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THERAPEUTIC TARGETS FOR MULTIPLE SCLEROSIS

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory disorder of the brain and spinal cord in which focal lymphocytic infiltration leads to myelin and axonal damage. Although first described in the 14th century, MS was untreatable until the 1990s, when interferon beta (IFN-β) reached the market for the first time. Four different IFNs and glatiramer acetate all obtained regulatory approval in the 1990s, radically influencing the treatment of MS. Since then, novel strategies have been developed for the treatment of MS, including targeting of leukocyte differentiation molecules, costimulatory molecules, antiadhesion molecules and chemotaxis; immunomodulatory agents, autologous stem cell transplantation, anti-infectious therapies and strategies for neuroprotection, neurorepair and remyelination have also emerged. However, there is still no cure for MS and existing treatments serve only to slow disease progression and mitigate symptoms. The search for effective treatments for MS continues, with researchers focusing on the identification of novel targets for therapeutic intervention. This article presents those drug targets that are currently under active investigation for the treatment of MS.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disorder of the brain and spinal cord characterized by focal lymphocytic infiltration that leads to myelin and axonal damage. Patients experience muscle weakness, visual disturbances and other neurological impairments, with severity ranging from relatively benign to devastating. In severe cases, patients are unable to walk, speak or write. The array and severity of symptoms vary widely among patients and within the same patient over the course of the disease (1, 2).

MS is the most common chronic central nervous system (CNS) disorder that affects young adults (aged 20-40 years) and occurs twice as often in women as in men. MS is believed to affect between 250,000 and 350,000 Americans and approximately 2.5 million individuals worldwide. The risk of developing MS within the general population is approximately 1 in 1,000. The European total mean MS incidence rate has been estimated to be 4.2 cases per 100,000/year, whereas a much lower prevalence has been reported in Asia (1-5 per 100,000) (3, 4).

There are several subclasses of MS. Relapsing–remitting MS (RRMS) is the most frequent form at first diagnosis and is characterized by unpredictable relapses or attacks, during which new symptoms appear or existing symptoms become more severe. The duration of these attacks varies and they can be separated by months or even years of partial or total remission. Secondary progressive MS accounts for approximately 40% of cases and occurs in patients who initially have RRMS and is characterized by a steady progression of clinical neurological damage with or without superimposed relapses and minor remissions and plateaus. Primary progressive MS affects between 10% and 15% of patients and is characterized by a lack of distinct attacks, a slow onset and steadily worsening symptoms without periods of remission. Deficits and disability tend to accumulate, although they may level off or continue to worsen over several years. This form of MS is unique in that it occurs more frequently in men than in women, and the average age at onset is approximately 10 years later than for RRMS (40 years vs. 30 years). Benign MS affects approximately 10% of patients and is characterized by one or two attacks with complete recovery. This form is generally associated with less severe symptoms at onset and does not worsen over time or cause permanent disability. The fifth form, progressive relapsing MS, is quite rare. It is progressive from outset and characterized by obvious acute attacks, with or without recovery (1, 2, 5).

During an MS attack, inflammation occurs in random areas of the white matter of the CNS, causing destruction of myelin coating nerve fibers in the brain and spinal cord. Myelin facilitates the smooth, high-speed transmission of neuronal electrochemical impulses. Demyelination, the loss of or damage to myelin, causes neurological transmission to be slowed or completely blocked and results in a decrease or total loss of function. Hardened, sclerotic patches of scar tissue, or plaques, replace lost myelin and appear at various points around the CNS, and the nerve fiber itself may also be damaged (1, 2).

Demyelination and resulting axonal destruction in MS may be acute or chronic. Acute demyelination leads to destruction of axons by inflammatory mediators (proteases, cytokines, T cells and free radicals). This process lasts for only a few days or weeks, although the accumulation of axonal destruction may contribute to a process in

which chronically demyelinated axons, devoid of the trophic support normally provided by myelin and oligodendrocytes, undergo more severe degeneration and irreversible structural damage. The damage to myelin in MS appears to result from an abnormal response by the immune system, which may be triggered by a virus or other pathogen; the virus or pathogen may even have been dormant for some time. Reactivation of the virus could cause lymphocytes in the bloodstream to penetrate the blood–brain barrier and, once inside the brain, to activate other elements of the immune system to attack and destroy myelin. Human herpesvirus type 6 (HHV-6) is frequently linked to MS. However, although HHV infection is more frequent in the cerebrospinal fluid (CSF) of MS patients, HHV-6 seems to be involved in the pathogenesis of MS only in a subset of patients. Other epidemiological studies have alluded to an association between Epstein-Barr Virus (EBV) infection and a risk of MS, since individuals with a history of symptomatic primary EBV infection (i.e., infectious mononucleosis) carry a moderately higher risk of MS. In addition, EBV-specific immune responses are altered in patients with MS (6-9).

Occasionally, neurological function fully recovers in spite of persistent demyelination. Thus, demyelination is not fully responsible for the functional impairment seen in MS. The "axonal hypothesis" was proposed as an alternative theory for chronic disability in MS. It states that axonal damage or loss is required for chronic functional impairment and disability. Axonal adaptation or repair may be the mechanism that enables recovery, and irreversible axonal damage and loss are therefore responsible for the persistent functional deficits encountered in MS. Significant axonal loss and injury in both acute and chronic lesions have been observed in post mortem brain samples of MS patients. Thus, it appears that the combination of loss of myelin and oligodendrocytes together with axonal injury eventually results in irreversible disability (6, 10).

MS is believed to begin with the migration of blood autoreactive lymphocytes to the CNS. Although patients with MS and healthy controls have similar numbers of peripheral blood T cells reacting to myelin, patients exhibit myelin-reactive T cells with a memory or activated phenotype. Injection of a modified myelin peptide has been shown clinically to exacerbate the disease, suggesting that myelin-reactive T cells lead to inflammatory demyelination. Myelin-specific T cells from MS patients produce Th1 proinflammatory cytokines and recent results obtained using the experimental autoimmune encephalomyelitis (EAE) model of MS have suggested that the Th17 subset and the cytokine IL-23 could be involved in this disease. Regulatory $CD4^+/CD25^+$ T cells are also affected in MS. These lymphocytes contribute to the maintenance of peripheral tolerance by active suppression, and a significant decrease in their regulatory function has been described in MS (11, 12).

B cells are also involved in the pathogenesis of MS. The CSF of MS patients contains high numbers of chronically activated B cells, plasma cells and immunoglobulin M (IgM), and IgG antimyelin antibodies and B-cell depletion therapy can significantly reduce MS relapses. Activated infiltrating lymphocytes produce different cytokines that can damage myelin-producing oligodendrocytes, resulting in myelin loss (13, 14).

