JCI The Journal of Clinical Investigation

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J Clin Invest. 2017;127(10):3577-3587. https://doi.org/10.1172/JCI90609.

Review Series

There is an increasing recognition that inflammation plays a critical role in neurodegenerative diseases of the CNS, including Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and the prototypic neuroinflammatory disease multiple sclerosis (MS). Differential immune responses involving the adaptive versus the innate immune system are observed at various stages of neurodegenerative diseases, and may not only drive disease processes but could serve as therapeutic targets. Ongoing investigations into the specific inflammatory mechanisms that play roles in disease causation and progression have revealed lessons about inflammation-driven neurodegeneration that can be applied to other neurodegenerative diseases. An increasing number of immunotherapeutic strategies that have been successful in MS are now being applied to other neurodegenerative diseases. Some approaches suppress CNS immune mechanisms, while others harness the immune system to clear deleterious products and cells. This Review focuses on the mechanisms by which inflammation, mediated either by the peripheral immune response or by endogenous CNS immune mechanisms, can affect CNS neurodegeneration.

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There is an increasing recognition that inflammation plays a critical role in neurodegenerative diseases of the CNS, including Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and the prototypic neuroinflammatory disease multiple sclerosis (MS). Differential immune responses involving the adaptive versus the innate immune system are observed at various stages of neurodegenerative diseases, and may not only drive disease processes but could serve as therapeutic targets. Ongoing investigations into the specific inflammatory mechanisms that play roles in disease causation and progression have revealed lessons about inflammation-driven neurodegeneration that can be applied to other neurodegenerative diseases. An increasing number of immunotherapeutic strategies that have been successful in MS are now being applied to other neurodegenerative diseases. Some approaches suppress CNS immune mechanisms, while others harness the immune system to clear deleterious products and cells. This Review focuses on the mechanisms by which inflammation, mediated either by the peripheral immune response or by endogenous CNS immune mechanisms, can affect CNS neurodegeneration.

Introduction

Increasing appreciation for the role of inflammation in neurodegenerative diseases of the CNS, including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and the prototypic neuroinflammatory disease multiple sclerosis (MS), has identified differential immune responses involving the adaptive versus the innate immune systems at various stages of disease. These responses may not only drive disease processes but could serve as therapeutic targets. An increasing number of immunotherapeutic strategies that have been successful in MS are now being applied to other neurodegenerative diseases. Some approaches suppress CNS immune mechanisms, while others harness the immune system to clear deleterious products and cells. This Review focuses on the mechanisms, cellular functions, signaling molecules, immune responses, and mediators through which inflammation affects CNS neurodegeneration, and identifies the therapeutic opportunities within these processes.

Mechanisms of neurodegeneration

Several basic mechanisms that drive neurodegeneration may be triggered by inflammatory cells and their mediators at various stages of the neurodegenerative cascade.

Apoptosis. Apoptosis is caspase-mediated programmed cell death (1) characterized by formation of membrane-enveloped apoptotic bodies that are rapidly phagocytosed by macrophages or neighboring cells. There is evidence of apoptotic mechanisms in animal models of several neurodegenerative diseases, but evidence in human tissues is limited. Caspase-1, -3, -8, and -9 activation and cytochrome *c* release seen in models of Huntington's disease (HD) were also demonstrated in human striatal brain tissue (2, 3). Similarly, caspase activation and neuronal apoptosis have been demonstrated in ALS (4) and HIV-associated neurodegeneration (5).

Necroptosis. Necroptosis represents a form of programmed cell death that is independent from the caspase activation and involves loss of plasma membrane integrity. Two main effector proteins of necroptosis are receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and mixed-lineage kinase domain–like (MLKL). Astrocytes release TNF-α, FasL, and TRAIL, which can trigger necroptosis through RIPK1 and MLKL activation, and this mechanism has been demonstrated in murine models of ALS (6). RIPK1-mediated axonal pathology was observed in pathological specimens from ALS patients (7). Necroptotic mechanisms were also observed in MS pathological samples (8).

Neuronal autophagy. Autophagy, also known as type II programmed cell death, is characterized by the accumulation of autophagic vacuoles during cell death, along with toxic components such as proteins or damaged organelles (9, 10). Excessive autophagy may lead to self-destruction and cell death. Autophagosomes were identified in affected neurons of patients with AD, HD, and PD (11–13). Many stimuli also induce autophagy, including nutrient starvation, mitochondrial toxins, hypoxia, and oxidative stress (14).

Retrograde degeneration. Retrograde degeneration of the proximal neuronal cell body may result from axonal injury or transection and may be associated with various pathological changes in the cell body, including apoptosis (15–17) and chromatolysis of the

Conflict of interest: T. Chitnis has received research funding from Verily, Serono, and Biogen. She has served as a consultant or on the advisory board for Novartis, Sanofi, Genentech, Bayer, and Biogen. H.L. Weiner has received research funding from Verily, Serono, Biogen, Genentech, and Sanofi-Genzyme. He serves on the scientific advisory board for Tiziana and MedDay.

Reference information: *J Clin Invest*. 2017;127(10):3577–3587.
https://doi.org/10.1172/JCI90609.

