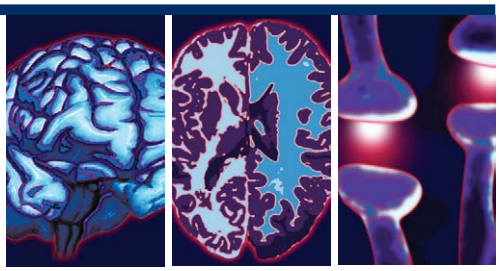
Review



Parasitic Worms for the Treatment of Neurodegeneration

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ABSTRACT

It is well established now that helminths mainly nematodes influence immune responses and provide protection and even inhibition of chronic inflammatory disorders. Nematode infection may be a consequence of the stimulation of specific immune responses including expansion of CD8(+) Tregs that actively contribute to a shift in immune response and gut microbiota. However, there is growing evidence for alternative CD8 (+) T cell fates influencing CD4(+) T-cell-mediated responses in the context of allergy and autoimmunity including neurodegeneration spectrum disorders. It seems to be that CD8 (+) iTregs play a unique role in restoring immune homeostasis. The article discusses the current status and prospects of clinical use of immunomodulatory parasitic worms in the treatment of autoimmune and inflammatory diseases, including MS and AD. Here, we present our current understanding of the diversity of CD8 (+) iTregs effector cells and possible contribution of nematode factors to this process.

Keywords

Chronic inflammatory disorders, Neurodegeneration, Parasitic infection, CD8 Tregs

Introduction

■ Worm Dependent Treatment Options for ADs

The phylum Nematoda comprises an ancient and biologically diverse group of moulting animals. It is the second largest phylum in the animal kingdom. Molecular phylogenetic analyses have found parasitism of animals to have arisen on at least five independent occasions in vertebrate hosts. Nematode infections in humans include ascariasis, trichuriasis, hookworm, enterobiasis, strongyloidiasis, filariasis, and trichinosis. These parasitic diseases, with the exclusion of filariasis, are provoked mainly in the gastrointestinal tract. Understanding the evolutionary origins of animal parasitism, and the mechanisms by which parasites locate and invade their hosts, avoid host immunity, and acquire nutrition are important goals, especially for medical and veterinary science [1].

Intestinal nematodes are highly prevalent in humans and animals. The modulation of the immune response in specific hosts by parasites allows them to survive for long periods. Nematodes have developed several effective mechanisms to regulate host immunity, thus ensuring a beneficial environment for survival with reduced morbidity and pathogenesis in the host. In many infections, parasite invasion is often unnoticed until dispersive forms appear**.** Upon infection, the larvae migrate to the targeted tissue, molt and, after relocation and settling in a particular part of the intestine, reproduce and lay eggs. After temporary localization of larvae in the tissue, an inflammatory response is elicited by antigens secreted by the parasite. The cells of the

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host innate immunity are vigorously entangled in the destruction of larvae [2].

Parasites are able to evade the action of the immune system. However, to explain their effect on innate immunity, which impedes development of effective adaptive immunity against adult stages, there is a need for greater research on parasite molecules. It seems that from the beginning of infection, nematodes are able to control the level of host inflammation by molecules expressed on their surface or by their excretory/secretory products, which have been described as a pathogen-associated molecular pattern [3]. A greater understanding of how nematodes afflict the immune cells of the host may allow the development of treatments that specifically interfere with inflammatory disorders. Parasitic molecules could operate as ligands for extracellular and intracellular receptors in antigen-presenting cells and regulate the activation of dendritic cells (DC) [4]. Depending on the strength of antigen signals and molecular characteristics, dendritic cells may induce protection or tolerance to ongoing infection. The affinity of the T cell receptor (TCR) to antigen, the strength of the co-stimulatory signals provided by antigen-presenting cells and cytokine production, DCs activated by parasitic antigens might induce $CD4^+$ cells that expand as either specific T helper or T regulatory cell populations. In inflammation that is not fully developed, e.g. in *milieu*, when innate immunity is not fully expressed, DCs are arrested in a semi-mature state and promote the development of naive T cells into Treg cells. Ultimately, these cells induce a suppressive environment [5].

The regulation of immune response is not only restricted to infective larvae; it is also valid for all parasite stages, which may use their own specific molecules to bring about the most advantageous circumstances for successful invasion, migration, settlement, development or reproduction [6]. Common molecules typical for PAMP, which are present in the tissue invaded by larvae, may be crucial for the induction of a long-lasting tolerance required for incoming stages of the nematode and which would optimize the conditions for most abundant infection.

