HHS Public Access

Author manuscript

J Mol Biol. Author manuscript; available in PMC 2020 April 19.

Published in final edited form as:

J Mol Biol. 2019 April 19; 431(9): 1805–1817. doi:10.1016/j.jmb.2019.01.045.

Microglia in Alzheimer's disease: Exploring how genetics and phenotype influence risk

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Abstract

Research into the function of microglia has dramatically accelerated during the last few years, largely due to recent genetic findings implicating microglia in virtually every neurodegenerative disorder. In Alzheimer's disease, the majority of risk loci discovered through genome-wide association-studies were found in or near genes expressed most highly in microglia leading to the hypothesis that microglia play a much larger role in disease progression than previously thought. From this body of work produced in the last several years, we find that almost every function of microglia has been proposed to influence the progression of Alzheimer's disease (AD) from altered phagocytosis and synaptic pruning to cytokine secretion and changes in trophic support. By studying key Alzheimer's risk-genes such as TREM2, CD33, ABCA7, and MS4A6A, we will be able to distinguish true disease-modulatory pathways from the full range of microglial related functions. To successfully carry out these experiments, more advanced microglial models are needed. Microglia are quite sensitive to their local environment, suggesting the need to more fully recapitulate an *in vivo* environment to study this highly plastic cell type. Likely only by combining the above approaches, will the field fully elucidate the molecular pathways that regulate microglia and influence neurodegeneration, in turn uncovering potential new targets for future therapeutic development.

Keywords

Neurodegeneration; Microglia; Alzheimer's disease; neuroinflammation; genome-wide association studies

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Alzheimer's Disease and the Amyloid Cascade Hypothesis

Alzheimer's Disease (AD) is the most common form of dementia and the sixth leading cause of death in the Unites States[1]. Unlike most other causes of death, the incidence of AD continues to rise, and cases are expected to double within the next 30 years as our population ages. Basic and translational science coupled with medical advances, have greatly increased human lifespan, but with this comes increased risk of developing age-related diseases, such as AD. Thus, it is critically important to focus our research efforts on increasing the healthy years of life in older individuals.

Alzheimer's disease was first identified in 1908 by a German Neurologist, Alois Alzheimer who described patients who exhibited disorientation, confusion, and progressive memory loss. His pathological examinations further revealed brain atrophy and the accumulation of key pathologies including intraneuronal neurofibrillary tangles, extracellular plaques, and morphological changes in microglia, the primary immune cells of the brain[2]. One hundred years past this original characterization, the diagnosis of AD remains largely similar, though somewhat more precise. Clinicians look for insidious onset of amnesic presentation, difficulties finding words, impaired facial recognition, and deficits in problem solving[3]. Researchers today are still searching for validated biomarkers through neuroimaging, cerebrospinal fluid (CSF), blood, or urine tests as well as genetic risk profiling, but none have yet proved to be reliably conclusive in large-scale clinical trials.

While the majority of AD occurs 'sporadically' in aged individuals, much can be learned from the rarer familial forms of AD (fAD). fAD accounts for around 2% of all AD cases, and often occurs earlier in life with onset in the 30s or 40s. Familial Alzheimer's disease occurs due to inherited genetic mutations within the genes presenilin-1, presenilin-2, or amyloid precursor protein (APP). Each of these mutations effects the production and processing of beta-amyloid (Aβ), which is the primary component of the extracellular plaques that were first described by Alois Alzheimer. The identification and subsequent understanding of the functional effects of these mutations, led to the proposal by Hardy and Higgins in 1992 of the 'amyloid cascade hypothesis' of AD[4]. This hypothesis posits that Aβ accumulation is the initial cause of AD that in turn induces a series of downstream pathological cascades including neurofibrillary tangle formation, inflammatory responses, as well as synaptic and neuronal loss. In strong support of this hypothesis, imaging studies have now clearly shown that Aβ begins to accumulate some 10–15 years prior to diagnosis. As a response to this hypothesis and the evidence that Aβ pathology is one of the first recognizable signs of AD, many drugs have been developed to clear Aβ from the brain in an attempt to relieve the symptoms of AD and potentially halt disease progression. To date, many therapies targeting Aβ synthesis or clearance have been tested in clinical trials (bapineuzumab, solanezumab, tarenflurbil, phenserine, gammagard etc.) but unfortunately none have yet proved to be effective in reducing memory deficits or halting disease progression in late stage trials. Famously, one compound; PF-04494700 a drug licensed by Pfizer, actually caused AD patients to deteriorate faster than their placebo counterparts.

A likely issue with Aβ centered treatments may be that patients are treated too late in the disease process. Since Aβ has already been accumulating for ~10 years by the time patients

are first diagnosed with AD or mild cognitive impairment (MCI), removal of Aβ from the brain is unlikely to resolve the additional downstream consequences of AD neuropathology. In others words, once neuroinflammation, tau pathology, and neurodegeneration begin, it may make little difference in disease progression to remove the initial insult of Aβ plaques. Instead, therapies that better target these downstream processes may be far more effective at later stages of disease. Yet, Aβ therapies could still be useful if treatments can be begun during the prodromal phases of the disease. Thus, research into earlier diagnosis and accurate biomarkers remains critical.