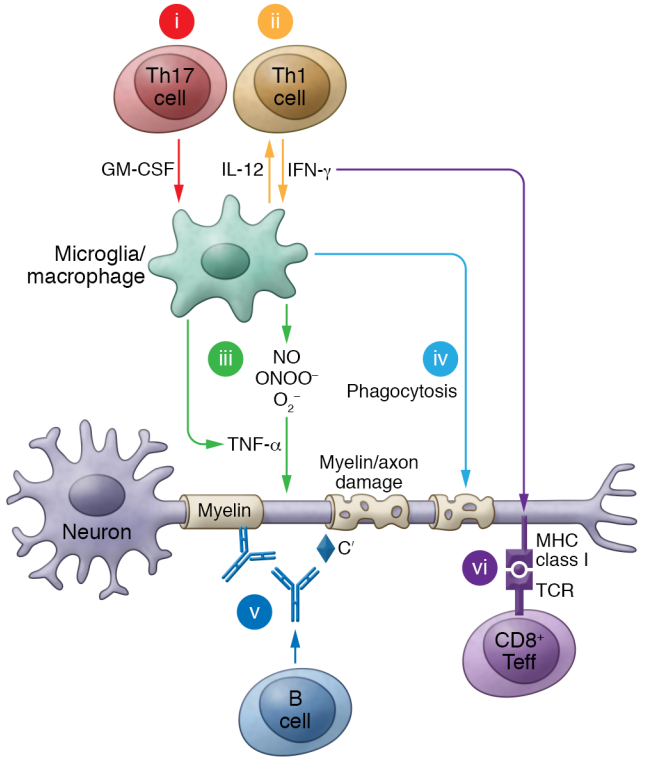

Figure 1. Immune-mediated attack on axons and myelin sheath. During MS and EAE, axonal damage and demyelination are initially mediated by the inflammatory response within the CNS. (i) $CD4^+$ Th17 cells produce GM-CSF, which activates macrophages and microglia. (ii) $CD4^+$ Th1 cells invade the CNS and produce IFN-γ, which activates macrophages and microglia to produce the cytokine IL-12 (the major promoter of Th1 cytokine production). (iii) Macrophages and microglia also produce nitric oxide (NO), peroxynitrite ($ONOO^-$), and superoxide (O_2^-), which are each capable of mediating cellular damage. This capability is enhanced by microglia- and macrophage-derived TNF-α production. (iv) Activated macrophages and microglia may also consume damaged myelin sheaths and axons. (v) B cells produce antibodies that bind to myelin sheaths and may promote complement-mediated damage (C′). (vi) IFN-γ upregulates the expression of MHC class I by resident CNS cells, potentially inciting a $CD8^+$ cytotoxic T cell response.

neuronal perikaryon (18, 19). The association of neuronal apoptosis with axonal damage suggests that inflammation-induced axotomy may produce retrograde (secondary) death of neuronal cell bodies via apoptosis.

Wallerian degeneration. Proximal damage to the neuron or axon may result in anterograde degeneration of the distal axon, termed Wallerian degeneration. Wallerian degeneration is a cascade of events that includes granular degeneration of the axonal cytoskeleton, accumulation of activated macrophages and microglia, and local changes in the immune environment. Evidence that Wallerian degeneration occurs in MS is seen in a histopathological study demonstrating inflammatory cervicomedullary junction lesion with distal axonal atrophy in the absence of demyelination (20), as well as in MRI studies (21, 22).

Demyelination. The relationship between the oligodendrocyte and axon is complex, with the provision of mutual support through trophic factors that can be disrupted in demyelinating diseases. In addition to protecting the axon from immune-mediated damage (23, 24), myelin integrity protects the integrity of the developing axon (25). Myelin-associated glycoprotein (26) and proteolipid protein (27) deliver essential myelin-derived trophic signals to axons (28). Mice lacking proteolipid protein developed axonal swellings and degeneration (27), suggesting that local oligodendroglial support is critical for axon survival.

Astrogliopathy. Dysfunction of astrocytes has broadly been termed astrogliopathy. Aging-related tau astrogliopathy (ARTAG) describes the pathological accumulation of abnormally phosphorylated tau protein in astrocytes that is found in AD, frontal temporal lobe dementias (29), and corticobasal degeneration (30). Neuromyelitis optica (NMO) presents with optic neuritis and myelitis and can mimic MS. NMO is associated with the presence of aquaporin-4 (AQP4) antibodies, which target astrocyte water channels. Pathologically NMO is characterized by extensive loss of immunoreactivity for the astrocytic proteins AQP4 and glial fibrillary acidic protein (GFAP), perivascular deposition of immunoglobulins, and activation of complement even within lesions with relative preservation of myelin (31).

Immune cells involved in neurodegeneration

Endogenous CNS immune cells. Inflammation-mediated neurodegeneration may result from dysfunction of endogenous or exogenous immune cells. The two major endogenous cells in the CNS that drive inflammation are astrocytes and mononuclear phagocytes, which include microglia and perivascular macrophages. The mechanisms by which astrocytes and macrophages/microglia drive the neurodegenerative process are outlined in Figure 1, A and B.

Astrocytes. Astrocytes account for almost half of CNS volume. Their normal functions include providing trophic support for neurons, facilitating synapse formation and function, and synaptic pruning by phagocytosis. Astrocytes also help control extracellular ion and neurotransmitter concentrations and maintain the blood-brain barrier (32). One of their most important homeostatic functions is maintaining glutamine-glutamate balance. Glutamate transporters, including EAAT1–EAAT3, are predominantly expressed on astrocytes and neurons and regulate the glutamate uptake and metabolism. Downregulation of these transporters can result in increased extracellular glutamate, potentially enhancing neurotoxicity. Glial expression of these transporters is altered in MS (33) and ALS (34).

