Immunoregulation of Multiple Sclerosis by Helminth Therapy: A Literature Review

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Artikel Review

Abstract: *Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) which is characterized by the recruitment of T cells into the CNS, leading to demyelination and axonal damage. Currently, there are limited options for MS therapy, thus researchers start to use helminths therapy as a new therapeutic agent. Helminths are promising organisms to treat autoimmune diseases like MS by interfering the host's immune responses. Several helminths, including Trichinella spiralis, Trichuis suis, Fasciola hepatica, Schistosoma japonicum and Schistosoma mansoni are under investigation in animal models for MS, experimental autoimmune encephalitis (EAE). Furthermore, Trichuis suis, Fasciola hepatica and Schistosoma mansoni are being examined in patients. This review outlines basic insight of MS, immunoregulation mechanisms induced by helminths, current helminths therapy for MS as well as helminths therapy for MS application in the future.*

Keywords: *multiple sclerosis, helminth therapy, immunoregulation, immune system, autoimmunity*

Abstrak: Multiple sclerosis (MS) adalah penyakit inflamasi kronis pada sistem saraf pusat (SSP) yang ditandai dengan perekrutan sel T ke dalam SSP, yang menyebabkan demielinasi dan kerusakan aksonal. Saat ini, pilihan terapi MS masih terbatas, sehingga peneliti mulai menggunakan terapi cacing sebagai agen terapi baru. Cacing adalah organisme yang menjanjikan untuk mengobati penyakit autoimun seperti MS dengan mengganggu respons imun inang. Beberapa cacing, termasuk *Trichinella spiralis, Trichuis suis, Fasciola hepatica, Schistosoma japonicum* dan *Schistosoma mansoni* sedang diselidiki pada model hewan untuk MS, ensefalitis autoimun eksperimental (EAE). Selanjutnya, *Trichuis suis, Fasciola hepatica* dan *Schistosoma mansoni* telah dilakukan pemeriksaan pada pasien. Ulasan ini menguraikan pengetahuan tentang MS, mekanisme imunoregulasi yang disebabkan oleh cacing, terapi cacing saat ini untuk MS serta terapi cacing untuk aplikasi MS di masa depan.

Kata kunci: multiple sclerosis, terapi kecacingan, imunoregulasi, sistem imun, autoimunitas

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Introduction

Multiple sclerosis is a chronic inflammatory disease of the central nervous system (CNS) which is characterized by the recruitment of T cells into the CNS, leading to demyelination and axonal damage (1, 2). Patients developing this disease will have major motor and sensory deficiency and show cognitive dysfunction, like memory deterioration. Both interaction of chemokines and the binding of leukocyte integrin with endothelial molecules will stimulate the entry of leukocytes to the CNS by crossing the blood brain barrier. This process is followed by the firm adhesion of leukocytes onto the vascular endothelium. Once the leukocytes are activated, it will stimulate myelin phagocytosis leading to demyelination (3).

Current treatment for multiple sclerosis focusses on immunosuppressive or immunomodulatory activities in relapsing-remitting multiple sclerosis (3). Laquinimod, for instance, is currently being tested as therapeutic drug in multiple sclerosis since it reduces the aggression of pathogenic effector T cells in the CNS tissue. The decreased aggression leads to a significant decrease in the migration of memory T helper type 1 (Th1) and T helper type 17 (Th17) lymphocytes across the blood brain barrier (4). However, this treatment may have severe side effects and can potentially interfere with immune balance (3). Currently, there are limited options for the therapy of multiple sclerosis, therefore researches related to this area is still ongoing. Current researches also investigate the treatment of multiple sclerosis by using helminth therapy.

Parasitic helminths can cause a chronic infection in humans by regulating host immune responses. Interestingly, this mechanism can also cause a positive effect as it can protect against several inflammatory immune disorders in humans, such as multiple sclerosis, inflammatory bowel disease, and allergies (5). Helminth infection suppresses immunopathology by triggering the induction of regulatory T (Treg) cells and Th2 responses. The responses causing the suppression of bystander responses to self-antigen (6). This mode of action can be a potential solution to treat several immune diseases in humans.