MS was first described as early as the 14th century, although it was virtually untreatable until the 1990s, when interferon beta (IFN-β) reached the market for the first time. Four different IFNs and glatiramer acetate all obtained regulatory approval in the 1990s, radically influencing the treatment of MS, and in many cases changing the clinical course of the disease. During the past decade, a number of new strategies have been developed for the treatment of MS. These include targeting leukocyte differentiation molecules, costimulatory molecules, antiadhesion molecules and chemotaxis. Immunomodulation, autologous stem cell transplantation, anti-infectious therapies and strategies for neuroprotection, neurorepair and remyelination have also emerged as promising therapeutic options. However, despite the abundant research dedicated to MS, there is still no cure. Those treatments that are currently available function at best only to slow disease progression and mitigate symptoms (1, 15-20).

The search for effective treatment strategies for MS continues, with research focusing on the identification of novel targets for drug development. Those targets which are currently under active investigation are discussed below (see Figure 1). Table I provides a selection of products under active development for each target and Table II includes selected patents.

TARGETS

Adenosine deaminase

Adenosine deaminase (EC 3.5.4.4) is a member of the adenosine and AMP deaminase family of enzymes that are responsible for nucleoside metabolism. Adenosine deaminase carries out the irreversible deamination of adenosine to inosine, which is subsequently deribosylated by purine nucleoside phosphorylase and converted to hypoxanthine. Deficiency of adenosine deaminase results in an accumulation of deoxyadenosine, which causes an increase in *S*-adenosylhomocysteine; both substances are toxic to immature lymphocytes, which as a consequence of exposure fail to mature. The immune system becomes severely compromised or is completely lacking. The result is severe combined immunodeficiency (SCID) and multiple organ damage. Purine nucleoside analogues inhibit adenosine deaminase and thus can interfere with the behavior and the proliferation of certain white blood cells, particularly lymphocytes, which are involved in the pathological process of MS. Through its differentiated mechanism of action, an adenosine deaminase inhibitor may offer an option for MS treatment (21-23).

AMPA receptor

The AMPA receptor is a non-NMDA-type ionotropic transmembrane receptor for the excitatory neurotransmitter glutamate that is involved in learning and memory; AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) is the synthetic ligand for these receptors. Studies have shown that changes in the number of synaptic AMPA receptors may be responsible for synaptic plasticity (i.e., the neuronal mechanism required for learning and memory). AMPA receptor modulators could potentially help combat the memory loss seen in Alzheimer's disease (AD) and the cognition deficits associated with schizophrenia. In MS, key alterations that contribute to chronic necrosis of axons may include defective axonal AMPA receptors. Moreover, studies have shown that the inflammation seen in chronic inflammatory diseases of the CNS such as MS can enhance

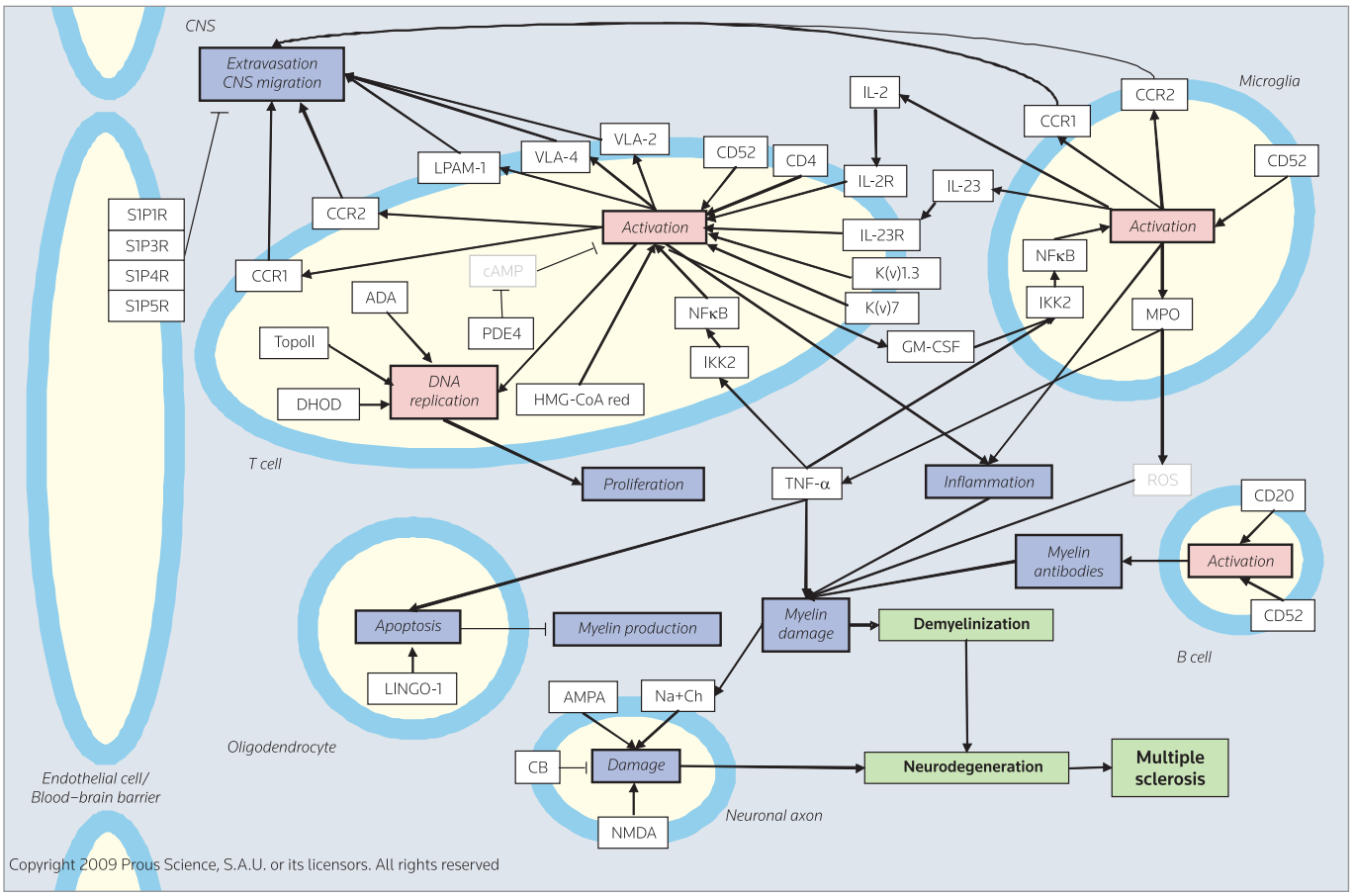

Figure 1. Multiple sclerosis targetscape. A diagram showing an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of multiple sclerosis and their biological actions. Arrow: positive effect; dash: negative effect. Abbreviations: ADA, adenosine deaminase; AMPA: AMPA receptor; cAMP: 3',5'-cyclic adenosine monophosphate; CB: cannabinoid receptor; CCR: chemokine (C-C motif) receptor; DHOD: dihydroorotate dehydrogenase; GM-CSF: granulocyte–macrophage colony-stimulating factor; HMG-CoA red: hydroxymethylglutaryl-CoA reductase; IKK2; I-kappa-B kinase beta; IL-2: interleukin-2; IL-2R: interleukin-2 receptor; IL-23: interleukin-23; IL-23R: IL-23 receptor; LINGO-1: leucine-rich repeat neuronal protein 1; LPAM-1: integrin $\alpha_4\beta_7$; MPO: myeloperoxidase; NMDA: *N*-methyl-D-aspartic acid receptor; NFκB: nuclear factor NF-kappa-B; PDE4: phosphodiesterase 4; ROS: reactive oxygen species; S1P: lysophospholipid receptors; Na+Ch: sodium channel; TopoII: topoisomerase II; TNF-α: tumor necrosis factor alpha; VLA-2: integrin $\alpha_2\beta_1$; VLA-4: integrin $\alpha_4\beta_1$.

glutamate transmission in the striatum and promote synaptic degeneration and dendritic spine loss. The effects are independent of demyelination and occur early in the course of MS. Thus, neuroprotective therapies such as AMPA receptor antagonists may suppress the early neuronal damage seen in MS (24-26).

