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REVIEW

The Evolving Biology of Microglia in Alzheimer's Disease

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Abstract Alzheimer's disease (AD) is typified by a robust microglial-mediated inflammatory response within the brain. Indeed, microglial accumulation around plaques in AD is one of the classical hallmarks of the disease pathology. Although microglia have the capacity to remove β-amyloid deposits and alleviate disease pathology, they fail to do so. Instead, they become chronically activated and promote inflammation-mediated impairment of cognition and cytotoxicity. However, if microglial function could be altered to engage their phagocytic response, promote their tissue maintenance functions, and prevent release of factors that promote tissue damage, this could provide therapeutic benefit. This review is focused on the current knowledge of microglial homeostatic mechanisms in AD, and mechanisms involved in the regulation of microglial phenotype in this context.

Keywords Alzheimer's disease · Microglia · Inflammation · Phagocytosis · Abeta

Microglia are Important for Normal Brain Function

Microglia account for approximately 5–12 % of the total number of cells in the brain, depending on the anatomic region [1]. While microglial origins have historically been controversial, Alliot et al. [2] and Ginhoux et al. [3] demonstrated that microglia are not derived from progenitors in the bone marrow—a surprising finding as it was thought that these cells had the same embryonic origins as other mononuclear phagocytes. Rather, they originate from primitive hematopoetic precursors in the yolk sac. These cells enter the central nervous system (CNS) over a brief interval around E9.5, and, at least under homeostatic conditions, are primarily self-renewing throughout the life of the organism.

Microglia also have unique functions in the maintenance of CNS homeostasis. Microglia in a healthy CNS are uniformly distributed throughout the brain where they display a ramified morphology characterized by long, thin, and elaborate processes, and a small cell soma. Although these microglia were often described as being in a resting or quiescent state, key studies using 2-photon imaging [4, 5] showed that microglia constantly survey the brain parenchyma by constantly extending and retracting their processes within a circumscribed domain of about 80 μm^3, and are more properly termed "surveillant". Importantly, these studies also demonstrated the ability of microglia to make contacts with other neighboring cells, such as astrocytes, neurons, and vascular endothelial cells. These contacts allow microglia to monitor synaptic function, which has been shown to be important for complement-mediated synaptic pruning in both development and following learning [6]. This constant surveillance also allows microglia to respond rapidly to injury. Microglia migrate to sites of injury in a manner dependent on adenosine triphosphate and initiate tissue repair functions [5, 7], including the phagocytic removal of dead cells and debris. They also directly interact with neurons in these settings, and have been reported to prune presynaptic boutons following ischemia [8].

Microglia in the Alzheimer's Disease Brain

The recognition of the significance of the microglial response in the Alzheimer's disease (AD) brain is owing largely to early studies, which showed accumulation of microglia around

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amyloid plaques and initial success using nonsteroidal anti-inflammatory drugs to modulate AD pathology (reviewed by McGeer and Rogers [9]). Since these landmark studies, our understanding of microglial biology in the context of AD has advanced dramatically. However, we are still far from understanding the finely tuned responses of these cells in the diseased brain.

Microglia accumulate in the vicinity of dense-core plaques and a few are also found surrounding diffuse β-amyloid (Aβ) deposits [10, 11]. Microglia are converted into a proinflammatory, "activated" phenotype by exposure to extracellular soluble and fibrillar Aβ [12], and through direct interactions with neurons that exhibit accumulation of intraneuronal Aβ [13]. Once recruited to plaques [14, 15], microglia undergo morphologic changes, such as increased soma size and thickened processes that they invest into the plaques [15]. These changes are accompanied by production of various inflammatory mediators and changes in cell surface receptor expression.

The role of microglia in the progression of AD is controversial. It is generally appreciated that patients with AD have increased levels of inflammatory cytokines [16]. Moreover, it has been argued that the microglia-derived cytokines are responsible for the neuronal death observed in the AD brain, though this linkage remains both unproven and controversial. Excessive production of proinflammatory mediators also increases Aβ levels. Proinflammatory cytokines can increase Aβ accumulation by promoting secretion of amyloid precursor protein (APP) and stimulating the amyloidogenic cleavage of APP by γ- and β-secretase in astrocytes [17–19]. In addition, increased inflammation lowers the levels of Aβ-degrading enzymes, such as neprilysin and insulin-degrading enzyme [20], the expression of Aβ-binding receptors [21], and the phagocytic capacity of microglia [22, 23], thus reducing Aβ clearance. Together, these phenomena offer potential explanations as to why microglia fail to prevent the accumulation of Aβ in the diseased brain.

While the relative contribution of these different factors is still unknown, it is indisputable that microglia in the AD brain are unable to remove effectively fibrillar forms of amyloid from the brain, resulting in the progressive accumulation of extracellular, deposited forms of amyloid in the brain parenchyma. It is enigmatic that despite their inherent capacity to eliminate foreign material, they fail to clear amyloid from the AD brain. This is not due to a generalized impairment of the phagocytic response, as microglia in the AD brain can readily phagocytose cargo that has been opsonized by antibodies, including amyloid [24, 25].