Studies have shown that there is a link between helminth infection and the prevalence of multiple sclerosis. Multiple sclerosis patients infected with helminths have a lower number of recurrences compared to the uninfected patients (7). Helminths may induce strong immunomodulatory effects on the host for a longer time period (7). These studies generated increased interest in the mode of action by which helminth can trigger immunomodulatory effect in humans. There are several regulatory mechanisms of the immune system which are triggered upon helminth infection, such as Treg cells, regulatory B (Breg) cells, alternatively activated macrophages, and tolerogenic dendritic cells.

The primary objective of this paper is to review recent research papers on helminth therapy of multiple sclerosis (MS). First, this paper will focus on MS disease. After that, immunoregulatory mechanisms induced by helminths will be investigated. Eventually, current helminths therapy followed by helminths therapy for MS in the future will be discussed.

Multiple Sclerosis (MS)

Multiple sclerosis (MS) is the most common cause of neurological disability in developed countries. As an autoimmune disease, MS is initiated by dysregulated T cells on myelinated nerve cells (8), followed by irregular periods of remission and relapse (9). The major histological abnormality of MS is the plaque formation, inflammation and tissue damage. These can lead to several complications. It begins with the attacks by immune system lead to of neurological dysfunction, such as loss of vision in one eye, weakness, numbness, double vision, incoordination (Fleming, 2013), speech disorder, seizure, action tremor and depression (9).

The pathology of multiple sclerosis is characterized by the presence of inflammation (10) and demyelinated areas in white and grey matter in coronal brain, spinal cord (11), thalamus, hypothalamus, hippocampus or cerebellum (10).

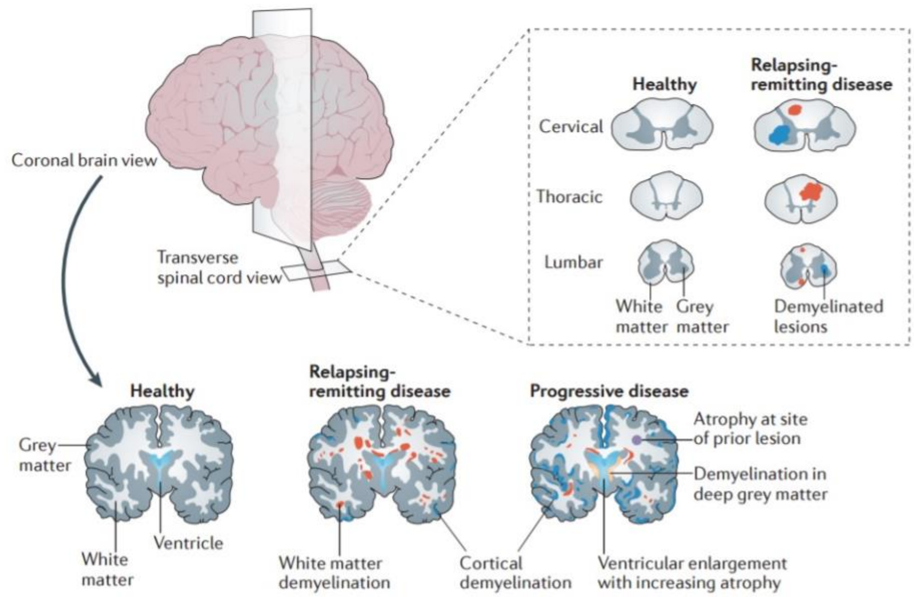

Figure 1. The pathology of multiple sclerosis. ***Adapted from Dendrou et al., 2015***

These demyelinated areas called plaques or lesions. In the coronal brain of patients with progressive disease, the characteristic feature is not only in cortical demyelination, but also demyelination in deep grey matter, atrophy at site of prior lesion as well as ventricular enlargement with increasing atrophy as can be seen in **Figure 1** (12). The cause is possibly due to axonal injury and loss occur in the disease lesions which correlates with inflammation (13). Demyelinated areas in the white matter can partially be remyelinated and repaired. Furthermore, relapsing-remitting disease in the spinal cord also shown demyelinated lesions area as the characteristic feature in the brain of patients with MS (12).

