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Microglia in Brain Development, Homeostasis, and Neurodegeneration

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Abstract

Advances in human genetics have implicated a growing number of genes in neurodegenerative diseases, providing insight into pathological processes. For Alzheimer disease in particular, genome-wide association studies and gene expression studies have emphasized the pathogenic contributions from microglial cells and motivated studies of microglial function/dysfunction. Here, we summarize recent genetic evidence for microglial involvement in neurodegenerative disease with a focus on Alzheimer disease, for which the evidence is most compelling. To provide context for these genetic discoveries, we discuss how microglia influence brain development and homeostasis, how microglial characteristics change in disease, and which microglial activities likely influence the course of neurodegeneration. In all, we aim to synthesize varied aspects of microglial biology and highlight microglia as possible targets for therapeutic interventions in neurodegenerative disease.

INTRODUCTION

Advances in health care have extended life spans globally, but with an aging population comes a rising prevalence of neurodegenerative disorders. Alzheimer disease (AD) is the most common cause of late-onset neurodegeneration worldwide and the sixth leading cause of death in the United States. The risk of AD increases exponentially starting at age 65, doubling roughly every five years to reach a prevalence of 25–50% in 85-year-olds. Considering the current lack of effective therapies, there is an urgent need for better understanding of AD mechanisms and disease-modifying treatments.

AD brains accumulate extracellular beta-amyloid (Aβ) deposits (plaques), intracellular Tau aggregates (tangles), and reactive glia (gliosis) that together conspire to drive synapse loss, neuronal death, and brain atrophy. Glia [the nonneuronal cells of the central nervous system (CNS)] support normal brain function through various functions such as trophic or metabolic support, but some glial responses may ultimately exacerbate disease-associated damage. Gliosis has been historically assumed to be a secondary result of the disease, but recent human genetics studies point to a crucial role for microglia in AD pathogenesis. Here, we review the genetic contributions to AD, the roles of microglia in the developing CNS, the changes in microglial state in neurodegenerative disease, and the functional roles of microglia in disease pathogenesis. We focus on AD, for which microglial involvement is best characterized, but draw comparisons with other neurodegenerative disorders.

GENETIC RISK FACTORS FOR NEURODEGENERATIVE DISEASES

Most neurodegenerative diseases have a large heritable component. In early-onset AD (EOAD), which is typically familial, rare autosomal dominant mutations in *PSEN1*, *PSEN2*, or *APP* result in amyloid plaque buildup and dementia in midlife (126). All three genes participate in the process by which APP (amyloid precursor protein) is cleaved by a PSEN1/PSEN2 complex to produce Aβ peptide, the principal component of amyloid plaques. Transgenic overexpression of various AD-linked *APP* or *PSEN1/PSEN2* mutant alleles (singly or in combination) amplifies Aβ production and drives amyloid plaque formation in mice, resulting in neuronal injury over the course of months (66). Insofar as there is limited neuronal death and brain atrophy in these mice, they likely best model amyloidosis in early stages of AD.

Risk Genes for Late-Onset Alzheimer Disease

EOAD represents only a small fraction (<5%) of AD cases; the more common late-onset AD (LOAD) exhibits similar brain pathology but manifests decades later (148). This similarity fuels the amyloid hypothesis, which posits that accumulation of Aβ aggregates is a primary pathogenic agent in AD (126), driving downstream events such as tauopathy and neurodegeneration. Unlike EOAD, for which excessive production of Aβ seems to be key, LOAD is thought to result from impaired Aβ clearance, arising from multiple genetic and environmental factors, including aging (126). Amyloid accumulation typically occurs one to two decades before obvious cognitive impairment (152); accordingly, individuals with high plaque loads may have no dementia symptoms, and plaque load correlates only modestly with cognitive deficits (108).

LOAD risk is strongly influenced by heritable factors, with over 40 risk-modifying alleles identified to date. The known alleles with strongest impact alter disease risk by approximately threefold in a heterozygous dose, such as the common *APOE* (apolipoprotein E) variant known as *APOE-ε4* and rare loss-of-function mutations in *TREM2* (triggering receptor expressed on myeloid cells 2) (58, 158). Homozygous loss-of-function of *TREM2* or *TYROBP* (which encodes a critical adaptor

SOURCES OF HUMAN GENETIC INFORMATION

Genome-wide association studies (GWASs) are a major method for linking genetic variants to disease. In these studies, common genetic variants are tested for enrichment within patient populations relative to healthy controls. Most of the causal variants for common diseases act by altering the expression levels of genes as opposed to altering the protein sequences. Genetic variants may therefore be located far from the gene whose expression modifies disease risk. Nomination of a candidate gene is not always straightforward. Nearby genes are prioritized by numerous analyses, such as expression quantitative trait loci (eQTLs), which correlate genetic variants to altered gene expression, or three-dimensional chromosome positioning. Given that eQTL linkages and chromosome structure can change with cell type, gene assignments can remain ambiguous and may be revised as a locus is studied further. Linkage disequilibrium, the tendency for noncausal genetic variants to cosegregate with nearby causal variants, further complicates matters. Adjacent genetic variants with opposite effects on disease risk can also occur (e.g., a variant driving higher expression of an important gene near a different variant driving lower expression of the same gene). Future GWASs incorporating more diverse genetic backgrounds, rare-variant analyses, and new techniques to integrate eQTL data and functional annotations with GWAS results promise to identify additional candidate genes and provide further insights into neurodegenerative disease processes.

mediating TREM2 signaling) causes Nasu–Hakola disease, which is characterized by early-onset neurodegeneration in white matter and bone lesions (72). *TREM2* is expressed highly and selectively by microglia in the human and mouse CNS (78, 137, 164, 165), although it is present at low levels in certain myeloid populations in the periphery (81). The discovery that neurodegeneration is associated with mutations in what is essentially a microglia-specific gene in the brain (*TREM2*) has spotlighted microglia in the pathogenesis of neurodegenerative disease.

Beyond *TREM2* and *APOE*, there are many genetic variants with relatively modest effects, affecting the risk of LOAD by 25% or less (see the sidebar titled Sources of Human Genetic Information). These risk genes repeatedly implicate endocytosis, lipid transport, and immune pathways in AD pathogenesis (50, 67, 76). Only a subset of LOAD risk genes are directly linked to Aβ production (e.g., *ADAM10*, *ACE*, and *APH1B*), much like *APP*, *PSEN1*, and *PSEN2*. Remarkably, many genes identified from AD genome-wide association studies (GWASs) have highest expression in microglia relative to other major CNS cell types (50, 67) (**Figure 1**). For instance, the AD GWAS hit *SPI1* (63) encodes PU.1, a principal transcription factor in macrophage fate specification and cytokine response that is expressed only by microglia and other macrophages in the CNS (164, 165). In general, AD GWAS single-nucleotide polymorphisms are more frequently found within open chromatin of microglia than within that of other CNS cells, although unlike *SPI1* and *TREM2*, the expression of most of these genes is not exclusive to microglia (78).

