Immunomodulation in multiple sclerosis: from immunosuppression to neuroprotection

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Multiple sclerosis (MS) is the most common disabling neurological disease of young adulthood. Following advances in the understanding of the immunological mechanisms that underlie the pathogenesis of MS, a growing arsenal of immunomodulatory agents is available. Two classes of immunomodulators are approved for long-term treatment of MS, the efficacy of several promising new concepts is being tested in clinical trials and classical immunosuppressive agents used in MS treatment have been shown to exert specific, immunomodulatory effects. Furthermore, two recent observations have changed our basic understanding of the pathogenesis of MS. First, immune cells in MS lesions have neuroprotective activity, which indicates a beneficial role of neuroinflammation. Second, there is evidence that axonal loss, rather than demyelination, underlies the progression of MS and, hence, constitutes a therapeutic target.

'The surest way to lose a reputation in neurology is to advocate a treatment for multiple sclerosis.' (H. Houston Merritt)

Multiple sclerosis (MS) is the most common inflammatory disorder of the CNS and the leading cause of neurological disability in young adults [1]. Many immune abnormalities have been described in MS, which indicates that the immune system plays a central role in its pathogenesis [2–4]. Although immune responses contribute to the formation and maintenance of MS lesions [5], neuroinflammation might have neuroprotective effects [6,7]. This crucial role of the immune system in disease pathogenesis has important therapeutic implications. For a long while corticosteroids were the only proven therapy for MS. However, these only shorten an acute attack and effective, long-term drug treatment was not available. Although several immunosuppressive agents (i.e. inhibitors of crucial components of the immune system that cause generalized immune dysfunction) were used off-label, the adverse systemic effects, such as increased risk of cancer and infection, limited the potential benefits in MS. More recently, two classes of immunomodulatory agents, interferon β (IFN-β) and glatiramer acetate (GA), have been approved for the treatment of MS [8–10]. Immunomodulators, which do not cause general suppression of the immune system, shift immune responses from pro-inflammatory autoimmune conditions [mediated by T helper 1 (Th1) cytokines that are released by autoreactive T cells] towards more beneficial anti-inflammatory circumstances (mediated through Th2 cytokines that are secreted by regulatory T cells). Both IFN-β and GA have been proven to be partially effective in clinical trials [1,11–14]. In the search for more efficacious agents, many new drugs are under investigation in preclinical and clinical trials, but several promising approaches have failed [15]. In parallel there have been advances in understanding the underlying pathogenesis of the disease as well as modes of action of the different agents.

Here, we summarize current concepts about the mechanisms of action of therapies already approved for MS and the most promising future candidates.

Disease-relevant immune processes

Since the first description of MS in 1835 by J. Cruveilhier as 'sclérose en taches, en îles par masses disséminées' [16], the concepts of its pathogenesis have been adapted continuously [1,2,4,17]. Although unproven, the current consensus is that MS pathogenesis comprises an initial inflammatory phase, which fulfils the criteria for an autoimmune disease [18], followed by a phase of selective demyelination and last, a neurodegenerative phase [4,17]. Subjects with genetically determined susceptibility to MS [19] harbor T cells that react with CNS autoantigens. Although these can remain dormant for decades, at some point they are activated in the periphery, probably by molecular mimicry (i.e. recognition of epitopes that are common to autoantigens and microbial antigens as exogenous triggers [20,21]). This enables them to migrate through the blood–brain barrier to the brain and spinal cord. Reactivated in the CNS, these T cells of either $CD4^+$ helper or $CD8^+$ cytotoxic phenotype [22] release pro-inflammatory Th1 cytokines and orchestrate the destruction of the myelin sheath by various types of immune cells. Destruction follows the first two of four pathological patterns [5]: (1) T-cell- and macrophage-mediated demyelination; (2)