The main cause of MS is not known. Nevertheless, there are several etiologic factors involved such as genetics, race, environment, infections, toxins, the immune system, allergies (14), and smoking (15). Major heritable genetic factors found for MS patients, for instance, is the human leukocyte antigen (HLA) class II region. It is shown that there is HLA class II deficiency in patients with MS. The reason behind this remains unclear (16). The main characteristics of MS are periodic neurologic attacks, disability and reduced physical, socio-economic and health conditions during the age of 30 (14). Females and younger people aged 18-27 have higher risk for developing MS. Moreover, family history with MS will also increase the risk of MS (17).

The periods of relapse and remission in MS occur for several days or weeks and are followed by partial or complete recovery, which is the characteristic of the relapsing-remitting subtype of MS (RRMS). After a period of time, the speed of the disease may change to the steady progression of disability and form the secondary progressive subtype of MS (SPMS). Primary progressive MS (PPMS) can be formed in the minority of patients characterized by dispiteous progression (18).

Immunoregulatory Mechanisms Induced by Helminths

Immunoregulation can be defined as the activity of integrated control systems which balance the individual components of immunity. Normally, immunoregulation urges immune homeostasis in several ways. First, it ensures that all the immune response work optimally and at the right time. Secondly, it promotes active tolerance to control excessive immune responses to parasites and pathogens, thus the immune-mediated damage to host tissue is limited (18). The immune system will protect the body from reacting to self-antigens, which otherwise cause autoimmune diseases. Autoimmune diseases have a correlation to self-tolerance in the host.

Self-reactive T and B cells in the central lymph organs are controlled to maintain self-tolerance (19). Tolerance to self-antigens is preserved by preventing the maturation of self-antigen-specific lymphocytes T and B cells. The maturation of self-antigens T and B cells are developed in the thymus and bone marrow and are normally inactivated by peripheral mechanisms. The pathologic response of autoimmune diseases directly against self-antigens due to a failure of mechanism in T or B cell tolerance (20). In order to against autoimmunity, T cells with autoreactive T cell antigen receptors (TCRs) will be eliminated during the development in thymus. This process called negative selection (21). Failure in negative selection T and B cells during maturation create an immune response to the host which finally results in autoimmune diseases.

The process take place in autoimmune diseases includes pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) and danger-associated molecular patterns (DAMPs). PAMPs are released during virus or bacterial infections while MAMPs are released from inflammatory commensal bacteria and DAMPs are released by dying cells during inflammation. These molecules bind to the pathogen recognition receptors (PRRs) on innate immune cells leading to the maturation of dendritic cells (DCs). The mature DCs will finally induce Th2 and Th17 cells which are responsible in the induction of allergies and autoimmune diseases respectively (22).

The development of autoimmune diseases, including MS are inversely correlated to helminth infection (22). This inverse correlation can be explained by hygiene hypothesis. According to the hygiene hypothesis, MS is associated with early life normal infections in high levels of sanitation (18, 23). The long co-adaptation between parasites and humans affects human immune responses. Helminths can be categorized as beneficial rather than harmful parasites (15). This is due to the fact that human infections with helminths can diminish the incidence of MS (18) in which correlates to Th1, Th2 and Th17 response, Treg cells as well as B cells as can be seen in **Figure 2** (24).