Microglial Phenotypes

Modulation of microglial activation to promote phagocytosis and limit proinflammatory cytokine release has been under intensive investigation as a potential treatment strategy to combat AD. Microglial activation states have been classically described as being either classically activated (M1) or alternatively activated (M2). These activation states have been characterized based on the cytokine secretion profile or alterations of cellular gene expression. Typically, M1-polarized microglia secrete an array of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-12, IL-18, nitric oxide, and prostaglandins, and are relatively poor phagocytes. Conversely, M2-polarized microglia secrete anti-inflammatory cytokines, such as IL-4, IL-10, IL-13, and transforming growth factor-β, are highly potent phagocytes, and upregulate their expression of arginase 1, Fizz1, and Ym-1. However, this current system for categorizing microglial phenotype arises from studies of peripheral macrophages, which fail to recapitulate fully microglial functions and is an oversimplification. Microglia do not conform to these binary phenotypes, but rather assume a broad range of functional roles that constantly change in response to the microenvironment, as reviewed by Gertig and Hanisch [26].

Microglial Recruitment to Plaques

When microglia encounter tissue damage or pathogens during their constant surveillance of the brain parenchyma, they are attracted to and undergo directed migration toward those sites of the damage. Aβ itself can attract microglia, and this response is likely to reflect the interaction of Aβ with both microglia and astrocytes, which are then stimulated to secrete chemokines [27, 28]. The C-C chemokine receptor type (CCR) 2–chemokine (C-C motif) ligand (CCL) 2 pathway has been shown to be especially important in mediating microglial recruitment to the plaques. Levels of CCL2 are increased in transgenic mouse models of AD and in the human AD brain [29–32]. Likewise, microglia in CCR2 knockout mice show impaired migration towards the site of Aβ plaques and have higher Aβ levels in the brain [33, 34]. Once attracted to the plaque, microglia secrete chemokines, including chemokine (C-X-C motif) ligand 2, CCL3, chemokine (C-X-C motif) ligand 8, and CCL5, resulting in further microglial recruitment. Plaque-associated microglia also release other cytokines, such as IL-6, TNF, macrophage inflammatory peptide-1α, and macrophage colony-stimulating factor [34], which can also serve as chemoattractants [28, 32, 33].

Receptors Involved in Microglia Aβ Recognition

Microglia detect fibrillar forms of Aβ though a variety of cell surface pattern recognition receptors, including class A1 scavenger receptors (SCARA1) [35], class B2 scavenger receptors, CD36 [36, 37], and Toll-like receptors (TLRs) 2, 4, and

6, and their co-receptor CD14. The role of various microglial scavenger receptors in AD has been thoroughly reviewed in Wilkinson and El Khoury [38]. These receptors act as ensembles to bind fibrillar forms of amyloid and to initiate downstream signaling [38]. In addition, intracellular receptors, such as nucleotide-binding oligomerization domain-like receptors (NLRPs) [39], interact with Aβ, although this has been less well studied.

Stimulation of these microglial receptors by Aβ results in activation of multiple parallel signal transduction cascades that ultimately stimulate the nuclear factor kappa B (NF-κB)-dependent transcription of proinflammatory cytokines, production of reactive oxygen species , and phagocytosis [40–45]. These responses can be both beneficial and detrimental in the context of AD. Pharmacological upregulation of SCARA1 in monocytes increases their clearance of Aβ [35]. However, SCARA1 also mediates proinflammatory cytokine production, which can have deleterious effects. Similarly, TLRs promote proinflammatory signaling through myeloid differentiation primary response gene 88 or Toll–interleukin receptor-domain-containing adapter-inducing interferon (IFN)-β-dependent pathways [46]. Deletion of the microglial TLR2/4 co-receptor CD14 reduces the inflammation and amyloid pathology in a transgenic mouse model of AD [47]. Likewise, disruption of TLR signaling by knockout of a common downstream signaling element, IL receptor-associated kinase 4, leads to decreased levels of brain Aβ and attenuated microglial reactivity [48]. However, others have shown that mice lacking functional TLR4 have increased microglial activation and brain Aβ levels [49]. Another pathway for proinflammatory microglial activation is the Aβ-dependent activation of the inflammasome through NLRP. Microglia in APP/presenilin 1 (PS1) mice deficient in NLRP or caspase-1 are more phagocytic [40]. Genetic deletion of CD36 or antibodies directed to CD36 have been shown to dampen the Aβ-induced production of proinflammatory mediators [36, 41], although CD36 has been shown to mediate microglial Aβ phagocytosis and an increase in CD36 has been linked to clearance of Aβ from AD mouse brain [50, 51]. Together, these Aβ recognition receptors can have dual roles in the disease process, promoting phagocytosis on the one hand, but enhancing inflammation on the other. Because of these dual roles, it is not surprising that the effect of modulating the expression or activation of these receptors has a complex effect on overall amyloid pathology.

