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Purely systemically active anti-inflammatory treatments are adequate to control multiple sclerosis

Abstract Collective evidence supports the notion that multiple sclerosis is principally an autoimmune disease. Much of it stems from models of experimental autoimmune encephalomyelitis, generated by inoculation of animals with central nervous system antigens such as MBP, PLP, S100 and MOG or peptides thereof. Different ways of immunization and different animal species and strains mirror different aspects of the neuropathology of multiple sclerosis, such as inflammation, demyelination or axonal damage, and reflect different clinical courses. In all these models, the first immune reactions take place in lymph nodes from which immune cells migrate into the circulation and then to the central nervous system. Adoptive transfer of myelin-reactive T cells from these animals produces pathology and disease in the central nervous system of naïve healthy recipients. In the human disease, autoreactive T and B cells specific for a variety of central antigens are present in the immune repertoire. These cells appear to be activated in the periphery through a number of mechanisms which causes them to home to the central nervous system. Contact with the local immune circuitry of the brain stimulates clonal expansion of autoreactive T cells, initiating a cascade of immuno-inflammatory events *in situ*. Numerous ways of disrupting this complex sequence of events, either by non-specific immunosuppression or by targeting specific checkpoints, abrogate or ameliorate disease in animal models. All approved disease-modifying drugs have an impact on components of the systemic immune compartment. All have been shown to reduce the number of gadolinium-enhancing T1 lesions observed with magnetic resonance imaging, an index of acute inflammatory invasion of the central nervous system.

Key words multiple sclerosis · immunology · autoimmune disease · treatment.

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Introduction

Multiple sclerosis (MS) is an autoimmune disorder targeting the central nervous system (CNS), which affects worldwide some 2 million patients and is the leading cause of neurological disability in young adults. It involves an attack on myelin in the CNS by autoreactive T and B cells. The hypothetical pathophysiological process [27, 44] is illustrated in Fig. 1 and can be understood as follows. Autoreactive cells are present in the immune repertoire and circulate in low numbers in the blood whence they can be retrieved. These cells can be activated if they encounter their respective or a structurally related antigen displayed along with major histocompatibility molecules (MHC) and co-stimulatory molecules by antigen-presenting cells such as dendritic cells. Following activation, T cells release a cocktail of cytokines as well as matrix metalloproteases which act on the endothelial lining of the vasculature allowing the T cells to bind to adhesion molecules, cross the endothelial cell layer to leave the vasculature and cross the blood-brain barrier into the CNS. Here, the cells are reactivated *in situ* by local antigen displayed on pericytes, perivascular dendritic cells or microglia. They release cytokines that initiate a local inflammatory response and the recruitment of additional macrophages that attack the oligodendrocytes and the myelin sheath. In par-

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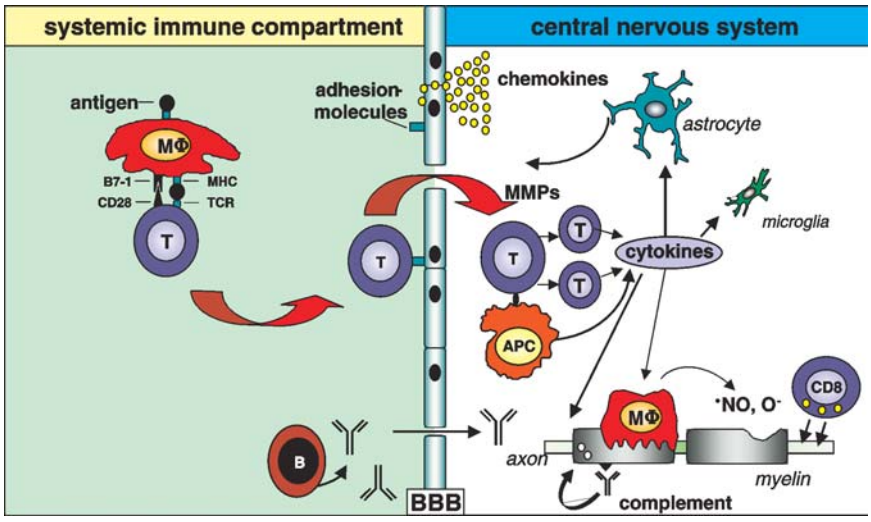

