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# Granulocyte-Macrophage Colony-Stimulating Factor as a Therapeutic Target in Multiple Sclerosis

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## ABSTRACT

Multiple sclerosis is an inflammatory neurodegenerative disease of the central nervous system (CNS) and the most frequent cause of non-traumatic disability in adults in the Western world. Currently, several drugs have been approved for the treatment of multiple sclerosis. While the newer drugs are more effective, they have less favourable safety profiles. Thus, there is a need to identify new targets for effective and safe therapies, particularly in patients with progressive disease for whom no treatments are available. One such target is granulocyte-macrophage colony-stimulating factor (GM-CSF) or its receptor. In this article we review data on the potential role of GM-CSF and GM-CSF inhibition in MS. We discuss the expression and function of GM-CSF and its receptor in the CNS,

as well as data from animal studies and clinical trials in MS.

**Keywords:** Granulocyte-macrophage colony-stimulating factor; Experimental autoimmune encephalomyelitis; Multiple sclerosis

## INTRODUCTION

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a haematopoietic growth factor and proinflammatory cytokine with pleiotropic functions. The aim of this review is to highlight the role of GM-CSF in multiple sclerosis (MS) and the reasons it may be a therapeutic target. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

## EXPRESSION AND FUNCTION OF GM-CSF AND ITS RECEPTOR IN THE CENTRAL NERVOUS SYSTEM

Granulocyte-macrophage colony-stimulating factor is a glycosylated protein with a molecular weight of 23 kDa [1]. It is produced by many cell types (Table 1), including both CD4+ and CD8+ T cells, and its production is regulated differently in each cell type. For example, naive

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**Table 1** Expression of granulocyte-macrophage-colony stimulating factor and granulocyte-macrophage colony-stimulating factor receptor subunits reported in published studies

Cell type	References on GM-CSF expression <sup>a</sup>	References on GMR expression <sup>a</sup>
Activated Th1, Th2, Th17 cells	+ [14–16] (human and mice)	
Monocytes/macrophages	+ [17] (human)	+ [18] (human)
Smooth muscle cells	+ [19] (human)	
Chondrocytes	+ [20] (human)	
Parenchymal cells in kidney	+ [21] (mice)	
Endothelial cells	+ [22] (mice)	+ [23] (human)
Epithelial cells	+ [24, 25] (human)	
Fibroblasts	+ [26, 27] (human)	+ [28, 29] (human)
Tumour cells		+ [18, 30] (human)
Keratinocytes	+ [31] (human and mice)	+ [32, 33] (human)
CD8+ cells	+ [2] (mice)	
B cells	+ [34] (human)	+ [34] (human)
NK cells	+ [35] (human)	+ [36] (human)
NKT cells	+ [2] (mice)	+ [37] (mice)
Epidermal T cells	+ [2] (mice)	
γδ T cells	+ [38] (EAE mice)	
	– [2] (mice)	
Astrocytes	+ [7] (mice)	+ [9] (mice)
Tregs	– [2] (mice)	
Dendritic cells	+ [39] (mice)	+ [39] (mice)
Microglial cells	+ [40] (human)	+ [41] (mice)
Neutrophils	+ [42] (human)	+ [43, 44] (human)
Eosinophils	+ [42] (human)	+ [45] (human)
Neurons		+ [9] (mice)
Oligodendrocytes	+ [46] (human)	+ [9, 46] (human and mice)

*GM-CSF* Granulocyte-macrophage colony-stimulating factor, *GMR* GM-CSF receptor, *Th cells* T helper cells, *NK cells* natural killer cells, *NKT cells* natural killer T cells, *Tregs* regulatory T cells, *EAE* experimental autoimmune encephalomyelitis

<sup>a</sup> +, Present in the cell type specified according to the study referenced; –, absent in the cell type specified according to the study referenced

CD4+ cells do not secrete GM-CSF, while naive CD8+ cells do [2]. Researchers have recently identified a new T helper (Th) cell subset that

produces GM-CSF and which is distinct from the other Th cells identified to date, such as Th1 and Th17 [3, 4]. GM-CSF is able to cross the

blood–brain barrier (BBB), blood–spinal cord barrier and blood–testis barrier in mice. This occurs via a selective and saturable transport system in addition to simple diffusion and leakage [5, 6]. GM-CSF has been detected in the normal central nervous system (CNS) of humans and mice and is produced mainly by astrocytes. It may have a role in the regulation of microglial functions [7] and in the stimulation of microglial priming for antigen presentation [8].