Regulation of Microglial Phenotype by Nuclear Receptors

Nuclear receptors are a class of ligand-activated transcription factors that act to regulate a wide array of cellular responses. Type I nuclear receptors include estrogen and progesterone receptors. The predominant type II receptors are peroxisome proliferator-activated receptors (PPARs) γ, δ, and α, and liver X receptors (LXR) α and β. PPARs function as lipid sensors and are involved in fatty acid metabolism by binding to dietary lipids and their metabolites, while LXRs act as cholesterol sensors. The type II nuclear receptors form obligate heterodimers with retinoid X receptor to form a functional transcription factor.

In addition to their canonical metabolic roles, PPARs have also been shown to act as "master regulators" of microglial activation. PPARγ expression is required for conversion of macrophages into an anti-inflammatory, prophagocytic phenotype. Arginase 1, a regulator of arginine metabolism which is also commonly associated with an anti-inflammatory microglial phenotype, is a direct target for PPARγ [52, 53]. In turn, the prototypic anti-inflammatory cytokine IL-4 induces PPARγ expression through a signal transducer and activator of transcription 6 signaling pathway [54]. In the context of AD, the activation of PPARγ has been shown to promote clearance of Aβ by "licensing" microglia as competent phagocytes, in part by upregulating CD36 [54]. Consequently, activation of PPARγ leads to rapid reductions in the levels of Aβ in mouse models of AD [51, 55–57].

Activation of PPARδ has similar influences on microglial reactivity. When macrophages engulf apoptotic cells, the catabolism of membrane lipids activates PPARδ [58]. Similar to PPARγ [52], genetic inactivation of PPARδ [58] and LXRs [59] shift macrophage phenotype towards a proinflammatory state and block their ability to convert into an anti-inflammatory, prophagocytic phenotype. This occurs, in part, through PPAR-mediated transactivation of genes with anti-inflammatory properties, such as IL-10 and TGF-β. PPARs and LXRs can also promote repression of proinflammatory genes. A subpopulation of PPARs and LXRs can be sumoylated, which enables their interaction with NF-κB and activator protein 1 (AP1). This interaction leads to stabilization of the nuclear receptor corepressor 1/histone deacetylase 3 complex with NF-κB preventing the transcription of NF-κB-regulated proinflammatory genes [60]. As a result, genetic deletion of PPARδ leads to reduced expression of anti-inflammatory cytokines and impaired phagocytosis. Conversely, activation of PPARδ leads to a rapid reduction in brain Aβ deposits in a transgenic mouse model of AD [61].

Microglial Phagocytosis and Other Endocytic Mechanisms

Microglia use distinct mechanisms to clear different Aβ species, including phagocytosis, autophagy, and pinocytosis. Impairment of these pathways can result in reduced Aβ clearance in AD [62, 63] (Fig. 1).

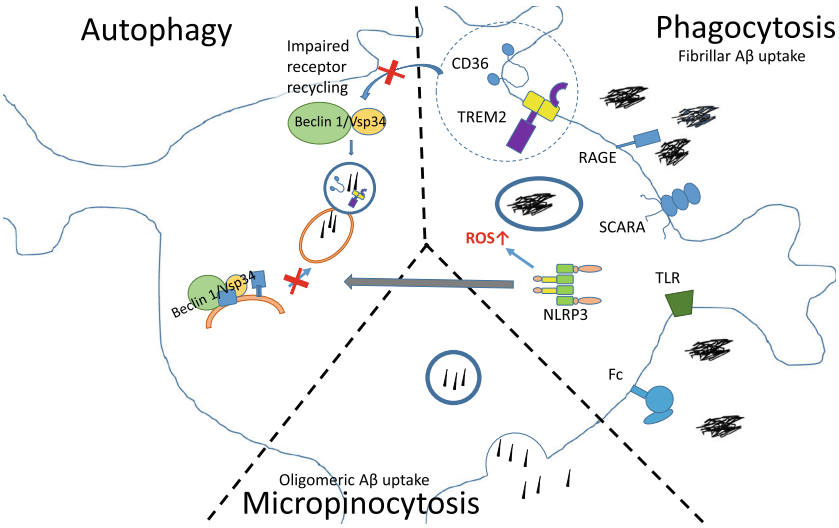

Fig. 1 Multiple pathways for microglial uptake of β-amyloid (Aβ) are impaired in Alzheimer's disease (AD). Microglia clear Aβ by phagocytosis, micropinocytosis, and autophagy. The figure indicates different receptors capable of recognizing Aβ. In AD the level of these receptors is downregulated, leading to reduced Aβ uptake. Reduced levels of beclin 1 lead to impairment in autophagic processes. As beclin 1 also regulates the receptor recycling of CD36 and Trem2, and may promote phagocytic clearance, reduced levels of beclin 1 may also cause defects in phagocytosis. However, inflammasome activation leads to increased formation of reactive oxygen species (ROS), and may also impair autophagy through interaction with beclin 1. NLRP3 = nucleotide-binding oligomerization domain-like receptor 3; SCARA = class A1 scavenger receptor, TLR = Toll-like receptor; RAGE = receptor for advanced glycation endproducts; Fc = Fc receptor