Cannabinoid receptors

The cannabinoid receptors CB_1 and CB_2 are seven-transmembrane-spanning, G protein-coupled receptors (GPCRs) identified as receptors for cannabinoids. CB_2 is highly expressed in immune cells (e.g., B cells, natural killer [NK] cells, monocytes, microglial cells, neutrophils, T cells, dendritic cells, mast cells), although its biological functions are still unclear; it may be responsible for the anti-inflammatory and possibly other therapeutic effects of cannabis. CB_2-selective agonists, inverse agonists and antagonists have been shown preclinically to suppress inflammation and may alleviate inflammatory and neuropathic pain and emesis. CB_1 is preferentially expressed in brain, where it mediates the psychoactivity of cannabinoids. High levels of CB_1 receptors are found in the basal ganglia, hippocampus, cerebellum and cortical structures. CB_1 receptors are coupled through the $G_{i/o}$ family of proteins to signal transduction mechanisms that include inhibition of adenylyl cyclase and activation of mitogen-activated protein (MAP) kinase. Activation of presynaptic CB_1 receptors inhibits N-type Ca^{2+} channel activity, which in turn reduces excitatory neurotransmitter release to the synaptic cleft, thus allowing the excitatory signals to activate the postsynaptic cell. Cannabinoids have been speculated to exert neuroprotection against excitotoxicity and acute brain damage. Thus, cannabinoid receptor activation would protect hippocampal or granule cerebellar neurons from excitotoxicity and from hypoxia and glucose deprivation, and may prevent neurodegenerative progression in AD and MS. Moreover, results from preclinical studies suggest that peripheral CB_2 receptors may play a protective role in MS pathology. They appear to mediate myeloid progenitor cell trafficking toward the inflamed spinal cord and its contribution to microglial activation,

Table I. *Selected targets and products launched or being actively investigated for multiple sclerosis (from Prous Science Integrity®).*

Target	Product	Source	Phase
Adenosine deaminase	Cladribine	Ivax/Merck Serono	III
AMPA receptor	Perampanel	Eisai	II
Cannabinoid receptors	*Cannabis sativa* L.extract Dronabinol	GW Pharmaceuticals University of California, Davis	Prereg. I/II
CD4	TRX-1	Tolerx	Preclinical
CD20	Rituximab Ocrelizumab Ofatumumab	Biogen Idec/Genentech Genentech/Roche Genmab/GlaxoSmithKline	III II II
CD52	Alemtuzumab	Bayer Schering Pharma/Genzyme	III
Chemokine CCR1 receptor	PS-031291	Pharmacopeia	Preclinical
Chemokine CCR2 receptor	INCB-8696 CGEN-54 OPL-CCL2-LPM	Incyte Compugen Osprey Pharmaceuticals	I Preclinical Preclinical
Dihydroorotate dehydrogenase	Teriflunomide	sanofi-aventis	III
Granulocyte–macrophage colony-stimulating factor (GM-CSF)	MT-203	Micromet	Preclinical
Hydroxymethylglutaryl-CoA reductase	Simvastatin Atorvastatin	Alpharma National Institute of Allergy and Infectious Diseases	III II
I-kappa-B-kinase beta (IKK-beta)	MLN-0415	Millennium Pharmaceuticals	I
Integrin $\alpha_2\beta_1$ (VLA-2)	CHR-1103	Glenmark Pharmaceuticals	Preclinical
Integrin $\alpha_4\beta_1$ (VLA-4)	TV-1102	Teva Pharmaceuticals	II
Integrin $\alpha_4\beta_7$ (LPAM-1)	Firategrast	GlaxoSmithKline	II
Interleukin-2 receptor alpha chain	Daclizumab	Biogen Idec/PDL BioPharma	II
Interleukin-23	Anti-IL23 antibody	Lilly	I
K_v7 channels	Flupirtine maleate	Bayer	II
$K_v1.3$ channel	PAP-1 ShK-186	Airmid Airmid	Preclinical Preclinical
Leucine-rich repeat neuronal protein 1 (LINGO-1)	Anti-LINGO-1	Biogen Idec	Preclinical
Lysophospholipid receptors	Fingolimod HCl BAF-312 CS-0777 KRP-203 ONO-4641	Novartis Novartis Daiichi Sankyo Novartis Ono	III I I I I
Myeloperoxidase (MPO)	AZD-5904	AstraZeneca	I
NMDA receptor	HT-1001 ABS-75	Oregon Health and Science University Harvard Medical School/Tel Aviv University	II Preclinical
Nuclear factor NF-kappa-B	Dimethyl fumarate	Biogen Idec	III
Phosphodiesterase 4 (PDE4)	GRC-4039 Lamotrigine Riluzole Nerispirdine HCl	Glenmark Pharmaceuticals University College London University of California, San Francisco sanofi-aventis	Preclinical II II II
TNF-α	COG-112 GRC-4039 Tranilast	Cognosci Glenmark Pharmaceuticals Nuon Therapeutics	Preclinical Preclinical Preclinical

and administration of CB_2 agonists in an animal model of MS reduced disease severity. This supports the potential use of nonpsychoactive CB_2 agonists in the treatment of MS and other neuroinflammatory disorders. Cannabinoid agonists are currently under development for the treatment of neuropathic pain and spasticity in MS. In addition, CB_1 is implicated in learning and memory, and antagonism of this receptor may improve cognitive deficits in AD, schizophrenia and other neurodegenerative diseases such as MS (27-30).

CD4

CD4 is a transmembrane glycoprotein and a member of the Ig superfamily of receptors that is expressed on the surface of T helper (Th)

Table II. *Selected patents for targets being pursued or explored for multiple sclerosis (from Prous Science Integrity®).*

Target	Patent	Source	Phase
Cannabinoid receptors	WO 2008152086	Solvay	Biological testing
	WO 2009024819	AstraZeneca	Biological testing
Chemokine CCR1 receptor	WO 2007022257	ChemoCentryx	Biological testing
	WO 2007129960	AstraZeneca	Biological testing
	WO 2008011392	Pharmacopeia	Biological testing
Chemokine CCR2 receptor	WO 2007014008	GlaxoSmithKline	Biological testing
	WO 2007014054	GlaxoSmithKline	Biological testing
	WO 2007053499	Millennium Pharmaceuticals	Biological testing
	WO 2007053498	Millennium Pharmaceuticals	Biological testing
	WO 2007053495	Millennium Pharmaceuticals	Biological testing
	WO 2007067875	GlaxoSmithKline	Biological testing
	WO 2007115713	Georg-August-Universitaet/ Universitaet Regensburg	Preclinical
	WO 2008008431	ChemoCentryx	Biological testing
	WO 2008008374	ChemoCentryx	Biological testing
	WO 2008008375	ChemoCentryx	Biological testing
	WO 2008014360	Bristol-Myers Squibb	Biological testing
	WO 2008045564	Epix	Biological testing
	WO 2008060621	Abbott	Biological testing
	WO 2008157741	GlaxoSmithKline	Biological testing
Chemokine CXCR3 receptor	WO 2007002701	Amgen	Biological testing/Preclinical
	WO 2007002742	Pharmacopeia	Biological testing
	WO 2007062175	Amgen	Biological testing
	WO 2007064553	Merck & Co.	Biological testing
	WO 2007070433	Merck & Co.	Biological testing
	WO 2007084728	Abbott	Biological testing
	WO 2007090826	Janssen	Biological testing
	WO 2007100610	Merck & Co.	Biological testing
	WO 2007109238	Schering Corp./Pharmacopeia	Biological testing
	WO 2008008453	Pharmacopeia/Schering Corp.	Biological testing
	WO 2008079279	Schering Corp./Pharmacopeia	Biological testing
	US 2007082913	Schering Corp.	Biological testing
Dihydroorotate dehydrogenase	WO 2009021696	Laboratorios Almirall	Biological testing
I-kappa-B-kinase beta (IKK-beta)	WO 2007097981	Millennium Pharmaceuticals	Biological testing
Integrin $\alpha_4\beta_1$ (VLA-4)	JP 2007023029	Mitsubishi Tanabe Pharma	Biological testing
	WO 2007101165	Elan	Biological testing
	WO 2007041270	Wyeth/Elan	Biological testing
	WO 2007041324	Wyeth/Elan	Biological testing
	WO 2008064823	UCB	Biological testing
	WO 2008064830	UCB	Biological testing
Integrin $\alpha_4\beta_7$ (LPAM-1)	WO 2008064823	UCB	Biological testing
	WO 2008064830	UCB	Biological testing
$K_v1.3$ channel	WO 2008040057	Bionomics	Biological testing
	WO 2008040058	Bionomics	Biological testing
Lysophospholipid S_1P_1 receptor	WO 2007089715	University of Virginia	Biological testing
	WO 2008016692	Praecis Pharmaceuticals	Biological testing
	WO 2008019090	Praecis Pharmaceuticals	Biological testing
	WO 2008024196	Praecis Pharmaceuticals	Biological testing
	WO 2008074820	GlaxoSmithKline	Biological testing
	WO 2008074821	GlaxoSmithKline	Biological testing
	WO 2008076356	Abbott	Biological testing
	WO 2008079382	Abbott	Biological testing
	WO 2008016674	Praecis Pharmaceuticals	Biological testing
	WO 2008128951	GlaxoSmithKline	Biological testing
	WO 2008142073	Novartis	Biological testing
Lysophospholipid S_1P_3 receptor	WO 2007092638	University of Virginia	Biological testing

Continued

Table II (Cont.). *Selected patents for targets being pursued or explored for multiple sclerosis (from Prous Science Integrity®).*

Target	Patent	Source	Phase
Lysophospholipid S_1P_4/S_1P_5 receptor	WO 2008142073	Novartis	Biological testing
Matrix metalloproteinase 12 (MMP-12)	WO 2007048788	Applied Research Systems	Biological testing
	WO 2007060132	Applied Research Systems	Biological testing
	WO 2008023336	Ranbaxy	Biological testing
	WO 2009007747	AstraZeneca	Biological testing
Myeloperoxidase (MPO)	WO 2007120097	AstraZeneca	Biological testing
	WO 2007120098	AstraZeneca	Biological testing
	WO 2007142576	AstraZeneca	Biological testing
	WO 2007142577	AstraZeneca	Biological testing
	WO 2008152420	AstraZeneca	Biological testing
Mitogen-activated protein kinase	WO 2007011762	Applied Research Systems	Preclinical
	WO 2007141224	Laboratories Serono	Preclinical
	WO 2008095943	Eisai London Research Laboratories	Biological testing
	WO 2008095944	Eisai London Research Laboratories	Biological testing
Phosphodiesterase 4 (PDE4)	WO 2008113881	CSIC/Instituto de Salud Carlos III	Biological testing
TNF-α	WO 2007048788	Applied Research Systems	Biological testing
	WO 2007084455	Schering Corp.	Biological testing

cells, regulatory T cells, monocytes, macrophages and dendritic cells. It is a coreceptor that together with the T-cell receptor (TCR) activates T cells following interaction with MHC class II molecules present on the surface of antigen-presenting cells. CD4 amplifies the signal generated by the TCR by recruiting the tyrosine kinase LCK. It has four Ig domains (D1-D4) exposed on the extracellular surface of the cell and uses the D1 domain to interact with the β2 domain of MHC class II molecules. T cells expressing CD4 molecules (not CD8) on their surface are MHC class II-restricted, specific for antigens presented by MHC II and not by MHC class I. In MS, myelin antigen-specific $CD4^+$ T cells become activated in the peripheral immune compartment and cross the blood–brain barrier to trigger the disease. The commitment of T cells to proinflammatory effector Th cell lineages (e.g., IL-17-producing $CD4^+$ T cells, or Th17 cells) appears to be an important inducer of organ-specific autoimmunity and studies suggest that Th17 cells are the dominant pathogenic cellular component in MS and other autoimmune inflammatory diseases. Decreasing myelin-specific $CD4^+$ T cell responses with an anti-CD4 antibody, for example, could reduce demyelination and decrease immune cell infiltration into the CNS, and thus reduce subsequent initiation and progression of the autoimmune response (31-33).

CD20

CD20 is a 33- to 37-kDa transmembrane glycoprotein of the Ig superfamily that is expressed on the surface of normal and malignant B cells, residing within lipid rafts of the phospholipid membrane, where it functions as a store-operated calcium channel following the ligation of the B-cell receptor with antigen. No natural ligands of CD20 have been identified. However, CD20 has been shown to participate in antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cellular cytotoxicity (CDCC) and cell growth. Antibodies directed against CD20 could eliminate pathogenic B cells and may therefore be effective in the treatment of autoimmune inflammatory conditions such as rheumatoid arthritis, systemic lupus erythematosus and MS (34-36).

CD52

CD52 is a 21-kDa cell-surface glycoprotein that is expressed by B and T lymphocytes, NK cells, monocytes, macrophages, dendritic cells, red blood cells, platelets and hematopoietic progenitor cells. Engagement of CD52 induces lysis via activation of complement- and direct cell-mediated cytotoxicity; however, the biological function of CD52 remains unknown. CD52 is expressed in the majority of low-grade B-cell lymphoproliferative disorders (e.g., chronic lymphocytic leukemia [CLL]/small lymphocytic leukemia, acute myeloid leukemia [AML], follicular lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, mucosal-associated lymphoid tissue lymphomas and B- and T-cell lineage acute lymphocytic leukemia [ALL]). Antagonism of CD52 leads to rapid and profound lymphopenia, and the T cells believed to be responsible for initiating the destructive process seen in MS are eliminated. CD52 antagonism may therefore be an effective therapeutic strategy for MS (37-39).