Microglia in AD pathogenesis

As mentioned previously, signs of microglial activation in AD, as assessed by broad morphological analysis, was first described by Alois Alzheimer in 1908[2]. Since then, many groups have clearly demonstrated the close spatial-temporal relationship between Aβ plaques and activated microglia in both AD patients and mouse models (Figure 1). Several studies have also further visualized beta-amyloid itself within microglia cell bodies, suggesting an important role for microglia phagocytosis in the clearance of beta-amyloid[5]. Because microglia are preferentially activated in close proximity to Aβ plaques, many groups hypothesized that the plaques are responsible for activating microglia, further explaining the prominent hypothesis that beta-amyloid initiates the Alzheimer's disease cascade. Yet, we now have evidence that microgliosis occurs *prior* to visible Aβ plaque deposition[6]. Furthermore, recent evidence suggests that microgla may even contribute to the seeding of plaques as pharmacological depletion of microglia leads to a significant reduction in plaque pathology in 5xfAD transgenic mice[7]. The next big questions are: what process leads to this microglial activation, and what are microglia doing to promote plaque formation or to inhibit plaque clearance?

Because microglia are highly sensitive to changes in their environment, these cells have proven difficult to study. Thus far, murine models have served as the primary tool to study microglial genetics and function. While these model systems have led to important discoveries of microglial ontogeny and function, it has also become clear that there are important differences between murine microglia and human microglia which are particularly evident in aging and disease[8,9]. Thus, we must be careful not to simply conclude that findings in mouse models will necessarily translate to human microglia. In order to study human microglia, several labs have developed techniques to isolate human microglia from brain tissue removed during surgical resection of epileptic foci or brain tumors[10–12]. This approach provides one of the very few methods to study viable human brain-derived microglia, but remains logistically very challenging. Another innovative technique to overcome the difficulty of studying human microglia has been to isolate microglia or their nuclei from postmortem brain tissue. These techniques have allowed researchers to discover important human-specific changes that occur as microglia age[13]. Still, it is likely that the agonal state preceding death, co-morbid infectious or inflammatory conditions such as pneumonia, or post-mortem delay influence microglial gene expression and activation state which may may obscure and greatly complicate data interpretation. Given these complications, several groups including our own have developed protocols to differentiate human microglia from pluripotent stem cells[14–20]. Producing human microglia *in vitro*

allows scientists to study these cells using better-controlled and more mechanistic approaches including the use of drug libraries and genetic manipulation such as CRISPR.

Although a fully defined microglia differentiation protocol is extremely useful for experiments that aim to study the mechanistic functions of human microglia, microglia in isolation may function quite differently than those in the brain environment. More comprehensive models of human microglia in a brain-like environment continue to be developed and include studies that involve engrafting human iPS-derived microglia into 3D neuronal cultures, brain organoids, or murine brains[14,21,22] In order to recapitulate how human microglia react to realistically complex disease environments such as beta-amyloid plaques, neurofibrillary tangles, or traumatic brain injury, etc., a chimeric xenotransplantation system is likely to best mimic human disease and thus help narrow the focus of pre-clinical targets to ones which most accurately reflect what occurs in patients.

Genome-wide Association Studies

Some clues as to how microglia may be effecting the progression of Alzheimer's disease can be found by studying which microglia-specific gene variants cause risk for or protection from AD. In recent years, the power of genomics has allowed geneticists to uncover many single nucleotide polymorphisms (SNPs) that are correlated with differential AD risk. These studies have confirmed the previously established importance of Apolipoprotein E (APOE), while also uncovering many new risk-SNPs. SNP variants may occurr within gene coding regions, or influence disease risk through known promoters, enhancers. Additionally SNPs may be sign posts which are inherited alongside mutations which are in map linkage disequilibrium. For this review, we will only discuss SNPs which are correlated with actual changes in gene expression or protein function. Surprisingly, around two thirds of these new AD-risk SNPs are exclusively or most highly expressed in microglia. This data has been corroborated by many groups including a recent study of over 300,000 individuals that reported 48 AD-risk SNPs (FDR < 10^{-5}), 29 of which are most highly expressed by microglia (60.4%)[23,24] (Figure 2). This data hints that changes in microglial function may influence differential risk for AD, suggesting that these brain-resident immune cells play a far greater role in disease development and progression than previously thought. While this review will not cover the role of every microglial specific risk gene, we provide a broad overview of all current AD-risk SNPs in Table 1.

Of the GWAS-risk genes, SNPs within Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) are associated with the highest risk of developing AD, increasing disease risk by 2–4 fold. As the name suggests, TREM2 is exclusively expressed on cells within the myeloid lineage. Thus, in the brain, TREM2 expression is dominated by microglia. Additionally, recent comparisons of human peripheral blood monocytes and both iPSC-derived and brain-derived microglia further suggest that TREM2 expression is greatly enriched in microglia versus other monocyte lineages[10]. Several of the AD-associated SNPs occur within the Trem2 coding region, including R47H, R62H, and H157Y. R47H TREM2 mutations, in particular have been ardently studied, uncovering relationships between carriers of this variant and increased CSF biomarkers such as tau, p-tau181, and soluble Trem2 (sTrem2), each of which have been associated with worse disease progression[11–13].

Research on the function of R47H and other TREM2 variants thus far suggests that AD-risk is incurred through a partial loss of function[28], however, the localization of the R47H and R62H mutations within the ligand binding domain of TREM2 suggest perhaps a more nuanced alteration in specific microglial responses.