Phagocytosis

Phagocytosis is one of the key mechanisms for microglia-mediated fibrillar and oligomeric Aβ removal [22, 41, 50, 64–67]. Large particles like fAβ are taken up by phagocytosis through engagement of innate immune surface receptors and activation of the cell's phagocytic machinery. After internalization, the phagosomes containing Aβ are trafficked to lysosomal compartments and degraded by proteases such as cathepsin B [66]. It is of particular interest that microglial phagocytosis is reliant upon beclin 1, a protein whose actions were thought to be restricted to autophagy. The beclin 1/Vsp34 complex has been shown to be required for the clearance of apoptotic bodies by macrophages and fibroblasts, and beclin 1-deficient cells are unable to do so [68]. Beclin 1 might promote phagocytic clearance by regulating CD36 and Trem2 expression, as beclin 1 deficiency impairs recycling of these receptors [69]. Lucin et al. [69] have recently reported that beclin 1 levels are reduced in microglia in the AD brain, consistent with the inability of these cells to phagocytose efficiently amyloid deposits.

Autophagy

Autophagy is an ancient process important for the catabolism of dysfunctional organelles and response to starvation [70]. The principal form of autophagy relevant to neurodegenerative diseases is *macroautophagy* [71], a process in which molecules are isolated in bulk by autophagosomes. One of the most important mediators of autophagy is the beclin 1/Vsp34 complex. Beclin 1 is an adaptor molecule that stimulates autophagy through promoting vesicle nucleation [72]. Autophagy can be induced by TLR4-mediated activation of myeloid differentiation primary response gene 88 and Toll–interleukin receptor-domain-containing adapter-inducing IFN-β [73, 74]. These proteins activate TNF receptor associated factor 6, which induces ubiquitination of beclin 1, resulting in stimulation of autophagy. However, inflammasome-associated receptors NLRP3, NLRC4, and NLRP4 bind to beclin 1 and reduce autophagy [75]. Several lines of evidence indicate that autophagic processes are impaired in AD, including the fact that beclin 1 levels are reduced in AD brains. Overexpression of beclin 1 reduces APP levels and leads to reduced intracellular and extracellular Aβ accumulation in AD mice [63, 76]. These findings link receptor mediated phagocytosis and autophagy, both of which are impaired in AD.

Macropinocytosis

Pinocytosis is a mechanism for removal of soluble molecules from the extracellular space, and can occur via 2 pathways, micropinocytosis and macropinocytosis. Micropinocytic vesicles are <0.1 μm in diameter and are typically associated with caveolin. Micropinocytosis does not require actin and occurs within cholesterol-rich lipid domains of the plasma membrane [77]. In contrast, *macropinocytosis* occurs when membrane ruffles are formed and closed in a process requiring both actin and

tubulin [78]. *In vitro* studies have shown that microglial uptake of soluble Aβ is not dependent on the cell surface receptors mediating the uptake of fAβ such as scavenger receptors A and B, CD36, and CD47, and nor does it require the endocytic receptor low-density lipoprotein receptor-related protein-1 [79]. Instead, soluble Aβ is internalized through nonsaturable, fluid-phase macropinocytosis requiring microtubules and actin, and is degraded through the endolytic pathway. Thus, macropinocytosis is a mechanism selective for uptake of soluble and oligomeric Aβ.

The Effect of Microglial Depletion on Plaques

While subtle alterations in microglial phenotype can have an impact on amyloid pathology, recent studies have used techniques to deplete microglia from the brain to determine the impact of total loss of microglia on AD pathology. Grathwohl et al. [80] demonstrated depleted microglia from APP transgenic mice expressing thymidine kinase of herpex simplex virus under the CD11b promoter (CD11b–HSVTK). In this model microglia could be selectively eliminated on administration of the drug gangciclovir. Surprisingly, microglial depletion had very little effect on plaque formation, plaque maintenance, or neuritic dystrophy [80]. Interestingly, depletion of microglia from the brains of CD11b–HSVTK transgenic mice created a niche for the subsequent infiltration of monocytic cells from the bloodstream [81]. There are caveats to using this genetic model, including myelotoxicity and off-target effects of ganciclovir on microglial phenotype [80, 82]. Work by Parkhurst et al. [83] took a different approach to depletion of microglia from the brain using a microglial-targeted diphtheria toxin receptor. This study showed that depletion of microglia induced multiple deficits in learning and behavior, and caused a significant reduction in motor learning-dependent synapse formation [83]. This effect was largely due to lack of microglial brain-derived neurotrophic factor signaling as the authors demonstrated that deletion of brain-derived neurotrophic factor specifically in microglia abrogated similar reductions in the levels of GluN2B and VGluT1, as well as impairments in fear conditioning [83]. An important new study has recently demonstrated that microglia can be eliminated from the brain using an orally delivered colony-stimulating factor 1 receptor antagonist [84]. This method for eliminating the microglia did not affect the cognitive functions of the mice. The ability to eliminate these cells easily will allow analysis of the roles of microglia in a number of experimental disease models. The effect of microglial depletion on AD-related behavioral impairments and the role of their blood-derived counterparts remains to be determined.