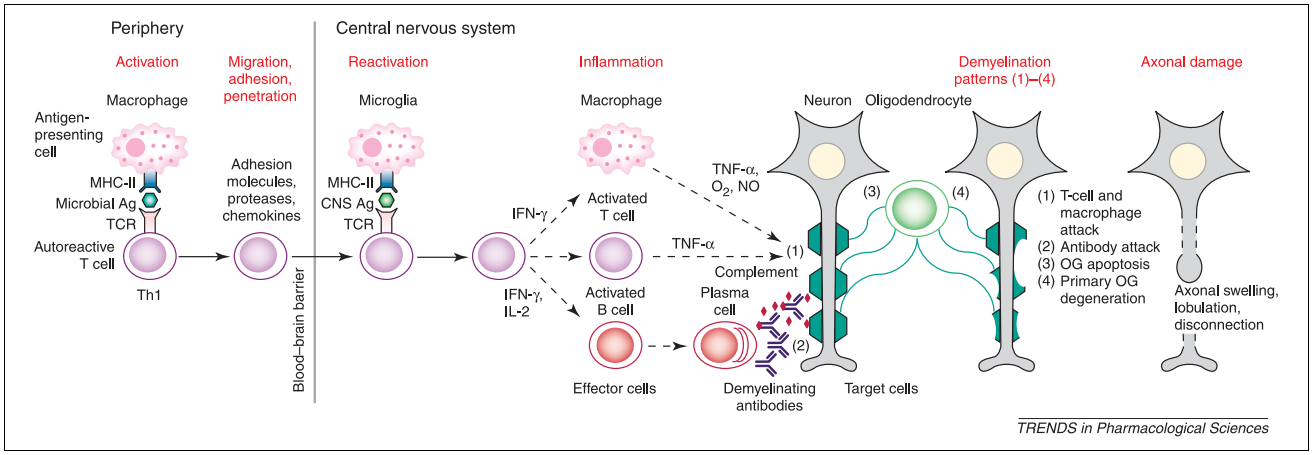


Fig. 1. Pro-inflammatory T cells in the periphery are activated when foreign antigens (Ags) and self-antigens that are presented on major histocompatibility complex class II (MHC-II) by Ag-presenting cells, such as macrophages, bind to T-cell receptors (TCRs). Activated T cells migrate to, adhere at and penetrate through the blood–brain barrier, steps that are mediated by adhesion molecules, proteases and chemokines. In the CNS, the T cells are reactivated by CNS Ags presented on MHC-II by other Ag-presenting cells, predominantly microglial cells. The reactivated T cells secrete pro-inflammatory cytokines, such as interferon γ (IFN-γ) or interleukin 2 (IL-2), which induce CNS inflammation by subsequent activation of macrophages, other T cells and B cells. Macrophages and T cells attack the myelin sheath of oligodendrocytes (OGs) by cytotoxic mediators, mainly tumor necrosis factor α (TNF-α), O_2 radicals and nitric oxide (NO). B cells differentiate into plasma cells. These secrete demyelinating antibodies that can guide and activate macrophages, and ignite the complement cascade, which causes assembly of the membrane attack complex and causes pore formation in myelin membranes. Demyelination occurs by four different pathological patterns (1–4), as described in the main text.

oligodendrocyte apoptosis; and (4) primary oligodendrocyte degeneration. The mechanisms of the latter two patterns remain elusive.

In addition to this autoaggressive inflammatory phase, axonal loss, which causes irreversible disability, occurs early in the course of the disease [24,25]. It is unclear whether axonal damage is the consequence of a primary active destructive process executed by, for example, macrophages and cytotoxic molecules derived from CD8 cells [26], or a (patho)physiological response that occurs secondarily to demyelination and is based on increased vulnerability [24,27]. Axonal damage appears to be initiated by increased membrane permeability followed by enhanced Ca^{2+} influx. Disruption of axonal transport alters the cytoskeleton and leads to axonal swelling, lobulation and, finally, disconnection [28].

The different molecules involved in each phase of MS are summarized in Fig. 1 [1,17,29].

MS therapeutics: immunomodulatory profile *in vitro* and *in vivo*

The structural features of the therapeutic agents are shown in Fig. 2. Interferon β1a (IFN-β1a), IFN-β1b and glatiramer acetate (GA) are approved for the treatment of relapsing-remitting (RR) MS, IFN-β1b for secondary-progressive (SP) MS, IFN-β1a for SP MS with superimposed relapses and mitoxantrone for worsening forms of RR and SP MS.

Glucocorticosteroids

Methylprednisolone and prednisolone are the mainstays of treatment for acute attacks in MS [30]. Because of side-effects in long-term treatment regimens and superior efficacy compared with oral application, steroids are mostly delivered in intravenous pulses. Most of the immunological effects of glucocorticosteroids are mediated molecules of heat shock protein 90 (HSP90), one molecule of HSP70, one molecule of HSP56, one so-called immunophilin, and other, less well characterized, proteins. After binding lipophilic glucocorticosteroid, the receptor dissociates irreversibly from the rest of the complex, and the steroid–receptor complex ligates specific glucocorticoid-responsive elements in nuclear DNA. This either upregulates or downregulates the transcription of target genes. The nuclear factor κB (NF-κB) transcription factor induces the expression of multiple inflammatory and immune genes [31]. Glucocorticosteroids inhibit NF-κB, both by direct binding of the activated receptor to NF-κB and by inducing expression of the specific inhibitory protein IκB in lymphocytes.