T cells perform as a primary role in modulating autoimmune diseases. Naïve T cells can differentiate into helper (Th) and regulatory (Tregs). The three main subsets of T helper cells are Th1, Th2 and Th17 cells. Th1 cells produce proinflammatory cytokines like tumor necrosis factor alpha (TNFα), interferon gamma (IFN-γ) and interleukin (IL)-12. Moreover, proinflammatory responses during autoimmune diseases are also mediated by Th1 (24). On the other hand, Th2 cells generate cytokines like IL-4, IL-5, IL-10 and IL-13 which are strongly induced by helminths (22). Furthermore, Th2 cells can prevent Th1 cells activities in mediating autoimmune diseases. Th17 cell differentiation are involved in the immune system response during inflammation. Th17 lymphocytes secrete IL-17 as a pro-inflammatory cytokine which is highly found in MS (24). The alteration of Th responses from Th1 and Th17 to Th2 is a possible underlying mechanism for the protective effect of helminths towards MS (22).

Helminth's tolerance is characterized by the production of anti-inflammatory cytokines (24). Anti-inflammatory cytokines, such as IL-10 and TGF-β are produced during helminth infection and they will promote the induction of FoxP3 in T cells+ which is transcription factor characteristic of Treg cells (22). IL-10 and TGF-β lead to a reduction of Th2 cytokines, ablate the Th1 cytokines and suppress T cell proliferation against helminths (24). Furthermore, the replenishment of CD4+ CD25+ FoxP3+ Treg cells in peripheral blood can also be associated to the immunomodulatory effects of helminth infections. Treg cells, which are induced by helminths, can suppress the Th1 and Th17 response (22).

A recent study has shown that apart from Treg cells, B cells also take an important role in MS (2). Moreover, B cells are also able to produce antibodies, including autoantibodies (24). It was concluded that B cells can significantly inhibit the proliferation of activated CD4+ CD25^{-} T cells. Furthermore, the secretion of IL-10 and TGF-β are also involved in the regulatory effects of B cells. B cells from MS patients have a reduction in IL-10 production and an increased secretion of pro-inflammatory cytokines such as TNFα (2).

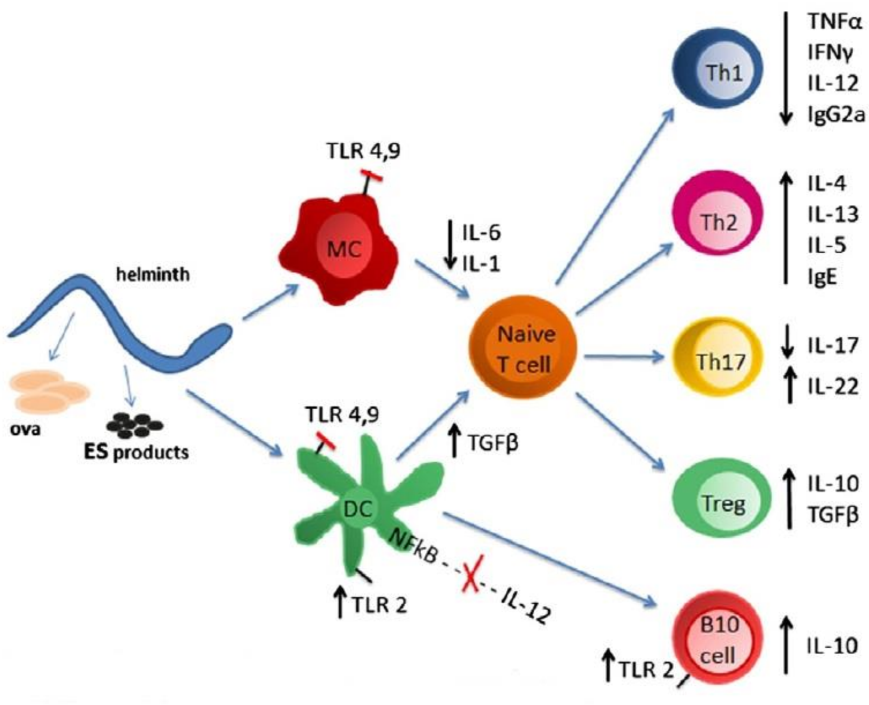

Figure 2. Immunoregulatory mechanisms induced by helminths. ***Adapted from Bashi et al., 2015***

Nevertheless, there are B cells which can downregulate immune response by generating regulatory cytokines and directly interacting to pathogenic T cells called B regulatory cells (Bregs) (24). The protective roles by helminths are important in the development of Bregs. Eventually, the increased production of IL-10 by Treg cells, Th2 cells, B cells and innate immune cells such as macrophages are involved in the helminth mediated the suppression of inflammatory diseases (25).