Effect of Microglial Activation on Neuronal Function in AD

The effect of microglia on neurons in the context of AD pathology has been described as detrimental owing to the microglial release of cytotoxic molecules. Elimination of microglia from mixed cultures makes neurons more resistant to the toxic insults elicited by Aβ *in vitro* [85]. The toxic role of microglia activation on neurons is supported by studies using AD mouse models that exhibit direct neuronal loss. In one such study using a model of P301S tauopathy, Yoshiyama et al. [86] reported that microglial activation and synapse loss occur before the appearance of tangles and neuron loss. One mechanism underlying the detrimental effect of microglial activation is the induction of cell cycle events in neurons via TNF-α. These cell cycle events are toxic to terminally differentiated neurons [87].

However, the study showing that depletion of microglia does not alter neuritic dystrophy makes it difficult to draw definite conclusions [80]. Indeed, microglial activation may be causing neuronal death and microglia may get activated in response to compromised neuronal function. Most likely, both phenomena exist at the same time, and the vicious inflammatory cycle created by the AD environment contributes to neuronal loss. Nevertheless, proper communication between neurons and microglia, which is essential for normal brain function, is disrupted in AD.

Regulation of Microglial Phenotype by Neurons

The interaction between microglia and other cell types is essential for their phenotypic regulation. Neurons regulate microglial activation through interaction of chemokine (C-X3-C motif) ligand 1 (CX3CL1) with its receptor CX3CR1, and CD200 with CD200R. Carbohydrates on the neuronal glycocalyx also interact with various siglec receptors on microglia, including the identified AD-associated risk factor CD33. While it does not yet have a definitive ligand, the AD-associated receptor Trem2 also likely regulates microglial function through contact with cell surface components on other CNS cell types. Together, these interactions are crucially important determinants of microglial phenotype. Not surprisingly, modifying this cell–cell communication has important implications for AD pathology.

CX3CR1/CX3CL1

CX3CR1 is a chemokine receptor, expressed exclusively on myeloid cells, that interacts with CX3CL1 (also known as fracktalkine), expressed primarily by neurons [88, 89]. The expression pattern led researchers to posit that the interaction of CX3CL1/R1 played an important role in communication

between neurons and microglia [90]. CX3CL1 exists in a membrane-bound form, which acts to promote cell adhesion [91], and a soluble form, which mediates microglial chemoattraction [92]. Hatori et al. [93] also demonstrated that exogenenously applied CX3CL1 could increase microglia proliferation, suggesting that CX3CL1/R1 signaling could also play a role in neuronal control of microglial abundance. However, perhaps the most dramatic effect of CX3CL1/R1 signaling is the inhibition of microglial inflammatory responses. Cardona et al. [94] showed that deficiency in CX3CR1 caused increased microglial activation and neurotoxicity in multiple neuroinflammatory contexts.

Because CX3CL1/R1 signaling was demonstrated to modify potently microglial phenotypes, Lee et al. [95] examined the effect of knocking out CX3CR1 in APP/PS1 and R1.40 models of AD. As expected from previous findings, CX3CR1 deficiency increased levels of the proinflammatory cytokines TNF-α, IL-1β and monocyte chemotactic protein 1 (also known as CCL2). Unexpectedly, however, this resulted in reduced amyloid pathology. These findings were confirmed in additional AD mouse models [96, 97]. Using *in vivo* 2-photon imaging, Fuhrmann et al. [98] showed that in the 5XFAD model of AD, homozygous loss of CX3CR1 impaired microglia recruitment to areas of neuronal death. This resulted in reduced neurodegeneration, again improving pathology. However, in the hTau model of AD, while CX3CR1 deficiency still enhanced inflammation, it also promoted increased levels of phosphorylated and insoluble tau species [99]. CX3CR1-deficient AD mice also had impaired performance in spatial learning tasks [96, 99]. Thus, CX3CR1/L1 signaling shifts microglia away from a pro-inflammatory phenotype, but this has disparate effects on different aspects of AD pathology—reducing amyloid pathology on the one hand, while enhancing tau pathology on the other.

CD200/CD200R

The interaction of CD200 and CD200R is also an important mediator of neuronal regulation of microglial function. In the CNS, CD200 is expressed primarily by neurons, but can, in some inflammatory contexts, also be unregulated on reactive astrocytes [100]. This ligand interacts with the myeloid-cell specific CD200R. Hatherley et al. [101] used targeted mutations to examine the nature of CD200/200R interactions, and showed that the interaction of the ligand receptor pair results in a distance between cells similar to that observed in the immunological synapse. This suggests that CD200/200R binding could facilitate other contact-dependent signaling. Whether by this mechanism or direct signaling through the immunoreceptor tyrosine-based inhibitory motifs on the intracellular domain of CD200R, CD200/200R signaling largely results in downregulation of microglial activation. In CD200-deficient mice, microglia express uncharacteristically high levels of the activation markers CD11b and CD45 [102]. The effect of CD200/200R signaling has been well studied in experimental autoimmune encephalomyelitis (EAE) where blocking the CD200 receptor using a monoclonal antibody increases CNS levels of the proinflammatory cytokines IL-6 and IFN-γ [103], and inducible nitric oxide synthase [102], resulting in worse clinical scores [104]. Conversely, overexpression of CD200 is protective against EAE induction [105].