Genome-wide association studies (GWASs): analyses of large human cohorts testing genetic variants enriched or depleted in patients versus healthy controls

Immunoreceptor tyrosine-based activation motif (ITAM): an amino acid motif commonly found in receptors that promote immune responses

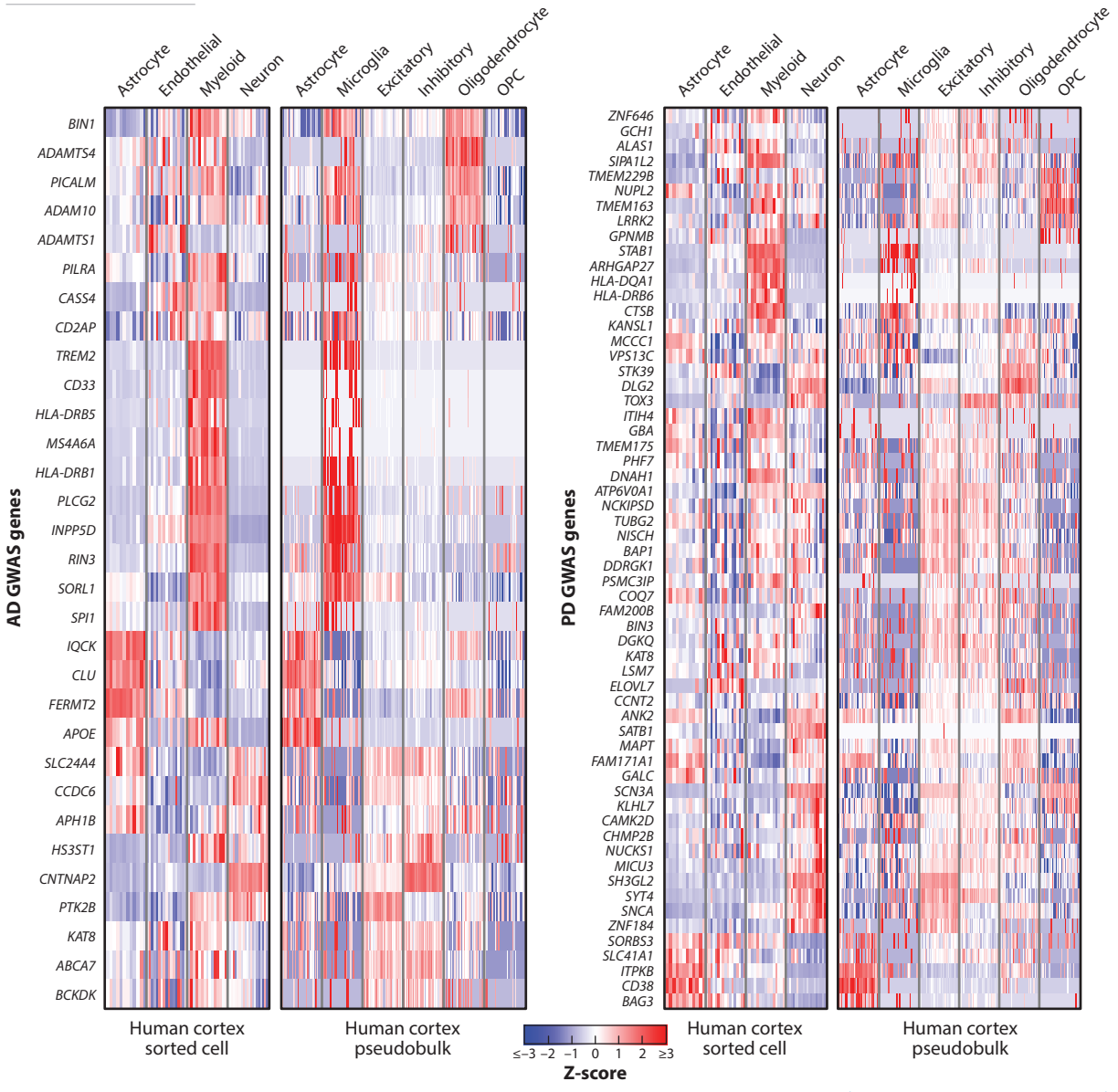
ITAM and ITIM Signaling

TREM2 encodes a transmembrane cell surface receptor that signals through the adaptor protein TYROBP/DAP12, which initiates Syk tyrosine kinase signaling through an immunoreceptor tyrosine-based activation motif (ITAM) upon TREM2 activation by extracellular ligands (144, 158). Aβ aggregates bind directly to recombinant TREM2 extracellular domain (83, 166, 167), although other studies suggest that certain phospholipids are the relevant ligand for TREM2 (15, 150). TREM2 also binds to APOE or APOJ when they are lipidated and facilitates uptake of APOE/APOJ-containing lipoparticles. Furthermore, microglial uptake and degradation of Aβ is accelerated when Aβ oligomers are bound to APOE/APOJ lipoparticles, a process that is partially

mediated by TREM2 (159). After ligation, TREM2 elicits a DAP12/Fyn/Syk signaling cascade that influences a host of microglial processes, including phagocytosis, endocytosis, chemotaxis, CSF1-mediated survival, aggregate degradation, and metabolic changes (144, 158). Thus, TREM2 is central to microglial activity in response to amyloid and lipids, and the functional relevance of these pathways is discussed below.

Immunoreceptor tyrosine-based inhibition motif (ITIM): an amino acid motif commonly found in receptors that inhibit immune responses

Besides *TREM2*, multiple other genes encoding ITAM- or immunoreceptor tyrosine-based inhibition motif (ITIM)-related proteins influence LOAD risk. *CD33* and *PILRA* encode ITIM-containing transmembrane receptors, and *INPP5D* and *PLCG2* encode intracellular signal



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Expression of AD and PD GWAS risk genes across different cell types. Heat map showing relative expression of GWAS locus-associated genes for AD (*left*) (67, 79, 97) and PD (*right*) (18) in various cell types of human cortex. Expression data are from bulk FACS-sorted cells analyzed by RNA-Seq (GSE125050) or by single-nucleus RNA-Seq profiles aggregated into pseudobulks (GSE97930) (78) for each cell type and sample, as indicated. For sorted cell RNA-Seq data, gene expression values were normalized by sample using DESeq2 size factors followed by Z-score normalization for each gene. Pseudobulk profiles were calculated as the sum of UMI counts over all cells of the given type within a single sample and then normalized using DESeq2 size factors for each pseudobulk followed by Z-score normalization for each gene. Oligodendrocyte lineage cells were not collected in GSE125050; endothelial cells and pericytes were omitted from GSE97930 owing to low cell number. Excitatory and Inhibitory denote glutamatergic and GABAergic neuronal populations, respectively. For some SNPs, multiple nearby candidate genes were included. Genes with low expression levels across all cell types were omitted. These data highlight that a striking number of genes associated with LOAD GWASs have highest expression in microglia compared with other CNS cell types. Such an association is not notable in PD. Abbreviations: AD, Alzheimer disease; CNS, central nervous system; FACS, fluorescence-activated cell sorting; GWAS, genome-wide association study; LOAD, late-onset AD; OPC, oligodendrocyte progenitor cell; PD, Parkinson disease; SNP, single-nucleotide polymorphism; UMI, unique molecular identifier.

transduction enzymes downstream of ITAM receptors (**Figure 2**). Human genetics analyses imply that ITAM signaling serves a protective function in AD, whereas ITIM signaling does the opposite: Loss-of-function mutations in *TREM2* (which signals through ITAM-containing DAP12) increase AD risk, alleles associated with elevated expression of ITIM-bearing CD33 elevate AD risk, and function-disrupting mutations in ITIM-bearing PILRA lower AD risk (9, 44, 117, 158). ITAM- and ITIM-related signaling proteins are highly expressed by microglia and can influence several cellular events implicated in AD through other risk genes. For instance, *ABI3*, *CD2AP*, *PTK2B*, *FERMT2*, and *CASS4* all regulate cortical actin rearrangements of the sort required for migration and phagocytosis, processes that are highly influenced by ITIM- and ITAM-triggered signaling cascades (**Figure 2**).

Lipoproteins and Lipids

TREM2 can interact with APOE/APOJ lipoparticles and Aβ (see above). APOE is a lipid-binding protein that is synthesized in the CNS by astrocytes, oligodendrocyte progenitor cells (OPCs), microglia, and other macrophages, where it is complexed with cholesterol and phospholipids before secretion as a lipoprotein complex (77). APOJ, another lipoprotein encoded by the AD GWAS risk gene *CLU*, and APOE are the major protein constituents of cerebrospinal fluid lipoparticles (77). Like high-density lipoprotein and low-density lipoprotein in the blood, these APOE/APOJ lipoparticles transport lipids and other hydrophobic molecules in the extracellular space, where they can be ingested by other cells via clathrin-mediated endocytosis. Interestingly, several genes that participate in endocytosis and downstream vesicular sorting (e.g., *PICALM*, *BIN1*, *SORL1*, *CD2AP*, and *RIN3*) are AD GWAS hits and are highly enriched in microglia.