Chemokine CCR1 receptor

CCR1 is a GPCR of the CC chemokine receptor subfamily that signals through increases in intracellular calcium levels. It acts as a receptor for macrophage inflammatory protein 1-α (MIP-1-α), RANTES (or CCL5), monocyte chemoattractant protein 3 (MCP-3, or CCL7) and myeloid progenitor inhibitory factor 1 (MPIF-1, or CCL23). CCR1 is essential for the recruitment of effector immune cells to sites of inflammation. Monocyte infiltration is implicated in a variety of diseases, including multiple myeloma, rheumatoid arthritis and MS, and CCR1 activation results in mediation of monocyte trafficking to sites of inflammation. Antagonism of CCR1 may therefore be effective in the treatment of MS (40-42).

Chemokine CCR2 receptor

CCR2 is a 7-transmembrane GPCR expressed on monocytes and mast cells, as well as B cells, T cells, megakaryocytes, basophils, eosinophils, fibroblasts, astrocytes, dendritic cells, chondrocytes,

colonocytes, endothelial cells, enterocytes, Langerhans cells, epithelial cells, smooth muscle cells and synoviocytes. Two isoforms have been identified (CCR2A and CCR2B) that bind MCP-1; agonist-induced activation of the receptors results in calcium mobilization and inhibition of adenylyl cyclase. MCP-1/CCR2 interactions are responsible for mediating monocyte recruitment to sites of inflammation, such as that seen in MS. Antagonism of the receptor may be effective for the treatment of rheumatoid arthritis and MS (42-44).

Dihydroorotate dehydrogenase

Dihydroorotate dehydrogenase (DHOD; EC 1.3.3.1) is a mitochondrial enzyme that catalyzes the fourth step in the de novo biosynthesis of pyrimidine-containing ribonucleotides; it catalyzes the ubiquinone-mediated oxidation of dihydroorotate to orotate. As rapidly proliferating human T cells have an exceptional requirement for de novo pyrimidine biosynthesis, small-molecule DHOD inhibitors constitute an attractive therapeutic approach to autoimmune diseases, immunosuppression and cancer. Inhibition of this enzyme would prevent T- and B-cell proliferation, and thus inflammatory responses, and may be effective in the treatment of MS (45, 46).

Granulocyte–macrophage colony-stimulating factor (GM-CSF)

GM-CSF is a hematopoietic growth factor and a proinflammatory cytokine secreted by macrophages, T cells, mast cells, endothelial cells and fibroblasts. It acts as a white blood cell growth factor and can stimulate stem cells to produce granulocytes (i.e., neutrophils, eosinophils and basophils) and monocytes, and is thus involved in immune and inflammatory responses. GM-CSF is lacking in normal brain tissue but is expressed under pathological conditions and is correlated with the presence of dendritic cells. This hematopoietic growth factor plays a central role in maintaining chronic inflammation in diseases such as MS. GM-CSF has been implicated in the development of inflammatory demyelinating lesions and the control of migration and/or proliferation of leukocytes within the CNS. Antagonism of this cytokine may be an effective therapeutic option for MS (47-49).

Hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase)

HMG-CoA reductase (EC 1.1.1.88) is a key enzyme that catalyzes the rate-limiting step in the biosynthetic pathway leading from mevalonate to cholesterol and isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which plays a role in protein prenylation, a crucial step in multiple cellular processes. Protein prenylation (i.e., farnesylation and geranylgeranylation) is a post-translational modification of proteins involving the addition of isoprenoids. Geranylgeranylation allows the activation of the small GTP-binding proteins Rho and Rac. Activated Rho regulates the activity of nuclear transcription factors such as nuclear factor NF-kappa-B, controls the actin cytoskeleton and induces stress fiber formation. This affects intracellular transport, migration, membrane trafficking, messenger RNA stability and gene transcription. Farnesylation allows the activation of Ras protein. Activated Ras stimulates cytoplasmic signaling pathways such as the MAP kinase pathway that regulates gene transcription and thus growth, proliferation, differentiation and survival of cells. Moreover, studies have shown that statins, which inhibit HMG-CoA reductase, are effective in the EAE model and are promising candidates for MS therapy. Statins have been exploited for their immunomodulatory characteristics for the treatment of MS patients, with inhibition of this enzyme offering neuroprotection and decreased disease severity via attenuation of inflammation, axonal loss and demyelination. However, statins also exert pleiotropic effects that are independent of their cholesterol-lowering actions. These include effects on endothelial function, cell proliferation, the inflammatory response, immunological reactions, platelet function and lipid oxidation, which appear to be beneficial in the context of brain injury. The mechanism of action underlying the neuroprotective effects of statins may involve heat shock proteins (HSPs) and the the survival-related phosphatidylinositol 3-kinase (PI3K)/Akt pathway (50-54).

I-kappa-B-kinase beta (IKK2, IKK-beta)

IKK-beta is a protein serine/threonine kinase (EC 2.7.11.10) that phosphorylates I-kappa-B (a cytoplasmic inhibitor) at specific residues, targeting it for proteasomal degradation via the ubiquitination pathway and allowing the nuclear translocation of I-kappa-B. Subsequent degradation of the I-kappa-B complex (IKK) activates nuclear factor NF-kappa-B (NF-kappa-B), a translation factor that plays an important role in inflammation, immunity, cell proliferation and apoptosis. If the serine residues are replaced by threonine residues, the activity of the enzyme is decreased considerably. It is composed of alpha, beta and gamma subunits, the latter not having kinase activity but presumed to play a regulatory role. IKK-beta regulates several inflammatory genes, including TNF-α, interleukin (IL-1) and cell adhesion molecules, and two proinflammatory mediators, NF-kappa-B and transcription factor AP-1, appear to be activated in airways diseases and inflammatory diseases such as MS. Inhibitors of IKK-beta block the NF-kappa-B activation cascade, thereby attenuating inflammatory responses. NF-kappa-B is inhibited by binding to I-kappa-B and polymorphisms of the *NFKB* and *NFKBI* genes and an imbalance in NF-kappa-B and I-kappa-B have been associated with the development of many inflammatory diseases, including MS, ulcerative colitis, Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus, psoriatic arthritis, giant cell arthritis, type 1 diabetes, celiac disease and Parkinson's disease, as well as susceptibility to several cancers (55-57).