Following publication of these findings, researchers began to explore a possible role for CD200/200R signaling in AD. *In vitro*, the presence of neurons in a microglial culture reduces their inflammatory response to Aβ. However, when CD200/200R signaling is blocked with a CD200 antibody, the presence of neurons no longer attenuates Aβ-induced microglial activation [106]. These findings suggest that, as in EAE, CD200/200R signaling might be neuroprotective in AD. However, CD200 expression is downregulated in rats following intraventricular injection of Aβ [106], and both CD200 and CD200R levels are reduced in patients with AD, particularly in parts of the brain that exhibit the highest plaque burden [107]. Thus, downregulation of CD200/200R signaling may provide a permissive environment for development of AD pathology. Possible mechanisms for Aβ-induced downregulation of CD200 and CD200R have recently been explored. Dentesano et al. [108] found that following the administration of lipopolysaccharide (LPS), CEBPB CCAAT/enhancer binding protein-β and histone deacetylase 1 bind to the CD200R promoter, resulting in reduced CD200R expression. They later demonstrated that PPARγ also binds upstream of the CD200R and CD200 genes [109], and that activation of PPARγ prevents the downregulation of CD200R following exposure to inflammatory mediators. In culture, this translated to enhanced neuroprotection in mixed microglial/neuronal cultures following LPS treatment. While these findings need to be confirmed in the context of AD, these findings suggest potential therapeutic strategies for preventing or reversing the AD-related reduction in CD200/200R signaling.

Siglecs/Sialic Acids

Siglecs (sialic acid binding immunoglobulin-like lectins) are a subset of lectins that specifically recognize carbohydrates modified with sialic acid residues. The CNS contains high levels of sialic acids, which densely populate the neuronal glycocalyx [110]. During homeostatic conditions, sialic acids on the neuronal cell surface signal that the neuron is healthy and consequently inhibit microglial activation [111].

In addition to their role in homeostasis, siglecs also have been shown to play an important role in neurodegenerative diseases. While there are numerous siglecs involved in the phenotypic regulation of myeloid cells specifically in AD [112, 113], the siglec CD33 has been the best characterized. Upon binding its sialic acid ligand [114], CD33 was shown to

promote downregulation of myeloid cell activation in the periphery [115]. CD33 function in the CNS, however, was not well studied until a series of genome-wide association studies identified CD33 variants that modified risk for developing AD [116–118]. It was later shown that CD33 expression levels correlate with cognitive decline in AD patients [119], and that the protective CD33 variant reduces CD33 expression [120]. Taken together, these data suggest that CD33 promotes AD pathogenesis. Consistent with this interpretation, Griciuc et al. [120] showed that upregulation of CD33 *in vitro* results in reduced Aβ phagocytosis, while microglial cultured from $CD33^{-/-}$ mice exhibit enhanced Aβ uptake. This was reflected in reduced amyloid pathology in CD33-deficient APP/PS1 mice. Bradshaw et al. [121] reported similar results in monocytes cultured from patients with AD with a variant of CD33 that increases risk for developing the disease. Monocytes from patients with the CD33 variant had reduced capacity for Aβ phagocytosis, and these patients had increased amyloid accumulation in positron emission tomography imaging studies compared with control patients. There are still many aspects of CD33 biology that remain to be explored, including determining a more thorough phenotypic profile of microglia and monocytes expressing the different CD33 variants. Additionally, it will be important to assess the effect of CD33 variants in the context of tau pathology. As studies in CX3CR1-deficient mice illustrated, modulation of microglial phenotype can have divergent impact on these 2 pathological hallmarks.

Trem2/Trem2 Ligand

Trem2 is a single-pass membrane protein expressed in the brain exclusively by microglia, but is also found on peripheral myeloid cells. Studies have yet to identify definitively the ligand of Trem2 and the cell type on which it is expressed. Stefano et al. [122] used a Trem2–Fc construct to identify heat shock protein 60 (Hsp60) as potential ligand present on neuroblastoma cells. While Hsp60 is normally located in the mitochondria, it can be expressed on the cell surface during cell stress [123], potentially making it accessible to Trem2 in the context of neurodegeneration. In this study, however, it took a very high concentration of Hsp60 to activate Trem2, even on N9 cells, which express unusually high levels of the Trem2 receptor. So, whether a functional interaction occurs between Trem2 and Hsp60 *in vivo* remains to be determined. Other groups have identified additional potential Trem2 ligands, including anionic residues on bacteria and lipid molecules [124, 125], as well as undefined species that are abundantly expressed on apoptotic neurons.