Fig. 1 Schematic representation of the pathogenesis of multiple sclerosis. *APC* antigen-presenting cell; *BBB* blood-brain barrier; *MHC* major histocompatibility complex; *MΦ* macrophage; *MMP* matrix metalloprotease; *NO* nitric oxide; *TCR* T cell receptor

allel, B cells infiltrate the perivascular cuff adjacent to the inflammatory focus, releasing antibodies that contribute to myelin destruction through complement-mediated opsonization and assembly of the terminal lytic complement complex C5b-9. Toxic substances discharged from T cells also damage the axons of neurons that have become demyelinated. In this way a focal lesion is formed in the nervous system in which a varying degree of demyelination and axonal damage may persist after the acute inflammatory event is over. The accumulation of these lesions is most probably responsible for the characteristic neurological manifestations of MS.

Experimental autoimmune encephalomyelitis – EAE

An important contribution to our understanding of the pathophysiology of multiple sclerosis has been made by studies in an animal model of autoimmune demyelinating disease, experimental autoimmune encephalomyelitis (EAE) [53]. This model consists of the inoculation of rodents, guinea-pigs, rabbits or primates with crude extracts of spinal cord or with proteins isolated from myelin, most commonly myelin basic protein (MBP), but also myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP) together with an adjuvant. After a couple of weeks, these animals develop extensive focal demyelination of the spinal cord and brain as well as motor and sensory deficits. The pathology has been demonstrated to be due to an immune response mounted against the injected myelin proteins. A crucial advance came with the demonstration that clonally expanded T cells recognizing MBP epitopes could be isolated from the blood of affected animals and that these cells, when injected into healthy recipients, can reproduce the disease [6]. This finding demonstrated that circulating T cells in the periphery are sufficient to generate central demyelinating disease. It has since been demonstrated that adoptive transfer of experimental autoimmune encephalomyelitis can be achieved with both CD4 + and CD8 + T cell lines [16, 24, 48].

Myelin autoantigens in multiple sclerosis

Several proteins associated with myelin have been proposed as potential epitopes for autoreactive T cells and for antibodies in multiple sclerosis. These include MBP, MOG, and PLP. However, epitopes unrelated to myelin may also potentially be important. T cell clonotypes whose receptor Vβ repertoires specifically recognize myelin epitopes have not yet been unequivocally identified in active lesions from biopsy or autopsy samples from patients with multiple sclerosis, although such lines have been identified in the nervous system of animals presenting an experimental autoimmune encephalitis [36]. On the other hand, antibodies recognizing MBP or MOG have been described in multiple sclerosis, are present in early disease and have been localized in lesions [15, 18, 40].

It has been suggested that the primary antigen that activates lymphocytes to trigger disease in multiple sclerosis is not a myelin-related protein itself, but rather an exogenous antigen whose three-dimensional structure resembles that of myelin proteins. This concept of molecular mimicry has been postulated as a general principle for the emergence of autoimmune disease [37]. For example, Epstein-Barr virus infections have been associated with multiple sclerosis in epidemiological studies [31] and an increased immune response to viral proteins has been implicated in the pathogenesis of multiple sclerosis. A recent study has shown that common epitope specificities of antibodies found specifi-

cally in the cerebrospinal fluid of patients with multiple sclerosis correspond to Epstein-Barr viral proteins and that immunoreactivities to these proteins were significantly higher in the serum and cerebrospinal fluid of patients with multiple sclerosis compared to control donors [11]. Moreover, CD8 + T cell responses to Epstein-Barr viral proteins were also higher in patients than in controls. T cell lines that recognize both myelin basic protein and viral proteins have also been isolated [28].