Thus far, the majority of studies examining TREM2 in relation to AD have utilized TREM2 deletion that in general appears to reduce microglial activation in response to varying stimuli. For example, murine AD models with TREM2$^{-/-}$ exhibit decreased microglial activation resulting in less microglial migration to beta-amyloid plaques and delayed plaque clearance[29]. In addition, plaques in TREM2$^{-/-}$ mice are less compacted, leading to increased plaque-associated neuritic dystrophy[30,31]. These data collectively suggest that microglial activation is necessary for clearance of plaques, and that suppression of these activation programs may accelerate plaque accumulation. Interestingly, total microglial numbers are also decreased in TREM2$^{-/-}$ mice potentially due to their inability to initiate activation-related proliferation and/or impaired microglial survival. Indeed, Trem2 expression normally decreases with some forms of microglial activation such as LPS treatment, but is conversely elevated in microglia adjacent to beta-amyloid plaques. Furthermore, microglia that lack trem2 do not seem to activate normally in response to injury[18,19]. In addition, as a transmembrane protein, recent studies have demonstrated that TREM2 can be proteolytically cleaved, resulting in sTREM2 which may serve as a promising biomarker for AD and may also provide additional immunomodulatory functions[34–36].

In addition to specific mutations in Trem2, other microglial AD-risk genes, membrane spanning 4-domains subfamily A members 4A and 6A (MS4A4A, MS4A6A) have recently been associated with altered sTrem2 levels in patient CSF. The MS4A family is itself linked to altered AD risk; in autopsied AD brains and blood samples from AD-patients with MS4A risk SNPs, expression of both MS4A4A and MS4A6A is increased. Importantly, these elevated expression levels also parallel increasing Braak tangle and plaque scores[37–39]. Interestingly, an AD-risk SNP (rs6591561) associated with increased expression of both MS4A genes, is also correlated with reduced levels of sTrem2. Conversely, rs1582763, a SNP associated with decreased MS4A4A and MS4A6A is linked to increased sTrem2 and protection from Alzheimer's disease[27]. However, MS4A proteins likely also influence disease risk independently of their effect on sTrem2. For example, unpublished data from our lab suggests these proteins play a role in regulating phagocytosis. Furthermore, other members of the MS4A family such as CD20 (MS4A1) have previously been implicated in immune regulation independent of Trem2 signaling.

CD33 or Siglec-3, is another myeloid cell specific receptor that has been significantly associated with Alzheimer's disease[40,41]. Sialic acid binding triggers Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM) signaling through Siglec proteins such as CD33, which has previously been shown to induce SYK-mediated signaling cascades that lead to changes in phagocytosis that are similar to those triggered by TREM2/DAP12 signaling[42]. CD33 expression is also increased in human AD brains and correlates with increased plaque burden as well as swifter disease progression[40]. Within BV2 immortalized microglia and murine CD33 knockout models of AD reduced expression of CD33 is associated with impaired clearance of beta-amyloid[43]. Extrapolation from these data may seem confusing

given that they suggest increased expression of CD33 in microglia would be predicted to increase beta-amyloid phagocytosis while also leading to increased plaque burden. However, this combination can be resolved if we again consider the microglial seeding hypothesis whereby increased phagocytosis of beta-amyloid would lead to higher levels of plaque seeding leading to increased plaque load.

In addition, an elegant recent study of monocyte-derived microglia-like6 (MDMi) cells recently demonstrated that the CD33 AD risk SNP rs3865444 is associated with increased expression and membrane localization of full-length CD33 and decreased expression of a shorter splicing variant that lacks the immunoglobulin V-set domain, which together lead to reduced phagocytic activity[44]. In parallel, it was discovered that a protective SNP (rs12459419) leads to increased splicing of exon 2 leading to a shorter length protein.[45] While our understanding of CD33 biology continues to improve, additional research is still needed to determine whether the main role of CD33 in AD is through modulation of Aβ phagocytosis or whether additional immune regulatory aspects of altered CD33 signaling play a more important role in disease pathogenesis.

Of additional interest, ATP-binding cassette transporter A7 (ABCA7) is a membrane transporter expressed highly by neurons, microglia, oligodendrocytes, and endothelial cells, but still seems to have the largest effect on disease risk through microglia[24]. In AD, SNPs in ABCA7 seem to be associated with a gain of function that may enhance phagocytosis of apoptotic cells and beta-amyloid[38,46–49]. On a broader scale, human post-mortem tissue analysis has shown that SNPs in ABCA7, which increase ABCA7 expression, correlate with increased hippocampal atrophy. Inversely, when ABCA7 was deleted from the J20 amyloid model of AD, a decrease in plaque deposition was observed. These data again suggest that changes in microglial phagocytosis of beta-amyloid may underlie the effects of microglial risk genes on disease. On the other hand, our studies to date have been guided by the existing knowledge in the field and the somewhat biased expectation that any studies of AD-associated microglial function should by definition examine beta-amyloid phagocytosis. Yet, a growing number of studies suggest that phagocytosis of other CNS-derived substrates such as synapses or myelin could be at least as important to disease progression and we and others are finding that microglial genes can differentially effect phagocytosis of differing substrates. Likewise, many other less studied functions of microglia, could also be critically involved in this disease. Thus, it seems a more comprehensive, unbiased analysis of the effects of AD risk genes on human microglial function and gene expression are desperately needed to improve our understanding of these cells and their role in AD.