The immunological effects of glucocorticosteroids include: (1) inhibition of T-cell activation and production of pro-inflammatory cytokines, such as interleukin 2 (IL-2) and IFN-γ; (2) increased production of anti-inflammatory Th2 cytokines by T cells; (3) inhibition of IFN-γ-induced major histocompatibility complex (MHC) class II expression on macrophages; (4) decreased production of pro-inflammatory cytokines, prostaglandins and leukotrienes by macrophages; (5) diminished adhesion of neutrophils to endothelial cells; and (6) inhibition of endothelial-cell activation, and expression of MHC class II and adhesion molecules. Taken together, these actions underlie the sealing effect of steroids on the blood–brain barrier, which prevents further access of immune cells and molecules to the brain.

In addition to the genomic effects mediated by steroid receptor activation, there is recent evidence that high doses of glucocorticosteroids induce apoptosis of target cells by a direct, non-genomic effect [32]. In this case, apoptosis is thought to be mediated by a direct effect on cellular membranes, which influences transmembranous ion transport and, subsequently, reduces the availability of

IFN-β

IFN-β is highly species specific. In humans, the IFN-β polypeptide is produced and secreted by fibroblasts, but virtually all mammalian cells can produce IFN-β on stimulation. Two recombinant IFN-β preparations, IFN-β1a and IFN-β1b, are approved for treatment of MS. The optimal dosage and route of administration (subcutaneously versus intramuscularly), the resulting pharmacodynamic properties of IFN-β and the role of neutralizing antibodies are controversial [33–37].

IFN-β has immunomodulatory properties, antiviral and anti-proliferative effects, and promotes cell differentiation [38]. Although the mechanisms of action of IFN-β are not fully understood, there is agreement that the major effects are mediated by activation of a transmembrane IFN receptor, which leads to either the upregulation or downregulation of target genes [39,40]. Unlike IFN-γ, the 'type II interferon', IFN-β and IFN-α (type I interferons) share a receptor, which consists of two chains, IFN-αR1 and IFN-αR2, or several subvariants. Binding of IFN-β (and other type I interferons) to the extracellular domain of the receptor induces an intracellular signal transduction cascade that involves: (1) recruitment and activation of the cytoplasmic tyrosine kinase 2 by IFN-αR1, and Janus kinase 1 by IFN-αR2; (2) subsequent phosphorylation and recruitment of signal transducers and activators of transcription (STAT1 and STAT2) to form a STAT1–STAT2 heterodimer; (3) migration of the STAT1–STAT2 heterodimer to the nucleus; (4) association of STAT1–STAT2 with the p48 protein, to form the active 'IFN-stimulated gene factor 3'; (5) binding of IFN-stimulated gene factor 3 to promoter elements and initiation of the transcription of target genes. The variations in this rough scheme of signal transduction that lead to different effects of IFN-β in different target cells are based on variations in each of the steps outlined above, which are not yet fully understood [39].

The range of immune effects attributed to IFN-β is wide and ever broadening [38,41]. It suppresses T-cell proliferation, diminishes IFN-γ-induced upregulation of MHC class II expression, induces the production of Th2 cytokines and reduces synthesis of Th1 cytokines, and inhibits monocyte activation. In addition, IFN-β downregulates matrix metalloproteinases (MMPs), decreases surface-expressed adhesion molecules and increases the release of soluble adhesion molecules, which combine to reduce the migratory potential of T cells.

GA

GA is the acetate salt of a standardized, randomized mixture of synthetic polypeptides. After subcutaneous administration, GA is quickly degraded to free amino acids and small oligopeptides and, thus, most probably initiates its major immunological effects in the periphery. Unlike the multiple immunological effects of IFN-β, which are antigen nonspecific, the immunomodulatory potential of GA is based on immune cells that are specific for myelin basic protein (MBP) and, probably, other myelin antigens [38,42]. Four major mechanisms of GA activity have been GA–MHC complexes and MBP–MHC complexes for binding to the T-cell receptor (TCR); (3) activation and tolerance induction in MBP-specific T cells through an altered peptide ligand; and (4) induction of GA-reactive, Th2-like regulatory cells that mediate local bystander suppression.