T cells in multiple sclerosis

Autoreactive T cells undergo clonal expansion and clonotypes with unique T cell receptor repertoires can be recovered from central lesions in multiple sclerosis patients [5]. In addition, clonally-expanded CD8 + T cells have been identified in the cerebrospinal fluid. These clonotypes are more frequently observed in cerebrospinal fluid than in peripheral blood and appear to persist for months, if not years [25, 42]. These cells could provide a central reservoir of memory T cells which could be mobilized during acute attacks. Such an enrichment of clonally-expanded T cell lines was not observed for lymphocytes with a CD4 + phenotype (helper cells). Importantly, a case study of two patients from whom brain biopsies had been obtained revealed that the same CD8 + T cell clones are present both in active lesions in the brain and in the cerebrospinal fluid and persist for years in the repertoire [5, 42]. Some of the cell lines retrieved from the lesions were also CD38 +, a marker of recent activation, suggesting that these cells may play an active role in lesion activity.

The origin of autoreactive T cells recognizing myelin-related epitopes has been the subject of much speculation. Myelin-specific T cells can be generated in the thymus during the formation of the immune system. T cells that are directed at host proteins are normally deleted during ontogeny by the process of central tolerance. Nonetheless, a few such cells may escape this process and T cells that recognize myelin can indeed be retrieved from the peripheral circulation of healthy adults without multiple sclerosis as well as from MS patients [38, 39]. The fate of these cells depends on the interplay of a number of regulatory factors. It is believed that peripheral tolerance involves a population of regulatory T cells which maintain autoreactive T cells in a 'dormant' state in the circulation in adults [51]. The autoreactive T cells can be 'awoken' from this state by local or environmental stimuli, such as exposure to endogenous or exogenous antigens in the circulation. In multiple sclerosis, these cells become activated, either because of failure of the mechanisms of peripheral tolerance or due to priming by antigens (Fig. 2). Intervention at the level of the regulation of peripheral tolerance is a promising strategy for new therapeutic interventions in multiple sclerosis.

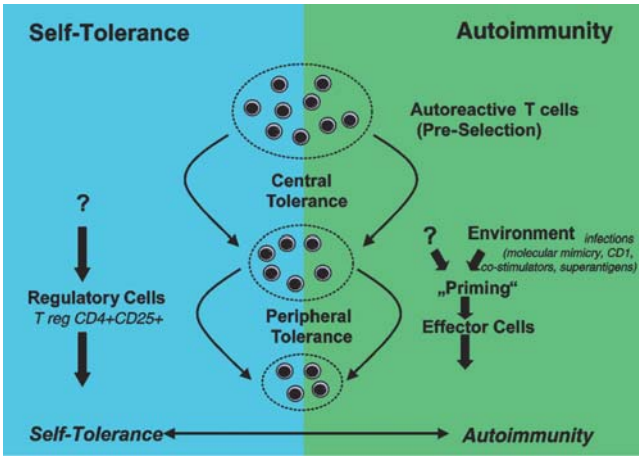

Fig. 2 Regulation of autoreactive T cells in the immune system

Once activated, autoreactive T cells release a cocktail of cytokines that are important for migration and homing of the cells to the target site and for initiation of the inflammatory response. Such cytokines include tumor necrosis factor-alpha (TNFα), important for promoting adhesion to the vascular endothelium and subsequent extravasation and for activation of macrophages and interleukin-1 (IL-1), also important for activation of macrophages and for recruitment of further T lymphocytes. However, different patterns of cytokine production by CD4 + T cells can be observed depending on the state of activation of the cell. These patterns represent a continuum between two phenotypes, the TH1 phenotype, characterized by the release of pro-inflammatory cytokines such as TNFα and IL-1, and the TH2 phenotype, characterized by the release of anti-inflammatory cytokines such as TGFβ and IL-4. The balance between the TH1 and TH2 phenotypes thus determines whether the overall T cell population has pro-inflammatory or anti-inflammatory activity (Fig. 3). In multiple sclerosis, the occurrence of acute relapses may be related to perturbation of this balance in favor of the TH1 phenotype [46]. Treatments that restore the balance or promote a TH2 phenotype are likely to be of use in preventing relapses, and this indeed seems to be an important aspect of the mechanism of action of glatiramer acetate (see below).

Role of B cells in multiple sclerosis

B cells also play an important role in multiple sclerosis, releasing antibodies directed against myelin-related antibodies. These antibodies can be observed as oligoclonal bands in the cerebrospinal fluid which have an

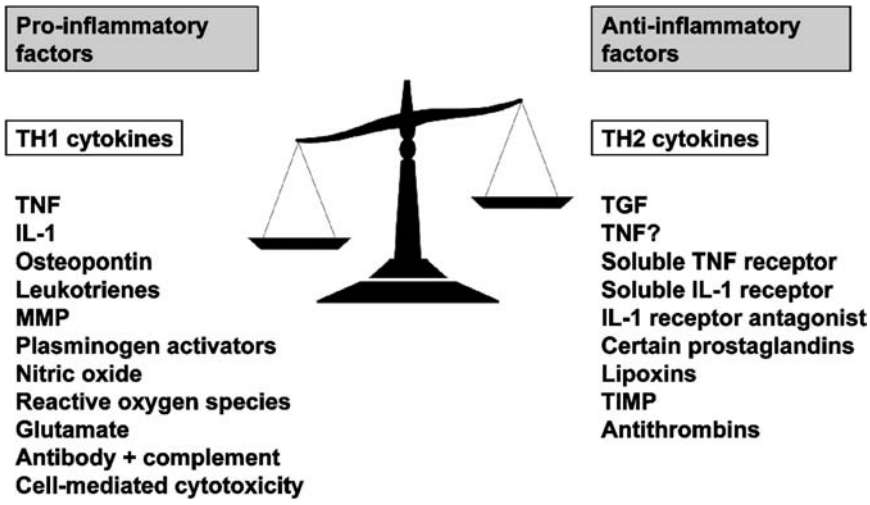

Fig. 3 The balance between TH1 and TH2 phenotypes of CD4 + lymphocytes and patterns of cytokine production. From Kerschensteiner et al. [26], with permission

important place in the diagnosis of MS. Unlike T cells, which appear to be activated and invade the CNS in an episodic fashion, B cells appear to be permanently activated and oligoclonal bands can be identified in the cerebrospinal fluid when the disease is clinically silent.

Autoreactive memory B cells recognizing myelin are generated spontaneously during the natural process of aspecific genetic rearrangement of precursor cells and are retained in the lymph nodes and spleen. Upon activation, these memory B cells undergo clonal expansion and migrate out of the lymph nodes into the circulation and thence across the blood-brain barrier into the CNS (Fig. 4). These mature B cells and, in particular, end-differentiated plasma blasts and plasma cells secrete large amounts of antibody. As with T cells, the factors that trigger activation and clonal expansion of B cells in the periphery are not known. Nevertheless, activated B cells and plasma blasts are observed in large numbers in the cerebrospinal fluid of patients with multiple sclerosis. Interestingly, the presence of this population correlates with local immunoglobulin production as well as with inflammatory disease activity [10].

B cell activity may be an important determinant of disease activity. An analysis of cerebrospinal fluid cytology from 60 multiple sclerosis patients by flow cytometry revealed that the B cell to monocyte ratio may be a trait marker of disease in patients with multiple sclerosis [9]. This ratio was correlated with disease progression but not with disability or disease duration, a predominance of B cells being associated with more rapid disease progression, and a predominance of monocytes with slower progression. These findings are in line with the observation that intrathecal IgM synthesis predicts progressive disease [49, 50].

Subsequently, a striking relationship has been demonstrated in patients presenting with a clinically isolated neurological syndrome between the presence of myelin-directed antibodies in serum and the probability of conversion to clinically-definite multiple sclerosis [7]. Relapses were observed in 95% of patients with both

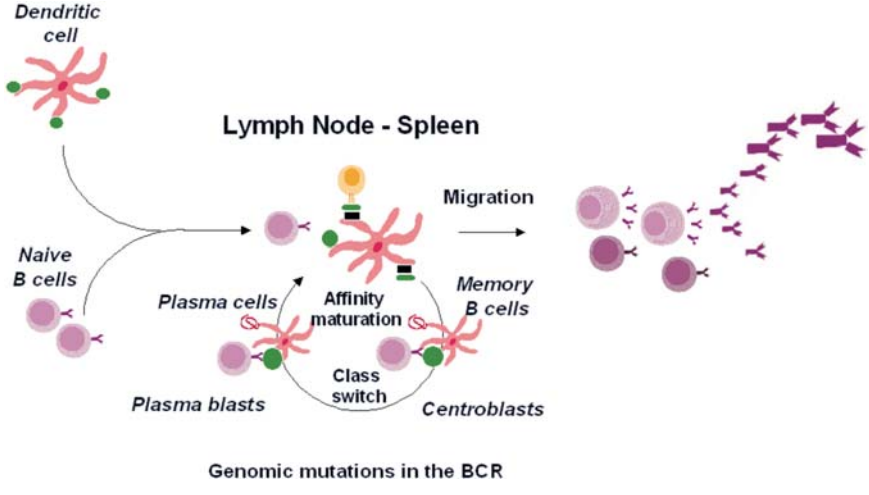

Fig. 4 Generation, maturation and migration of B cells in the immune system

anti-MOG and anti-MBP antibodies within a mean interval of 7.5 ± 4.4 months. In contrast, in patients without either antibody, only 23 % had a second relapse within the study period, which occurred after a mean interval of 45.1 ± 13.7 months (Fig. 5). The degree of sensitivity and specificity observed suggests that measurement of these antibodies may prove a suitable surrogate marker for prognosis of disease course in such patients.

Immunomodulatory treatments

All disease-modifying treatments that are available for the treatment of multiple sclerosis or that have shown activity in clinical trials interfere with the immune system in the periphery. These treatments differ in their selectivity for the points of the immune system that they target and in their selectivity for immune dysfunction specific to multiple sclerosis.

Three monoclonal antibodies that target different key macromolecules for immune system function have been evaluated in MS. These are alemtuzumab, rituximab and natalizumab. The last of these was approved for use in the treatment of relapsing-remitting disease, but subsequently withdrawn due to a case reports of fatal viral encephalopathy associated with its use. Alemtuzumab (Campath-1H) is a humanized monoclonal antibody directed against CD52 which induces depletion of T cells. In a series of 27 patients who received a single administration of alemtuzumab, a gradual but sustained decline in the number of new relapses and the number of new gadolinium-enhancing lesions was observed over the following 18 months [12]. Rituximab is a chimeric monoclonal antibody directed against CD20. This agent induces B cell depletion and has been studied extensively for the treatment of non-Hodgkins lymphoma. In neuromyelitis optica, a primarily B cell-driven MS variant, clinical efficacy of this drug has been demonstrated in eight patients [13]. In four patients with PPMS, it could be shown that treatment with rituximab depleted B cells in peripheral blood and the CSF compartment [30]. In a recent case-report, clinical efficacy of rituximab could be demonstrated in a patient with a fulminant course of RRMS. B cells were depleted in the CSF as well as in the peripheral compartment [47]. The third monoclonal antibody, natalizumab (*Tysabri®*) is directed at the α4 component of integrin on the lymphocyte, which when activated binds with an adhesion molecule of the vascular endothelium, a process that is essential for the docking of immune cells and their translocation across the blood-brain barrier. This agent thus prevents access of T and B cells to the CNS. Several studies have been performed with natalizumab and the agent has been shown to reduce dramatically relapse rates and also slow progression of disability [41, 45]. However, the non-specific normal T cell policing of the nervous system is also prevented and this may explain the cases of progressive multifocal leukoencephalopathy reported with this drug [1].