The GM-CSF receptor (GMR) is expressed by many cell types, including monocytes, macrophages, antigen presenting cells (APCs), neurons, astrocytes, and oligodendrocytes, which suggests that GM-CSF is involved in the physiological regulation of these cells [9]. Some studies have shown that for GMR to be activated, it must form a dodecamer structure through head-to-head assembling of two ligand–receptor hexamers; this induces intracellular signalling [10, 11]. GM-CSF concentration controls which signalling pathways are activated. Low GM-CSF concentrations lead to a selective survival-only signalling pathway, while higher GM-CSF concentrations result in dodecamer assembly and activation of survival and proliferation signalling pathways. Therefore, the GMR is considered to be a therapeutic target in the treatment of autoimmune diseases [12, 13].

GM-CSF expression is also regulated by a number of cytokines, as shown in Table 2.

## GM-CSF IN MS

To date, the exact pathogenic action of GM-CSF in MS is incompletely elucidated. High GM-CSF concentrations have been found in the cerebrospinal fluid (CSF) of active-phase MS patients, suggesting that GM-CSF may play a role in MS pathogenesis [56]. The possible mechanisms by which GM-CSF may exert pathogenic effects in MS are shown in Fig. 1.

GM-CSF enhances the migration of monocytes to the CNS through the BBB. GM-CSF is also known to activate macrophages to adopt a phenotype similar to those found in active MS lesions [57]. Th cells producing GM-CSF have an

enhanced ability to migrate to the CNS and are more abundant in the CSF of MS patients than in healthy controls [3]. Studies have shown varying results in terms of GM-CSF expression by T cells in peripheral blood in MS patients compared to controls, with the levels in the former ranging from being similar to the controls [3, 58] to increased levels in CD4+ T cells [59, 60] and CD8+ T cells [60]. The exact causes of elevated GM-CSF expression in MS are uncertain. A polymorphism in the interleukin (IL)-2 receptor alpha (IL-2RA) gene, which is one of the genetic risk factors for MS [59], dictates higher IL-2-induced GM-CSF in T cells of MS patients. Moreover, it has recently been shown that microglia and astrocytes have a higher expression of GMR in acute and chronic MS lesions [61]. The mechanism of immunoregulation of some MS therapies, such as interferon beta (IFN- $\beta$ ) and B-cell depleting therapy, may be due to the reduction of GM-CSF production or the removal of cells producing GM-CSF [60, 62].

GM-CSF-producing memory B cells are increased in the peripheral blood of MS patients and have myeloid cell-stimulating properties [62]. GM-CSF may also exert an autocrine effect on B-cell survival [34], which may be relevant to MS.

A newly identified subset of T cells producing predominantly GM-CSF but no or minimal other Th1 and Th17 signature cytokines may also be involved in MS pathogenesis. These cells have distinct migration properties, with an enhanced ability to migrate to inflamed CNS tissues [3]. Most of the Th cells in the CSF and blood of MS patients have recently been shown to be non-classical Th1 cells producing GM-CSF and Th cells producing only GM-CSF [63].

## GM-CSF IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

The role of GM-CSF in MS is supported by studies in the experimental model of experimental autoimmune encephalomyelitis (EAE), where detailed analysis and manipulation of the immunopathology is more feasible than in

**Table 2** Major cytokines that regulate granulocyte-macrophage colony-stimulating factor production

Cytokine	Effect on GM-CSF expression	Reference(s)
IL-2	Stimulatory effect	[3]
IL-12	Stimulatory effect in Th1 cells	[3, 47]
	Inhibitory effect in Th17 cells	[16]
IL-23	Stimulatory effect in Th17 cells	[16, 48]
IL-4	Inhibitory effect	[16, 49]
IL-10	Inhibitory effect	[50, 51]
TGF- $\beta$	Inhibitory effect	[52]
IFN- $\gamma$	No effect in Th1 cells	[3, 47]
	Inhibitory effect in Th17 cells	[16]
IL-27	Inhibitory effect in Th1 cells	[53]
IL-1 $\beta$	Stimulatory effect	[38, 54]
TNF	Stimulatory effect	[55]
IL-7	Stimulatory effect	[4]

*IL* Interleukin, *TGF* tumour growth factor, *IFN* interferon, *TNF* tumour necrosis factor

human tissue. Despite the previous suspicion that EAE depends on IFN- $\gamma$  and IL-17 for initiation and maintenance, recent experiments have found these two cytokines are redundant [64], while GM-CSF is crucial for the encephalitogenicity of CD4+ T cells infiltrating the CNS.