APOE binds Aβ and accumulates in amyloid plaques in a manner dependent on microglia and TREM2 (114). Genetic deletion or antibody-mediated depletion of APOE greatly reduces dense plaque load in mouse models of amyloidosis (58, 77, 88), suggesting that APOE lipoparticles somehow facilitate plaque seeding, alter amyloid aggregation state, or both. Replacing mouse *Apoe* with human isoforms partially reverses the effects of *Apoe* knockout, with *APOE-ε4* resulting in higher amyloid loads than *APOE-ε3*, the most common human isoform (58). Other studies suggest that *APOE-ε4* slows amyloid clearance (relative to *APOE-ε3*) through altered binding to Aβ and reduced clearance of APOE-ε4/Aβ complexes (58). These results suggest a model in which microglia contribute to plaque compaction or seeding by introducing APOE lipoparticles to early Aβ aggregates but contribute to plaque clearance through phagocytic or endocytic clearance of lipoparticles associated with Aβ (58, 114, 159). Additionally, *APOE-ε4* knock-in mice also show

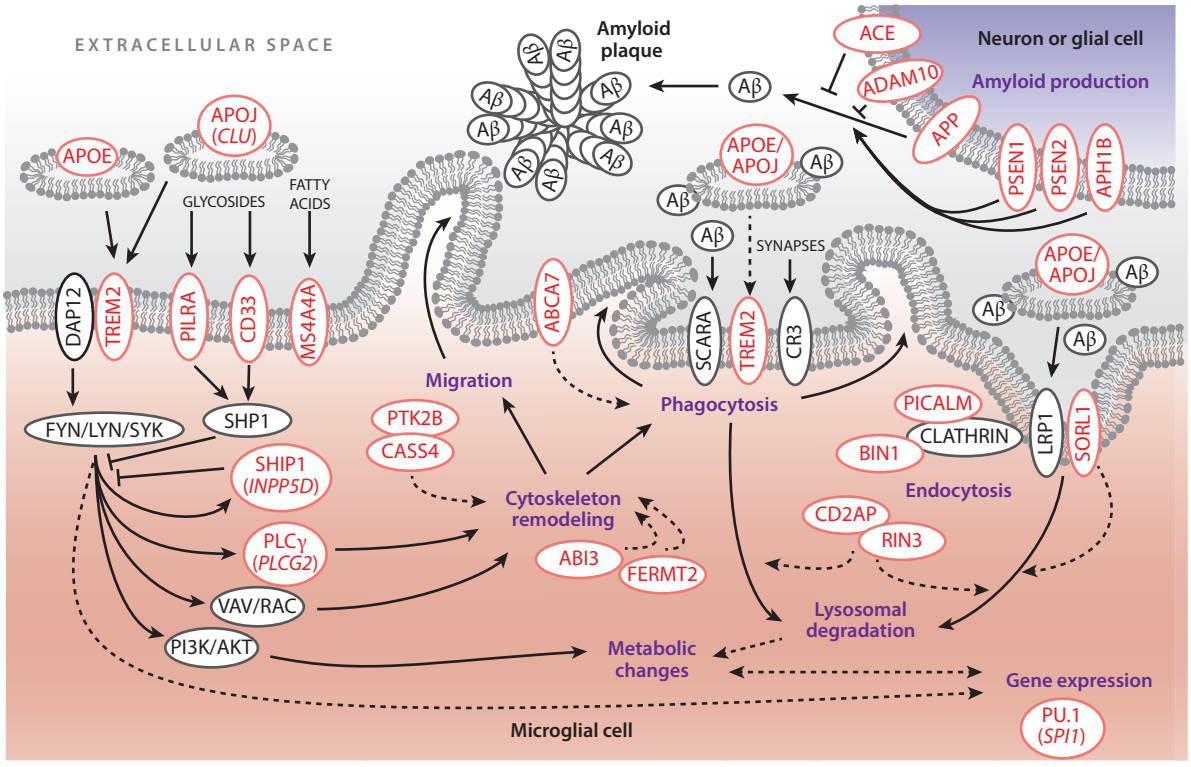


Figure 2

Potential relationships among AD risk genes and microglial functions. Schematic overview of functional relationships among AD GWAS risk genes (*red ovals*) and cellular processes (*purple text*). Related factors not identified as AD GWAS hits (*black ovals*) are provided for context. Dashed lines denote indirect or inferred relationships. Protein names are shown, with gene names in parentheses where relevant. Abbreviations: AD, Alzheimer disease; GWAS, genome-wide association study.

exaggerated neuronal loss and gliosis in tauopathy models (130), pointing to amyloid-independent mechanisms for *APOE-ε4* in AD. Further studies are needed to clarify the relationships among lipoproteins, microglia, and AD.

Risk Alleles for Other Neurological Diseases

Is the high prevalence of microglia-enriched genes among AD GWAS hits a general feature of neurodegenerative diseases? Genetic variants associated with Parkinson disease (PD) (18), the second most common neurodegenerative disorder, are generally not linked to AD, and as a class show broader expression across multiple CNS cell types (**Figure 1**). A fraction of genes (e.g., *CTSB*) show high microglial enrichment, but GWAS hits for PD more typically lie within open chromatin of excitatory neurons rather than microglia (78, 164, 165). By contrast, genes associated with bipolar disorder, schizophrenia, lysosomal storage diseases, and multiple sclerosis often lie within microglial open chromatin or show high microglial expression, but these are rarely the same microglial genes as those associated with AD (78). Thus, microglia likely have a leading role in a subset of brain diseases, with molecular mechanisms varying across disorders.

Parkinson disease (PD): a neurodegenerative disease characterized by dopaminergic cell loss and α-synuclein aggregates

MICROGLIA IN BRAIN DEVELOPMENT AND HOMEOSTASIS

Given the strong genetic evidence for microglial involvement in AD, we review in this section normal microglial functions in the developing and adult CNS to provide context for microglial changes in neurodegenerative disease.

Peritoneal macrophage (PTM): tissue macrophages residing in the lining of the peritoneal cavity

Specialized Macrophages for the Central Nervous System

Tissues typically contain parenchymal macrophages that are specialized to handle the demands of their home organ, and each tissue-resident population has a distinct gene expression profile that reflects their function within a particular niche (40, 41, 81). For instance, red pulp macrophages in the spleen degrade red blood cells, and high heme loads activate *Bach1*/*Spic*-dependent transcriptional changes that improve tolerance of accumulated iron and heme metabolites (73). Likewise, microglia express a set of microglial signature genes that are absent from or expressed at much lower levels by other macrophages (12, 40, 55, 81). Microglia have distinct chromatin states and sometimes exhibit low expression of genes that are highly expressed by other macrophages (41, 81). Macrophage signature gene expression is shaped by the tissue environment. For example, peritoneal macrophages (PTMs) transplanted into the lung largely convert to a pattern of gene expression akin to that of native alveolar macrophages (81). When microglia are taken out of the brain and maintained in vitro, microglial signature gene expression is lost within hours (8, 42) but can be recovered by engrafting the cells back into an intact CNS (6).

In addition to environmental cues, developmental histories also determine macrophage specialization. For instance, in PTMs retinoic acid supports signature gene expression but is insufficient on its own to induce the full signature in macrophages of a different origin; exposure to omental cues during PTM generation modifies chromatin to allow complete induction of PTM genes upon retinoic acid exposure (112). Similarly, the developmental history of microglia is required for full expression of the microglial signature. Microglia derive from yolk-sac progenitors during primitive hematopoiesis before the formation of bone marrow (85). Upon implantation in the brain (naturally or artificially), yolk-sac progenitors fully induce microglial signature gene expression, whereas bone-marrow-derived cells can induce this signature only partially, indicating that cellular ontogeny dictates how much microglial signature gene expression can be evoked by environmental cues (6, 11, 94, 128). CNS-engrafted cells of bone marrow origin fail to induce *Sall1*, which is a key microglial transcription factor that is continuously required for a highly ramified morphology and expression of a subset of microglial signature genes (6, 14, 128).