While the identity of its physiological ligand is not yet clear, studies have used Trem2 cross-linking antibodies or Trem2 fusion constructs to study downstream Trem2 signaling pathways and functions. Trem2 requires the intracellular signaling adaptor DNAX activation protein of 12 kDa (DAP12) [126], which, when phosphorylated by the tyrosine kinase src, provides binding sites for the SH2 domain-containing proteins Syk, phosphoinositide 3-kinase, and DAP10 (reviewed in [127]). Alternatively, SH2-containing inositol-5'-phosphatase 1 can bind to these same sites on DAP12 and prevent downstream signaling through these other pathways [128]. What causes DAP12 to interact with activating or inhibitory components is not yet known. It has been posited to be ligand-specific or to result from the integration of intracellular signals from other activated immune regulatory pathways, though this remains to be further explored.

Trem2 signaling through these pathways results in regulation of a diverse array of myeloid cell functions. Phagocytosis is the best characterized downstream function of Trem2 receptor activation. Melchior et al. [129] showed that the level of Trem2 expression in cells of the BV2 rat microglial cell line correlated with their phagocytic capacity. Conversely, knocking down Trem2 in primary microglia inhibits phagocytosis of apoptotic neurons [126]. N'Diaye et al. [130] transfected a Trem2–DAP12 fusion construct into normally nonphagocytic Chinese hamster ovary cells and found that this was sufficient to induce phagocytic uptake of *Escherichia coli* bioparticles. Trem2 activation has also been shown to promote anti-inflammatory cytokine production, reduce pro-inflammatory cytokine production [131], and enhance chemotaxis via upregulation of CCR7 in various immune cell types [132]. While these *in vitro* studies have determined some of the signaling components involved and possible functional outcomes of Trem2 signaling, these results need to be confirmed and expanded in an *in vivo* context.

A role for Trem2 in AD was first suggested by data that showed that Trem2 was upregulated in plaque-associated myeloid cells in AD mouse models [129, 133]. However, the recent identification of Trem2 variants that confer a high risk for developing AD in the human population has greatly accelerated research in this area [134, 135]. The R47H variant of Trem2 confers a 3-fold increase in risk for AD [132, 133], comparable to the risk conferred by the apolipoprotein E4 allele. Trem2 mutations are responsible for Nasu-Hakola disease [136]. These findings suggest that Trem2 may play a fundamental role in disease etiology. Since these studies were published, additional groups have identified Trem2 variants as genetic risk factors for frontotemporal dementia [137], Parkinson's disease [138], and amyotrophic lateral sclerosis [139]. This common genetic linkage suggests a more general role for Trem2 in modulating neurodegenerative disease pathology.

The human genetic data have instigated a number of studies to examine possible mechanisms by which these Trem2 variants confer increased risk for AD. Because a missense mutation is among the Trem2 variants identified as increasing AD risk, it has been posited that these Trem2 variants cause loss of Trem2 function [140]. While this still needs to be

explored experimentally, the studies published thus far focus on Trem2 deficiency in AD models. Jiang et al. [141] induced overexpression of Trem2 by intraventicular lentrivral injection in APPswe/PS1de9 mice. As expected, overexpressing Trem2 resulted in reduced levels of the proinflammatory cytokines TNF-α, IL-1β, IL-6, and inducible nitric oxide synthase; reduced amyloid pathology; and improved behavior. However, Ulrich et al. [142] showed trends but no significant changes in inflammatory markers or amyloid pathology in APPPS1;Trem2$^{+/-}$ mice. Jay et al. showed opposing data, namely that APPPS1;Trem2$^{-/-}$ mice exhibit reduced levels of proinflammatory cytokines, increased levels of anti-inflammatory cytokines, and ameliorated amyloid and tau pathology (unpublished data). These studies might have divergent results owing to alterations in the role of Trem2 throughout the progression of AD pathology, differences in acute *versus* chronic changes in Trem2 levels, or because of differences in the specific cell types targeted in these various studies. Others have examined changes in Trem2-deficient mice in the context of stroke and shown increased inflammation in Trem2$^{-/-}$ mice [143], whereas blocking Trem2 using a monoclonal antibody in the context of EAE blocked infiltration of peripheral immune cells and ameliorated pathology [144]. This provides additional evidence that the role of Trem2 might be context dependent. As these issues continue to be explored, the biology of Trem2 may have to be redefined to fit with these new *in vivo* findings.

Infiltration of Peripheral Monocytes into the AD Brain

It is generally accepted that microglia can become dysfunctional during aging and disease [145, 146]. In order to compensate for disease-related dysfunction or to respond to injury or pathogens, microglial density can increase dramatically in these contexts [33]. However, it is controversial as to how changes in microglial number in these settings is achieved. A number of studies suggested that peripheral monocytes might enter the CNS and differentiate into microglia, but lethal whole-body irradiation induced confounds that called these results into question [147, 148].

While the origin of microglia, particularly the expanded population evident in disease, has historically always been controversial, a series of studies in the mid-2000s suggested that brain macrophages derived from peripheral monocytes were important players in AD. Malm et al. [149] used irradiation and transplantation of bone marrow from an enhanced green fluorescent protein donor to label specifically peripheral hematopoietic cells in APP/PS1 mice. They found that some of these cells localized around Aβ deposits. This association was increased following LPS injection and resulted in reduced Aβ burden. Simard et al. [150] used the same technique and also found that peripherally derived cells were recruited to the brain in response to Aβ40 and Aβ42 species and, once extravasated, migrated to Aβ plaques. By depleting microglia using the HSVTK strategy discussed above, and allowing several months for repopulation to occur, they showed that these mice had improved amyloid pathology. The plaque-associated myeloid cells had reduced levels of the proinflammatory genes *IL1β* and *TLR2*, and actively phagocytosed Aβ, suggesting that these presumably peripherally derived cells were beneficial in this disease model. El Khoury et al. [33] showed that the trafficking of peripherally derived cells was greatly reduced in CCR2$^{-/-}$ mice. Furthermore, these mice had enhanced amyloid accumulation. These findings suggests that peripheral immune cells are responsible for the increase in myeloid cell number in AD, and play an important role in Aβ clearance. The importance of CCR2+ cells in AD was later confirmed in studies which showed that knocking out CCR2 in APP/PS1 mice reduced inflammatory markers in cells surrounding plaques, while enhancing soluble Aβ levels and resulting in worse performance on spatial memory tests [151]. Taken together, these results from chimeras and evidence from CCR2-deficient mice suggested that peripheral monocytes infiltrated into the AD brain, migrated to Aβ plaques, and modulated AD pathology and behavior.

However, the outcome of studies using bone marrow chimeras were later called into question. These studies relied on irradiation and bone marrow transplantation as a primary means to distinguish peripherally derived cells from resident microglia. Mildner et al. [147] showed that parenchymal microglia can arise from CCR2+Ly6Chi monocytes, but that this did not occur without preconditioning irradiation of chimeras. They showed that whole body irradiation was necessary to induce trafficking of peripheral cells into the CNS. These effects were long lasting, with increases in peripheral immune cell trafficking into the CNS several months after irradiation. Additionally, Ajami et al. [148] showed that bone marrow transplantation could cause aberrant cell infiltration into the CNS, even in the absence of irradiation. They posited that the presence of hematopoietic precursors in the bloodstream following bone marrow transplantation could artificially increase trafficking of peripherally derived cells into the CNS.

The controversy regarding peripheral cell infiltration in AD was echoed in studies looking at repopulation of microglia following depletion. Varvel et al. [81] depleted microglia by expressing HSVTK under the control of the CD11b promoter. They used bone marrow transplantation from a congenic wild-type mouse to prevent depletion of peripheral immune cells. They found that 2 weeks after microglial depletion, the brain was completely repopulated (2× tiling density) by CCR2+ peripheral monocytes. However, a recent study using a different depletion model showed that repopulating cells were derived from resident microglial progenitors rather than from

peripheral infiltrates [84]. It is possible that monocyte entry into the CNS is dependent on the particular experimental paradigm, but this controversy remains to be resolved.

Most of these studies suggest that, if peripheral monocytes play a role in AD, the proinflammatory CCR2+ subset of monocytes is likely important. Characteristically, $Ly6C^{hi}CX3CR1^{-}CCR2^{+}$ cells infiltrate tissue during inflammation [152], while the $Ly6C^{lo}$ CX3CR1+ $CCR2^{-}$ monocytes perform homeostatic functions [153]. The $Ly6C^{hi}$ cells downregulate Ly6C over time and change monocyte subtype in the periphery [153]. The role of CCR2 in infiltration of peripheral monocytes has been established in the genetic depletion models discussed above. Additionally, viral-mediated overexpression of its ligand CCL2 in the CNS mediates enhanced peripheral immune cell trafficking [154]. However, it remains to be seen whether the CX3CR1+ monocyte subtype could also play a role in AD pathogenesis.

A recent study provides novel evidence that microglia derived from peripheral monocytes could play a role in AD. Jay et al. (unpublished data) showed that Trem2+ cells are plaque-associated myeloid cells in human patients with AD cases and 3 AD mouse models. Using flow cytometry, they showed that these Trem2+ cells are exclusively CD45hi, a classic marker of peripherally derived macrophages in the CNS. While these cells are highly immunoreactive with CD45, they do not label with the marker P2RY12, a purinergic receptor found to be specifically expressed exclusively in microglia but not peripherally derived cell populations [155]. Interestingly, these CD45hi, plaque-associated cells are absent in APPPS1;$Trem2^{-/-}$ mice. While these data still require confirmation using additional techniques, if the Trem2+ cell population is peripherally derived, the high risk conferred by Trem2 variant mutations suggest a critical role for peripheral monocytes in AD etiology.

Conclusions

Microglial dysfunction is largely responsible for the overwhelmed inflammatory milieu in AD brain. Research has pinpointed key molecules with ability to shift microglial phenotype and induce phagocytosis. Successful engagement of these processes is likely to be of great therapeutic benefit.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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