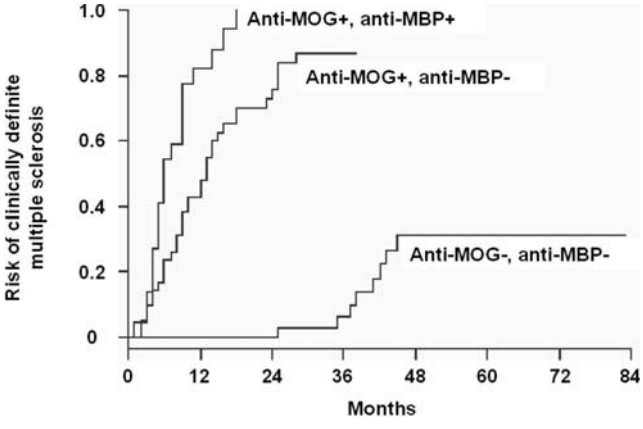

Fig. 5 Survival analysis of conversion of a clinically isolated syndrome into clinically definite multiple sclerosis as a function of the presence of myelin-directed antibodies. *MBP* myelin basic protein; *MOG* myelin oligodendrocyte glycoprotein. Data are expressed as Kaplan-Meier curves. Reproduced from Berger et al. [7] with permission

Mitoxantrone is a synthetic anticancer drug derived from doxorubicin that has been used for the treatment of leukemia and other cancer types for nearly 20 years. Mitoxantrone acts as a non-specific immunosuppressant, inhibiting proliferation of T cells, B cells, and macrophages as well as cytokine and antibody secretion [17, 35]. In placebo-controlled clinical trials in secondary progressive multiple sclerosis [14, 19], mitoxantrone was demonstrated to reduce relapse rates and attenuate progression of disability. As a result of these findings, mitoxantrone was approved by the FDA in 2000 for the treatment of worsening relapsing-remitting, secondary progressive and progressive-relapsing MS.

Beta-interferons act in the periphery to block T cell activation, release of pro-inflammatory cytokines and translocation of T cells into the central nervous system [8, 20, 32]. This is manifested as a rapid reduction or extinction of gadolinium-enhancing lesion activity in the brain. Several large, randomized, placebo-controlled clinical trials have shown that beta-interferons decrease relapse rates and, in some trials, slow progression of disability [52]. Together with glatiramer acetate, these drugs are now the mainstay of management of relapsing-remitting MS.

Glatiramer acetate (GA) is the only disease-modifying treatment to have an immunomodulatory action directed specifically against the autoimmune process occurring in multiple sclerosis [33] (Fig. 6). GA is a standardized mixture of synthetic polypeptides with a defined molar ratio resembling MBP. It was identified as a potential therapy for multiple sclerosis by the observation that it protected animals against experimental