Distinguishing features aside, microglia have many core properties in common with other macrophages. Among them is a reliance on continuous CSF1R (colony stimulating factor 1 receptor) activation for survival. *Csf1r*$^{-/-}$ mice have no microglia, and microglia can be almost completely eliminated with CSF1R inhibitors (93). Mice with deficiencies in the CSF1R ligands CSF1 or IL-34 show reduced microglial numbers, indicating that both ligands contribute to microglial numbers in the CNS (93). Artificial elevation of CSF1R activity is sufficient to drive microglial proliferation in vitro or in vivo, and elevated CSF1 production from injured cells is required for microglial expansion in multiple injury paradigms (45).

TGF-β Signaling in Microglia Maintenance and Central Nervous System Homeostasis

The physiological importance of microglial specialization can be appreciated from studies of microglial TGF-β (transforming growth factor β) signaling, which is critical for both microglial signature gene expression and CNS homeostasis. Relative to other macrophages, microglia express

high levels of TGFBR1 (12, 40, 55, 81), which collaborates with TGFBR2 to mediate responses to TGF-β1 or TGF-β2. Mice lacking TGF-β1 in the CNS have microglia with altered signature surface markers, showing skewed microglial maturation as early as embryonic day (E)14.5 (12, 96). Conditional ablation of *Tgfbr2* from adult microglia triggers major changes in signature surface markers and gene expression profiles, demonstrating that sustained TGF-β signaling is essential for microglia maintenance (14, 94).

Lipopolysaccharide (LPS): lipidated carbohydrates from bacterial cell wall that induce innate immune inflammatory responses

Long-term elimination of *Tgfbr2* from microglia leads to gradual loss of motor function that begins in the hind limbs and climbs rostrally to cause fatal paralysis (94). Concurrently, myelin-engorged microglia accumulate within descending axon tracts in the spinal cord white matter (94). Considering the importance of oligodendrocyte metabolic support for axon maintenance (132), these results suggest that microglia contribute to myelin maintenance and thereby influence long-distance corticospinal transmission. In contrast, deletion of *Tgfbr2* from approximately 80% of microglia in adults had no impact on mouse survival over months and only mild changes in microglial gene expression, suggesting that the impact of TGF-β signaling on CNS homeostasis may vary with context (3, 168). Mice lacking microglial *Nrros*, a factor involved in TGF-β1 maturation, show phenotypes that closely resemble those of *Tgfbr2* conditional knockout mice, including adult-onset climbing paralysis preceded by embryonic aberrations in microglial development (116, 155). In areas of neurodegeneration, microglial signature gene expression is typically compromised, which may contribute to loss of homeostatic signals and eventual neural dysfunction nearby.

How microglial specialization is normally sustained is not completely understood. TGF-β signaling is critical for microglial signature gene expression, but other CNS attributes also likely influence microglial specialization. The lung parenchyma and epidermis also exhibit constitutive TGF-β signaling, which is required for alveolar macrophage and Langerhans cell survival but fails to drive microglial signature gene expression (161). In culture, microglia rapidly lose expression of many microglial signature genes even in the presence of active TGF-β (8), although TGF-β activation does affect a subset of signature genes (12, 41, 168). Thus, TGF-β is not sufficient to instruct microglial identity, at least under the environmental constraints imposed by typical tissue culture environments or the lung or skin, and requires the presence (or possibly absence) of additional CNS environmental cues to drive microglial specialization.

Microglial Clearance of Apoptotic Cells

Apart from their specialized CNS properties, microglia engage in universal macrophage functions such as removal of apoptotic cells, which is important throughout the body for preventing inflammation and autoimmunity (2). In the CNS, this function is exemplified in neurogenic zones, where microglial density is highest and where microglial ablation leads to accumulation of apoptotic cells (27, 106, 119). Remarkably, microglial ablation also changes the abundance and migration of surviving neural progenitor cells, and lipopolysaccharide (LPS) stimulation or *Sall1* deletion has reciprocal effects (14, 27, 106, 119). Microglia also clear apoptotic cells in developing white matter tracts, where many differentiating OPCs undergo apoptosis, and in the cerebellum (5). Exposure to apoptotic cells alters the gene expression profile of microglia in vitro and in vivo, which could explain atypical gene expression patterns in a subset of cerebellar microglia and in postnatal microglia within nascent white matter tracts (5, 43, 48, 75, 86). Gene expression changes evoked by apoptotic cells share commonalities with microglial expression changes observed in neurodegenerative diseases (see the section titled Disease-Associated Microglia Signature in Alzheimer Disease).

Several different receptors and signaling pathways contribute to apoptotic cell clearance by microglia depending on the specific context (2). Zebrafish screens identified TIM4 as a critical

receptor for microglial phagocytosis of apoptotic cells in early development (101). However, TIM4 expression is strongly suppressed in mouse microglia a few days before birth (99); AXL and MERTK at least partially account for microglial phagocytosis in postnatal neurogenic zones (5, 36). TREM2 and the vitronectin receptor have also been implicated in apoptotic cell clearance in vitro or after CNS injury in vivo (74, 107, 140).

Synapse Pruning

During brain development, an abundance of synapses are formed and selective synapse elimination is required for network refinement. Synaptic material is detected within microglia in the developing hippocampus (113, 153), in the retinogeniculate system (124), and in response to visual experience (142) during a restricted developmental period (124, 139). Synapse removal is regulated in part by activity, with microglia preferring to engulf material from less active neurons (124, 142). Altered microglial uptake of synaptic material results in aberrant circuit refinement (113).

The complement cascade, part of the innate immunity system that facilitates the clearance of pathogens and cellular debris in the periphery, has an important role in synapse pruning (109). In the CNS, all complement components are expressed and secreted locally, mainly by microglia and astrocytes. C1q, the initiator of the classical complement cascade, and C3, a central downstream protein whose cleavage product iC3b opsonizes structures for phagocytic removal, are abundant in the developing brain (139). Whereas the expression of C3 wanes after development, C1q expression stays high and the protein accumulates in the brain with aging (138). Microglia, the only cell type in the brain that expresses CR3 (a complement receptor encoded by *Itgam* and *Itgb2*), can phagocytose complement-tagged synapses through the C3–CR3 pathway. Mice deficient in C1q, C4, C3, or CR3 have defects in synaptic connectivity (124, 125, 139). However, although deletion of C1q or C3 significantly reduces anatomical refinement of retinogeniculate connections, it does not completely prevent it (139), suggesting that additional pathways contribute to synapse removal (109), e.g., the neuronal chemokine CX3CL1 and its microglial receptor, CX3CR1 (20, 113). Astrocytes also engage in synapse pruning, utilizing receptors MEGF10 and MERTK, which are also involved in phagocytosis of apoptotic cells (23). Apoptotic cell removal and synapse elimination during development may share some common cellular and molecular mechanisms (1, 33).

Guidance and Support Roles for Microglia

Additional microglial contributions to CNS development have been discovered owing to their irregular anatomical distribution in the embryonic brain. Microglial density is high near the growing ends of embryonic dopaminergic axons, and interfering with microglia disrupts proper targeting of these axons (136). Elsewhere, microglia associate with neovascular tips and facilitate vascularization of the embryonic CNS (19). Microglia are conspicuously excluded from axon bundles in the developing barrel cortex, and disrupting microglial migration into these regions delays synapse maturation (62). Likewise, mislocalization of *Cx3cr1*$^{-/-}$ microglia to the outer retina is associated with retinal dysfunction and cone loss (68). The molecular mechanisms underlying these functions have not been fully elucidated but may involve phagocytosis, matrix-remodeling proteases, trophic cues, or a combination thereof.

In layer 5 of the cortex, a subpopulation of interneurons depend on microglial cues for survival in a process dependent on CX3CR1 and IGF1 (143). Microglia also influence the proper laminar organization of some cortical interneurons during development (136). At a subcellular level, microglial contact facilitates spine formation on neuronal dendrites, and microglial ablation leads to decreased synapses in layer 2/3 (87, 103, 153). Thus, microglia likely also influence neuronal circuits in ways unrelated to synapse engulfment.