Now that it has become clear that microglia are crucial in AD pathogenesis, the field needs to better understand *how* these cells influence disease risk and whether the normal function of microglia in disease is generally protective or pathogenic. Though many of these risk genes eventually effect production or clearance of Aβ plaques, it is not known whether this is the mechanism that confers altered disease risk or whether this is merely a byproduct of a more important pathway or our somewhat biased experimental designs. By understanding the broader role of microglia and the immune system in AD we will be able to gain insight into the elusive causes of late onset Alzheimer’s disease in order to better target disease-modifying therapies that can prove to be effective in clinical trials.

Microglia in Homeostasis and Disease

In homeostatic conditions, microglia are responsible for promoting neuronal health through secretion of trophic factors and synaptic remodeling as well as clearing pathogens, protein aggregates, myelin, and dead cell debris. These immune cells tile to form a grid through the brain, ensuring that no section goes unsurveiled. Homeostatic microglia are highly ramified and each of their processes is appreciably motile, constantly probing their environment for potential pathogens[50]. When a threat arises, microglia quickly become activated in order to address the insult. Activated microglia can secrete pro-inflammatory cytokines, clear pathogenic materials through phagocytosis and lysosomal degradation, and may also induce astrogliosis and astrocyte-associated changes to the blood brain barrier. After the pathogen has been cleared, microglia will typically return to a homeostatic state.

In some cases, however, microglia activation fails to resolve. In these circumstances, the constitutively active microglia often become detrimental to brain health. They may aberrantly over-prune synapses, kill neurons through phagoptosis, or induce unnecessary astrogliosis through pro-inflammatory cytokine secretion. Through prolonged, unnecessary microglial activation, severe neurodegeneration may occur. For example, aberrant inflammation in traumatic brain re-injury results in an inability for lesions to heal[51]. Chronic microglial activation has also been strongly implicated in many neurodegenerative diseases, playing a role in multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease and Alzheimer's disease[25,38,52].

As mentioned previously, problems can also arise if, conversely, microglia are unable to become appropriately activated in response to an insult, such as in Trem2 knockout models. When microglia are constitutively homeostatic, they may not be able to properly remove pathogens, debris, or dead cells. In this case, these hazardous materials may build up creating further imbalances in brain homeostasis. Because microglia are responsible for supporting brain health and homeostasis through many avenues, microglia may influence the onset of AD in various ways, some of which are explored below.

Migration, phagocytosis, and lysosomal degradation

Many of the Alzheimer's risk genes highly expressed in microglia effect microglial phagocytosis of beta-amyloid. Given the widespread interest in and adoption of the amyloid cascade hypothesis[4], it follows that the majority of research on microglia in AD has often begun with examinations of this question. However, amyloid targeted therapeutics have thus far failed to improve or delay cognition in late stage clinical trials, leading some to speculate that beta-amyloid deposition could be a sign post of other more detrimental issues rather than a pathogen directly. If therapies can be developed that can reset and enhance microglial-mediated clearance of beta-amyloid, many would predict that this might stop or delay disease progression. Yet, as with other amyloid targeting therapies such an approach would likely only be useful if initiated during very early prodromal phases of the disease.

Phagocytosis of beta-amyloid is a complex system which includes migration towards the beta-amyloid plaques, endocytosis of beta-amyloid and lysosomal degradation into its

constituent amino acids. The build up of beta-amyloid plaques observed in AD brains may be occurring from deficits in any or all of these components. These dysfunctions may be beta-amyloid specific or may also effect a broader range of phagocytosis of other substrates including apoptotic cells, myelin, or debris.

The ability of a microglia to migrate is crucial to its immune surveillance activity. In order to clear something from the brain, microglia must first follow chemotactic cues towards the debris or pathogens. This process is complex to study given that there are many chemokines, but often the mechanisms can be extrapolated from macrophage biology. When neurons die, for example, ADP and nucleotides released from the dying cell form a chemoattractive gradient sensed by the puranergic receptor P2RY12 on microglia[53–55]. When P2YR12 is chemically blocked, microglia are unable to activate in response to ADP/ATP and additionally do not migrate along their concentration gradient. *In vivo*, blockade of P2YR12 would likely inhibit microglial activation in response to dead neurons leading to a build up of apoptotic debris in the brain[56]. This is similar to what occurs with trem2 responses to beta-amyloid in which knockout of trem2 inhibits microglial migration toward amyloid plaques leading to increased beta-amyloid accumulation in AD mouse models. Correspondingly, it has been suggested that trem2 and its' co-receptor dap12 may act as an actual phagocytic receptor for beta-amyloid. However, a large number of receptors on microglia have been posited to bind beta-amyloid and thus additional research is needed to tease out which receptors are necessary for directed migration and which are more important for beta-amyloid internalization.

If a microglia cell is able to properly migrate towards its target, the cell will still need to express the receptors and machinery to complete phagocytosis of this substrate. We still do not fully understand all the components involved in microglial phagocytosis, but much has been learned from assuming homology with other myeloid cells. In terms of neural phagocytosis, one of the major signals for a microglia cell to engulf its target is exposed phosphatidylserine. This phospholipid becomes exposed on the cell surface during the early stages of apoptosis and in response to oxidative stress, ATP depletion, or increased calcium ion levels all of which are signs of cellular stress and increase with age[57–59]. Interestingly tau-laden neurons have also been shown to aberrantly expose phosphatidylserine[60,61]. Microglial recruitment to these neurons may be a partial mechanism for how tau causes neurotoxicity. Indeed, PET imaging in mice has shown tau accumulation to precede microglial activation which strongly correlated with a reduction in brain volume[62]. Other groups however cite microglia as the mediators of tau spreading though phagocytosis remains important in either case[60].