T-regulatory cells are primary generated in the thymus (tTreg), but also could be generated extrathymically at peripheral sites (pTreg) or induced in cell cultures (iTreg) in the presence of transforming growth factor β (TGFβ). Two cytokines: TGF-β and IL-10 have been identified that play key roles in the expansion and support of Treg cell function: these cytokines play the most crucial role for the development of a tolerogenic environment, both locally and systematically [7]. TGF-β promotes the induction of iTreg cells, accompanied by an increase in the expression of Foxp3, and Foxp3 (+) CD4 (+) cells, and increases the expression of CD25 and the CTLA-4 receptor to regulate the immune response [8-10]. CD4 (+) CD25 (+) Foxp3 (+) Treg suppress effector CD4 T cell activation and proliferation [11,12]. In nematode infection de novo Foxp3 is expressed in T cells along with IL-10 and TGF-β production and greater expression of CTLA-4 [13]. This cytokine is important for the resolution of nematode infection, and the neutralization of TGF-β in mice with monoclonal antibodies resulted in marked changes in the course of nematode infection accompanied with cytokine production. The nematode suppressed the immune response through pathways involving TGF-β, and activity of Th2-related immune response was not a target upon cytokine neutralization [14]. The induced Treg cells express their suppressive activity by influencing the production of TGF-β, IL-10 and IL-35 [15,16]. IL-10 acts as a positive autocrine factor in the development of IL-10-producing Treg cells [17]. The cytokine reduces the expression of major histocompatibility complex type II (MHC II) and the co-stimulatory molecules CD80/CD86, and inhibits cytokine production [13]. Following exposure to the action of IL-10, CD4 T cells become unresponsive to antigens, and are unable to produce cytokines and reach a state of anergy [18,19]. T regulatory cells create an immune homeostasis in filarial infections in an IL-10-dependent and dominated environment [20].

Long-term, and usually asymptomatic, infections with Ascaris, hookworm, and Trichuris species in human are significantly linked with hyporesponsiveness associated with regulatory cell activation, IL-10 and TGF-β production and a higher frequency of circulating CD4(+)CTLA-4(+) T cells [21-23]. It is clear that infection with nematodes exploits the TGF-β pathway to suppress host immunity and it is postulated that an immunoregulated state is a common outcome across a diverse range of helminths: a diverse group of multicellular parasitic worms divided into two major phyla, the nematodes and the platyhelminths [24,25]. An immunosuppressive environment might be also created by parasitic nematode proteins with enzymatic activity

[26]. Many proteins of different families have been found to have intense proteolytic activity and these may be involved in many evading mechanisms, also resulting in the degradation of cytokine, antibodies or receptor specificity. The structure of parasite molecules is a key factor in their immunoregulation; however, few studies have proposed nematode molecules with such potential and their function remains poorly known.

The pathogenic cause of autoimmune diseases is currently unknown. However, it is widely believed that susceptibility to autoimmune reactions is multifactorial and includes genetic predisposition, gender, ethnicity, age and environment. While no single factor has been identified as preeminent, the role of the environment has garnered increasing interest, as postulated by the National Institute of Environmental Health Sciences (NIEHS). The increasing incidence of autoimmune diseases, with their high prevalence in Western countries and rapid growth in hitherto low prevalence countries like Japan, has led to a range of explanations based around environmental triggers [27]. One such explanation is the "Hygiene Hypothesis", a term first used by Strachan in 1989. This hypothesis proposes that exposure to pathogens during childhood is essential for immune system development. The "Old Friends Theory" or the "Biome Depletion Theory", is an extension of the Hygiene Hypothesis [28], which proposes that the long association between host and parasite has resulted in a coevolutionary dependence, where parasitic helminths rely on their hosts for nutrients and a niche, and the host immune system is primed to expect signals from helminths [29]. However, until now, helminth infections have not been demonstrated as necessary for proper host immune maturation in either humans or in animal models, and co-evolution over millions of years involves a form of mutualism where both the host and the parasite derive some benefit from their relationship. In such long-lasting infections, immunosuppression is obviously beneficial for the parasite: it prevents parasite death and expulsion, improves the fitness of the parasite and benefits the host through inhibition of inflammatory reactions preventing local and peripheral pathology generated by itself and otherwise innocuous antigens [30].

Based on the observation that treatment of patients in helminth-endemic countries with anthelminthic drugs lead to exacerbated allergic disease, and conversely, an infection of allergic patients with helminths ameliorated allergic symptoms, inferred that nematode exposure could prevent autoimmune diseases [31]. Asa consequence, gastrointestinal nematodes are currently being evaluated as a novel form of therapy in the treatment of chronic human inflammatory disorders, due to their unique ability to induce immunoregulatory pathways in their hosts. The most exciting and promising option in the field is based on the identification and characterization of immune targets as being highly specific to well-tolerated molecules involved in the interaction between parasites and their hosts.

Clinical data, experiments in NOD mice and other animal models of inflammatory and autoimmune diseases have shown that nematode infections may not only prevent but also reverse allergy and asthma and autoimmune diseases such as inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis (UC), Type-1 and -2 diabetes (T1D, T2D), multiple sclerosis (MS), atherosclerosis and celiac disease by influencing both innate and adaptive immune reactions [32,33]. One of the advantages of helminth-induced immunosuppression is that it has the bystander effect of attenuating dysregulated and pathogenic immune responses to allergens and autoantigens. Consequently, individuals infected with helminth parasites have reduced susceptibility to developing allergies, asthma and autoimmune diseases. Th2 cell and forkhead box protein 3, ($Foxp3^+$) regulatory T cell expansion is associated with downmodulation of Th1 and Th17 responses and is the most common characteristic shared across different worm infections. However, the proposed induction of Th2, Treg, regulatory B (Breg) cells, regulatory macrophages (M2, alternatively activated AAMφs) and regulatory dendritic cells (DCs) cells provoked by infection only partly explains the beneficial effects in patients with such conditions as UC and MS [34,35].