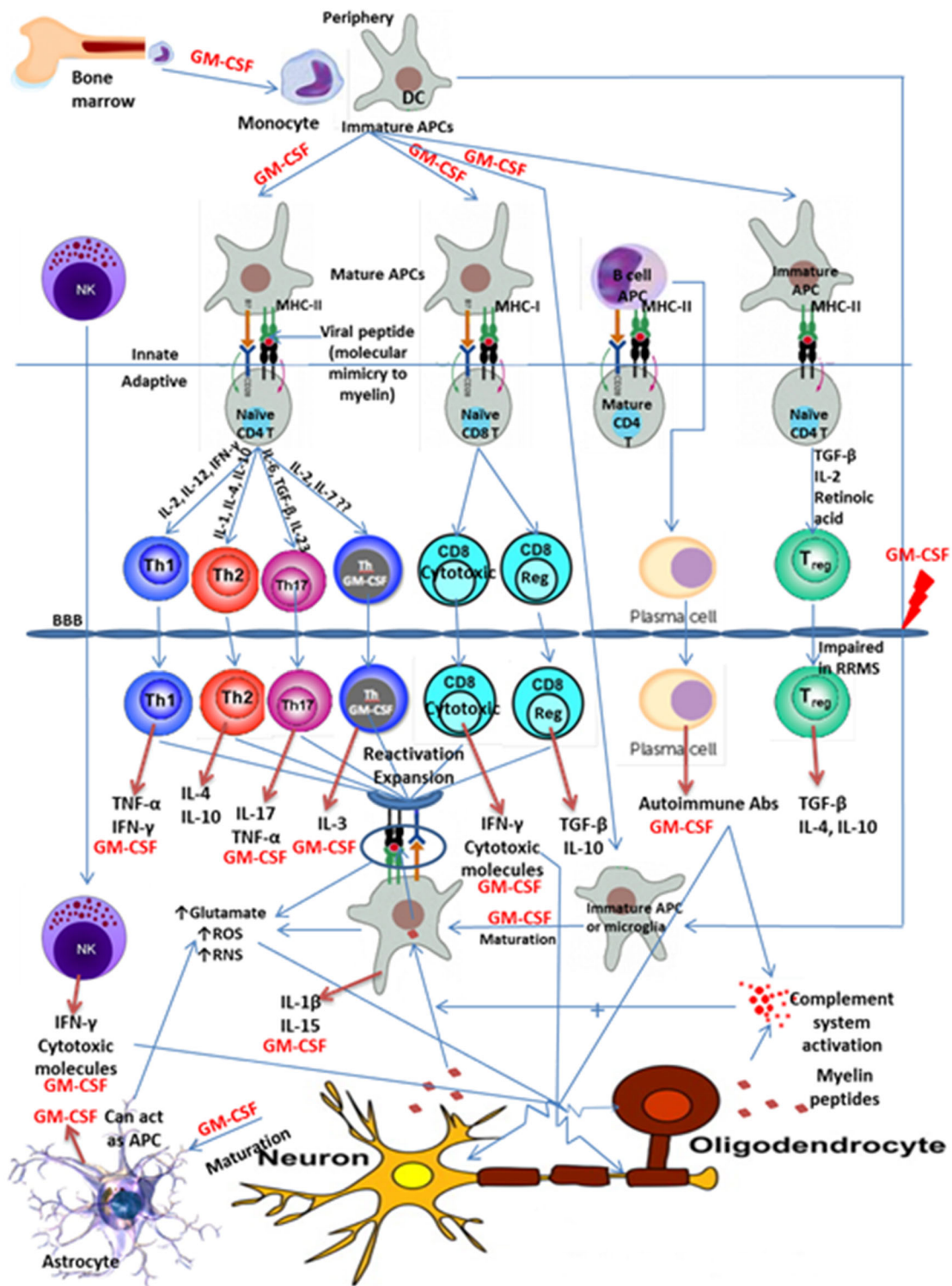
#### GM-CSF Knockout Mice are Resistant to EAE

Granulocyte-macrophage colony-stimulating factor knockout mice (GM-CSF $^{-/-}$ ) backcrossed onto an EAE-susceptible (NOD/Lt) background are essentially protected from clinical signs of EAE after immunization with myelin-oligodendrocyte glycoprotein, an encephalitogenic peptide that induces severe disease in wild-type (WT) controls [65]. CNS infiltration was reduced in the GM-CSF $^{-/-}$  mice and did not persist, unlike in their WT counterparts. Resistance to EAE has also been observed in other mouse strains following knockout of GM-CSF or the GMR, as well as chimeras lacking GM-CSF expression in the

bone marrow [16, 66, 67]. Furthermore, recombinant GM-CSF restores susceptibility to disease [67].