Protein aggregates, on the other hand, often must become opsonized before they can be recognized by a microglia cell. The most well-studied opsonins are IgG antibodies and the complement system both of which have been associated with AD[63–65]. Though, for beta-amyloid proteins, it has also been suggested that opsonization is not necessary. Many toll-like receptors, g-protein coupled receptors, and several AD-risk genes (trem2, abca7) have been proposed to serve as beta-amyloid receptors. For some of these receptors, it is likely that beta-amyloid does indeed bind, but rather than triggering phagocytosis of Aβ, this ligand may trigger downstream pro-inflammatory signaling cascades. It is difficult to

distinguish receptors necessary for activation from those necessary for engulfment since removal of the former may still inhibit beta-amyloid phagocytosis by causing the cells to remain in a homeostatic state. This may be the case with AD-risk genes such as TREM2 and ABCA7. However, cell culture based studies have begun to provide initial evidence that beta-amyloid can indeed be recognized by TREM2, albeit only when bound to Apolipoprotein E[66].

After a microglia cell has successfully sensed, migrated to, and engulfed a particle. It must still degrade the particle. For most substrates that have been engulfed, the phagocytic vesicle containing the cargo will merge with early and late endosomes to load digestive enzymes and acidify the pH before finally merging with a lysosome to form a phagolysosome[67]. Within the phagolysosome, particles are broken up by hydrolytic enzymes suitable for the low pH of the lysosome and can then be released from the cell. The specific proteins involved in this pathway differ depending on the cell type and the substrate being engulfed. Currently, the downstream signaling pathways involving specific processing of apoptotic cells[68] or beta-amyloid[69,70] have not been found to be linked to disease progression directly. However, more research into microglia-specific responses to phagocytic substrates in homeostatic or activated states will be required to better understand how these immune cells are able to respond to pathogenic stimuli in both early and later stages of disease progression. General knowledge from other immune cell types demonstrates that when phagolysosome formation or function is disrupted, this results in a build up of debris within enlarged phagolysosomes and can even result in cell death through necrosis[71].

Several groups have showed that activating microglia not only boosts the migration to and engulfment of Aβ, but microglia treated with pro-inflammatory cytokines or LPS can actually degrade Aβ more efficiently. This is in part because activation induces acidification of the lysosomes which encourages faster and more complete degradation of proteins and cellular debris. If a microglia is unable to properly activate in response to neuroinflammatory stimuli, lysosomal efficiency would not increase, resulting in further reduction of the ability of microglia to process pathogenic debris. It is possible that a cascade like this may be the multifactorial trigger promoting disease progression, however, there is also significant data suggesting that many other important microglia functions are also altered in Alzheimer's disease as discussed below.

Cytokine secretion, astrogliosis, and blood-brain barrier breakdown

Cytokines and chemokines are important mediators of neuroinflammation. Somewhat contradictory to the story surrounding Trem2 which concludes that hindering microglial activation increases AD risk, pro-inflammatory cytokines such as CCL2 and TNFα are increased in human AD brains. In addition, homeostatic cytokines such as CX3CL1 are dramatically decreased. CX3CL1 is secreted from neurons and acts as a homeostatic signal for the microglia receptor CX3CR1. In studies of AD models deficient for CX3CR1, AD brains displayed decreased beta-amyloid plaque deposition and substantially less neurodegeneration[72–74]. Not surprisingly, $CX3CR1^{-/-}$ mice showed increased levels of CCL2 and TNFα further confirming their activated state as a result of the absence of homeostatic signaling. Yet in stark contrast to this, deletion of CX3CR1 in tau transgenic

models leads to increased neurofibrillary tangle pathology and behavioral deficits[75]. Thus, the effects of microglia activation can be diametrically opposite between the two hallmark AD pathologies. A similar relationship has also been described following treatment of AD mice with LPS, which leads to increased microglial activation and reduced beta-amyloid plaques, but enhanced tangle pathology[76]. Effects from pro-inflammatory cytokines can of course be pleiotropic as cytokines may have autocrine and paracrine effects signaling both back to microglia as well as to astrocytes furthering the spread of neuroinflammation, perhaps providing a partial explanation for these findings. Alternatively, perhaps the key role of microglia in AD is as an intermediary that transduces the proinflammatory-inducing effects of beta-amyloid plaques into increased neuritic dystrophy and tau pathology. In support of this are recent findings regarding the influence of TREM2 deletion and mutations on plaque barrier formation[28].

In terms of pro-inflammatory cytokines, CCL2 levels are increased in patients with Alzheimer's disease and may potentially provide a reasonable biomarker for disease progression[77]. The mechanism of CCL2 in disease progression is still unclear though there is evidence that CCL2 expression alters phagocytosis of beta-amyloid plaques and effects disease progression through this axis[78]. Others propose that CCL2 is mainly effective through recruitment of peripheral mononuclear phagocytes though it remains unclear and controversial whether these cells actually migrate into the brain during human disease[79]. TNFα, is similarly increased in Alzheimer's patient brains as well as model systems and seems to also increase phagocytosis of beta-amyloid[80]. Though the effect of TNFα may be broader in that it is secreted by neurons as well and has independent effects on neuronal survival and proliferation[81].