Glutaminase converts glutamine to glutamate and is present in astrocytes. Glutaminase is upregulated in MS lesions, colocalizing with infiltrating macrophages and microglia (33), and its expression is correlated with axonal damage. The astrocytic enzymes glutamine synthetase and glutamate dehydrogenase are responsible for glutamate degradation. Both are reduced in MS lesions (33) and spinal cords from mice with experimental autoimmune encephalomyelitis (EAE) (35). Dysfunctional or damaged astrocytes may not perform this function, resulting in increased concentrations of glutamate in the milieu, which may induce NMDA receptor–induced cell death.

Astrocyte function and gene expression are heterogeneous and dependent on local inflammatory milieu as well as region-

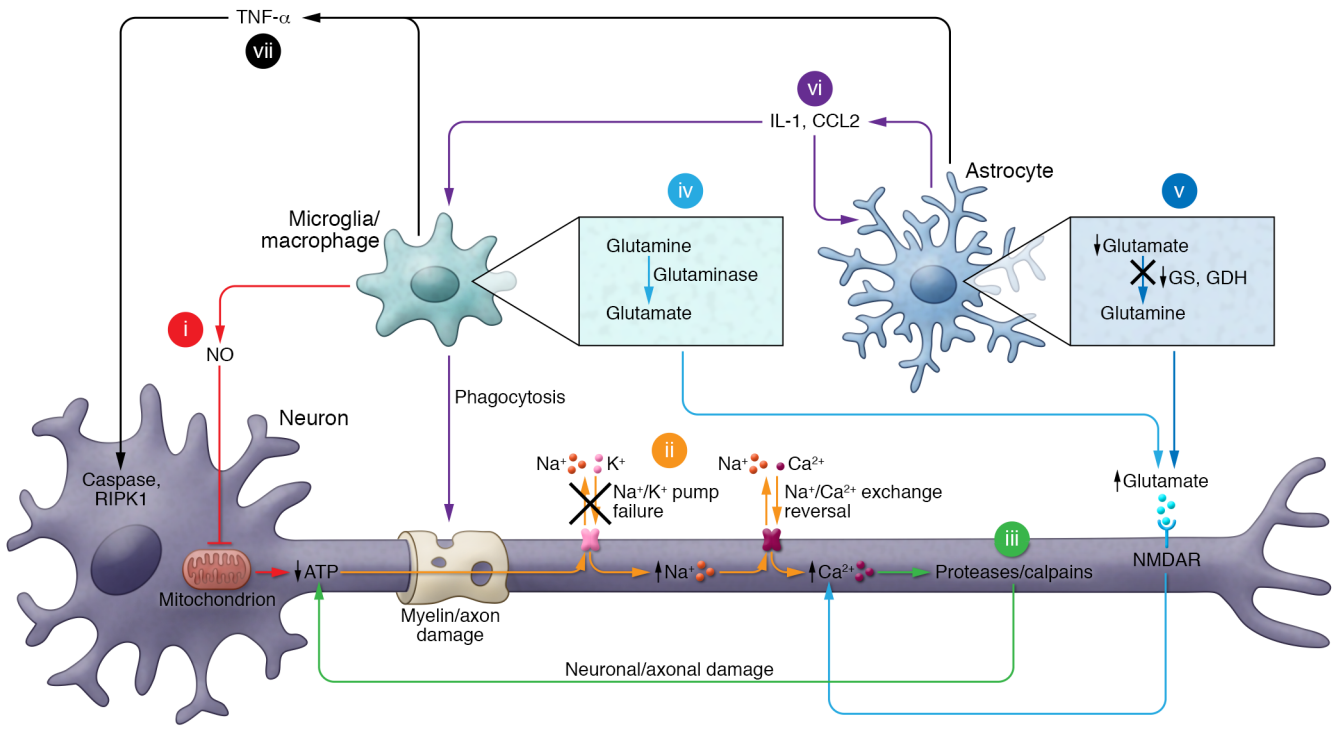

Figure 2. Secondary degeneration of the axon and neuronal cell body. Secondary degeneration may be mediated through several mechanisms. (i) Nitric oxide (NO) produced by macrophages and microglia may inhibit normal cellular respiration and mitochondrial ATP production. (ii) Reductions in neuronal ATP production may lead to failure of the Na^+/K^+ pump. The subsequent increases in intracellular concentrations of Na^+ lead to reverse operation of the Na^+/Ca^{2+} exchanger and opening of voltage-sensitive Ca^{2+} channels, resulting in a rise of intra-axonal Ca^{2+}. (iii) This, in turn, may activate degradative enzymes, including proteases, phospholipases, and calpains, resulting in further neuronal or axonal damage and impaired ATP production. (iv) Microglia and macrophages recruited to the area produce glutamate, which can interact with NMDA or AMPA receptors, which also cause a rise in intracellular Ca^{2+}. (v) Impaired glutamate uptake and degradation in the astrocyte, accompanied by downregulated expression of glutamine synthase (GS) and glutamate dehydrogenase (GDH), perpetuates increased extracellular glutamate levels. (vi) Astrocytes produce CCL2 and cytokines that further activate microglia and macrophages. In turn, microglia and macrophages consume damaged myelin sheaths and axons. (vii) Secondary neuronal cell body degradation can occur by apoptotic or necroptotic mechanisms, triggered in part by immune molecules (e.g., TNF-α, TRAIL) that are produced by microglia/macrophages or astrocytes.

and circuit-specific diversity (36–38). Inflamed astrocytes can produce proinflammatory cytokines and chemokines, including CCL2, which recruit monocytes into the CNS and may further activate astrocytes themselves (39). In EAE, astrocytes can have a different role depending on the stage of disease (40). Depletion of astrocytes during acute EAE worsens disease, as astrocytes play a protective role through glutamate metabolism. However, during chronic EAE, astrocytes become inflamed and acquire a pathogenic role; deletion of astrocytes at this stage improves EAE. Lactosylceramide synthesized by β-1,4-galactosyltransferase 6 is upregulated in the CNS, and lactosylceramide acted in an autocrine manner to control astrocyte transcriptional programs that promote neurodegeneration (40).