Pharmacological ablation of microglia from postnatal mice profoundly reduces the number of OPCs and impairs myelination (47). IGF1 (insulin-like growth factor 1), a factor critical for oligodendrocyte proliferation and survival, is upregulated by microglia that encounter apoptotic cells, as is the surface receptor CD11c (75). Knocking out IGF1 from the CD11c-positive subpopulation of microglia hinders normal myelination much like pharmacological ablation of microglia (154). Collectively, these findings suggest that microglia not only dispose of cellular corpses but also release trophic cues after engaging apoptotic cells, thereby providing a potential feedback mechanism to balance oligodendrocyte numbers.

Microglia and Aging

Aging is a dominant risk factor for neurodegenerative diseases. Many studies have described microglial changes in aged mice, reporting increased density in some regions, altered morphology, and induction of activation markers, particularly lysosomal CD68 (31, 51, 110, 115, 131). Single-cell RNA-Seq (scRNA-Seq) and cytometry by time of flight (CyTOF) mass cytometry studies found expansion of two microglial populations expressing high levels of genes associated with neurodegeneration and interferon signaling (48, 105). These single-cell studies extend prior immunohistochemical findings (110) and are generally consistent with bulk RNA-Seq analyses of purified mouse microglia (43, 55, 57, 123). Human microglia may age differently, however (39). Functionally, aged microglia exhibit reduced phagocytic capacity and fail to migrate to and proliferate in response to injury (17, 52).

What drives microglial changes with age? Elevation of microglial activation markers such as CD68 is most pronounced in white matter regions (51), and one key contributor is the accumulation of myelin debris in microglial lysosomes. Myelination continues throughout adult life, and in aged (~20–30 month old) mice myelin debris accumulates in large CD68-positive lysosomes within microglia (56, 123). Myelin is rich in various lipid species including cholesterol, and microglia burdened with lysosomal myelin accumulate crystals of precipitated cholesterol that disrupt lysosomal integrity and trigger the NLRP3 inflammasome (17). Interventions that improve cholesterol clearance are sufficient to reverse some age-related microglial deficits, improving recovery after lysolecithin-induced demyelination (17). Similarly, introduction of young monocytes into aged mice through heterochronic parabiosis accelerates remyelination postinjury (122). Thus, increased myelin load with age leads to the buildup of lysosomal myelin, which in turn alters microglial activation status and function. Surprisingly, age-associated microglial changes can be reversed largely by transient pharmacological ablation of microglia followed by repopulation (31, 111), suggesting that replacement of aged microglia (even from aged progenitors) may override environmental cues that drive age-related dysfunction.

MICROGLIAL CHANGES IN NEURODEGENERATIVE DISEASE

Microglia are highly plastic cells that respond to myriad stimuli. Indeed, transcriptomic studies of sorted cell populations from disease mouse models commonly find that transcriptional changes are more extensive in microglia than in other cell types. In this section, we discuss the multiple states of microglial activation in various models of brain disease, with an emphasis on whole-transcriptome mRNA profiling of microglia.

Disease-Associated Microglia Signature in Alzheimer Disease

Transcriptomic studies of bulk brain tissue usually find significant increases of microglial transcripts during injury or disease, which can be explained in part by expanded microglial numbers in

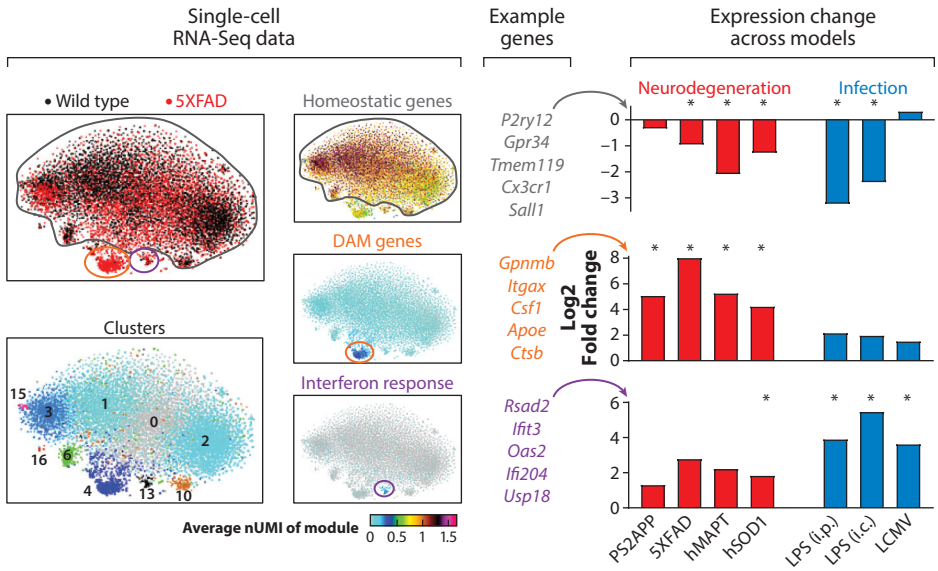


Figure 3

Microglial gene expression changes in mouse models of AD highlight the similarities and differences with other brain disease models. (*Left*) tSNE plots of scRNA-Seq data of microglia ($CD11b^+$ brain cells) from brains of wild type (*black dots*) and 5XFAD mice (*red dots*) reveal multiple discernable clusters (see numbers between 0 and 16 in the *bottom-left plot*). Clusters with elevated expression of homeostatic genes (clusters 0–6), DAM module genes (cluster 4), or interferon response genes (cluster 13) are highlighted in the right-hand tSNE plots, with example genes from each module listed to the right. tSNE plots represent wild type six-month 5XFAD $CD45^+$ cells from Reference 70, with nonmicroglial clusters (lymphocytes, monocytes, neutrophils, and macrophages) removed. Gene sets were taken from Reference 38 and colors depict average normalized UMIs (nUMIs) of all genes in the set for each cell. (*Right*) The fold change of expression of *P2ry12*, *Gpnmb*, and *Rsad2* is shown for sorted microglia from various neurodegeneration or infection models. DAM genes are induced in neurodegeneration models, interferon response gene induction is most pronounced in infection models, and homeostatic genes are downregulated in multiple models. scRNA-Seq data from GSE98969 and expression changes are from (in order) GSE89482, GSE65067, GSE93180, GSE43366, GSE75246, GSE67858, and GSE67858. Fold changes and *P*-values from Reference 38; *adjusted $P \leq 0.05$. Abbreviations: AD, Alzheimer disease; DAM, disease-associated microglia; i.c., intracerebroventricular; i.p., intraperitoneal; LCMV, lymphocytic choriomeningitis virus; LPS, lipopolysaccharide; scRNA-Seq, single-cell RNA-sequencing; tSNE, t-distributed stochastic neighbor embedding; UMI, unique molecular identifier. The five boxed tSNE maps on the left were adapted from Reference 38. They are available for reuse under a CC-BY 4.0 license.

the distressed CNS (137). However, transcriptomic studies of sorted (purified) microglia demonstrate that microglia in neurodegenerative models induce a common set of genes known as the disease-associated microglia (DAM) response (70) [also termed neurodegeneration-specific (22), neurodegeneration-related (38), MGnD (75), or primed (57)]. This upregulated DAM gene module was described first in the SOD1-G93A mouse model of amyotrophic lateral sclerosis (ALS) (22) and then in other disease models such as AD amyloidosis (70, 137), frontotemporal dementia tauopathy (38), and cuprizone-induced demyelination (115) (**Figure 3**). Some of the most robustly upregulated genes in microglia from these models include *Itgax* (CD11c), *Csf1*, *Cst7*, *Clec7a*, *Gpnmb*, *Mamdc2*, and *Apoe*. Overall, the DAM gene module contains many secreted and transmembrane proteins, and it is enriched for lysosome, phagosome, and antigen presentation gene ontology terms (38, 57). Compared with the robust upregulation of DAM genes, sorted microglia

Amyotrophic lateral sclerosis (ALS): a neurodegenerative disease prominently affecting motor neurons

from mouse models of neurodegenerative disease show relatively little induction of classical inflammatory genes (such as *Il1b*, *Tnf*, and *Saa3*) that can be readily triggered in microglia after LPS injection (22, 32, 38, 137).