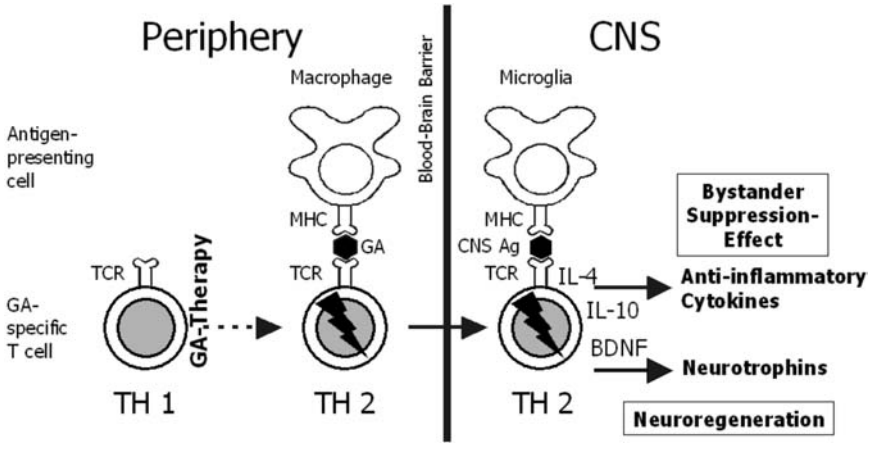

Fig. 6 Schematic representation of the mechanism of action of glatiramer acetate. *AG* antigen; *BDNF* brain-derived neurotrophic factor; *CNS* central nervous system; *GA* glatiramer acetate; *IL* interleukin; *MHC* major histocompatibility complex; *TH* T helper cell; *TCR* T cell receptor. Reproduced from Hohlfeld and Wekerle [22], with permission

autoimmune encephalomyelitis. GA acts in the periphery to recruit a population of GA-specific T cells which respond by clonal proliferation and a phenotype shift towards a TH2 anti-inflammatory phenotype, characterized by secretion of IL-4 [29, 34]. In the experimental autoimmune encephalomyelitis model, passive transfer of these GA-reactive T cells have been shown to protect against disease [4]. Moreover, in these animals, the cells migrate across the blood-brain barrier into the central nervous system [2]. Based on such experiments in animal models, it has been suggested that GA-reactive T cells also enter the brain in the human disease and home in on areas of active inflammation where they become re-activated by myelin-related antigens [3]. Upon activation, they release anti-inflammatory cytokines which exert a bystander suppressor effect on the inflammatory process, as well as neurotrophic factors that might promote repair to myelin and neurons. GA thus differs from the other immunomodulatory treatments of MS in that the key immune step that is regulated is within the nervous system at the site of the active inflammatory lesion. Although its primary action is on immune cells in the periphery, and indeed GA probably never enters the nervous system itself, the therapeutic effect may be carried into the site of the lesion by a specific population of T cells.

In addition to the activation of brain-penetrating TH cell lines, it has recently been demonstrated that GA can also induce a population of CD4 + CD25 + regulatory T cells [23]. These T cell types intervene in the regulation of peripheral tolerance of autoreactive T cells in the circulation [51]. If this is the case, then GA may have an important upstream role in maintaining autoreactive T cells in a 'dormant' state and thus preventing recurrence of acute flairs of inflammatory activity.

From a clinical point of view, GA provides a sustained decrease in relapse rate and a stabilization of neurological disability in a majority of patients [54]. Unlike many other immunomodulatory treatments, GA has also been shown to slow down the rate of brain atrophy [43], perhaps due a neuroprotective effect mediated by growth factors released in the brain from GA-reactive T cells [55].

Conclusions

Most researchers would subscribe to the view that multiple sclerosis, in the great majority of patients, is an autoimmune disease or, at least in part, immune driven. Several lines of evidence collected over several decades have converged to support this notion. The key pathological event corresponds to the generation of populations of autoreactive T and B cells in the periphery which subsequently enter the nervous system and attack oligodendrocytes. Although there remain many unanswered questions, such as the nature of the initial trigger of disease, the reason why tolerance fails, the precise epitopes recognized by T cell receptors, the fate of autoimmune cells in the nervous system and the triggers of individual relapses, the fundamental autoimmune nature of multiple sclerosis is clearly established. All effective therapies for multiple sclerosis target different steps of the autoimmune process and all act in the periphery to modify the properties, activity or trafficking of different autoreactive lymphocyte populations.

There is a clear rationale from studies in animals and patients that multiple sclerosis is primarily a disease resulting from aberrant systemic immune responses which can be controlled to a large extent by interfering with these. Whether this also pertains to the rarer forms of disease characterized by a relative paucity or absence of inflammatory cells in the central nervous system remains to be seen.

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