Whereas the first two effects are thought to take place only *in vitro*, the latter two are also likely to occur *in vivo* and could contribute to the anti-inflammatory effects of GA [42].

Intravenous immunoglobulins

Intravenous immunoglobulins (IVIgs) are pooled, purified, human Igs with virtually unlimited specificities. Consistent with their composition, several mechanisms of action of IVIgs have been suggested [43,44]. These include: (1) anti-idiotype antibodies (binding and inactivation of pathogenic antibodies by IVIgs); (2) blockade of Fc receptors on mononuclear phagocytes; (3) downregulation of the endogenous production of Igs; (4) attenuation and abrogation of complement-mediated effects (partially by 'consumption' of complement components); (5) neutralization of molecules (TCR, MHC, costimulatory molecules and cytokines) that are involved in inflammation; (6) induction of anti-inflammatory cytokines; and (7) induction of apoptosis. Experimental evidence indicates that IVIgs might also be involved in myelin repair, but clinical proof of this is lacking [44]. Currently, the role of IVIgs in treating MS is undecided.

Immunosuppressive agents

Mitoxantrone is effective in the treatment of severe active forms of MS [45,46]. Although mitoxantrone suppresses both T cells and B cells, *in vitro* experiments indicate that major sites of action in MS are antigen-presenting cells, which are induced to undergo apoptosis, and macrophages, the major effector cells of demyelination, which are deactivated [47].

Azathioprine is widely used in organ transplantation and autoimmune disorders [48] and is considered a second-line drug in MS. It mainly targets the activation, proliferation and differentiation of both T cells and B cells by competition between its metabolites and DNA nucleotides. Specific immunomodulatory properties have not been reported to date.

Cyclophosphamide is used to treat severe and rapidly progressive forms of MS, although evidence of its efficacy in clinical trials is conflicting. In addition to strong immunosuppression, cyclophosphamide exerts immunomodulatory effects that shift immune responses from Th1 towards Th2 by an unknown mechanism [49].

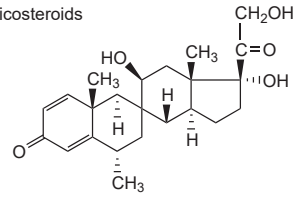
One study has shown methotrexate to convey some therapeutic benefit in progressive forms of MS [10], but its mechanisms of action in autoimmune diseases are largely unknown.

Potential new agents

Anti-adhesion molecules

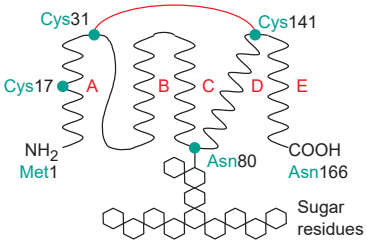
Adhesion molecules comprise several families of molecules that are essential in virtually all cellular inter-

(a) Glucocorticosteroids

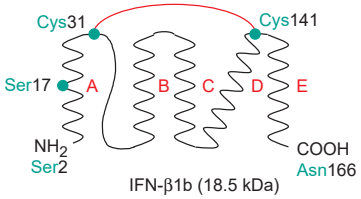


Methylprednisolone (374.5 Da)

(b) Interferon β (IFN-β)

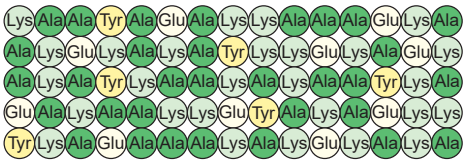


IFN-β1a (22.5 kDa) Disulfide bridge: Cys31–141



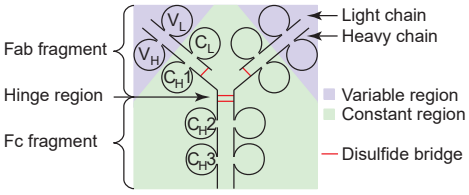
IFN-β1b (18.5 kDa)

(c) Glatiramer acetate (GA)



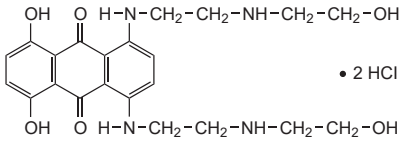
4.7–11.0 kDa

(d) Intravenous immunoglobulins (IVIgs)