#### GM-CSF Synthesis by CD4+ T Cells is Necessary for Encephalitogenicity

Although myelin-specific Th1 and Th17 cells can induce EAE after passive transfer, knockout and antibody-blocking experiments confirm that the dominant cytokines (IFN $\gamma$ , IL-17) are redundant and that the encephalitogenicity of these cells is proportional to the GM-CSF they produce [47]. Transfer of IL-17 $^{-/-}$ , IFN $\gamma$  $^{-/-}$ , or double knockout CD4+ T-cells cultured in conditions favouring GM-CSF expression results in EAE with similar kinetics to that seen after transfer of WT cells, whereas the transfer of GM-CSF $^{-/-}$  cells does not cause disease [16]. Cells polarized in vitro towards a Th1 or Th17 phenotype upregulate GM-CSF expression following entry to the CNS [16]. What is more, transferring myelin-specific CD4+ T cells into GM-CSF $^{-/-}$  recipients leads to disease



**Fig. 1** Diagram showing the potential mechanisms of GM-CSF involvement in MS. *APC* antigen presenting cell, *CD* cluster of differentiation, *IFN* interferon, *IL* interleukin, *MHC* major histocompatibility complex, *NK*

natural killer cell, *RNS* reactive nitrogen species, *ROS* reactive oxygen species, *TGF* transforming growth factor, *Th* T helper cell, *TNF* tumor necrosis factor, *Treg* regulatory T cell

indistinguishable from that seen in WT recipients [41], while transferring WT polyclonal CD4+ cells does not lead to EAE if they are co-transferred with myelin-targeted GM-CSF<sup>-/-</sup> T cells [68]. These results prove that GM-CSF is indispensable in EAE and that CNS-infiltrating Th cells are the source of GM-CSF.

### Overexpression of GM-CSF Exacerbates EAE

Treatment with recombinant GM-CSF exacerbates disease and increases the frequency of relapses in EAE. Passive transfer experiments showed that myelin-specific CD4+ T cells transduced with adenovirus-expressing GM-CSF caused a more severe disease than cells transfected with virus lacking GM-CSF [68]. Transgenic mice constitutively over-expressing GM-CSF in CD4+ T cells spontaneously developed CNS lesions, even in the absence of sensitization with myelin peptides [69]. Although the lung and liver were also infiltrated by cells in this study [69], they did not demonstrate pathology or symptoms, suggesting the CNS is particularly susceptible to the effects of dysregulated GM-CSF.

### Antibodies to GM-CSF Prevent Disease if Given Prior to Induction and Ameliorate Ongoing Disease

Anti-GM-CSF antibodies prevent the onset of chronic EAE if given prior to active induction and reduce CNS lesion load [65, 66]. They also reduce the number and severity of relapses in relapsing EAE [70]. However, cessation of treatment leads to disease with a similar evolution as that seen in untreated controls. Furthermore, treatment with antibodies targeting either GM-CSF [69] or its receptor [70] given after clinical disease onset results in partial (in the case of receptor antibody) or complete (in the case of cytokine antibody) clinical recovery and a significant reduction in CNS lesion burden. These results have implications for the treatment of MS when therapy is needed in established disease.

### GM-CSF Levels in the CNS Increase at Disease Onset

The expression of GM-CSF is very low in the spinal cords of mice at the time of disease induction, but it increases dramatically when disease becomes apparent [41, 64].

### What Does GM-CSF do in EAE?

Several research groups have demonstrated that the absence of GM-CSF impairs myelin-specific T-cell priming [65, 66] and prevents epitope spreading [70]. These effects are unlikely to be due to failure of autocrine effects of GM-CSF given the absence of GMRs from T cells. Rather, they are believed to be due to impaired antigen presentation by APCs, particularly monocyte-derived dendritic cells (moDCs), for T-cell priming in the periphery and reactivation in the CNS [71].

GM-CSF is a key factor in the differentiation and mobilization of inflammatory monocytes from the bone marrow prior to the onset of EAE [16, 67, 69]. The migration of these cells to the CNS is also believed to be influenced by EAE as the knocking out GM-CSF or its receptor reduces the number of granulocytes and moDCs in the CNS after induction [65, 66, 70] and over-expression increases recruitment [47]. Conditional GMR deletion restricted to the inflammatory monocytes prevents the onset of EAE if knockout occurs at disease induction and it ameliorates disease if knockout occurs after clinical signs develop [72]. However, the crucial role of GM-CSF appears not to be in the recruitment of inflammatory monocytes per se but rather in the further differentiation of these cells into moDCs and tissue macrophages within the CNS. For example, bone marrow chimeras have shown GMR expression is not an absolute requirement for the migration of these cells into the CNS [73]. GM-CSF can modulate cytokine and chemokine expression by mature myeloid-derived cells and upregulate costimulatory molecules and major histocompatibility complex (MHC) class II molecules [70–72, 74]. As the expression of GM-CSF restricted to CNS-infiltrating CD4+ T cells alone is sufficient to

cause disease [65, 68], its essential role may be effector cell differentiation within the CNS, establishing a complex pro-inflammatory feedback system involving APCs, neutrophils, microglia and endothelial cells [71, 75].

### GM-CSF is Necessary for Maintaining Disease

Preventing GM-CSF signalling results in milder disease with recovery in chronic models of EAE and fewer, milder relapses in relapsing–remitting models of EAE [16, 48, 65]. This action is reflected on a cellular level by pre-symptomatic lymphocyte and myeloid infiltration that persists in WT mice but regresses in GM-CSF<sup>-/-</sup> mice or mice treated with blocking antibodies [16].

However, results from animal models must be interpreted with caution. One problem intrinsic to EAE is that different host genotypes and methods of induction result in distinctive phenotypes, and factors found to be crucial in one model may not be in another. For example, C3HeB/FeJ mice develop an “atypical” EAE with neutrophilic brainstem and spinal cord infiltration and predominant ataxia, as well as the “classic” inflammation in the spinal cord and ascending paralysis. Using passive transfer of WT and GM-CSF<sup>-/-</sup> T-cells into WT and IL-17R<sup>-/-</sup> hosts, Pierson and Goverman demonstrated that either GM-CSF or IL-17 is required for brainstem inflammation, but neither is required for spinal cord disease [76]. This finding is in stark contrast to the plethora of similar experiments in different mouse strains demonstrating that GM-CSF, but not IL-17, is necessary for classic EAE [16, 48]. These differences may be accounted for by variation in methodologies and the mouse genotypes used, or by inadequate synthesis of IL-17 by alternative mouse lines, with subsequent failure to compensate for the absence of GM-CSF [76].

In conclusion, many experiments have demonstrated a critical role of GM-CSF in EAE. Although the importance of GM-CSF varies according to the model used, and the extrapolation of EAE results to MS can be problematic, the totality of the EAE evidence justifies

pursuing work targeting GM-CSF, its receptor and signalling pathways in MS.

## CLINICAL TRIALS TARGETING GM-CSF IN MS

Several agents targeting GM-CSF are being developed or currently under study in clinical trials. There are several ongoing or completed clinical trials targeting GM-CSF or GMR in rheumatoid arthritis (RA), psoriasis and MS. These have been reviewed elsewhere [77].

GSK3196165 (or MOR103) is a high-affinity recombinant human immunoglobulin (Ig) G1 antibody. It was developed by MorphoSys AG (Planegg, Germany) and in-licensed by GlaxoSmithKline (GSK; Brentford, UK) [78]. It blocks the interaction of GM-CSF with its receptor and thus averts subsequent signal transduction [79]. Its access into the CNS may be facilitated by the altered BBB in MS, which can potentially favour IgG penetration.

The study of MOR103 in RA included 96 patients with active disease [78]. The primary outcome measure was the adverse event (AE) rate and safety profile, while secondary endpoints included Disease Activity Score 28-joint assessment (DAS28), American College of Rheumatology (ACR) core set measures, European League Against Rheumatism (EULAR) response criteria and magnetic resonance imaging (MRI) of synovitis [78]. Three different dosages of MOR103 (0.3, 1.0 and 1.5 mg/kg) were compared to placebo, and significant clinical improvement was shown, most pronounced at 1.0 mg/kg. There were no safety and tolerability issues, and AEs were generally mild or moderate, with the most common AE being nasopharyngitis [78].

Based on these positive results regarding tolerability and efficacy in patients with RA, a phase 1b trial of MOR103 in patients with MS was performed (NCT01517282) [80]. The aim of this trial was to determine the safety, immunogenicity and pharmacokinetics (PK) of MOR103 in people with MS with clinical or MRI activity. This 20-week, double-blind, placebo-controlled phase 1b dose-escalation trial recruited 31 MRI-active relapsing–remitting or

secondary progressive MS patients. Participants were randomized to receive an intravenous infusion of placebo ( $n = 6$ ) or active treatment with 0.5 ( $n = 8$ ), 1.0 ( $n = 8$ ) or 2.0 ( $n = 9$ ) mg/kg antibody every 2 weeks for 10 weeks [80]. The antibody was overall well-tolerated, and no evidence of immunogenicity was found. Most treatment-emergent AEs were mild to moderate, with again the most frequent AE being nasopharyngitis. There were no AE-related trial drop-outs, infusion-related reactions or deaths [80]. The differences in AE between the trial arms were small. No clinically significant changes were observed in other clinical assessments or laboratory safety assessments. Nine patients from different groups experienced MS exacerbations: three in the placebo arm, five in the 0.5 mg/kg group, one in the 5 mg/kg group and zero in the 2.0 mg/kg group. MRI activity (T1 Gd-enhancing lesions and/or new or enlarging T2 lesions) were observed in all treatment groups. PK assessments showed dose linearity with low or no drug accumulation over time [80].

The results of this trial show that the antibody has a good tolerability profile, with no unexpected safety concerns in the treatment of MS. This finding is particularly important since immune therapies can have different or unexpected AEs in different autoimmune conditions. Although GM-CSF inhibition seems to be generally safe to date [79], active monitoring for potential side effects is needed.

Studies in GM-CSF-deficient mice show a potential increased risk of infections [81]. An imbalance in GM-CSF regulation can induce colitis [82], and inhibition of GM-CSF expression may exacerbate existing intestinal inflammation [83]. People with high-titre GM-CSF autoantibodies or the mutations of GMR may be at risk for pulmonary alveolar proteinosis [84].

The good safety and tolerability findings from the phase I trial of anti-GM-CSF antibody in MS are consistent with prior safety data reported for patients with RA [78] and warrant further clinical studies.

In planning further studies, one should bear in mind the complex relationship between GM-CSF effects and the specific immunological status in a given patient. Studies of GM-CSF as an

adjuvant in clinical trials of vaccination with autologous tumor cells, peptides and/or dendritic cells in patients with cancer have shown heterogeneous results in terms of the induction of vaccine-specific immune response and clinical response [85]. The authors of this study [85] noted that a dose-dependent effect was probable, with repeated low doses of GM-CSF increasing the vaccine-induced immune response and with an opposite effect with higher dosages. Indirect evidence of a possible role the clinical implications of GM-CSF in MS comes from a case report of a 51-year-old woman with stage-three melanoma who received subcutaneous recombinant human (rh) GM-CSF injections for 3 years in a phase two clinical trial [86]. During the trial, yearly MRI scans showed subtle stable demyelination. The patient was in remission of her melanoma at the end of the trial, and 7 months after discontinuing GM-CSF she had her first MS clinical relapse [86]. Development of anti-GM-CSF antibodies during rhGM-CSF therapy as a cause of the relapse was deemed unlikely, as white cell counts suggested persistent biological effect of GM-CSF [86]. Therefore, the enhanced activity could conceivably be due to persistent GM-CSF effects rather than to its withdrawal.

Further studies of anti-GM-CSF treatments in MS are warranted, and the identification of patients more likely to benefit is of crucial importance.

## CONCLUSION

Granulocyte-macrophage colony-stimulating factor is increasingly recognized as a pivotal cytokine in the pathogenesis of MS. Its production by multiple immune cell types is increased in MS, and its levels in the CSF correlate with disease activity. Moreover, GM-CSF is implicated in the pathological mechanisms of EAE. The blockade of GM-CSF signalling prevents or suppresses EAE. These studies strongly support interventions to block GM-CSF in MS, and results from phase I studies indicate a favourable safety profile of antibodies against GM-CSF. GM-CSF is, therefore, a plausible therapeutic strategy.

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**Compliance with Ethics Guidelines.** This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

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