Activated microglia in these neurodegenerative disease models show elevated lysosomal CD68 immunoreactivity and are concentrated at sites of pathology (i.e., clustered closely around plaques in mouse amyloidosis models and in AD patient brain) (64, 70). Microglia with elevated immunostaining for DAM markers such as CLEC7a, CD11c, or LPL are also concentrated at sites of pathology (70, 75). Furthermore, regions bearing CD68-high microglia during brain development also contain microglia exhibiting upregulation of DAM genes, which can be induced by introduction of apoptotic neurons, as discussed above (75). Foamy (lipid-laden) macrophages in atherosclerotic lesions also upregulate many genes common with the DAM cassette, suggesting that abnormal levels of lipids may be sufficient to induce a subset of DAM genes (24, 71). Further investigation into the induction of DAM genes by macrophages in other tissues and pathological contexts may help our understanding of the functional significance of the DAM activation state.

How is DAM gene expression regulated in microglia and is the microglial DAM response protective or damaging for disease pathogenesis? The regulatory network of the DAM module is poorly understood, but it is interesting that both *Trem2* and *Apoe*—each a high-odds-ratio risk gene for AD—are essential for the full expression of the DAM response in amyloidosis (APP-PS1, 5XFAD) and cuprizone demyelination models (38, 70, 75, 115, 145, 150). *Trem2*$^{-/-}$ mice show impaired microglial DAM gene induction in amyloidosis models; however, *Trem2* knockout microglia also fail to congregate around plaques, so the effects of *Trem2* on the DAM response could be either direct or indirect. *Apoe*$^{-/-}$ mice exhibit diminished microglial DAM gene induction after direct apoptotic neuron injection into the brain (75) and in the PS19 Tau-P301S tauopathy model (130). By contrast, *Apoe* is dispensable for inducing a DAM-like module in atherosclerosis models (24, 71), suggesting that the molecular mechanisms of DAM gene activation vary with context. A study using a series of knock-in mice carrying different human *APOE* isoforms demonstrated that DAM gene expression in PS19 tauopathy mice was more highly induced in PS19/*APOE-ε4* knock-in than in PS19/*APOE-ε3* knock-in microglia (130). Thus, genetic manipulations mimicking increased AD risk can enhance DAM gene induction (as in the case of *APOE-ε4*) or diminish it (as with loss-of-function of *Trem2*). The functional significance of the DAM gene response, and of microglial activation in general, remains a central question in the pathogenesis of AD and other neurodegenerative diseases.

Additional Microglial Gene Signatures in Disease

Concomitant with the upregulation of DAM genes, microglial cells in neurodegenerative disease models exhibit downregulation of a different module of so-called homeostatic genes, which include *Tmem119*, *P2ry12*, *Gpr56*, and *Cx3cr1* (38, 70). This homeostatic gene module is largely the same set as the microglial signature genes discussed above, which are expressed at low levels in other myeloid cells (81) or in immature microglia (99). In keeping with the inverse relationship between expression of homeostatic genes and DAM genes, TGF-β signaling and the transcription factor *Sall1* repress DAM induction in addition to LPS-induced transcripts while promoting microglial signature gene expression (12, 14). The homeostatic gene module seems primed for repression, as expression is reduced in microglia after many types of stressors, including neurodegenerative disease models, LPS treatment, viral infection, and transfer to cell culture after acute isolation (8, 42, 137) (**Figure 3**).

Aside from the DAM module, upregulation of interferon pathway–related genes such as *Ifit1*, *Isg15*, and *Stat2* is characteristic of microglia in mouse models of infection (38). Additionally,

scRNA-Seq experiments in mouse brain have revealed that the small fraction of microglia expressing high levels of interferon-related genes is modestly increased in amyloidosis models (38, 70). By contrast, interferon-stimulated genes are robustly induced after LPS treatment or virus infection and also in the CK-p25 model of severe neurodegeneration (32, 100, 137). Artificial induction of a microglial interferon response by IFN-β overexpression or injection is sufficient to drive microglial ingestion of synaptic material in the brain, which may contribute to sickness behavior and is likely detrimental during neurodegeneration (7, 146). Cytoplasmic leakage of mitochondrial nucleic acids after lysosomal stress and autophagy failure may contribute to the initiation of interferon signaling cascades in microglia (133). Expression of a proliferation-associated gene module is also elevated in the myeloid compartment in response to virus challenge and during early development (38, 86, 100). Despite the increased number of microglia in Aβ models, in fact very few proliferating microglia can be identified by scRNA-Seq analysis or immunohistochemistry (38, 70).

Given so many different types of microglial activation (as defined by gene expression patterns), one might wonder whether they can co-occur in the same cells or whether different microglia in the same brain enter into distinct states. scRNA-Seq analyses of amyloidosis models show that induction of DAM genes, interferon-activated genes, and proliferation-related genes rarely co-occur within the same cell (38, 70). However, the CK-p25 model of severe neurodegeneration is notable in that DAM genes are activated and interferon response genes are expressed within the same cells (100). Different types of activated microglia also exhibit reduced expression of the homeostatic gene module (100). Thus, depending on the stimuli, the various types of microglial activation can occur in different cell populations or within a single microglial cell.

Microglial Gene Expression Changes in Human Disease

The diversity, number, and resolution of microglial RNA-Seq studies in mouse paint a rich and detailed picture of varied microglial activation states in murine models of disease; however, the analysis of microglia in human disease tissue is lagging behind. Many immunohistochemical analyses of microglial markers have been conducted and generally highlight upregulation of CD68 and MHCII staining in AD brains (61). Gene expression profiles of human microglia have been reported (39, 42, 46, 78), but these commonly utilized surgical resections or fresh autopsy samples of brain and included few if any neurodegenerative disease cases. A recent study used scRNA-Seq profiling of myeloid cells from a small number of patients suffering from both multiple sclerosis and epilepsy, demonstrating gene expression changes (including induction of DAM genes) that parallel those observed in mouse demyelinating injuries (98). Additional expression profiles of purified microglia from human AD and control samples should be illuminating in the near future.

By contrast, several gene expression studies of bulk cortical tissue from large AD and control patient cohorts have already been published (28, 38, 151). With the important caveat that RNA-Seq signals in bulk tissue might reflect changes in cell-type composition in the brain or gene expression in nonmyeloid cells (137), these data sets can be mined to determine whether genes associated with microglial activation in mouse models show analogous changes in human disease. In bulk tissue from human AD cortex, DAM genes are elevated, albeit more modestly than in mouse models, and other gene modules, such as the interferon-related and classical inflammatory gene sets, are also induced, suggesting that some inflammatory aspects of AD pathology are not recapitulated in mouse models of AD (38) (**Figure 4**). Possible explanations for the apparent discrepancy include the fact that human samples come from postmortem brain, including patients with end-stage AD, which has multifactorial etiologies and comorbidities.

Furthermore, efforts to identify gene expression patterns in human AD brain that correlate well with cognitive decline have identified gene modules that are not enriched for markers of any single