150 kDa

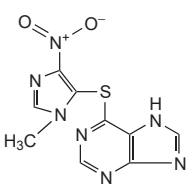
(e) Mitoxantrone



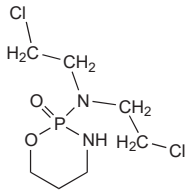
• 2 HCl

517.4 Da

(f) Azathioprine

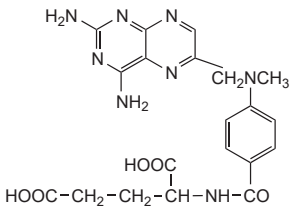


(g) Cyclophosphamide



279.1 Da

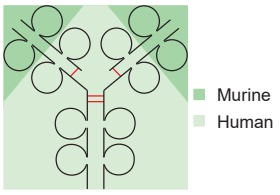
(h) Methotrexate



454.4 Da

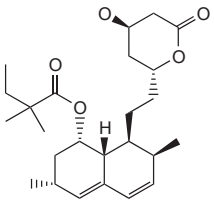
(i) Anti-adhesion molecule antibodies

150 kDa



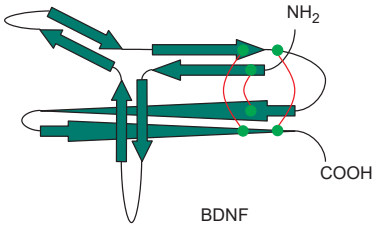
150 kDa

(j) Statins



Simvastatin (418.6 Da)

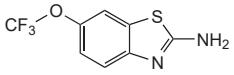
(k) Neurotrophic factors



BDNF (13.5 kDa)

Disulfide bridges: Cys13–80; Cys58–109; Cys68–111

(l) Neuroprotective agents



Riluzole (234.2 Da)

domain that binds to glycosylated and sialylated ligands, are involved in the 'rolling' of leukocytes. Integrins comprise α4β1, α5β1, α6β1 integrins [also known as very late antigen 4 (VLA-4) VLA-5 and VLA-6, respectively] and leukocyte function antigen 1 (LFA-1). VLA-4 and LFA-1 are present on circulating leukocytes and mediate their migration across the vascular endothelium; in addition, LFA-1 is a costimulatory molecule in T-cell activation. Cadherins (including classical cadherins, desmosomal cadherins and protocadherins) form molecular links between adjacent cells by zipper-like structures. Members of the Ig superfamily, such as intercellular adhesion molecule 1 (ICAM-1), ICAM-2 and ICAM-3, vascular cell adhesion molecule (VCAM), and LFA-2 and LFA-3 have Ig-like domains. ICAM-1 and VCAM-1 are the counter-receptors of LFA-1 and VLA-4, respectively, and are thus involved in leukocyte migration.

Antibodies against single adhesion molecules can potently inhibit crucial steps in the pathogenesis of MS, especially lymphocyte migration. Currently, the most promising candidate is natalizumab [52], a humanized monoclonal antibody against the α4 chain of α4β1 integrin that has been effective in Phase II clinical trials [53]. 'Humanized' means that a murine antibody clone has been grafted to a human IgG4 framework at the complementary determining region to reduce its immunogenicity [52]. Two large-scale, Phase III clinical trials in RR MS have been initiated recently and small-molecule antagonists, which offer advantages over monoclonal antibodies (oral availability and lack of antigenicity) will certainly be explored in the future [54].

Statins

Statins are effective lipid-lowering agents. Recent findings indicate that they have additional immunomodulatory effects *in vivo* in the animal model experimental autoimmune encephalomyelitis (EAE), and *in vitro* [55–57]. One proposed immunomodulatory mechanism of statins is based on the selective inhibition of the adhesion molecule LFA-1, an integrin that is involved in inflammation [58]. Furthermore, statins reduce IFN-γ-induced MHC class II expression by blocking transcription of the class II transactivator IV promoter [55]. In addition, statins curtail T-cell proliferation, lower expression of activation surface markers and induce production of the cytokine IL-4 [56]. Currently, simvastatin is being tested in a Phase II clinical trial in MS. The oral administration, extensive safety data, possible effects synergistic with IFN-β and simultaneous treatment of co-morbidity make statins particularly attractive candidate agents.