Recently, two distinct phenotypes of astrocytes have been described — A1 and A2 (41). A2 astrocytes were predominant in ischemia-induced CNS lesions, while A1 astrocytes predominated in inflammation-induced lesions. TLR4-activated microglia seem to be necessary for A1 astrocyte induction, which is dependent on IL-1α, TNF, and complement C1q in combination. A1 astrocytes expressing downstream complement C3 were identified in CNS specimens from MS, PD, AD, ALS, and HD and may play a key role as mediators of inflammation-induced neurodegeneration (41).

Mononuclear phagocytes

Perivascular and meningeal macrophages. The CNS contains an extensive network of bone marrow–derived (BMD) mononuclear phagocytes, including macrophages of the meninges, choroid plexus, and perivascular spaces. Perivascular macrophages differ from microglia and from BMD peritoneal macrophages or circulating monocytes. The transcriptional activator MYB is critical for development of BMD monocytes, but perivascular macrophage development depends on PU.1, IRF8, and CSF1R. The unique microenvironments of perivascular macrophages may work to shape their properties, including their CNS macrophage signatures (42–44). In MS, perivascular macrophages may function as antigen-presenting cells to T cells (45). The accumulation of perivascular macrophages observed in HIV encephalopathy may play an important role in the ensuing neurodegenerative process (46).

Microglia. Microglia are the resident macrophages of the CNS. Derived from the embryonic yolk sac, they migrate into the developing neural tube and subsequently colonize the brain parenchyma (47–49). Microglia turn over slowly and are present throughout the lifespan. They do not repopulate from the BMD myeloid precursors and are independent of blood-derived monocytes. Microglial precursors proliferate independently of MYB and myeloid-lineage hematopoietic stem cells (49). Unique markers and gene signatures

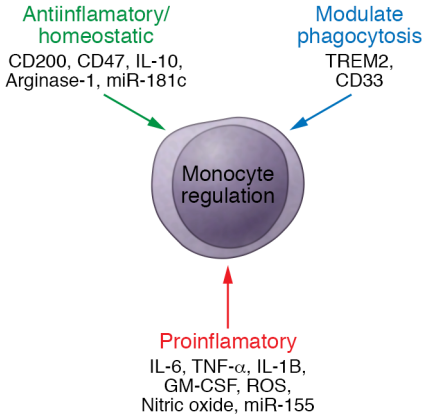

Figure 3. Schema of antiinflammatory, proinflammatory, and phagocytosis-regulating molecules involved in the regulation of CNS monocytes.

that distinguish resident microglia from peripheral mononuclear cells/macrophages (50–52) include FSRSL, P2Y12, TMEM119, CX3CR1, Siglec-H, and the microRNAs miR-99a, miR-125-5p, and miR-342-3p, which are highly expressed in both mouse and human microglia (50, 53). Homeostatic microglia are dependent on TGF-β, and CNS-specific TGF-β–KO mice develop late-onset motor dysfunction (50), which may be due to impairment of microglia-associated glutamate recycling and synaptic plasticity (54). TMEM119 immunoreactivity is expressed exclusively on a subset of microglia with ramified and amoeboid morphologies in the brains of neurodegenerative diseases, such as AD, whereas infiltrating macrophages did not express TMEM11 9 (55) in demyelinating lesions of MS and necrotic lesions of cerebral infarction (56).

Although microglia have previously been classified as M1 (proinflammatory) and M2 (antiinflammatory), it is now clear that microglia are more complex than this simple dichotomy, as many microglia demonstrate characteristics of both phenotypes (refs. 57, 58, and Figure 2). A phagocytic program plays an important role in clearing aberrant proteins, particularly in AD, PD, and ALS. More recent work identifies a microglia subtype called "dark microglia" with ramified and thin processes and prominent staining for IBA1, CD11b, and microglia-specific 4D4, as well as TREM2 that associates with amyloid plaques (59). Future studies examining the molecular signatures of microglia in health and disease may identify novel targets for diagnosis and modulation of neurodegenerative diseases.

Invading peripheral macrophages. Blood-derived monocytes can differentiate into macrophages and may invade the CNS to mediate CNS pathology, where they may be difficult to distinguish from resident microglial cells. Blood-derived macrophages associate with nodes of Ranvier and initiate demyelination, whereas microglia appear to clear debris (60). GM-CSF–activated mononuclear phagocytes that migrate into the CNS exhibit a more pathogenic signature than those that migrate to other organs, which may be due to the unique microenvironment of the CNS (61).

Major signaling molecules of CNS mononuclear phagocytes

CD200-CD200R. CD200 is primarily expressed on the surface of neurons in both the CNS and the PNS, as well as thymocytes, recirculating B cells, activated B cells, and follicular dendritic cells (62). CD200 receptor (CD200R) initiates tyrosine phosphorylation (63) and is expressed only on cells of the microglia/macrophage lineage (63, 64). Administration of a CD200R-blocking antibody to Lewis rats exacerbated EAE (63), and CD200-deficient mice experienced earlier onset of EAE, with increased microglia/macrophage accumulation and activation in the CNS as demonstrated by increased expression of inducible NOS (iNOS) (65). We found that administration of a CD200R1 agonist attenuated disease in a chronic MS model (66). Enhanced CD200 expression was associated with reduced Wallerian degeneration in the slow Wallerian degeneration (Wld^s) mouse (67), a spontaneously occurring mutant with the unique phenotype of protection against several forms of axonal injury (68–71). In vitro, Wld^s neuronal cultures were protected from microglia-induced neurotoxicity, which was abrogated by anti-CD200 antibody treatment (67).