Many important microglial-derived pro-inflammatory cytokines such as CCL2, TNFα, Il 1β, IL-6 and others also influence astrocyte activation or astrogliosis[82,83]. Even in injury models, removal of microglial cytokines inhibits astrogliosis from occurring further proving that microglia are often responsible for induction of astrocyte reactivity[84,85]. Like microgliosis, astrogliosis is particularly prevalent near plaques suggesting they play a role either in barrier formation to protect neurons and/or in the chemoattractive recruitment of microglia to the plaque environment[86,87]. Conversely there is also evidence that astrogliosis is detrimental in that increased astrocyte derived IL-1β, iNOS, and ROS secretion acts as a positive feedback mechanism to increase neuroinflammation and may even harm the blood brain barrier[85,87] which would allow for further recruitment of peripheral phagocytes into the brain via CCL2/CCR2 signaling.

Damage Associated Microglia

Since the direct pathways through which microglia influence Alzheimer's disease remain unclear, several groups have begun to study microglial biology using broader unbiased approaches. For example, Keren-Shaul et al. used single-cell RNA-sequencing to uncover a specific population of microglia whose temporal appearance mirrored the progression of plaque pathology in the 5x-fAD mouse model[30]. These Damage Associated Microglia (DAM) are formed via a two-step process the second of which appears to be TREM2 dependent since in TREM2$^{-/-}$ mice, microglia remain in the intermediate activation phase

throughout disease progression. Therefore, DAM have been hypothesized to be beneficial in the context of AD knowing that Trem2 loss of function mutations are known to exacerbate disease severity and age of onset.

Interestingly, Krasemann et al. have discovered a similar set of genes which they have denoted the microglia neurodegenerative phenotype or MGnD[31]. Here, the authors have described a more generalized phenotypic change associated with several neurodegenerative diseases and demonstrate that this activation state is influenced by APOE. Using mouse models of amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Alzheimer's disease, the authors highlight genes that are induced or repressed commonly across disease type. This list includes many of the same genes discovered in Keren-Shaul et al. including increased apoe, hla, clec7a, and cd11c expression as well as decreased p2ry12, cx3cr1 and tmem119 expression. Although the gene sets discovered in each paper are not identical, it seems likely that each group has independently discovered a similar set of cells. Indeed DAM microglia have been shown to be similarly occurring in ALS as well. Interestingly, MGnDs and the corresponding loss of more homeostatic microglia have been proposed to be detrimental in contrast to the subsequent conclusions of Keren-Shaul et al. Whether the MgnD and DAM phenotype is equivalent and more importantly whether they are detrimental or beneficial will likely depend on the nature of the disease process and timing. For example, one might predict that DAM phenotypes are protective against beta-amyloid given the effects of TREM2 deletion on DAMs and plaque load whereas DAM cells might conversely by detrimental in the context of tau pathology or synaptic pruning. Continued validation of these unbiased approaches and extension of these studies to include examination of human microglia are critically needed and will hopefully help narrow down the true roles of microglia in neurodegenerative disease.

Microglia as a therapeutic target

Since microglia effect so many crucial pathways in the brain, therapies which effect this cell type may have unexpected off-target effects. Fortunately some of the most important microglial functions, such as synaptic pruning, occur predominantly early in life and thus it may not be detrimental to dampen these processes in Alzheimer's patients. Another concern is that microglia share many transcriptional and functional pathways with peripheral monocytes and macrophages. For this reason, small molecule therapies may produce unwanted side effects on these peripheral targets. Currently, in AD, is not yet clear whether immune activation or suppression will be therapeutic as examples in this review have been presented in support of both possibilities. In either case, broad activation or suppression of myeloid cells would likely be detrimental for patients. Sustaining myeloid activation globally may cause chronic inflammation similar to macrophage activation syndrome[88,89]. On the other hand, general suppression of immune activation in aged patients who already experience an increased risk of infection and immune impairment would leave patients increasingly vulnerable to infectious disease. For these reasons, the most successful microglial therapies will need to be precisely targeted towards microglia but not other monocytes and thus need to capitalize on our growing understanding of the genetic and functional differences between these closely related cells.

If cell-specificity can be sufficiently achieved, it is possible that broad activation or suppression of microglia may be effective although the timing of these approaches will likely be critical. Recent data from mouse studies in which microglia are ablated using a CSF-1 blockade demonstrated no cognitive detriments from complete removal of microglia in otherwise normal WT mice[89]. While behavioral studies in mice are much less nuanced than human cognition, this research suggests that therapeutic microglia suppression, perhaps via more subtle means such as reduced proliferation[90], may be therapeutically tractable. Although ideally a specific pathway of microglia activity such as migration, phagocytosis, or cytokine signaling pathways could be isolated and specifically modulated, the effect of microglia on AD pathogenesis does not seem to be that simple. Indeed, this review has provided evidence for disruption in all three of those pathways in AD and likely further study of microglia enriched risk genes will uncover additional microglia functions that influence disease progression.