For now, research into worm-dependent treatment options has focussed on three options: Helminth therapy (HT), Helminth-derived product therapy (HDPT) and Helminth-induced immunoregulatory pathways (HIIP).

Trichuris suis ova (TSO) and the haematophagous hookworm *Necator americanus* were proposed as improved and effective HT treatments for inflammatory bowel disease

and MS, and two clinical trials have been initiated: NCT01040221 and NCT01070498 [36,37,38]. Although the results obtained from a small number of patients have indicated that TSO is safe, the findings are not conclusive. One recently published study did not find any such therapeutic effects [39]. Another reports clinical improvement, but amelioration of symptoms was only seen when the infection was present and removal of the parasites resulted in the remission of disease pathology and the inhibition of immunomodulatory response [33]. In addition, many patients feel uneasy about receiving live worms for therapy.

Aside from the ethical concerns, there are many practical considerations that may reduce the efficacy of this approach and even provoke pathological consequences, especially in an immunocompromised host [40]. Nematode L4 larvae invade tissues, exhibit aberrant migration in the human host and influence the physiology of their respective niches, and even small numbers of hookworms induce gastrointestinal pain in the early stages of infection. Live parasite infections result in the induction of danger signals and pro-inflammatory stimuli, thus leading to inflammation. Furthermore, the host is exposed to the full spectrum of helminth-derived products including potent antigens, inflammatory stimuli and potentially disease-causing allergens in addition to the desired helminth immunomodulators. In addition, for safety reasons, the trials used the minimum number of larvae, and potential clinical benefits could have been missed. It is also very difficult to conduct these trials using proper placebo-based controls as early infection is associated with obvious symptoms.

It has been argued that treatment of patients with living nematodes has disadvantages. Helminths can influence drug efficacy by modulating the host immune response, and colonization may worsen other pathogenic infections in immunocompromised hosts [37]. In addition, in order to survive for a long time in an adverse and aggressive environment, the nematodes secrete several soluble factors that interact with host cells; this may modify host-cell homeostasis and increase susceptibility to oncogenic transformation [41]. Factors secreted by helminths could be involved in neoplasma promotion and progression: *Schistosoma haematobium, Taenia taeniaformis*, *Spiromera mansonoides,* and *Taenia solium* all have significant tumor-promoting activity [42]. Excretory-secretory (ES) products from the small intestine nematodes Trichostrongylus vitrinus, Trichostrongylus colubriformis, Cooperia curticei, Nematodirus battus and the abomasal nematode Teladorsagia circumcincta have all been shown to produce over-proliferation in normal intestinal epithelial cells and/or cell lines [43]. Additionally, it has been found that intestinal nematode larval adaptation and growth significantly increase in mice with colitis [44,45].

An important area of HDPT study is the characterization of the key molecules responsible for immunomodulation. These could be used as drugs or as structural templates for future drugs with therapeutic potential to control inflammation and autoimmune diseases. It has been demonstrated that infection with live nematodes is not a prerequisite for suppression of inflammation: Treatment with soluble products from the nematodes *T. suis* and *Trichinella spiralis* also induces significant suppression of symptoms in murine EAE and colitis [46,47]. The best known example is the filarial molecule- phosphorylcholine product ES-62 from *Acanthocheilonema viteae*. ES-62 modulates dendritic cells and macrophage activity in a TLR-4 dependent manner and attenuates the symptoms of CIA, AHR, and DSS-induced colitis [48-50]. Other filarial *A. viteae* molecules (AvCystatin) have been shown to prevent asthma and colitis in animal models of the disease by induction of macrophage IL-10 production [51] as a consequence of phosphorylation of the mitogen-activated protein kinase signaling pathways ERK1/2 and p38 by macrophages [52]. The recombinant 53kDa protein from *T. spiralis* prevented experimental colitis in mice and upregulated Th2 and regulatory cytokines while downregulating the activity of some Th1 cytokines [53]. More potential options are discussed in many excellent review articles [54-57]. However, the results of studies on the use of single immune-active components isolated from nematode products as potential drugs are not as meaningful as previously believed. This comes as no surprise for several reasons: The live nematodes express and secrete copious quantities of antigens into host tissues with different immunomodulatory properties and the immunomodulatory effects must be multiple and complex. These mixtures of proteins might aid worm survival in a number of ways, minimizing or interfering with inflammatory processes, and selectively skewing the phenotype of the generated

immune response [58].

The protective immune responses to native antigens have been difficult to replicate using recombinant antigens with incorrect conformations and lacking post-translational modifications produced by most popular artificial expression systems such as bacteria and yeast. Furthermore, the use of single defined immunomodulatory products as therapeutics is doomed to failure as these can be neutralized and rendered ineffective by the host immune response.