Neurotrophic factors

Neurotrophic factors are secreted proteins that regulate the survival and differentiation of nerve cells [59]. They act via specific neurotrophin receptors [60]. Neurotrophic factors have been observed to shift the CNS cytokine balance from Th1 to Th2 by an undefined mechanism [61]. In addition, they might promote survival of neurons in MS lesions [6] and pharmacological neuroprotection by the exogenous application of neurotrophic factors provides a promising therapeutic approach [59,62]. Insulin-like growth factor 1 is currently explored in a Phase I/II study. Other attractive candidates are brain-derived neurotrophic factor, glial growth factor and ciliary neurotrophic factor [63].

Neuroprotective agents

Recent evidence indicates that axonal and neuronal degeneration occur early in MS and – as the disease evolves – predominate the underlying pathogenetic mechanisms [4,17,24]. This paradigm shift has obvious therapeutic implications. In addition to neurotrophic factors, other chemically defined neuroprotective agents that save neurons from toxic stress [64], such as riluzole (a potent K^+ channel activator used in amyotrophic lateral sclerosis) are either being investigated or are likely to be tested in clinical trials.

Similarities and peculiarities

Given the complexity of MS pathogenesis, there are multiple sites where immunomodulatory agents, either alone or in combination, might be effective. Current knowledge of the sites of action obtained from *in vitro* and *in vivo* data is summarized in Fig. 3. Although the

Fig. 2. Structures of immunomodulators. Interferon β (IFN-β), glatiramer acetate (GA) and mitoxantrone are currently approved for use in multiple sclerosis (MS). (a) Glucocorticosteroids are derived from cortisol, a naturally occurring adrenal hormone. Methylprednisolone is commonly used to treat acute relapses in MS. (b) IFN-β contains 166 residues that form five α-helices (A–E). Two preparations of recombinant IFN-β are approved for MS treatment. IFN-β1a is produced in Chinese hamster ovary cells and is pharmacologically identical to the natural form (i.e. it is glycosylated by oligosaccharides at Asn80). Compared with natural IFN-β, IFN-β1b is not glycosylated, it lacks Met1 (i.e. IFN-β1b has 165 residues) and there is a Cys to Ser substitution at residue 17. In both forms of recombinant IFN-β, there is a disulfide bridge between Cys31 and Cys141. Structurally important amino acids are shown in green. (c) GA is the acetate salt of a standardized, randomized mixture of synthetic polypeptides (average length 45–100 amino acids) that consist of L-glutamic acid, L-lysine, L-alanine and L-tyrosine in the molar ratio of 0.14:0.34:0.43:0.09. An example sequence is shown. (d) Intravenous immunoglobulins (IVIgs) are pooled, purified, human Igs prepared by cold ethanol fractionation of human plasma derived from 3000–10 000 donors. Igs share a common Y-shaped structure, which consists of two heavy chains (50 < hsp sp = 0.25 > kDa each) and two light chains (25 < hsp sp = 0.25 > kDa each) connected by disulfide bridges. The variable region is responsible for antigen recognition. The five heavy-chain isotypes determine the immunoglobulin classes. IVIgs contain ~95% IgG, 2.5% IgA and a minority of IgM. (e) Mitoxantrone is an anthracenedione derivative that is related to the anthracyclins doxorubicine and daunorubicine. It interacts with topoisomerase-2 and causes single and double string breaks by intercalating with DNA. (f) Azathioprine is a purine analogue that is metabolized rapidly to the cytotoxic and immunosuppressant derivatives 6-mercaptopurine and thioinosine acid, the latter competes with DNA nucleotides. (g) Cyclophosphamide is an alkylating agent of the nitrogen mustard group; its active metabolites, formed by the activity of hepatic cytochrome P450, induce DNA-string breaks. (h) Methotrexate interferes with DNA synthesis by inhibiting dihydrofolate reductase and, thus, thymidine biosynthesis. It reduces 1-carbon transfers to purines. (i) Monoclonal antibodies against adhesion molecules inhibit homing of T cells to the CNS. To reduce immunogenicity, newer approaches use 'humanized' antibodies, which are chimeras of murine variable regions and human constant regions connected at the complementary determining region. (j) Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Statins are effective lipid-lowering agents because HMG-CoA reductase is essential to the cholesterol biosynthesis pathway, but they also have immunomodulatory properties. (k) The structure of neurotrophic factors is dominated by four antiparallel pairs of β strands (green arrows) held in place by three disulfide bridges. Neurotrophic factors are active

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