Perspectives

This review presents a broad overview of the current data positing that the immune system, primarily microglia, plays a much larger role in disease development and progression than previously understood. With the rapid growth of research focusing on microglia in AD, many different functional pathways have been proposed to alter disease risk. Of these, most pathways can be broadly altered by changing microglial activation state. In order to separate these individual pathways from the pleiotropic effects of broad microglia activation, more research towards understanding the spectrum of human microglial activation states will be required. We have learned a great deal from studying peripheral macrophages, but given the key transcriptome and functional differences between peripheral macrophages and microglia, we must assume that microglial activation is likewise quite different. Furthermore, even murine microglia *in vivo* have been shown to significantly differ from human microglia and these differences are enhanced in aging, making it particularly difficult to study age-related human disease in traditional murine models. While mouse models are extremely useful for studying microglia in their natural environment, they are inherently biased based on what we currently understand to cause Alzheimer's disease and thus will always produce data related to those original assumptions. In order to create a more accurate model of microglia in Alzheimer's disease using patient derived iPS-microglia, one potential promising approach will be to utilze brain organoid models or generate chimeric mouse models to study the complex interactions between human microglia, neurons, astrocytes, and AD neuropathology.

Acknowledgements

The authors would like to thank Morgan Coburn for contributing the images for Figure 1. This work was supported by NIH grants AG048099, AG056303, and AG016573, CIRM RT3–07893 (M.B.J.) and NINDS T32 NS082174 (A.M).

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Highlights

- Microglia are associated with the progression of Alzheimer's disease.
- Key microglia functions in AD: cytokine secretion, phagocytosis, trophic support.
- Human *in vitro* models allow for controlled studies of molecular microglia function.
- Understanding human microglial function in AD may elucidate new, targeted therapies.

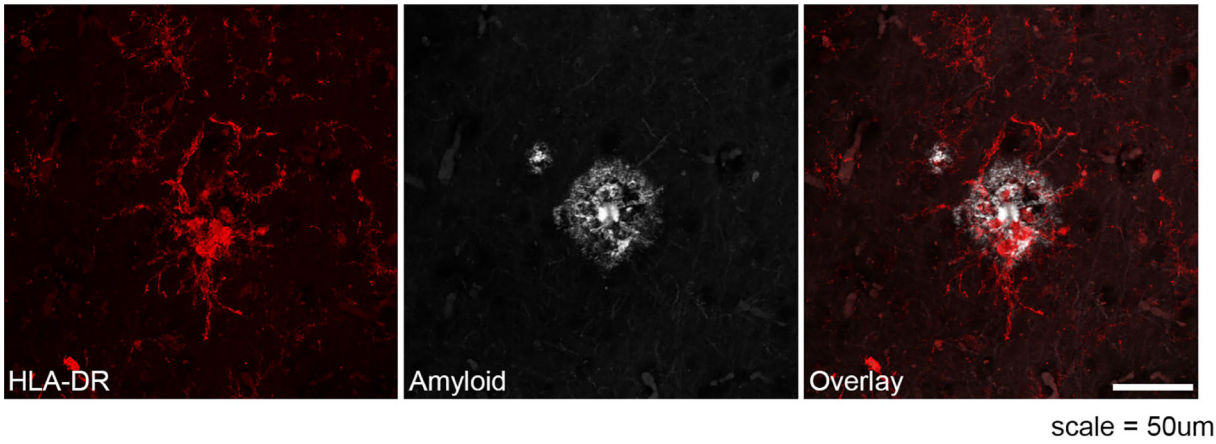

Fig. 1.

Disease-associated microglia surrounding Aβ plaques. Immunofluorescent stain of human Alzheimer's patient tissue demonstrates microglia (stained with DAM marker HLA-DR, red) surrounding Aβ plaques (gray). HLA is upregulated in microglia around plaques. The scale represents 50 μm.