Integrin $\alpha_2\beta_1$ (VLA-2)

VLA-2 is a β_1 collagen/laminin-binding integrin (also known as CD49b/CD29) involved in cell adhesion and cell surface-mediated signaling. It is a heterodimeric surface molecule composed of non-covalently bound α_2 (CD49b) and β_1 (CD29) integrin chains that mediate specific cell adhesion towards extracellular matrix components (ECM) such as collagen type I and IV. VLA-2 is expressed on many cell types, including monocytes, platelets, activated T cells, megakaryocytes, neuronal cells, epithelial cells, fibroblasts and osteoclasts. VLA-2 expressed on T cells has been shown to be involved in T-cell activation, cell anchorage on collagen, signal transmission for cell activation, proliferation and survival. VLA-2 plays a crucial role in cell–cell and cell–ECM interactions during inflammation. Blockade of the interaction between inflammatory cells and vascular endothelium can prevent cell entry into tissues and harmful inflammatory responses (i.e., autoimmunity), such as

those observed in MS. Studies suggest that blocking cell–ECM interactions via VLA-2 antagonism could be an effective therapy for MS (58).

Integrin $\alpha_4\beta_1$ (VLA-4)

VLA-4 is an α_4 integrin also known as CD49d/CD29. It is a key cell-surface receptor expressed on leukocytes (i.e., lymphocytes, monocytes, mast cells, macrophages, NK cells, dendritic cells, basophils and eosinophils, but not neutrophils) and is involved in both adhesion and T-cell costimulation. It binds to both vascular adhesion molecule 1 (V-CAM 1) expressed on cytokine-stimulated endothelial cells and the connecting segment (CS-1) domain of fibronectin, an ECM protein. During inflammatory reactions, VLA-4 regulates cellular adhesion and leukocyte migration into tissues through activation of cell–cell and cell–matrix interactions. Thus, antagonism of VLA-4 is an attractive strategy for the treatment of chronic inflammatory diseases such as inflammatory bowel disease (IBD), psoriasis and MS (38, 59-61).

Integrin $\alpha_4\beta_7$ (LPAM-1)

LPAM-1 is an α_4 integrin (also known as CD49d/beta7) composed of the α_4 and β_7 subunits that form a lymphocyte-homing receptor that mediates lymphocyte attachment within the ECM by adhering to the CS-1 site of fibronectin. It belongs to the Ig superfamily and is found on the majority of peripheral lymphocytes and subsets of thymocytes and bone marrow cells (including mast cell progenitors). LPAM-1 binds its ligands, V-CAM 1 (CD106), MAdCAM-1 and fibronectin, plays an important role in lymphocyte adhesion and helps direct the migration of blood lymphocytes to the intestine and associated lymphoid tissues. Inflammation leading to tissue damage and disease is mediated in part by the α_4 integrins $\alpha_4\beta_1$ and $\alpha_4\beta_7$, expressed on the leukocyte cell surface. Inhibition of leukocyte trafficking by antagonism of the α_4 integrin is a validated therapeutic approach for the treatment of inflammatory diseases such as MS and IBD, and monoclonal antibodies specific for α_4 integrins or their cell adhesion molecule (CAM) ligands can moderate inflammation in animal models, suggesting that such inhibitors may be useful for treating human inflammatory diseases such as MS (61).

Interleukin-2 receptor alpha chain (IL-2-RA, CD25)

IL-2 is a cytokine produced by $CD4^+$ T lymphocytes upon activation by antigens and costimulators. It promotes T-cell clonal expansion in the adaptative immune response and can activate B lymphocytes, monocytes and NK cells. Binding of IL-2 to its receptor activates the JAK/STAT, PI3K and Ras signaling pathways. Alpha-chain monomers conform a low-affinity IL-2 receptor. High-affinity and intermediate-affinity IL-2 receptors are conformed by alpha/beta heterodimers and beta-chain monomers, respectively, associated to a gamma chain. This receptor plays a role in both proliferative and activation-induced cell death signaling of T cells. MS is in part genetically determined and the gene encoding the alpha chain of the IL-2 receptor, *IL2RA,* harbors alleles associated with a risk for MS and other autoimmune diseases. In addition, *IL2RA* genetic variants correlate with the levels of a soluble form of the IL-2 receptor in subjects with type 1 diabetes and MS (31, 62, 63).

Interleukin-23 (IL-23)

IL-23 is a heterodimeric cytokine composed of a unique p19 subunit and the p40 subunit component of IL-12. It is secreted by activated dendritic cells, glial cells and macrophages and binds to memory T cells, NK cells, macrophages and dendritic cells. In particular, this cytokine is suspected to be involved in the activation and maintenance of the Th17 subset of inflammatory T cells. It promotes upregulation of matrix metalloproteinase MMP-9, increases angiogenesis and reduces $CD8^+$ T-cell infiltration. The IL-12 family of cytokines (IL-12, IL-23, IL-27) plays a critical role in the differentiation of Th1 cells and is believed to contribute to the development of MS. It has been hypothesized that the autoimmune actions of IL-12 are attributable to IL-23, since mice lacking IL-23p19 (only IL-23 absent) and mice lacking IL-12p40 (both IL-12 and IL-23 absent) were protected from autoimmune EAE and collagen-induced arthritis (CIA). On the other hand, mice lacking IL-12p35 (only IL-12 absent) developed more severe disease. Overexpression of IL-23 and/or IL-12 or a defect in their receptors may be involved in conditions such as rheumatoid arthritis, psoriatic arthritis, psoriasis, Crohn's disease, ankylosing spondylitis and MS. Monoclonal antibodies directed against both IL-12 and IL-23 may be effective treatment options for these diseases (64-66).

K_v7 channels

K_v7 channels include five subtypes of voltage-gated potassium channels: $K_v7.1$ (*KCNQ1*), $K_v7.2$ (*KCNQ2*), $K_v7.3$ (*KCNQ3*), $K_v7.4$ (*KCNQ4*) and $K_v7.5$ (*KCNQ5*). In cardiac cells, $K_v7.1$ mediates the slow delayed rectifier K^+ current that contributes to the repolarization of the cell, terminating the cardiac action potential and thereby myocardial contraction. $K_v7.2$ associates with $K_v7.3$ to form an M-current potassium channel, a slowly activating and deactivating potassium channel that determines neuronal excitability and responsiveness to synaptic inputs. M-currents can be inhibited by muscarinic acetylcholine M_1 receptors. Mutations in the *KCNQ3* gene are associated with epilepsy (e.g., benign familial neonatal convulsions). $K_v7.4$ is thought to play a critical role in the regulation of neuronal excitability, particularly in sensory cells of the cochlea, and defects in this channel are the cause of nonsyndromic sensorineural deafness type 2 (DFNA2), an autosomal dominant form of progressive hearing loss. $K_v7.5$ is differentially expressed in subregions of the brain and in skeletal muscle. The channel yields currents that activate slowly with depolarization and can form heteromeric channels with the protein encoded by the *KCNQ3* gene. Currents expressed from this protein have voltage dependencies and inhibitor sensitivities similar to those seen with M-currents. They are also inhibited by muscarinic M_1 receptor activation. In MS patients, many neurological signs and symptoms have been attributed to underlying conduction deficits. Neurological function might be improved if conduction could be restored in demyelinated CNS axons. Thus, K_v7 channel activators could be potentially effective in the treatment of MS (67).