Current Helminth Therapy for MS

The treatment of MS currently focuses on immunosuppressive compounds (3), for instance laquinimob (4). Moreover, the treatment also focuses on recombinant interferon (IFN)-β (26). Laquinimob reduces the aggression of pathogenic effector T cells in the CNS tissue. It will lead to the alteration of Th1 and Th17 across the blood brain barrier (4). In addition, the treatment with recombinant IFN-β, type I IFN, is also used for the first-line treatment for RRMS. This treatment results in the induction of anti-IFN-β neutralizing antibodies (NAbs). The NAbs are associated with the diminish of disease activity in magnetic resonance imaging (MRI) and increased expression of the immunoregulatory cytokine IL-10 and FoxP3+ in Treg cells. Furthermore, IL-10 plays a key role in decreasing the disease activity as assessed by MRI (26). The experiment of these therapy for MS further investigated using animal models.

Experimental autoimmune encephalitis (EAE) is the most frequently studied animal model of MS (8). It is a T-cell mediated inflammatory diseases associated with the development of demyelinating lesions in the central nervous system (CNS). EAE model is used to study the effect of helminths and bacteria in RRMS (27), such as *Trichinella spiralis, Trichuis suis*, *Fasciola hepatica*, *Schistosoma japonicum* and *Schistosoma mansoni*.

Trichinella spiralis is a parasitic nematode targeting mammals which provokes Th2 and anti-inflammatory type responses in an infected host. The balance of Th1 and Th2 responses affect the outcome of MS. *T. spiralis* induce Th2 and suppress Th1 cell-mediated diseases. Furthermore, *T. spiralis* infection also suppress the production of IL-17 which is responsible for EAE development and initiation. Treg cells are considered as an important regulator of immune response. Additionally, Treg cells are responsible for modulation and suppression of immune responses for helminths infection. In the case of MS, Treg cells are able to prevent EAE severity in rats (28). Treg cells induced in *T. spiralis* infection elevate the levels of IL-10 and TGF-β. IL-10 plays role in recovery the EAE and TGF-β is important for the survival of FoxP3+ Treg cells (29).

Trichuis suis ova (TSO) uses vital eggs of non-pathogen parasite *T. suis* which was studied in animal. Intriguingly, *T. suis* can colonize humans, but there is no report to cause human disease (30). TSO was effective for inflammatory bowel disease (IBD) treatment (25). This beneficial effect in IBD underlies the next investigations using the same therapy for MS. Fleming and colleagues studied that after TSO administration in patients with MS, the new active MRI lesions are decreased. Most of the patients develop an anti-inflammatory response, which is associated with augmented serum levels of IL-4 and IL-10 (18). Furthermore, TSO infections in MS not only increase IL-10 producing Breg cells, but also escalate Treg cells, alternatively activated macrophages (31). TSO infections also induce the production of Th2 related cytokines like IL-4, IL-5, IL-9 and IL-13 which gives anti-inflammatory effect (31). On the other hand, other study reported that there is no significant change in anti-inflammatory IL-10 levels during TSO therapy (25). This is because IL-10 is not the dominant player in immune suppression by helminths. However, the increase of IL-4 after 2 months of therapy in this study is reported. Moreover, the number of CD4+ and CD8+ T cells are slightly decreased after 2 months of therapy (25).

Infection with *Fasciola hepatica* in EAE has been studied and it showed that *F. hepatica* attenuated the symptoms of EAE through TGF-β-mediated suppression of Th1 and Th17 response (22). Furthermore, FhHDM-1, a 68-mer peptide secreted by *F. hepatica*, has a protective effect in relapsing-remitting EAE by modulating the function of macrophages to interfere with the release of pro-inflammatory cytokines and prevents autoimmune disease (15).