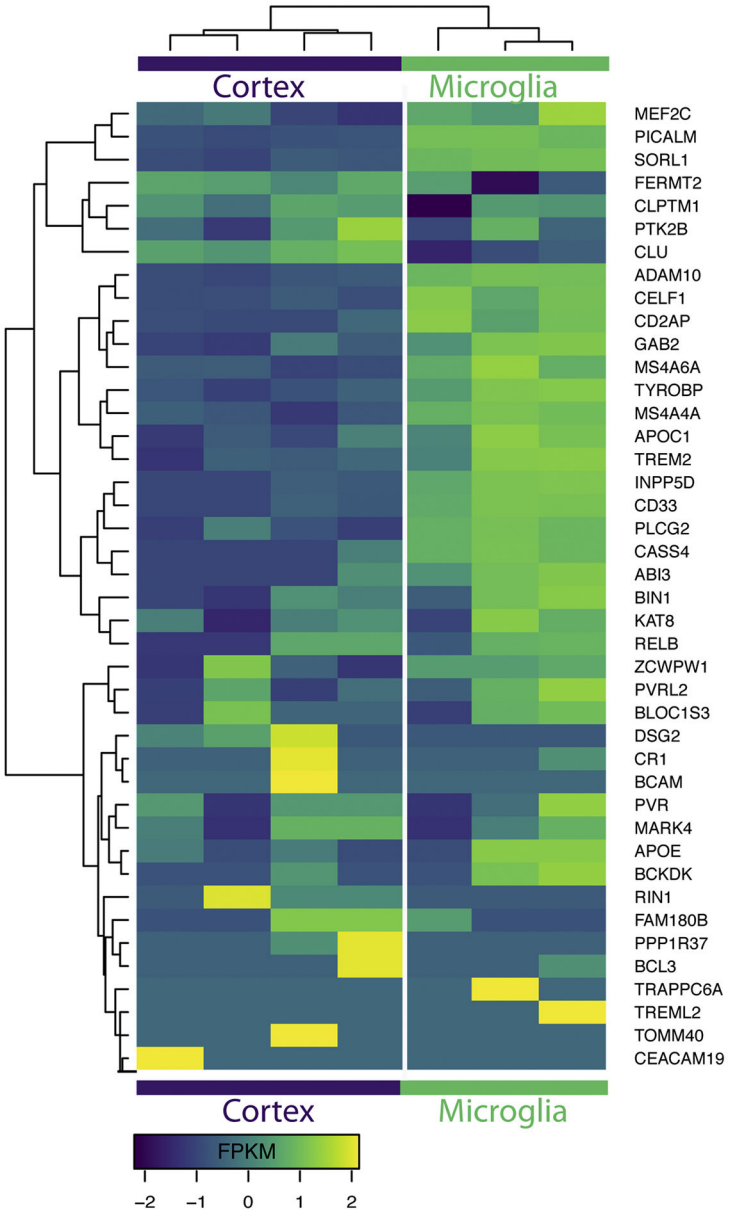

Fig. 2.

Alzheimer's risk genes are enriched in microglia over total cortex expression. Transcriptome data from Zhang et al. [24] was used to generate this heatmap of expression levels of each AD risk gene in the brain cortex (left) versus expression level in microglial cells (right). Data are displayed in frequency per kilobase million reads (FPKM).

Table 1.

Alzheimer's disease risk-loci and their proposed functions

SNP ID	Proposed gene affected	Function	Citation
rs3764650 rs3752246	ABCA7	lipid transport	Allen et al. Neurology 2012
rs616338	ABI3	actin polymerization	Sims et al. Nat Genet 2017
rs2305421	ADAM10	cleaved TNFa and E-cadherin	Akhter et al. Neurobio Aging 2018
rs4420638	APOC1	lipid metabolism	Lin et al. J Hu Genetics 2016
rs5167	APOC4	lipid metabolism	Allan et al. Genomics 1995
rs2075650	APOE	lipid metabolism	Lin et al. J Hu Genetics 2016
rs889555	BCKDK	unknown immune function	NA
rs2965101 rs2927438	BCL3	NF-kB immune regulation and survival	Poveda et al. Exp Mol Med 2017
rs744373 rs7561528	BIN1	endocytosis and phagocytosis	Prokic et al. J Mol Md 2014, Gold et al. J Exp Med 2004
rs597668	BLOC1S3	endosome and lysosome trafficking	Seshadri et al. JAMA 2010
rs7274581 rs6024870	CASS4	cell adhesion and axonal transport	Beck et al. Oncoscience 2014
rs9349407 rs9296559	CD2AP	cytoskeletal remodeling	Guimas et al. Cell Mol Life Sci 2018
rs3865444 rs3826656	CD33	phagocytosis	Griciuc et al Neuron 2013
rs2965109	CEACAM16	antigen cell adhesion	Kammerer et. Al J Biol Chem 2012
rs714948	CEACAM19	antigen cell adhesion	Kleita et al. Int J Oncol 2013
rs10838725	CELF1	transcription regulation	Dasgupta and Ladd Wiley Interdiscp Rev RNA 2013
rs35577563	CLPTM1	telomere regulation	Carkic et al. J Oral Sci 2016
rs11136000	CLU	complement, apoptosis, lipid transport	Karch and Goate Biol Psy 2015
rs679515 rs3818361	CR1	complement	Rogers et al. Neurobiol Aging 2006
rs8093731	DSG2	lysosomal function	Karch and Goate Biol Psy 2015
rs11767557 rs11771145	EPHA1	immune response, cell adhesion and motility	Misra et al. Indian J Med Res 2018, Aasheim et al. Blood 2005
rs10415983	EXOC3L2	exocytosis	Dayeh et al. PLoS Genet 2014
rs12287076	FAM180B	unknown	NA
rs17125944	FERMT2	actin polymerization	Yasuda-Yamahara et al. Matrix Biol 2018

SNP ID	Proposed gene affected	Function	Citation
rs1385600	GAB2	cell growth and apoptosis	Bagyinsky et al. Clin Interv Aging 2014
rs5848	GRN	lysosomal function	Paushter et al. Acta Neuropathol 2018
rs9271192	HLA-DRB5-DBR1	antigen presentation	Karch and Goate Biol Psy 2015
rs35349669	INPP5D	myeloid proliferation and survival	Efthymiou and Goate Mol Neurodegener 2017
rs7196161	KAT8	cell survival	Patillon et al. PLoS One 2012
rs8100183	MARK4	inflammasome	Li et al. Nat Commun 2017
rs190982	MEF2C	immune profliferation and antigen presentation	Sao et al. Phychiatry Clin Neurosci 2018
rs558678 rs554311	MS4A2	hematopoietic immune response	Keuk et al. Immuno Cell Bio 2015
rs610932 rs11824773	MS4A4A	signal transduction phagocytosis	Greer et al. Cell 2016 and unpublished data from our lab
rs10897011 rs7926729	MS4A4E	unknown immune function	Hollingworth et al. Nat Genetics 2011
rs610932 rs983392	MS4A6A	phagocytosis	unpublished data from our lab
rs17643262	NKPD1	lipid synthesis	Amin et al. