune system through different antigens and soluble products [66,140]. The interaction between host-helminth-secreted products induces the activation of dendritic cells (DCs) by Toll-like receptors, C-type lectin receptors or lectin type receptors [10,99,129]. The stimulator of OX40L on DCs is also required to trigger the differentiation of Naïve hosts T-helpers cells into Th2, activate the

regulatory T- (T-reg) cells [45,67,85,96,135], regulatory B (B-reg) cells, and alternatively activated macrophages (AAMs) [10]. Type 2 cells from helminthic infection promote a Type 2-cytokine profile of IL-3, IL-4, IL-5, IL-9, IL-10 and IL-13, and the differentiation and proliferation of the parasite-specific IgE, IgG and IgG4 antibodies [19,51,95]. Interleukins IL-4 and IL-13 are fundamental in host resistance against helminths, TGF- $\beta$  and IL-10 attenuate pathogenic autoreactive Th1 response and suppress the production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-12, IL-1, nitric oxide and IFN- $\gamma$  [51,66,78,156]. Helminthic therapy is based on the therapeutic administration of helminths or helminth-derivate products to block the Th2 allergen-specific response and the autoreactive-Th1 response in human [49,125]. A particular activation of cells IL-10/T-reg dependent or independent can be activated depending on each particular parasitic species [50,150]. There are still, not enough studies detailing the molecular mechanism by which DCs regulatory are primed by helminths [50], (Fig. 1).

### Helminth therapy

Helminth therapy is based on the infection with living stages of helminths as a therapeutic tool for patients suffering immune-related diseases [55,81,131]. Although helminth therapy has been successfully used in a series of autoimmune diseases, not all the helminthic infections protect against excessive inflammatory response [46,103]. When considering helminthic therapy to treat immune-based diseases several questions might arise: Do all helminth species have the same immunomodulatory effect? What helminth species are the best options for the treatment of immune diseases? What treatment works better, human-hosts helminths or animal-hosts helminths? Regarding these questions, literature offers a variety of points of view. Some authors advocate for the use of human-specific gastrointestinal helminths, while most prefer non-human host helminths as a tool to treat human disease [49]. For example, epidemiological studies showed the lack of protective effects and an enhanced allergic response in patients infected with *Ascaris* spp., *Toxocara* spp., *Fasciola hepatica*, and *Enterobius vermicularis* human-specific helminths [10,49]. Meanwhile, *Trichuris suis*, a gastrointestinal parasite hosted by swans and pigs, has been proposed as a novel and safe therapy for allergic inflammatory diseases and autoimmune diseases in human, being able to suppress the clinical symptoms of some complex autoimmune diseases [26]. During host-parasite infestation, helminths go through distinct life stages while interacting with the host's immune system. These interactions could mechanically or chemically damage the host cells [5]. Chemical interactions are induced by the active release of large amount of Excretory/Secretory Products (ESPs) and/or by the surface antigens [5,66,73,102,140]. However, the composition of helminth products responsible of the immunosuppressive mechanism remains unknown, but different protein, lipids, and glycoconjugates are suspected to be involved [90,140]. For example, studies of the brain response to infection by the helminth responsible of neurocysticercosis (*Mesocestoides corti*) have shown that helminth-soluble factors modulate microglia activation and the inhibition TLR signaling-induced inflammatory cytokines production in mice [6]. Other examples include the filarial nematode *Acanthocheiloneema viteae* ES-62 protein that has been shown to reduce Fc $\epsilon$ RI-mediated responses in human bone marrow-derived mast cells [104]. In addition, the AvCystatin protein, a cysteine protease inhibitor secreted by some helminths, modulates the host's immune system in an allergic-induced mouse model [37,127]. Based on the particular modulatory properties of helminths, helminth derivate molecules can serve as templates for the design of novel anti-inflammatory drugs or vaccines [50,70,73,118,131]. A recent publication showed that the immunization with the recombinant schistosome protein P28GST with allhydrogel as adjuvant reduced the local expression of pro-inflammatory mediators including TNF- $\alpha$  and IL-17 in induced-colitis in rats and mice [43]. These examples confirm the efficacy of immunogenic helminth proteins in the treatment of autoimmune diseases.

### *Trichuris suis*: the porcine whipworm

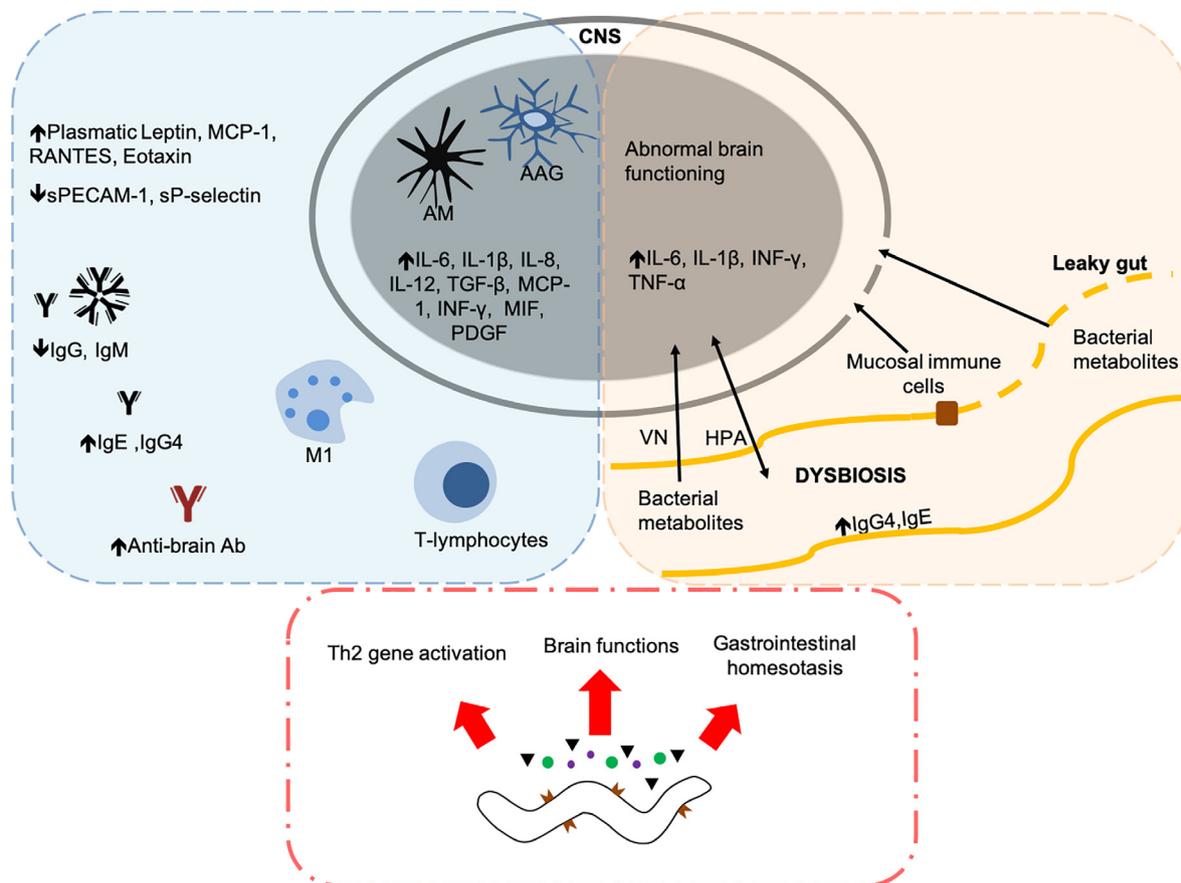
*Trichuris suis*, the porcine whipworm, belongs to the superfamily Trichuroidea whose members are parasites of a large number of domestic animals [80] and induces a strong Th2 type immune response responsible of a rapid worm expulsion [8]. In human, *T. trichiura* and three other related species are associated with Trichuriasis [75]. Infection with *T. trichiura* induces the inflammation of the large intestine manifested in bloody diarrhea, malnourishment and stunting in children. Paradoxically, the zoonotic *T. suis* is considered as a substantial promise for the treatment of human autoimmune disorders [79], due to its abilities to infect human with a self-limited colonization [45,135]. *T. suis* has a direct life cycle, where the unembryonated bipolar eggs are present in the feces, requiring 3 or more weeks to develop to infective stage, the larvae 1 (L1). The infective eggs are ingested by the definitive host. After ingestion, eggs hatch in the small intestine and L1 is liberated and migrate to the large intestine mucosa penetrating the mucosal glands [7,139]. L1 penetrates the epithelium lining the crypts of Lieberkühn, molting three times inside (L2, L3, and L4), and arising to adulthood in approximately 41 days after infection [7]. After the last molt, the adult *T. suis* embeds a mouth stylet, named stichosome, into the mucosa [7,139]. Then, the parasite secretes several molecules that are characteristics to the stage of its life cycle [79].

### *Trichuris suis* OVA therapy in gastrointestinal disorders

Based in the predominant Type 2 response and suppressed Th1 profile associated to *T. suis* ova (TSO), therapy with TSO has been performed in several chronic allergic and autoimmune human diseases [19,124,135,136]. Patients received an oral administration of embryonated eggs that hatched in the small intestine and molted until reaching adulthood [19,124,135,136]. Specifically, clinical trials have demonstrated the effectiveness the TSO therapeutic infection on immune-mediated inflammatory diseases as immune bowel disease, Crohn's disease and Ulcerative colitis. The administration of TSO to immune bowel disease patients decreased the intestinal inflammation and induced a remission and improvement in the Crohn's disease activity index [88,134,135], and a remission in ulcerative Colitis patients [136]. TSO, in pigs induce damages on the epithelium and significantly affects gut microbiota composition. The characterization of gut microbiota composition showed alterations in richness and abundances of species increasing levels of the genus *Campylobacter* or *Mucispirillum*, but a decrease in *Ruminococcus* or *Fibrobacter* [93,155]. In addition, primates affected by idiopathic chronic diarrhea, infected with *Trichuris trichiura*, showed improvements in wealth conditions, Inflammatory gut processes were increases in phylum Tenericutes while reverted Cyanobacteria to control levels [22]. Effects on GI bacterial affects their associated metabolic pathways [93,155]. In addition, Phase-I studies as well as controlled trials have also confirmed the safe clinical effects of TSO on multiple sclerosis patients [56,124]. In addition, TSO has also been hypothesized to contribute to the improvement of the mucosal barrier function on ASD [131,154]. However, there are several limitations and questions that remain unclear: Is *T. suis* able to establish and mature in human intestines? Do *T. suis* live infections require multiple infections or higher doses of infection to have therapeutic effectiveness? Which are the mechanisms through which *T. suis* modulate the host immune system? How can we manage the safety and "fear factor" that can condition a helminth therapy in autism? Additional research is needed in order to answer these questions.

### Excretory/secretory helminth products

Some authors hypothesize that the immunogenicity and efficacy of helminthic therapy on autoimmune diseases rely on helminth-derived soluble molecules like excretory and secretory products (ESPs). In pigs, the exposure to helminth-derivate products rich in glycans induced the



**Fig. 2.** Left upper side: Immune Th1/Th2 dysfunctions in autism: A Th1 type profile can be detected in autistic brain and cerebrospinal fluid: AM (Activated Microglial) and AAG (Activated Astroglia). Pro-inflammatory cytokines: IL-6, IL-1 β, IL-8, IL-12, TGF-β (Transforming Growth Factor Beta), INF-γ (Interferon gamma), MCP-1 (Monocyte chemoattractant protein-1), TNF-α (Tumor necrosis factor), MIF (Macrophage inhibitory factor), and PDGF (Platelet-derived growth factor). High levels of plasmatic Leptin, MCP-1, RANTES, and Eotaxin in contrast to lower levels of adhesion molecules sPECAM-1 and sP-selectin. A decrease of IgG and IgM. High levels of IgG4, allergen type IgE and M1 (classic activate macrophage) and the presence of Anti-brain antibodies were also measured in plasma. Less responsive of NK (Natural Killer cells), and an increase of Total lymphocytes, T-lymphocytes, and Cytotoxic lymphocytes levels, can configure the pool of neuroinflammatory markers found in autistic phenotypes. CNS: Central Nervous System. Right upper side: Gastrointestinal affections and dysbiosis in autism: Gastrointestinal (GI) affections in autistic patients can be associated with behavioral dysfunctions. Patients can present a “leaky gut” condition, enabling bacterial neuroactive molecules and GI antigens, to cross the gut mucosa, reach the bloodstream and CNS, altering brain functions. Microbiome dysbiosis is also associated with ASD symptoms. Bacterial metabolites can reach the CNS (Central Nervous System) via enteric nervous system VN (Vagus nerve), the neuroendocrine system by the HPA (hypothalamic-pituitary-adrenal axis), or by the activation of the mucosal immune cells. Mucosal immune cells can initiate pro-inflammatory immune responses releasing IL-1β, IL-6, INF-γ, TNF-α. Th1 mediators reach the CNS through an altered BBB (blood-brain barrier) contributing to a neuroinflammatory process, altering normal brain functions. Furthermore, an increase in the expression of IgG4 and allergen-specific IgE in the gut mucosa of autistic patients can be detected. Bottom side. Helminth regulatory mechanisms under gut-brain axis: A TsSPs therapy can revert host Th1 expression inducing a Th2 gene activation, contributing to ameliorate autistic Th1/Th2 disbalances increases of helminth specific antibodies IgG, IgG4 and IgE production. TsSPs can restore gastrointestinal homeostasis, and gut microbiome, affecting brain functioning under the gut brain axis.

activation of host adaptive Th2 immune response mediated by dendritic cells [8]. *T. suis* ESPs can be collected from *in vitro* cultures and used in animal or human treatments avoiding live parasitic infection [11,73]. Experiments in mice with ESPs obtained from adult *T. suis* showed an increased IL-6 and IL-10 production in swine digestive epithelial cells [117]. Moreover, *in vitro* trials showed that proteins bigger than 10 kDa secreted by *T. suis* have antibacterial activity against *Campylobacter jejuni*, *Escherichia coli* and *Staphylococcus aureus* [1,2]. This antibacterial activity was also confirmed a posteriori in pigs [93]. ESPs from adult *T. suis* also showed a significant suppression of the symptoms of autoimmune encephalomyelitis in mice. Moreover, the administration *in vivo* in a murine model of L1 stage *T. suis* ESPs elicited immune regulation in allergic airway hyperreactivity [44]. *In vitro*, data collected from human showed a suppression of the production of pro-inflammatory cytokines in dendritic cells cultured in a *T. suis* ESPs medium [85,89]. Recently, complete genome analyses of different stages, gender, and body portions of *T. suis* rendered a series of molecules that participate in *T. suis* immunomodulation, revealing an

important amount of ESPs as parasitic cysteine protease inhibitors, serpins, thioredoxin peroxidase calreticulin and cytokine homologues [79]. For example, parasitic calreticulins bind dendritic cell receptors to induce an anti-inflammatory response through the secretion of IL-4 and IL-10 together a limiting T-reg (Fox<sup>3+</sup>) cell differentiation. Calreticulins and apyrases block the conversion of T-reg (Fox<sup>3+</sup> to pro-inflammatory T-cells. Thioredoxin peroxidase induces alternative activation of host macrophages, inhibiting nitrous oxide production and stimulating IL-4 and IL-10 production. Serine proteases-inhibitors block neutrophils; cathepsin G and elastase limits tissues destruction and reduce the inflammation [79]. The available data on the effect of HSP in immune-related diseases indicate a potential treatment for autism.

#### TSO therapy in autism

Due to the particular immune and neural dysregulation observed in autism, *Trichuris suis* OVA (TSO) administration was proposed as a potential treatment for autism [131]. To the best of my knowledge,

there was/is only one clinical trial using TSOs to treat autism (NCT0104022) by Dr. Hollander laboratory at the Albert Einstein College of Medicine, New York. They were/are assessing the efficacy and safety of TSO to treat autistic behavior in 10 different high functioning ASD patients, with a familiar history of allergies and/or immunoinflammatory illness. They were/are further analyzing the relationship of TSO with the immune response in the host. No preclinical studies in animals have published to date.

Even though, Helminth therapy with living infestations may induce several contraindications including potential complications related to zoonoses like maladaptive responses [90], aberrant migrations to non-intestinal regions, and possible pathological side effects on host physiology and tissues [101,107]. In addition helminth infection can reduce the antibacterial and antiviral response, making host more susceptible to opportunistic infections [29,101,103]. For example, in the treatment of allergic rhinitis *T. suis* TSO induce significant gastrointestinal symptoms, including diarrhea during the initial phases of therapy in humans [20]. Moreover, it is unclear what the ability of *T. suis* is to establish and mature in human [44,89,107].

### Hypothesis: *Trichuris suis* esps a therapy in autism

In order to avoid complications related to TSO, I propose a therapy with no living agents as soluble excretory and secretory products, obtained after the culture of larvae and adult worms. A postnatal treatment with TsSPs might modulate aberrant autistic core behaviors, contribute to balance Th1/Th2 immune response, helping to control gastrointestinal homeostasis. TsSPs revert host Th1 expression towards Th2, contributing to increasing the production of parasitic specific IgG, IgG4 and IgE [19] and effector cells (mast cells, eosinophils and basophils) [88]. TsSPs, affect the production of human pro-inflammatory cytokines IL-6 and TNF- $\alpha$ , and classic activated macrophages M1, prompting the production of IL-10, and the anti-inflammatory M2 macrophages (alternative activated macrophages pathway) [62,74]. While in autism the immune balance tips towards Th1 activation and GI disbalances, helminths preferentially produce a Th2 activation that counteracts Th1 immune response, contribute gastrointestinal homeostasis, and brain functioning. In other words, helminths polarize the immune response in autism toward Th2. Specifically, specific pro-inflammatory cytokines increased in autism such as IL-6, IL-1 $\beta$  and IL-12 can be partially or totally reverted by increased production of anti-inflammatory cytokines such as IL-3, IL-4, IL5 and IL10 due to the helminthic action. In human cells, induce Rab7b, a negative regulator of the inflammatory response of TLR4 receptor on DCs [86]. *T. suis* exerts an antibiotic activity, This potential immunogenic properties already tested in the treatment of other autoimmune diseases observed in humans, represent an opportunity to treat the inflammatory profile of autism [121,122]. A treatment for gut dysbiosis and gut altered permeability can be helpful in regressive autism [42], so a therapy with *T. suis* can contribute to restore altered mucosal epithelium, balance GI dysbiosis, reducing the GI immune response associated to the reactivity to GI microbiome as in Chron diseases helminths therapies.

Overall, data indicate that *T. suis* SPs can restore gastrointestinal epithelium, reestablish gut microbiome to healthier levels, reverts pro-inflammatory Th1 immune response inducing an anti-inflammatory Th2 response, contributing to revert behavioral autistic behaviors, as a holistic therapy for autism (Fig. 2).

Further complications such as difficulties in purification and isolation of HSPs immunogenic molecules may arise, due to the small production [49,85] and the high concentration probably needed for a treatment. The isolation and characterization of immunogenic ESPs and other helminth derivate products might be the key to the development of novel treatments for autism. Preclinical studies with helminths or derived molecules to treat autism must be performed shed light on the development of new immunotherapeutic strategies.

## Conclusion

A therapy with TsSPs represents a feasible treatment for autism. TsSPs contributes to reestablish gastrointestinal epithelium, restore gut dysbiosis to healthier levels, reverts a pro-inflammatory response and induces behavioral changes improving autistic behavioral cores. Preclinical studies are needed to confirm this hypothesis.

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## Conflict of interest

Author declare no conflict of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.02.042>.

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