SIRPα-CD47. SIRPα (also known as SHPS-1, p84, and BIT) is a regulatory membrane glycoprotein expressed mainly by myeloid cells, stem cells, and neurons. It binds CD47 on microglia to deliver an inhibitory signal (72, 73). SIRPα activation was linked to the inhibition of cell activities, including decreased cytokine production, reduced monocyte adhesion to the extracellular matrix, reduced phagocytosis, and maturation arrest in dendritic cells (74–76). SIRPα engagement inhibits macrophage phagocytosis by recruiting SHP-1 (77). Fcγ receptor (FcγR) activation stimulates the association between SHP-1 and SIRPα, which in turn inhibits FcγR- and complement receptor–mediated phagocytosis (75, 78). CD47 expression is downregulated in MS brain lesions (79). Although CD47-deficient mice are resistant to EAE, primarily owing to lack of APC activation, blocking CD47 enhances disease progression by increasing myelin phagocytosis in the CNS (79).

TREM2. TREM2 (triggering receptor expressed on myeloid cells-2) is an activating phospholipid-binding receptor that couples with TYROBP, an adaptor protein, to attenuate inflammatory activation and increase phagocytic clearance of cell debris. DAP12 forms a receptor-signaling complex with TREM2 and triggers activation of immune responses to macrophages and microglia. A rare missense mutation in the gene encoding TREM2 confers risk of late-onset AD (80, 81) and frontotemporal dementia (82), possibly due to impaired clearance of proteins by macrophages and microglia.

CD33. CD33 is an immunoglobulin-like lectin that binds sialic acids and delivers inhibitory signals to human microglia. CD33 is increased in microglial cells in AD brain and inhibits uptake of amyloid by microglial cells (83). The minor allele of the CD33 SNP rs3865444 has been shown to confer protection against AD (84).

Purinergic metabolites. Dying cells in the brain may release extracellular purinergic metabolites such as ATP and NAD, leading to both innate and adaptive immune activation (85, 86). The uptake of double-stranded DNA can induce activation of TLRs. The purinergic receptor P2Y12 is a TGF-β–responsive molecule uniquely expressed on mouse and human microglia and not on perivascular macrophages or blood-derived monocytes (50), and is expressed predominantly in homeostatic-type microglia (87). ADP is the endogenous ligand of P2Y12, and homeostatic microglia have increased ligand-mediated calcium responses, which are blocked by selective P2Y12 antagonism (87).

GM-CSF. GM-CSF stimulates BMD stem cells to differentiate into granulocytes and monocytes. A variety of cells, including macrophages, T cells, mast cells, NK cells, endothelial cells, and fibroblasts, produce GM-CSF, and it can enhance ROS production as well as myeloid cell–mediated phagocytosis. GM-CSF was increased in the cerebrospinal fluid of MS patients (88). In EAE, GM-CSF production by Th17 cells activates effector mononuclear phagocytes (89). GM-CSF–activated mononuclear phagocytes that migrate into the CNS exhibit a more pathogenic signature than those that migrate to other organs, which may be due to the unique CNS microenvironment (61).

MicroRNAs. MicroRNAs (miRNAs) are small noncoding RNAs that function in RNA silencing and posttranscriptional regulation of gene expression (90). While the majority of miRNAs are located within the cell, some miRNAs, commonly known as circulating miRNAs or extracellular miRNAs, function in the extracellular environment. Several miRNAs are associated with microglial function. We have shown that in the SOD1 model of ALS, miR-155 expression was associated with loss of homeostatic microglia signature molecules including P2Y12, TMEM119, OLFM13, the transcription factors EGR1, ATF3, Jun, Fos, and MAFB, and the upstream regulators CSF1R, TGFB1, and TGFBR1, which are essential for microglial survival (91). miR-Let-7a participates in the reduction of nitrite production and the expression of iNOS and IL-6. It also increases expression of brain-derived neurotrophic factor (BDNF), IL-10, and IL-4 in microglia (92). The miRNA miR-181c controls microglia-mediated neuronal apoptosis by suppressing TNF (93).

T cells and neurodegeneration. Th1 and Th17 cells play a major role in the initiation of neurodegeneration in MS by creating an inflammatory milieu and recruiting inflammatory monocytes that lead to axonal damage. T cells may also have a neuroprotective role: we have shown that intranasal administration of anti-CD3 monoclonal antibody in chronic EAE induces an IL-10–secreting Tr1-like cell in the cervical lymph nodes that migrates to the brain, dampens astrocyte and microglial inflammation, and ameliorates neurodegeneration.

$CD8^+$ T cells. $CD8^+$ T cells can directly mediate neurodegeneration (Figure 1A). Under inflammatory conditions, IFN-γ can induce neuronal expression of MHC I molecules. This may be important in virally mediated reactions as well as in autoimmune diseases, including MS, in which $CD8^+$ T cells are found in pathology specimens (94). $CD8^+$ T cells may also mediate collateral killing of neurons, in part as a result of the release of perforin (and granzymes) independently of Fas/FasL signaling (95). Upon stimulation, $CD8^+$ T cells can upregulate the molecular repertoire for vesicular glutamate release, including glutaminase, which is required to generate glutamate, vesicular proton-ATPase, and vesicular glutamate transporters (96), and thus may be key in immune-mediated neurodegeneration.

$CD4^+$ T cells. Until recently, a direct role of $CD4^+$ T cells in neurodegeneration was not appreciated, though they can indirectly initiate neurodegeneration by recruiting inflammatory monocytes. Thus, Th1 and Th17 may be targets to potentially minimize a hostile neuronal microenvironment. The ability of $CD4^+$ T cells to directly mediate cytotoxicity was recently demonstrated in the MPTP-induced mouse model of PD (97). Similar attenuation of MPTP-induced dopaminergic cell death was seen in mice lacking CD4 as well as in *Rag1*$^{-/-}$ mice reconstituted with FasL-deficient splenocytes. However, CD8-deficient or *Rag1*$^{-/-}$ mice reconstituted with IFN-γ–deficient splenocytes were not protected.

Regulatory T cells. Regulatory T cells (Tregs) include $FoxP3^+CD25^+CD4^+$ T cells as well as IL-10–producing Tregs and may play a role in dampening or controlling inflammatory processes within the CNS. Considerable evidence reveals that these cells play a role in the early stages of MS (98). More recent work found that Tregs from ALS patients were less effective in suppressing responder T lymphocyte proliferation and were increasingly dysfunctional with disease progression (99, 100).

Downstream inflammatory mediators of neurodegeneration

Inflammatory mediators are known to enhance or affect several neurodegenerative mechanisms through key downstream mediators. Others may play neuroprotective roles, as discussed in the next section.

Reactive oxygen and nitrogen species. ROS are chemically reactive chemicals containing oxygen and include peroxides and superoxides. Activated immune cells, particularly macrophages, can produce ROS, which contributes to mitochondrial dysfunction and ultimately neuronal apoptosis. Superoxide dismutase (SOD) binds free superoxide radicals for conversion into molecular oxygen and hydrogen peroxide, the latter of which is then broken down by catalase (101). Over 160 mutations in the *SOD* gene have been found in forms of ALS (102, 103).

Nitric oxide (NO) is a key mediator of neurotoxicity with multiple roles in the CNS. NO is synthesized from L-arginine by NOS and can potently inhibit mitochondrial respiration (Figure 1B). Constitutive forms of NOS are produced by endothelial cells (eNOS) and neurons (nNOS), while the inducible form (iNOS) is produced by a variety of cell types, including macrophages and microglia, in response to inflammatory stimuli. iNOS accounts for the majority of NO production. NO can regulate a variety of cellular processes, including the generation of the highly cytotoxic superoxide anion ($O_2^{\cdot-}$) and peroxynitrite anion ($ONOO^-$) (104, 105). Collectively, NO and its products have been shown to mediate neurotoxicity in vitro (106–108), and selective inhibitors of iNOS reduce microglia/macrophage-mediated neurotoxicity (109).

NO appears to act in concert with other factors in increasing susceptibility to neurotoxicity. In a cerebral ischemia model, the combination of TNF-α and IL-1β was neurotoxic in the presence of iNOS, while in the absence of iNOS, this combination mediated neuroprotection and plasticity (110). Conversely, TNF-α increased neuronal sensitivity toward NO (111). NO inhibits neuronal respiration, which in turn enhances release of glutamate, causing excitotoxic death of neurons (112). Inhibition of iNOS blocked NMDA-mediated neurotoxicity (113, 114), reinforcing the concept that NO plays a role in excitotoxicity. Further, arginase can compete with NOS for their common substrate, L-arginine, and thus inhibit NO production. This regulatory mechanism may be important when the extracellular supply of L-arginine is limited (115). Electrically active axons exposed to high concentrations of NO have enhanced susceptibility to persistent conduction block and axonal degeneration (116, 117).

Cytokine-mediated neurodegeneration. It is now well recognized that cytokines secreted by immune cells may have direct neurotoxic properties.

IL-1 is produced by the activated mononuclear phagocyte and upregulated in the CNS during EAE induction (118–120). Injection of its isoform, IL-1β, into the rat brain at the time of experimental ischemia or traumatic injury caused increased neuronal cell death and edema (121). Overexpression of IL-1 receptor antagonists in the CNS blocked these effects (122). Addition of IL-1 in vitro results in neuronal apoptosis (123), and its neurotoxic effects appear to be dependent on iNOS expression (124, 125). Thus, IL-1, alone or in combination with iNOS, may be an important factor in neuronal and axonal damage in the CNS. However, IL-1 has also been shown to induce the production of nerve growth factor (NGF) in vitro (126, 127), suggesting some neuroprotective effects.

IL-3 is a cytokine growth factor produced by $CD4^+$ T cells and microglia. It exerts both trophic (128) and toxic effects on neurons (129, 130). Systemic overexpression of IL-3 in the CNS of mice results in severe neurological dysfunction characterized by degenerated, vacuolated neurons (131), predominantly motor neurons of the spinal cord, with an increase in inflammatory infiltrates surrounding neurons in the absence of demyelination. In contrast, transgenic IL-3 expression under the astrocytic GFAP promoter resulted in a predominantly demyelinating disease with minimal axonal pathology (132).

IL-6 is secreted by mononuclear phagocytes, T cells, vascular endothelial cells, and fibroblasts in response to IL-1 and TNF-α. Overexpression of IL-6 in the CNS resulted in neurodegenerative pathology characterized by dendritic vacuolization (133, 134). IL-6 orchestrates the transition between innate and adaptive immune responses through the recruitment of monocytes and T cells. In particular, IL-6 in the presence of TGF-β induces Th17 cells (135). IL-6 also enhances astrogliosis and angiogenesis needed for the tissue remodeling (136).

TNF-α is a proinflammatory cytokine. Local TNF production in the CNS induces oligodendrocyte apoptosis and demyelination in EAE models (137, 138). However, TNF-α may play either neuroprotective or neurotoxic roles, depending on expression of other factors including NO (111, 123, 124), upregulation of NF-κB (139), timing of exposure of damaged neurons to TNF (139), and the presence of excess NMDA receptor agonists (127). In addition, TNF-α may exert neurotoxic effects through inhibition of growth factors such as IGF (140). Interaction of TNF-α with TNF receptor 1 (TNFR1) versus TNFR2 may result in different and even opposite effects. This may explain the unexpected finding that TNF-α–deficient mice develop a more severe form of EAE than controls (141). In addition, TNF-α has been shown to promote the proliferation of oligodendrocyte progenitors (142).

Antibody- and complement-mediated neurodegeneration. Antibodies can induce neurodegeneration through two mechanisms, antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In ADCC, effector immune cells lyse a target cell, whose membrane-surface antigens have been bound or opsonized by specific antibody. The effector cells in this case are typically NK cells, but macrophages, neutrophils, and eosinophils can also mediate ADCC. CDC is a function of the complement system that kills without the involvement of antibodies or cells. Three pathways of complement activation lead to the deposition of membrane attack complexes onto the target, which eventually causes colloid-induced osmotic swelling and lethal membrane damage.

Several studies have demonstrated that complement activation may have neuroprotective effects in specific situations. C3a protects neurons against NMDA toxicity (143). In an in vivo mouse model, C5a protected neurons from kainic acid–induced apoptosis, which was associated with the inhibition of glutamate-mediated caspase induction (144). Some neuronal subtypes, including dentate gyrus granule cells, hippocampal pyramidal hilar cells, and cerebellar Purkinje cells, constitutively express receptors for C3a and C5a (145, 146), which may play a role in development.

Neurons express particularly low levels of CD59 and DAF, molecules that normally confer cellular resistance to complement-mediated damage in other organs. Complement deposition is noted in several neuroinflammatory conditions, including NMO (147) and subtypes of MS (148), and may be a downstream therapeutic target.

Immune-directed therapies for neurodegenerative diseases

Immune-directed therapies for neurodegenerative diseases generally fall into two categories: (a) therapies that target components or cells of the immune system determined to cause disease, such as T cells in MS and microglia in ALS; and (b) therapies that utilize the immune system to clear or target aberrant proteins, including vaccine or passive antibody therapies for AD and more recently PD. As outlined above, the immune system, and particularly CNS immunity, is composed of many complex interrelated and dependent pathways. Therefore, consideration of upstream and downstream effects of immune-directed therapies is important and can affect a therapy's mechanism of action as well as the risk of adverse events. Below, we discuss lessons learned from the successes and failures of specific immunotherapies for neurodegenerative diseases that provided important insights for developing specific targets of therapy.

Multiple sclerosis. In MS, the initial target is the myelin sheath in the CNS; however, later stages are characterized by microglial activation, astrocytic dysfunction, and secondary neurodegeneration (149). Current immunotherapies for MS target a diverse array of immune cell types of the peripheral immune system, including those of the lymphoid lineage (T cells and B cells) and those of the myeloid lineage (macrophages and dendritic cells). These therapies are most effective during the early relapsing-remitting stage of disease and have only limited efficacy during the later secondary progressive stage of disease, which is thought to be mediated by intrinsic CNS inflammatory and neurodegenerative mechanisms (149) including oxidative stress and axonal degeneration. Some oral immunotherapies, including S1P1 modulators, may penetrate into the CNS, and a recent trial has demonstrated effects in secondary progressive MS (150). S1P1 receptors on astrocytes suppress astrocyte- and microglia-mediated inflammatory pathways (151, 152). B cell–directed therapies have a modest effect in primary progressive MS, but it is unclear whether this is due to effects within the CNS or potent modulation of peripheral immune mechanisms (153). An ongoing clinical trial in secondary

progressive MS evaluates ibudilast, which inhibits macrophage migration inhibitor factor and phosphodiesterase-4 and -10 and thus may modulate microglia and macrophage function (154). A challenge for halting progressive MS is successful targeting of multiple immune-dependent and immune-independent neurodegenerative cascades initiated within the CNS.

Amyotrophic lateral sclerosis. Motor neuron death in ALS often occurs in association with protein inclusions. Familial ALS accounts for 10% of cases and has been associated with mutations in the *SOD1* gene and ubiquitin-related genes (155). Glutamate levels are elevated in ALS patient cerebrospinal fluid, possibly because of aberrant expression of the astrocytic glutamate transporter EAAT2 (156). Glutamate inhibition is the mechanism of riluzole, the first approved therapy for ALS. There is evidence for both central and peripheral immune system activation in ALS. In early disease, there is evidence of homeostatic microglia and Treg infiltration, while in later disease stages, M1 microglia and activated astrocytes predominate (157). Tregs themselves are defective in ALS (99, 100). TLR4, which activates microglia, is upregulated in the spinal cord of ALS patients, and could thereby mediate neurodegeneration (158). Immune-directed therapeutic trials to date have largely targeted microglia, and include minocycline, masitinib, NP001 (a modulator of NF-κB function on monocytes), and celecoxib. Of these, only masitinib, a tyrosine-kinase inhibitor targeting microglia and mast cells, has demonstrated positive results. Interim analysis showed that masitinib met both its primary endpoint, a change in the ALS function rating scale revised score, and its secondary endpoint, which included respiratory function and combined assessment of function and survival (159). Minocycline modulated microglial function and delayed disease progression in a mouse model of ALS but failed to demonstrate benefit in human studies. A potential explanation was later demonstrated in animal models: in later stages of ALS, in the setting of activated microglia, minocycline induces end-stage GFAP-biophotonic signals and increases connexin-43 expression levels, thereby altering astrocyte function and inducing microgliosis (160). Edaravone is a free radical scavenger with antioxidant effects that was approved for ALS in the US in 2017 based on a small randomized controlled clinical trial with people with early-stage ALS in Japan. It failed two earlier trials in people with all stages of ALS. Other immune-directed strategies currently under investigation include ibudilast, anti–IL-6 (tocilizumab), and IL-1 receptor antagonist (anakinra) therapies (157). We have found that both microglia and peripheral monocytes have an inflammatory phenotype in the SOD model and in ALS and that targeting miR-155 ameliorates disease in this model (91); targeting of miR-155 is now being evaluated in clinical trials.

Alzheimer's disease. AD is characterized by the presence of extracellular amyloid plaques, formed mainly from amyloid-β (Aβ) 1–42 peptide and intracellular neurofibrillary tangles that contain hyperphosphorylated tau, both of which are neurotoxic. Additional pathological changes include gliosis, inflammation, neuritic dystrophy, neuronal loss, and changes in neurotransmitter levels. Solomon et al. demonstrated that monoclonal antibodies could dissolve Aβ plaques in vitro (161, 162). Schenk et al. attenuated and prevented disease in Aβ-transgenic mice by administering Aβ antibodies (163). Over the past 10 years, Aβ immunotherapy has transitioned from preclinical studies to human studies, with at least 13 different trials stratified into passive antibody administration and active vaccination with Aβ. In the first vaccine trial, using AN1792Aβ, 6% of patients developed meningoencephalitis (164), likely because of increased Th1 cell responses to Aβ (165, 166). Eight vaccinated patients died, and autopsy examinations revealed profound reductions in amyloid load; however, they exhibited severe dementia at the time of death, suggesting that vaccination may not be sufficient to stop ongoing neurodegenerative processes. Newer active-immunization trials have either modified the Aβ peptide to be less immunogenic or used only the B cell–reactive epitope (167).

At least seven trials using passive transfer of Aβ antibodies have been performed. Post hoc analysis demonstrated marked cognitive benefits largely in patients who did not carry the apolipoprotein E (APOE) ε4 allele; however, only a trend toward benefit was observed in APOE ε4 carriers, possibly because of accelerated pathogenesis in these individuals (167). Vasogenic edema and cerebral hemorrhage have occurred in cases after passive anti-Aβ immunotherapy (168). A large phase III clinical trial of the anti-amyloid antibody aducanumab is under way based on encouraging initial results (169).

Newer strategies for AD aim to enhance microglial phagocytosis of Aβ, for instance by targeting TREM2 and CD33, but limit potentially deleterious microglial responses. We have found that nasal administration of a proteosome-based adjuvant (Protollin) induced monocytes that cleared fibrillar amyloid, insoluble Aβ, and soluble Aβ fragments successfully in aged amyloid precursor protein–transgenic (APP-transgenic) mice (170). A clinical trial in AD subjects is planned.

Lessons learned and future directions

(a) Treat early: It is clear that immune-targeting therapeutics for MS and AD are most effective when targeting patients early in their disease course, when neurodegeneration and activation of secondary inflammatory pathways are not pronounced. Immunotherapy for AD has been more successful in earlier patients with minimal cognitive decline, but there is evidence from imaging that Aβ deposition has already occurred at this point (171).

(b) Different immune mechanisms predominate depending on the stage of disease and thus require different approaches: This is exemplified in ALS, in which minocycline was ineffective in the later stages of disease and may have even triggered astrogliosis in the setting of diffuse CNS inflammation. In MS, antiinflammatory medications have little effect in the secondary progressive phase of disease, which is mediated by innate and CNS-compartmentalized immune mechanisms.

(c) Microglia, macrophages, and astrocytes play important roles: As with T cell subsets, it is becoming clear that microglial, astrocyte, and macrophage subsets with unique molecular signatures can have both pathogenic and disease-ameliorating functions in neurodegeneration. Further work is required to elucidate the role of molecules involved in these subsets to target them therapeutically.

(d) Use caution in interpreting animal studies: One shortcoming of animal studies is the testing of therapies at early disease stages, which is unlike the situation in human clinical trials, where disease is only recognized in later stages.

(e) Precision medicine approaches will enhance immune-based therapeutics: Given the heterogeneity of disease course as well as key pathogenic differences according to disease stage, precision approaches combining genomics, biomarkers, imaging markers, and targeted therapeutics may enhance immune-based management for CNS diseases.

In conclusion, inflammatory processes play an important role in neurodegeneration, both in triggering and amplifying degeneration and in providing avenues to limit neurodegeneration. Immune processes that impinge on neurodegeneration exist in both the periphery and the CNS. Because immune cells traffic to the CNS, some therapies can access periphery inflammatory processes to affect the CNS. It is more difficult to target local CNS inflammatory processes, such as microglia and astrocytes. It was recently shown that microglia (44) and astrocytes (172) may be affected by the microbiome, providing a potential new avenue to modulate CNS inflammation and degeneration. It must be realized, however, that neurodegenerative processes may become inflammation-independent, especially in later stages. There is great promise in ongoing studies of inflammation-targeted neuroprotective strategies, which may ultimately be used across neurodegenerative diseases.

Acknowledgments

We thank the Ann Romney Center for Neurological Diseases for support of this work. We thank Belinda Kaskow for assistance with the figures.

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