HIIP studies aimed at the identification and trial of key helminth- derived immunomodulatory molecules and identification of therapy targets according to might provide focused safe therapy. This is a relatively recent approach developed as a result of long-term study of the topic. Recently Terrazas et al. [59] identified the critical role played by PD-L1 (+)/PD-L2 (+) alternatively activated macrophages in the regulation of autoimmune disorders following tapeworm *Taenia crassiceps* infection. Adoptive transfer of PD-L1 (+)/PD-L2 (+) AAMϕs into mice in whom experimental autoimmune encephalomyelitis [60,61] had been induced reduced disease incidence, delayed disease onset and reduced clinical disability [59]. The PD-L1 and PD-L2 macrophages recognized and activated by AvCystatin **of A**. **viteae** have also been found to transfer adoptive protection against allergy and colitis [62]. At this aspects clinical trials use macrophages to treat MS could be expected. In our last study, an induced population of CD8 regulatory T cells has been proposed as participating in the intervention by the intestinal nematode *Heligmosomoides polygyrus* in chronic EAE in mice [63]. We found that infection with *H. polygyrus* (*H.* **polygyrus** as a useful laboratory model for the hook worm *N. americanus* infection study [55]) dramatically diminished EAE symptoms at the early pre-patent phase, while nematode L4 stage colonized the small intestinal wall. At this stage of exposure to L4 stage we observed extensive leukocyte infiltration into the CNS, consisting mainly of CD8 (+) CD25 (+) (IL-122) FoxP3 (-) T cells [63]. In the cerebrospinal fluid (CSF) of EAE mice infected with nematode, CD8 T cell subpopulation contained several times more CD25 expressing cells than EAE control mice that were not exposed to nematodes. The accumulation of CD8(+)CD25(+)FoxP3(-) T cells in the CNS correlated with visible regeneration of brain edges and an increased quantity of blood vessels, reduced proliferation of T cells and reduced demyelination, as well as an increased concentration of nerve growth factor (NGF) [63] vascular endothelial growth factor (VEGF) and myelin basic protein (MBP) (unpublished data). Interestingly, nematode infection in unimmunized mice did not enhance the proportion of the CD8(+)CD25(+)FoxP3(-) T cell population, which can confirmed the regulatory potential of myelin/nematode-specific CD8(+) CD25(+)FoxP3(-) T cells in inflammation. Enhanced expression of a CD8(+) regulatory T cells in the intestine of *H. polygyrus* infected Rag mice (T and B cell deficient) with piroxicam-induced colitis was reported previously. *H. polygyrus* required total CD8 (+) T cells *in vivo* to reverse the disease process and the regulatory cells required cell contact but did not need IL-10 or TGF- signalling through the T cell [64]. Although much is to determined, the above observation reveals that the suppression of this autoimmune disease by parasitic worms is strongly associated with the induced CD8 (+) regulatory T lymphocytes and, importantly, the CD8 (+) lymphocyte subpopulation induced by helminths seems to be different from Tregs CD8 (+) physiologically induced by regulatory cytokines [65,66].

CD8(+) Tregs In The Treatment Of Neurodegenerative Spectrum Disorders

Classical CD8(+) T cells serve as a critical component of the cellular immune response. In particular, cytotoxic T lymphocytes (Tc1 cells) play a crucial role in the control of viral infection and the elimination of cells with malignant potential, and constitute the best characterized subpopulation of CD8 (+) effector T cells.

Similar to CD4(+) Treg cells, CD8(+) Treg subpopulations and their mechanisms of suppression are quite heterogeneous, and a small number of experimental models have confirmed that the phenotype and activity of diverse CD8(+) T cell subsets differentiate in accordance with the physiological state of the body and the needs of homeostatic protection. One relatively well-defined CD8 Treg cell subset comprises CD8(+) Treg cells restricted by the Qa-1 (mouse) or HLA-E (human) non-classical MHC class Ib molecules. The cells recognize peptides complexed with Qa-1 molecules expressed by effector CD4 (+) T cells, and then eliminate the CD4(+) T cells in a perforin-dependent manner [67-69]. The Qa-1-restricted

CD8(+) Treg cells, representing 3–5 % of all CD8(+) T-cells, become functional after re-encountering the antigen, suggesting that it may involve differentiation of the CD8(+) T cells to cytotoxic T cells.

A second subpopulation that has been described in some detail includes CD8 (+) CD28 (-) CD152 (+) CD62L (+) T cells. This subpopulation expresses the Foxp3 **transcription** factor. Functionally, it targets APCs and renders them tolerogenic. The exposure of APCs to this subset of T cells results in increased expression of genes encoding the Ig-like transcripts ILT3 (CD85K) and ILT4 (CD85D), these being members of the NK-cell inhibitory-receptor family associated with inhibition of NF-kB activation. They also reduce the capacity of APCs to transcribe NF-kB- dependent costimulatory molecules [70].