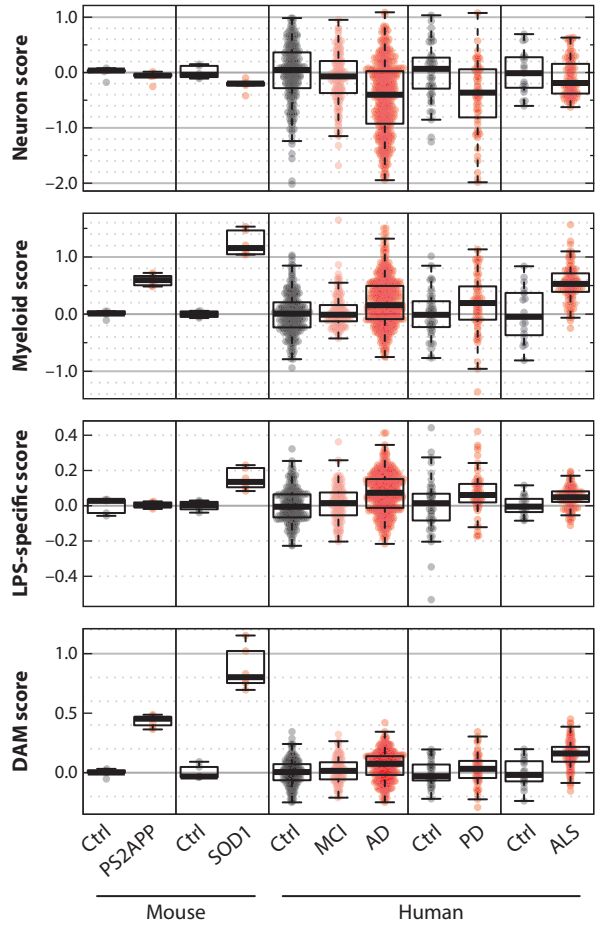


Figure 4

Changes in specific gene sets in bulk brain or spinal cord RNA-Seq data from mouse models of amyloidosis (PS2APP) or ALS (SOD1) compared with human postmortem AD, MCI, PD, or ALS patient tissues. Each point corresponds to a single sample, with the *y*-axis showing gene set scores calculated as described (38). Expression values of each gene were log2-transformed and normalized to within–data set controls. Then, sample-wise gene set scores were calculated as the sum over all the genes in the set of these control-centered expression values. Gene set scores are used to estimate cellular abundance (neuron and myeloid scores) or microglial activation status (LPS-specific and DAM scores). Gene sets are taken from Reference 38, except genes in the myeloid gene set, which were further restricted to those detected in unpublished human and mouse purified microglia data sets and not induced in LPS-treated (GSE75246) or PS2APP brain microglia (GSE89482). Data sets are (from left to right): PS2APP, GSE75357; SOD1G93A, GSE18597; MCI/AD, GSE95587, GSE125583, and ROSMAP-DLPFC; PD, GSE7621, GSE8397, GSE20163, GSE20164, GSE26927, and GSE49036; ALS, Target ALS consortium. These data highlight that mouse models recapitulate some disease-associated responses discernable in human tissue, such as the DAM response, which is induced much more modestly in human models. Other responses (such as induction of LPS-specific genes) are not as well captured in these rodent models. Abbreviations: AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; DAM, disease-associated microglia; LPS, lipopolysaccharide; MCI, mild cognitive impairment; PD, Parkinson disease.

brain cell type or activation state (104), ultimately showing little overlap with gene expression patterns described in mouse models of AD. The knowledge gap between microglial activation states in human neurodegenerative disease and those in mouse models remains significant.

FUNCTIONAL ROLES OF MICROGLIA IN DISEASE

Do microglia protect against or exacerbate neurodegenerative disease? In a quintessentially biological way, the answer depends on specific characteristics of the disease, the microglial pathway in question, and the disease stage. In AD models, pharmacological or genetic ablation of microglia at intermediate to late stages (i.e., following plaque formation) generally has little effect on amyloid load (93). However, long-term pharmacological ablation of microglia from the time when plaques start to form in 5XFAD mice leads to a strong reduction in plaque burden (135). Microglial ablation can also ameliorate behavioral changes and anatomical abnormalities such as dendritic spine loss in amyloidosis mice (93, 135).

Thus, microglia ablation studies imply a neutral or detrimental net impact of microglia on pathology progression in mouse models of AD. By contrast, microglial signaling pathways in humans appear to protect against AD: Loss-of-function mutations in *TREM2* greatly elevate LOAD risk and interfere with microglial clustering around amyloid plaques, suggesting that TREM2 signaling is required for protective microglial functions (162). To fully understand the myriad contributions of microglia, it is important to consider individual microglial effector functions, how they influence disease, and which mechanisms are at play during different disease stages.

Interaction with Amyloid and Amyloid Plaques

A subset of microglia intimately associate with amyloid plaques in AD patients and mouse models of amyloidosis. Whereas microglia normally dynamically extend and retract processes, microglial processes in contact with plaques remain fixed for weeks (25, 162). Plaques can have distinct morphologies: Compact plaques are more frequently in close contact with surrounding microglia, whereas diffuse plaques tend to be in regions devoid of microglia and are associated with dystrophic neurites, which are manifestations of axonal injury (25, 162). Microglia in *Trem2*$^{-/-}$ or *Apoe*$^{-/-}$ mice fail to congregate around plaques, resulting in a less compact and more fibrillar plaque structure and more severe local axonal injury (144, 145, 158). Mice heterozygous for *Trem2* or carrying AD-linked *TREM2* mutations exhibit similar defects to a lesser degree (21, 134, 162). Notably, *Trem2* and *Apoe* knockout mice also show reduced microglial responses in tauopathy and demyelination models, suggesting microglial contributions beyond direct plaque interaction (16, 84, 115, 130). *TREM2* overexpression in 5XFAD mouse microglia leads to increased microglial coverage per plaque, lower total plaque burden, and amelioration of behavioral deficits (82). In all, microglia seem to serve as a protective barrier between established amyloid deposits and neuronal processes, compacting plaques and shielding neurons from damaging signals emanating from amyloid plaques.

Aβ aggregates have been observed within endosomes and lysosomes inside plaque-associated microglia (91). Besides potential contributions from TREM2 in uptake of lipoparticle-associated Aβ or noncomplexed Aβ aggregates (discussed above), cultured macrophages utilize scavenger receptors to phagocytose Aβ oligomers, particularly SCARA1 (scavenger receptor A1), which is encoded by the *Msr1* gene (37). *Msr1* expression is absent from parenchymal microglia in vivo but is abundant in perivascular macrophages (155) and is highly upregulated by cultured microglia (8). Despite relatively low expression by parenchymal CNS cell types, *Msr1*$^{-/-}$ mice have elevated amyloid burden and a shortened life span (37). Knockout of the chemokine receptor *Ccr2*,

which has low expression in parenchymal microglia but is important for perivascular macrophage maintenance, leads to elevated vascular plaque deposits that accelerate death in mouse models of amyloidosis (30). Intriguingly, disruption of TGF-β signaling in $CD11c^+$ cells (which includes DAMs and perivascular macrophages) greatly reduces plaque burden in APP-Swe amyloidosis mice (141); similar manipulations disrupting TGF-β signaling in resting microglia lead to the acquisition of perivascular macrophage characteristics, including induction of the *Msr1* transcript (94). Together, these studies suggest that peripheral or perivascular macrophages help stave off plaque deposition and that they may utilize distinct amyloid recognition and phagocytosis machinery compared with microglia. Additional receptors may also contribute to plaque recognition or clearance. For instance, the transmembrane protein–encoding gene *Tm2d3* participates in Aβ oligomer phagocytosis in cultured human macrophages and cell lines (49), and rare *Tm2d3* variants greatly increase AD risk (65).

Microglia and perivascular macrophages counteract plaque expansion through clearance of Aβ oligomers and plaque barrier formation, but paradoxically, it is possible that microglia also contribute to the seeding of new amyloid plaques. NLRP3 inflammasome activation in macrophages or microglia, which occurs in AD patient tissue and mouse models of amyloidosis (54, 147), leads to the formation of cytoplasmic fibrils composed of NLRP3 and the adaptor protein ASC, which can be released into the extracellular space. These ASC specks bind to Aβ and may seed plaque formation, as *Asc* or *Nlrp3* knockout reduced plaque load and behavioral changes in mouse models of amyloidosis (54, 147).

Release of Neurotoxic Factors

Classical activation of macrophages (e.g., by LPS) triggers release of reactive oxygen species and many cytokines that are detrimental to neuronal health. Several studies have found that activation of microglia through pattern recognition receptors [for example, TLR4 (Toll-like receptor 4) activation with LPS] is injurious to neurons and oligodendrocytes in coculture and conditioned-medium experiments (13, 53). Candidate toxic factors include reactive oxygen species, TNF (tumor necrosis factor), and IFNγ (interferon gamma) in various models (53, 127). Microglial damage to neurons need not be direct, as TLR-induced cytokines such as TNF and IL-1α (interleukin 1 alpha) activate astrocytes, disrupting homeostatic astrocyte functions and stimulating release of neurotoxic factors (89).

LPS is a common stimulus for driving classical activation responses in microglia, but it is unlikely to be a relevant agent in the context of AD. However, Aβ oligomers and fibrils can directly activate TLR signaling in cultured microglia through TLR2 and CD14 (80). Transcriptomic studies of mouse models of AD do not show clear induction of classical inflammatory TLR response genes, although this signature is readily observed acutely in the brain after peripheral LPS exposure and seems more prominent in human AD brain tissue (22, 38). In mouse models of amyloidosis, the inhibition of this pathway through knockout of *Tlr2* or *Cd14*, or through heterozygous knockout of the critical signal transduction protein *Myd88*, leads to delayed plaque deposition but accelerated behavioral deficits (102, 118, 120). Knockout of genes encoding downstream inflammatory cytokines such as *Tnf*, *Il6*, and *Il1b* has been generally reported to have beneficial effects in mouse models of neurodegeneration, but further work is needed to clarify its contributions and mechanisms (149).

Although microglia are generally regarded as phagocytic cells that degrade toxic protein aggregates (thereby protecting against disease progression), recent reports suggest that microglia may also facilitate the neurotoxicity or spread of protein aggregates. For instance, microvesicles secreted by microglia alter the aggregation of Aβ to promote the formation of soluble neurotoxic

oligomers (69). Microglia are required for cell-to-cell propagation of pathological phosphorylated Tau after viral overexpression of Tau in the brain, and microglia modify Tau to a transmittable misfolded form in vitro after ingestion (4, 60). A complete understanding of how microglia propagate toxic aggregate species is lacking, but these mechanisms illustrate again the multifaceted nature of microglial contribution to disease pathogenesis.

Complement Pathway in Neurodegenerative Disease

Microglia collaborate with the complement pathway, another major arm of the innate immune system, in the elimination of apoptotic cells and synapses during normal development. Aberrant complement pathway activation is evident in animal models of AD and in postmortem AD brains (29, 59). Components of the classical complement cascade (C1q and C4) are upregulated and deposited around Aβ plaques and, to a lesser extent, neurofibrillary tangles (121, 163). Expression of complement genes is increased in AD patient cerebral cortex as well as mouse models, where C1q and C3 proteins accumulate at synapses (and perhaps other structures) in brain regions affected by amyloid or Tau pathology (29, 38, 59). Indeed, synaptic material accumulates in a complement-dependent manner within microglia in these AD models, suggesting pathological reactivation of microglial synapse engulfment in the neurodegenerative state (29, 59). Importantly, genetic deletion of C1q, C3, or CR3 rescues synapse loss and provides neuroprotection in amyloidosis models of AD (35, 59). However, C3 deficiency also increases plaque burden (95, 129, 157), suggesting that complement pathways may have a role in clearance of Aβ plaques. Whereas reports consistently show neuroprotection upon inhibition of C1q, loss of C3 function seems generally protective only in early stages of amyloidosis; some C3-deficient amyloidosis models show prominent neurodegeneration at advanced age, perhaps because the continuous increase in plaque load over time outweighs the benefits of C3 deficiency (95, 157).

The complement system is also overactive in response to Tau pathology in AD and other tauopathies. Inhibition of C3 or of the C3a receptor C3aR or blocking the receptor for the downstream proinflammatory complement activation product C5a decreases phospho-Tau levels and neuronal and synapse loss in tauopathy models (10, 34, 90). An unbiased proteomic analysis identified C1q as one of the most highly increased proteins at excitatory synapses of TauP301S transgenic mice (29). Accumulation of synaptic C1q correlated with phospho-Tau infiltration into synapses and occurred prior to frank neurodegeneration, suggesting that, similar to its role in amyloidosis, C1q acts downstream of Tau pathology and mediates synapse loss. Accordingly, a C1q-blocking antibody reduced microglial synapse engulfment and rescued synapse density in hippocampi in TauP301S mice (29). Synaptic C1q levels were higher in TauP301S mice than in PS2APP amyloidosis model mice, arguing that Tau pathology is a stronger inducer of complement activation in AD (29). In support of this idea, C1q is highly increased in synaptic fractions of AD patients, correlating with the degree of Tau pathology (29). Thus, similar to microglia, complement has homeostatic functions or damaging activities depending on the context and disease state.

Complement receptor CR1 is a GWAS hit for AD, providing some genetic evidence for complement involvement in AD pathogenesis. Additionally, APOE can inhibit the classical complement cascade, which may partially explain contributions of *APOE* variants to AD risk (160). Intriguingly, an increased copy number (and expression) of complement factor C4A is a significant risk factor for schizophrenia (125), underscoring the possible impact of overactive complement on other diseases of synaptic and cognitive dysfunction, including autism and epilepsy (26, 156). Besides AD, aberrant complement activation might cause synapse loss and neuronal damage in multiple neurodegenerative diseases, including progranulin-deficient frontotemporal dementia (92), interferon- or virus-induced memory impairment (146), and glaucoma (139), all of which show activation of microglia and complement early in disease course.

CONCLUSION

Changes in microglial morphology and activation status are characteristic of neurodegenerative diseases. Human genetics studies indicate that microglia exercise substantial influence over the risk of CNS disorders, particularly LOAD. Although much remains to be learned, on the basis of our understanding of microglial function and genetic drivers, we propose a multifactorial and multiphase model of microglial contributions to AD pathogenesis. Preceding and in the early stages of LOAD when amyloid deposits are beginning to form, microglia are a primary cellular force combating amyloid accumulation through clearance and lysosomal degradation of Aβ aggregates. Later, as amyloid plaques are deposited and grow, microglia operate at plaque borders to compact plaque and to shield neighboring neurons from toxic influences (such as oligomeric Aβ) emanating from plaques. Concomitantly, microglia may paradoxically contribute to plaque formation and the spread of amyloid and Tau pathology via secretion of APOE, microvesicles, seeding complexes, or a combination thereof. With aging, deterioration of microglial capacity to handle and dispose of Aβ may shift the balance toward a pathological net effect. In later disease stages, altered activation status of microglia may cause harm through secretion of neurotoxic factors and reactivation of developmental programs such as complement-mediated synaptic pruning.

Given the range and importance of microglial contributions to, and their great plasticity in the face of, brain pathology, microglia represent an attractive node for therapeutic interventions in neurodegenerative diseases. Macrophages have been clinically targeted in the context of cancer, autoimmunity, and infection; similar strategies may prove beneficial in the context of neurodegeneration, but it is also worth considering the specialized functions of microglia and the unique demands of the CNS environment. Although general similarities among microglial responses across neurodegeneration models are beginning to emerge, specific nuances of microglial responses in different diseases and at different disease stages may also provide valuable insights. Many unexpected roles for microglia in health and disease have been uncovered in recent years, and many more are likely waiting to be discovered.

SUMMARY POINTS

1. Human genetics studies highlight microglia-enriched pathways as important for Alzheimer disease (AD) risk.
2. Microglial immunoreceptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based activation motif (ITAM) signaling, endocytosis, migration, and phagocytosis likely participate in AD.
3. Environmental and developmental cues trigger microglial specialization, which is important for central nervous system (CNS) homeostasis.
4. Microglia exhibit multiple activation states reflected by expression of distinct gene modules.
5. Common microglial activation signatures have been observed in varied models of neurodegeneration.
6. Multiple microglial functions participate in neurodegenerative disease, some beneficial and some detrimental.
7. Microglial contributions may evolve with aging and disease stage.
8. Some microglial disease processes are reminiscent of developmental events.

FUTURE ISSUES

1. Genome-wide association studies of diverse populations and rare variant analyses should identify additional genetic links to neurodegeneration.
2. Continuing studies will deepen mechanistic insights into the relationships among risk genes and microglial functions.
3. Detailed characterization of microglial changes in human aging and in neurodegenerative disease will be imperative for justifying microglia-targeted treatments.
4. Identification of molecular drivers of microglial gene expression modules will enable experimental manipulations to determine the functional impact of specific gene sets.
5. Understanding stage-specific contributions from microglia throughout the disease course of different disorders will be essential for determining appropriate therapeutic strategies.

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