$K_v1.3$ channel

The voltage-gated potassium channel $K_v1.3$ is a delayed rectifier ion channel that regulates membrane potential and calcium signaling in human effector memory T cells. $K_v1.3$ is a member of a large family of potassium channels involved in diverse functions, such as reg-

ulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction and cell volume. The channel allows the repolarization of nerve cells after an action potential and plays a role in T-cell proliferation and activation; it maintains the membrane potential of effector memory T cells, which are important mediators of MS, type 1 diabetes and rheumatoid arthritis. The functional $K_v1.3$ channel in T cells is composed of four identical subunits that are physically coupled through a series of adaptor proteins to the TCR signaling complex; it traffics to the immunological synapse during antigen presentation. Blockade of $K_v1.3$ channels in effector memory T cells suppresses calcium signaling, cytokine production (IFN-γ, IL-2) and cell proliferation, thus preventing inflammatory responses. In MS, disease-associated myelin-specific $CD4^+$ T cells in patient blood and post mortem MS brain lesions express high numbers of $K_v1.3$ channels. Targeting $K_v1.3$ could suppress effector memory T-cell responses, thereby ameliorating autoimmune diseases like MS without compromising the protective central immune response (67-70).

Leucine-rich repeat neuronal protein 1 (LINGO-1)

LINGO-1 is a nervous system-specific transmembrane protein that contains a fibronectin type III domain, an Ig-like domain and 11 leucine-rich repeats. LINGO-1 forms a signaling complex with the ligand binding Nogo-66 receptor NgR1 and the signal-transducing neurotrophin receptor p75NTR to activate RhoA; NgR1 and p75NTR alone are unable to activate RhoA, but require LINGO-1. LINGO-1 is expressed in oligodendrocytes and is an important negative regulator of oligodendrocyte-mediated control of myelination. Studies have shown that attenuation of its function results in differentiation and increased myelination competence, with downregulation of RhoA activity implicated in oligodendrocyte differentiation. Conversely, LINGO-1 overexpression leads to activation of RhoA and inhibition of oligodendrocyte differentiation and myelination. CNS myelination has been demonstrated in vitro using LINGO-1 antagonists and the in vivo using *LINGO1* knockout. Thus, LINGO-1 signaling appears to be critical for CNS myelination, and enhancing this repair mechanism with LINGO-1 antagonists may promote remyelination and represent a potentially effective therapeutic option for the treatment of MS (71-73).

Lysophospholipid (sphingosine 1-phosphate, S1P) receptors

Sphingosine 1-phosphate (S1P) is a signaling sphingolipid derived from the aliphatic amino alcohol sphingosine. It is released from ceramides via ceramidase-mediated catalysis. Phosphorylation of sphingosine is catalyzed by sphingosine kinase found in the cytosol and endoplasmic reticulum of various types of cells; S1P can be cleaved by sphingosine phosphatase. It is a blood-borne lipid mediator, in particular in association with lipoproteins such as high-density lipoprotein (HDL); it is present to a much lesser extent in tissues. S1P is an extracellular ligand for the lysophospholipid receptor family, a group of GPCRs important for lipid signaling; lysophospholipid receptors are also referred to as Edg (i.e., endothelial differentiation gene). Eight lysophospholipid receptors have been identified to date: S_1P_1 (Edg-1), LPA_1 (Edg-2), S_1P_3 (Edg-3), LPA_2 (Edg-4), S_1P_2 (Edg-5), S_1P_4 (Edg-6), LPA_3 (Edg-7) and S_1P_5 (Edg-8). Through the lysophospholipid receptors, S1P regulates angiogenesis, vascular stability and permeability. In the immune system, it is a major regulator of B- and T-cell trafficking. Inhibition of S1P receptors was shown to be critical for immunomodulation and targeting of lysophospholipid receptors could be effective in controlling autoimmune diseases such as MS (74-76).

Myeloperoxidase (MPO)

MPO (EC 1.11.1.7) is a member of the heme peroxidase–cyclooxygenase superfamily that is highly expressed in neutrophil granulocytes and also at lower levels in monocytes and certain macrophages. This lysosomal enzyme requires heme as a cofactor and produces hypochlorous acid (HOCl) from hydrogen peroxide (H_2O_2) and chloride anion (Cl^-) during the neutrophil respiratory burst and oxidizes tyrosine to tyrosyl radical using H_2O_2 as an oxidizing agent. Hypochlorous acid and tyrosyl radical are cytotoxic and used by neutrophils to kill bacteria and other pathogens. Extensive axonal damage observed in patients with progressive MS can be caused by several factors, including the release of proteolytic enzymes and cytotoxic oxidants by activated immune cells and glia within the lesion. Macrophages and microglia are known to express MPO and generate reactive oxygen species (ROS) during myelin phagocytosis in white matter. Studies have shown that in patients with MS, MPO is expressed mainly by macrophages within and adjacent to inflammatory lesions, and that demyelination is associated with increased activity of MPO. Because the production of microglial ROS contributes to axonal injury within lesions, inhibition of MPO may be a strategic option for the treatment of MS (77-79).

NMDA receptor

The NMDA receptor is a subtype of glutamate receptor that binds the excitotoxic amino acid NMDA (*N*-methyl-D-aspartic acid) in neurons. Activation of the NMDA receptor results in the opening of an associated ion channel pore, allowing influx of Na^+, K^+ and Ca^{2+}, of which the latter is thought to play a critical role in synaptic plasticity. The receptor mediates long-term potentiation (LTP) of the signaling involved in learning, memory and cognition, but it has also been implicated in causing the cell damage observed in MS, as well as AD and Parkinson's and Huntington's diseases. NMDA receptor expression has been shown to be increased in the murine EAE model of MS. The excitotoxicity seen with increased NMDA receptor activity plays a critical and detrimental role in chronic neurodegenerative disorders. Synaptic overactivity results in excessive glutamate release, thus overstimulating postsynaptic cell membrane receptors (i.e., NMDA receptor), which upon activation, open associated ion channel pores and increase ion influx. The consequence is neuronal cell injury and death. Antagonism of the NMDA receptor may therefore be effective in preventing neurodegeneration in AD and MS (80-82).

Nuclear factor NF-kappa-B (NF-kappa-B)

NF-kappa-B is a protein transcription factor and intracellular mediator of the inflammatory cascade involved in the generation of adhesion molecules (ICAM-1, V-CAM 1), inducible nitric oxide synthase (iNOS synthase), cyclooxygenase COX-2, cytokines (i.e., IL-1β, IL-2, TNF-α, IL-6, IFN-γ) and chemokines (IL-8). Other genes that are regulated by NF-kappa-B include those encoding the IL-2 receptor, the IL-12 p40 subunit and c-Myc. NF-kappa-B provides a mechanistic link between

inflammation and cancer, controlling the ability of preneoplastic and malignant cells to resist apoptosis-based tumor surveillance mechanisms and regulating tumor angiogenesis and invasiveness. NF-kappa-B activity is closely associated with IKK, and aberrant or constitutive NF-kappa-B activation has been detected in many human malignancies. It has also been reported that constitutive activation of the tyrosine-protein kinase receptor FLT3 is responsible for IKK activation. Moreover, TNF activation results in NF-kappa-B activation and plays a role in inflammation and is an important signaling factor for cytokines that appear to participate in several pathological conditions, such as MS, Parkinson's disease and depression. NF-kappa-B has been implicated in various aspects of neuroplasticity, including LTP and cellular apoptosis and differentiation. Macrophages of MS patients have been shown to display heightened activation of STAT6, STAT1 and NF-kappa-B and a corresponding inflammatory profile that may be responsible for controlling macrophage-mediated demyelination. Inhibitors of NF-kappa-B activation may therefore be effective in preventing demyelination in MS (56, 83-85).

Phosphodiesterase 4 (PDE4)

The phosphodiesterase (PDE) isozymes (EC class 3.1.4) degrade cAMP and cGMP and thereby modulate signal transduction mediated by these second messengers. The PDE4 isozyme is characterized by high affinity for cAMP and poor affinity for cGMP. Four PDE4 isoforms have been identified (A, B, C and D) and are abundant in immunocompetent cells, where an increase in cAMP leads to the inhibition of the synthesis and release of proinflammatory mediators, cytokines and ROS. Since PDE4 is the primary cAMP-hydrolyzing enzyme in inflammatory and immune cells (e.g., macrophages, eosinophils, neutrophils), inhibition of this isozyme would increase cAMP levels, consequently downregulating the inflammatory response. Thus, PDE4 inhibitors may be effective as a treatment for inflammatory diseases such as chronic obstructive pulmonary disease, asthma, rheumatoid arthritis, atopic dermatitis and inflammatory bowel disease. Moreover, PDE4 inhibitors may also be a novel therapy for the treatment of MS and AD, which both involve neuroinflammation. PDE4 inhibitors have been shown to counteract deficits in long-term memory in preclinical models and they also exert neuroprotective, neuroregenerative and anti-inflammatory activities, all of which would be beneficial in the treatment of AD and MS (50, 51, 81).

Sodium (Na^+) channels

The Na^+ channels are plasma membrane-bound ion channels permeable to sodium ions. Ubiquitous, the channels are classified as either voltage-gated (expressed on central and peripheral neurons, skeletal muscle, cardiac myocytes) or ligand-gated, such as nicotinic receptors in neuromuscular junctions that bind acetylcholine. The fast voltage-gated sodium channel is composed of an α and β subunit. The α subunit contains four repeat domains (I-IV) each containing six membrane-spanning regions (S1-S6); the S4 segment is the voltage sensor. The channel's voltage sensitivity is mediated by positive amino acids located at every third position. When stimulated by an alteration in transmembrane voltage, this region moves toward the extracellular side of the cell membrane and the channel becomes more permeable to ions. The ions are conducted through a pore, which can be broken into two regions comprising a largely extracellular portion of "P-loops", which is responsible for ion selectivity, and the more cytoplasmic portion, formed by the combined S5 and S6 regions of the four domains. The region that links domains III and IV serves to physically plug or block the channel after extended activation, thereby inactivating it. Inhibition of voltage-gated Na^+ channels results in the stabilization of neuronal membranes and the subsequent modulation of presynaptic transmitter release of excitatory amino acids (e.g., glutamate). Na^+ channel regulation is thus important in diseases associated with neurotransmitter deregulation and Na^+ channel blockers may have potential for use in the treatment of epilepsy, pain, anxiety, angina pectoris, arrhythmia, depression and bipolar disorder. Moreover, in MS, damage to axons may occur due to a combination of energy failure and axonal sodium overload. Sustained sodium influx in turn triggers calcium ion influx, which produces axonal injury in neuroinflammatory disorders such as MS. Thus, partial blockade of axonal sodium channels may afford protection against inflammatory axonal injury (81, 86-88).

TNF-α

TNF-α, a proinflammatory cytokine also known as cachectin, is a member of the tumor necrosis factor (TNF) family of cytokines. It is released by activated macrophages and lymphocytes. It acts via receptors belonging to the TNF family of receptors, among which TNF-R1 and TNF-R2 trigger several signal transduction pathways, resulting in the activation of transcription factors such as NF-kappa-B and c-fos/c-jun. TNF-R1 (also known as CD120a and p55) is expressed in most tissues and is fully activated by both the membrane-bound and soluble trimeric forms of TNF. TNF-R2 (also known as CD120b and p75), however, is found only in cells of the immune system and is activated by the membrane-bound form of the TNF homotrimer. Activated factors induce the transcription of antiapoptotic, proliferative, immunomodulatory and inflammatory genes. NF-kappa-B is the major survival factor in preventing TNF-α-induced apoptosis and inhibition of this transcription factor may improve the efficacy of apoptosis-inducing cancer therapies. TNF-α is also a crucial cytokine in the establishment and maintenance of inflammation in multiple autoimmune diseases. Studies have reported elevated TNF levels at the site of active MS lesions in post mortem brain samples from patients with MS, and CSF and serum TNF levels in individuals with MS are elevated compared to healthy individuals, with TNF levels correlating to the severity of the lesions. In addition, peripheral blood mononuclear cells (PBMCs) from MS patients just prior to symptom exacerbation display increased TNF secretion after stimulation as compared to cells from the same patient during remission. Inhibition of the TNF-α signaling pathway (e.g., TNF-α blockers, blockers of p38, JNK and/or ERK kinases, inhibitors of NF-kappa-B activation) is an attractive therapeutic strategy for the treatment of MS, as well as Crohn's disease, psoriasis, psoriatic arthritis, uveitis, sarcoidosis, Behcet's syndrome, graft versus host disease and ankylosing spondylitis (89-91).

Topoisomerase II

DNA topoisomerase II (EC 5.99.1.3) is an enzyme that plays a critical role in maintaining the proper topology and physical integrity of DNA. It makes transient double-strand breaks and allows the passage of a second DNA duplex across the break. DNA topoisomerase

II is usually ATP-dependent and the alpha isoform has been found to have preference for positive DNA supercoils. Since it participates in the processes of DNA transcription, chromosome disentanglement, recombination and repair, targeting DNA topoisomerase II has become a valid approach for the design of anticancer therapeutics. DNA topoisomerase II antagonism is used to treat cancer, including breast, colorectal, lung, prostate and head and neck cancer, melanoma and hematological cancer, including acute myeloid leukemia. Moreover, inhibition of topoisomerase II may be an effective therapeutic option for the treatment of autoimmune diseases such as rheumatoid arthritis and MS. Mounting interest has been shown in the chemical reaction performed by topoisomerase, which is able to trigger movement of DNA segments several orders of magnitude larger than the size of the protein. Studies have shown that inhibition of topoisomerase II results in cytotoxic effects on both proliferating and nonproliferating cells, suggesting a lack of cell cycle phase specificity. Moreover, inhibition of the enzyme has been shown to inhibit B-cell, T-cell and macrophage proliferation and to impair antigen presentation, as well as to inhibit the secretion of the proinflammatory cytokines IFN-γ, TNF-α and IL-2. In MS, topoisomerase II inhibition, and thus immunosuppression, may slow the progression of the disease or the frequency of relapses (92-95).

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