Soluble egg antigen (SEA) form *Schistosoma japonicum* prevents EAE in animal models. SEA, a complex extract of soluble molecules from disrupted eggs, is able to induce Th2 responses. The mechanisms behind this still unclear. Preimmunization of mice with SEA from *S. japonicum* create a switch from Th1 to Th2 immune response after EAE induction. This probably due to the glycosylated carbohydrates from SEA which give immunological properties of egg antigens (32). Apart from *S. japonicum*, the mice infected with *Schistosoma mansoni* can reduce the incidence of EAE due to Th1 response downregulation, not the Th2 switching (32).

The study of helminths infection in patients with MS is still ongoing, for instance in *T. suis*, *F. hepatica* and *S. mansoni*. First, Rosche and colleagues investigated the efficacy and tolerance of TSO in MS patients. The outcome parameters fulfil the criteria for clinical phase II trial in MS. It was found that there are reductions of lesions in MRI under therapy with TSO in MS patients after three months of therapy (31). Secondly, FhHDM-1, a peptide secreted by *F. hepatica*, improve relapsing-remitting immune-mediated demyelination in MS patient (15). Eventually, SEA from *S. mansoni* modulates intracellular pathway leading to escalation of IL-10 and Treg cells development. It is indicated that IL-10 producing capacity by B cells are diminished in patients with MS. On the other hand, B cells isolated from MS patients infected with SEA from *S. mansoni* may produce higher levels of IL-10 compared to MS patients uninfected to helminth. IL-10 production by B cells can be induced by stimulation with sugar molecules present in *S. mansoni* eggs (33). Ultimately, the increased production of IL-10 by B cells will be responsible in the suppression of inflammatory diseases (25).

Helminth Therapy For MS In The Future

The beneficial effects of helminth infections in reducing the induction of auto-immunity have been proven in some studies in EAE. These discoveries are a great starting point in developing the potential of helminths as MS therapy in humans. However, more studies related to the safety and efficacy need to be further investigated to be implemented in MS patients.

TSO, for instance, has a well-tolerated administration in RRMS patients (18) since it can be administered for 12 weeks without safety concerns (30) and no early major toxicity observed (18). However, based on Voldsgaard and colleagues, it is indicated that neither clinical nor immunological outcome of TSO gives beneficial effects. This is probably due to the fact that patients in this study were exposed to other helminths (30).

Despite the beneficial effects of helminth infection as a therapy for autoimmune diseases, the drawbacks can be potentially harmful. There is a limitation from this type of therapy as helminths infection cannot specify the immune system and can potentially interfere the mode of actions in human's immune system. Thus, it can suppress the immune response that is required for immunity against other pathogens. On the other hands, helminths can also have an effect on the development of proper immune responses upon vaccination. Another safer and more effective alternative therapy is to deliver specific immune-modulatory molecules from helminth parasites. Thus, it will increase therapeutic effectiveness (15).

Conclusion

Helminths produce an exceptional potential to treat multiple sclerosis due to their ability to interfere with host's immune system. *Trichinella spiralis*, *Trichuis suis*, *Fasciola hepatica*, *Schistosoma japonicum* and *Schistosoma mansoni* are up to now to be promising helminths for reaching this purpose. Th2 responses, Treg cells and Breg cells are regulated by these helminths to give anti-inflammatory activity and at the end these mechanisms can be used as MS treatment. Although helminths therapy could be one of many options to treat MS, controversies still exist whether it is safe and effective for humans. Despite all beneficial activities generated by helminths for MS treatment, the safety and efficacy of this potential pharmaceutical application are crucial and need to be investigated prior to the implementation in humans. Furthermore, several factors like issues relating to *in vivo* stability and pharmacodynamics of helminth-derived molecules as well as delivery method to patients need to be studied in order to develop new therapeutic products.

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