Another recognized subpopulation of CD8 (+) Tregs produces IL-10 and expresses high amount of CD122, the IL-2 receptor β-chain. These CD8 (+) CD122 (+) Tregs cells are Foxp3 (-). The percentage of CD8 (+) CD122 (+) T cells is high (50%) in young mice; however, this value decreases to 10% at 7–10 weeks of age, before increasing again in older mice [71]. As CD8 (+) CD122 (+) T cells inhibit the activation of T cells *in vitro* in the absence of APC [72], it can suggest that the IL-10 produced by CD8 (+) CD122 (+) T cells is responsible for suppression of proliferation and IFN-γ production.

CD8(+) iTreg cells also use various other mechanisms for limiting the activity of CD4(+) T cells, including those based on indoleamine 2,3-dioxygenase (IDO), a potent inhibitor of tryptophan metabolism [73].

Studies on the role of Treg in autoimmune disorders were largely limited to CD4 (+) Tregs; however, recent studies have shown the participation of CD8 (+) Tregs in immunoregulation in these groups of diseases [74]. Genetic associations between MHC class I alleles and MS have now been established, and both CD8 (+) and CD4 (+) T cells have been found to invade and clonally expand within inflammatory plaques in the central nervous system. Interestingly, the depletion of total CD8 (+) T cells from mice following recovery from EAE renders them susceptible to the development of EAE upon reimmunization with myelin basic protein (MBP); in addition, CD8 PL/J mice develop more chronic EAE than wild-type PL/J mice. The mechanism of suppression used by these CD8 (+) T cells is unknown; however, it has been suggested that it may involve the differentiation of the CD8 (+) T cells into suppressive/regulatory T cells, whose function is restricted by HLA-E (MHC-1) [75].

Recently CD8 (+) Tregs have been closely associated with disease protection and recovery from EAE in mice. A study based on an autoimmune EAE model with experimental colitis and cGVHD identified autoregulatory CD8 (+) Treg cells with low Foxp3 expression that demonstrated *in vitro* and *in vivo* suppression activity equivalent to, or even better than, CD4 (+). The activity of CD8+ iTregs is dependent on cell contact, as the cells do not express granzyme A, B or perforin A [65].

Among the phenotypic markers expressed in CD8 (+) Foxp3 (-) cells, αEβ7 integrin-CD103 expression is crucial for the generation and function of this lymphocyte subset and both IL-10 and TGF-β signals were found to mediate the suppressive effect of this population [66]. The lack of detectable expression of Foxp3 does not affect the effector functions of this lymphocyte subpopulation. $CD8^{+}Foxp3^{+}CD103^{+}$ iTreg and CD8 (+) Foxp3 (-) CD103 (+) iTreg have been found to share a similar capability to suppress the Th cell response [66]. Recent research based on a chronic graft-versus-host disease model with typical lupus syndrome found that CD8 (+) CD103 (+) iTreg cells suppress not only T helper cells, but also B cell responses which involve both TGF-β and IL-10 signals [76].

Dysfunction of CD8 (+) Tregs in humans has been implicated in the development of MS [77]. In the peripheral blood of MS patients, CD8(+) Tregs were identified which killed myelin-reactive CD4 (+) T cells by a granule-mediated cytolytic process and which were restricted by the non-classical MHC class Ib molecule HLA-E during exacerbations of MS [38,78]. Confirmation of the role of CD8 (+) in the pathomechanism of MS may be the FDA approved glatiramer acetate (GA), a synthetic copolymer activity, for the use in immunotherapy of MS: The substance is known to induced CD8 (+) iTregs upregulation which killed GA-loaded CD4 (+) T cells in a perforin-dependent manner [79].

However, further clarification is required of the mechanisms invoked by CD8 (+) Treg cell subsets, including a population induced by nematodes under EAE autoimmune inflammation conditions. CD8(+) Treg cells can play a number of other unique roles in nematode-induced immuneregulation; for example,

CD8(+) T cells can upregulate the neuronal expression of vascular endothelial growth factor (VEGF) in the CNS, thus facilitating disruption of the blood-brain barrier by a process requiring perforin expression [80]. Interestingly, the level of CD8(+)CD25(+)FOXP3(-) iTregs induced by intestinal nematode has been found to correlate with inhibition of EAE relapses and greater BBB permeability [63], as well as an increased number of veins in the brain (our unpublished data). In a controversial hypothesis, Zamboni proposes that chronic cerebrospinal venous insufficiency (CCSVI) is a causal factor of multiple sclerosis (MS): CCSVI has not been associated with other neurological conditions or observed in healthy controls, and it has been found to have a significant impact on brain pathophysiology, particularly the balance of intracranial fluids [81]. Further studies are necessary to understand the possible mechanisms behind these processes including parasites and possible behavioural consequences.

Chronic Inflammation, Autoinflammation and Autoimmunity in Neurodegeneration

In many CNS diseases, neurological inflammation is an important disease-enhancing factor; as documented in amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), disease (AD), and also in the remitting-relapsing multiple sclerosis (RRMS) [82,83]. Chronic, recurrent or persistent neurouinflammation could lead to autoinflammation in genetically predisposed people, in which inflammatory reactions are self-sustaining, and the primary triggering factor is no longer involved. An exemplary molecular mechanism of self-sustaining inflammatory reactions could be a vicious cycle of TLR-RAGE receptor activation, discussed in AD [84].

Autoinflammatory syndromes are characterized by the innate immune system hyperactivation which could leads to damage to cells and tissues, and eventually, to cell death, There are several categories of autoinflammatory diseases, caused by polymorphism and mutations of genes associated with an innate immune response, and which define persons susceptible to the development of auto-inflammation, including neuroautoinflammation [85,86]; among them, already recognized genetic defects lead to impaired activation of IL-1β (inflammasomopathy), NF-κB activating syndromes, toTNF-α relaxation disorders, complement cascade regulation and macrophage activation [86]. The polymorphisms and mutations of the NRLP3 inflammasome-related genes are relatively well-known, result in permanent overactivation of IL-1β that stimulates and sustains inflammatory reactions. In subjects with such defects in immune regulation auto-inflammation can be induced and sustained even without the involvement of infectious agents [87]. However, a number of factors, including psychological stress, trauma, cold exposure, and improper diet, may trigger some of these illnesses, but in all cases the expression of inflammatory symptoms refers to people with genetic predisposition for autoinflammation [86]. It is now clear that the neuroinflammatory reactions carried out by the innate immunity system primary by microglia and astrocytes, are pathogenetically important in AD and belong to the central mechanisms that determine the progression of the disease [82,87].

Excessive activation of the innate immune system leads to hyperactivity of the adaptive response system, because both systems work closely together in both health and disease conditions. Therefore, the analysis of neuroimmune axis abnormalities in neurodegenerative syndromes must consider both the neuroinflammatory mechanisms (excessive activation of the innate immune system) and the autoimmune response (hyperactivity of the adaptive immune system) as important components jointly responsible for the progression of the disease [88, 89]. Although autoimmunity is conceptually perceived as a defect in the immune system of B and T lymphocytes that recognize and respond to own body antigens and may lead to tissue damage in autoimmune diseases, however, it should be emphasized that low self-immunity is present in all people in health conditions and plays an important, beneficial role in fine-tuning to the needs of an adaptive immune response [90]. For example, the autoimmune recognition of MHC molecules on those pre-T cells whose TCR receptors generate a vigorous but not excessive signalling response to foreign antigens is important to accelerate the maturation and proliferation of the effector lymphocyte clone in the early stage of infection when the availability of the foreign antigen is relatively low, poorly recognizable [91].

Multiple line of evidence suggest that under neurodegenerative condition circulating myeloid cells penetrate to the brain through the brain choroid plexus gateway (the epithelial layer that forms the blood-brain barrier) enabling recruitment of monocyte-derived macrophages,

T lymphocytes and neutrophils to help fighting off AD-connected pathology [92-95]. Importantly, it was found that circulating CD4 (+) lymphocytes capable of recognizing their own brain antigens support neural plasticity exert a protective, renewing and regenerative effects on brain cells in neurodegeneration and also potentiate blood-borne monocyte transmigration through the blood-brain barriers [96-99]. The name "protective autoimmunity" was proposed for these autoreactive CD4(+) cells, emphasizing the beneficial function of T cells recognizing their own cerebral tissue antigens (internal T cell threats) and distinguish them from T lymphocyte subsets that recognize non-self-antigens, that fight external danger [98,99]. Autoimmune responses to brain antigens are rigorously controlled, however, in the case of a significant increase in exposure of their own antigens (e.g., extensive destruction and death of cells) or due to regulation disorders within the lymphocyte subpopulation an increased autoimmune reactions may be damaging to the brain tissue.

Although autoinflammatory and autoimmune disorders are currently subdivided into two different groups, however, similarities in their course, numerous mutual regulations/activations and close cooperation in immune responses suggests that they should be treated as one common group of immune response diseases with a large spectrum of immunological abnormalities that together determine the progression of neurodegeneration [88]. For the above reasons, new directions of therapy should aim both at anti-inflammatory effect and, simultaneously, at lowering the level of autoimmune reactions for effective control of immunopathological components of neurodegeneration. At the same time, the applied therapy cannot inhibit important homeostatic aspects of inflammatory and autoimmune reactions (neuroprotection, neuroregeneration, cell differentiation, plasticity of neurons), therefore precise tuning of immune responses instead of total inhibition should be the recommended choice for effective treatment of neurodegeneration.

Neuroinflammation and Autoimmunity in Ad

Significant impact of neuroinflammatory reactions on the progression of AD symptoms is already well documented [82-87], whereas the contribution of autoimmune reactions, in particular dysregulations of lymphocyte subsets in these syndromes are just beginning to be recognized. Among them, it was shown that the blood serum of AD patients contains significantly higher antibody titers against Aβ 1-42 and RAGE proteins (3-4 fold higher, compared to age-matched control group) [100]. Because the leakage of the blood-brain barrier is a typical feature of an aging human brain that strongly contributes to the progression of AD [101,102] it is not surprising that the brain parenchyma contains the anomalous presence of blood-derived IgG, which are particularly close to neurodegenerative and apoptotic neurons [103]. In addition these IgG-positive damaged/dying neurons contain also classical complement components C1q, C5b-which were spatially more associated with reactive microglia [104]. IgG immunoreactivity, particularly pronounced near damaged neurons and its association with complement activation strongly suggests the involvement of autoimmune mechanisms in neuronal cell death in AD [103-105].

Several papers reveal that the total number of circulating CD4(+) and CD8(+) lymphocytes does not vary in AD compared to age matching healthy controls [106]; on the other hand, changes of specific subsets of cells are evident; for instance increased number of circulating CD8(+) CD71(+) cells and CD8(+)CD28(+) cells were reported in AD, although did not correlate with the disease severity [107,108]. In patients with moderate disease state according to MMSE scale (10-25 points) the percentage of CD8(+) CD27(+)CD28(+) as well as CD4(+)CD27(+) CD28(+) was decreased while those of CD(8+) CD27(-)CD28(-) and CD4(+)CD27(-)CD28(-) was decreased in AD [108,109]. Similarly, it was documented that the proportion of activated HLA-CR(+) lymphocytes CD4(+) and CD8(+) was elevated in peripheral blood of mild (MMSE ≥19) AD patients [106]. In cerebrospinal fluid of AD patients the proportion of activated CD4(+) and CD8(+) lymphocytes was increased and was correlated with neuronal damage especially in the hippocampal region [106], also CD45(+) IL-17(+) lymphocyte level in CSF were elevated in demented AD patients [108].

As established on the mouse model of AD, amyloid-specific Th1 cells have enhanced the progression of neurodegeneration. [27,29,30]. In particular the number of lymphocytes that release IFNγ, increased in the brain of APP/PS1 mice with animal age, and was associated with glial activation, enhanced cytokine release, accumulation Aβ and reduced synaptic plasticity

[110,111]. In contrast, the transfer of Aβ-specific Th2 cells to 11-month APP / PS1 mice improved cognitive function and decreased amyloid deposition [108,112], which was consistent with other papers that indicate a beneficial effect of IL-4 on cognitive function [113,114]. However the effect was age-related in mice and in aged mice Th2 cells could increase neuroinflammation which reduced neuronal plasticity and cognitive function [115]. A beneficial impact of Tregs systemic transplantation on neurodegeneration in APP/PS1 mice comprise reduced plaque deposition, decreased soluble Aβ and lowered microglia activation and enhanced cognitive function [108,116]. On the other hand, the higher frequency and elevated Treg damping activity is a common feature in older people, which can be interpreted as contradictory to the generalization of Treg's beneficial role in neurodegeneration [117]. It was also determined in the 5XFAD AD mouse model that transient depletion of the $Foxp3^+$ Tregs content or pharmacological inhibition of their activity leads to clearance of Aβ platelets, decreased neuroinflammatory response and improved cognitive functions [118]. This group of authors strongly suggests that transient suppression of Tregs improves the function of the choroid plexus barrier and facilitates the transmigration of effector lymphocytes to brain parenchyma where they reveal their neuroprotective and neuroregenerative functions [118-120].

Undoubtedly, further research is necessary to explain the role of Tregs in AD. Particular attention should be paid to the considerable heterogeneity of phenotypes and functions in the Tregs subpopulation; both CD4 (+) Tregs and CD8 (+) Tregs contain lymphocytes that differ phenotypically, secrete different cytokines and acting on different phases / stages of the immune response.. For instance, it seems that the CD8 (+) Tregs target activated T cells whereas the nonspecific CD4 (+) Tregs primary suppress the naïve or unstimulated T cells, and experiments in mice suggest that CD8 (+) Tregs kill only activated but not resting T cells [121,122]. Importantly, CD8 (+) Tregs preferentially suppress Th1 cells not Th2 cells, thereby shifting the overall immune response from self-antigen reactive IFN-γ-producing Th1 cells to IL-4 and IL-10 –producing Th2 cells, which are considered non-pathogenic , potentially beneficial in AD [121,122]. Thus, one can conclude that CD8 (+) Tregs control autoimmune response by regulating the Th phenotype of CD4 (+) cells. The considerable level of complexity of the Treg lymphocyte subgroup, the differences in their function and mutual interactions, indicate that the therapeutic strategy of immunomodulation in AD should achieve the effect of fine tuning the lymphocyte subpopulations. It seems a promising use for this immunomodulation of the work of our "old friends": gut microbiotic bacteria and parasitic helminths.

Helminth parasite infections exert a broad spectrum of host immunity modulation that affects almost all facets of the immune response. The host can benefit from suppression of allergic, inflammatory and autoimmune reaction [123,124]. Nevertheless, helminth infection may also be harmful to the host, increasing the susceptibility to the coinfection (e.g. Mycobacterium tuberculosis, malaria etc), the potential decreasing tumor immunosurveillance, secretion of potentially procarcinogenic factors and increasing low-grade inflammation potentiate in tumor growth [123]. Most experimental studies suggest that the beneficial effect of parasitic worm infections is by inhibiting the Th1 inflammatory response, and because many modern vaccines are aimed at inducing Th1, therefore helminths infections significantly reduce the effectiveness of vaccination [123]. Evidently helminths may not be suitable for all patients, for instance some helminth infections increased inflammatory response in infected humans [125]. Different reactions of some people to parasite infections may depend on the type and amount of parasites, the way/method of infection, but also to a large extent depends on individual characteristics of host's immune response ("i*mmunome*"), also on whether it was the first infection or further infections with parasites [124].

In order to avoid the potentially harmful effect of parasitic infections on the human body, protect the body against harmful substances expelled by parasites, but at the same time to broadly expose the immune system to the repertoire of parasite antigens and released products, an *ex vivo* procedure can be used. Lymphocytes isolated from the patient's venous blood will be cultured in the presence of parasites separated from them by the polycarbonate membrane. Then, after thoroughly washing the cultured cells, the patient can be re-injected intravenously with lymphocytes containing subpopulations stimulated by antigens and parasitic products. It seems that the proposed procedure is more secure and can also be used in patients with advanced AD dementia.

Although the molecular mechanism of synaptic damage and neuronal loss in Alzheimer's disease (AD), Parkinson's disease dementia (PD) and Lewy body dementia (LBD), frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) is poorly understood and differ among different types of neurodegenerative processes, however, the presence of neuroinflammation is a common feature of all these dementia. In the advanced stage of neurodegenerative diseases of the late onset, both innate and adaptive immunity are key determinants of the progression of clinical symptoms of neurodegeneration. Therefore, it can be suggested that immunomodulation of chronic inflammation along with attenuation of humoral and cellular autoimmune reactions may be a universal strategy aimed at suppressing the progression of clinical symptoms and improving the current neuronal function in various neurodegenerative diseases [120]. A promising direction for the development of symptomatic neurodegenerative therapy may be the use of immunomodulatory capabilities of our "old" friends" - parasitic worms and intestinal microflora. Both intestinal bacteria and parasitic worms have evolved together with the immune system of mammals for millennia and have become equisitely powerful immunomodulators, capable of altering and suppressing host immune responses, contributing to slow down excessive inflammatory and autoimmune responses [126,127]. More recent studies also show that the interaction between intestinal parasites and intestinal microflora significantly changes their immunomodulatory capacity; microflora help helminths modulate host immunity [128-130]. Presumably human lymphocytes after the *ex vivo* cultures in the presence of intestinal parasites and gut microbiota ("ménage à trois" system) will be more beneficial for the treatment of patients with dementia. Undoubtedly, many basic and preclinical studies must precede the development of procedures and recommendations for the treatment of late-onset neurodegeneration in humans with the help of parasitic worms and intestinal microflora. It seems, however, that this can be a very promising universal therapy, as the dysregulation of inflammatory and autoimmune reactions is significantly present in various neurodegenerative diseases.

Conclusion

The treatment of MS and other autoimmune diseases improved greatly during the second half of the 20th Century, with a number of improvements seen in the standard of care; however, the new treatments do not address all patient needs. Although corticosteroid drugs for treating MS relapses are universally available, the availability of expensive second-line therapy is more limited, being restricted to approximately 50% of countries, almost all of which are in the higher income groups.

As patients present differently, very little is known about the pathophysiology of the disease, which hinders the identification of targets and pathways for new drug development. Although CD4 (+) Foxp3 (+) regulatory T cells (Treg) serve as a key part of autoimmunity prevention, their therapeutic effect on neurodegenerative disorders remains unsatisfactory. Therefore, there is considerable incentive to describe the cellular and molecular mechanisms involved in neuroimmune diseases, as well as their response to nematode therapy. Future studies characterizing the functions and mechanisms controlling the transitions of CD8(+) effector cells to induced CD8(+) regulatory cells, the effector-regulatory molecules that induce CD8 Tregs, as well as their interactions with other cells, could lead the way to devising new, real therapeutic options for the modulation of immune responses. As nematodes represent a potential target for parasite-derived molecules, it is highly desirable to obtain a detailed description of the process of inflammation regulation by nematodes at the cellular and molecular level. In this article, we presented literature evidence showing a significant contribution of autoimmune mechanisms and inflammatory reactions that worsen the progression of clinical symptoms of dementia caused by AD. It has been confirmed that the subpopulation of CD8 (+) Treg cells is particularly promising in obtaining an effective suppression of the autoimmune and inflammatory response. Based on the recently published paper [63] the Polish research team has developed a system to obtain a Treg CD8 (+) subpopulation after exposure of animals to infection of intestinal nematode. The nematode-induced CD8 (+) Treg seems to be very strong suppressors of the **autoimmune** response: a very small dose of these cells was sufficient to strongly modulate EAE disease severity in vivo. If this method is also effective in an ex vivo system, it will be possible to use it in the treatment of patients with advanced AD dementia. Given the beneficial effects of parasitic worm infections in many patients with autoimmune diseases in MS, one can hope that the proposed treatment

method will bring significant improvement in neurological function and immune status in patients with AD.

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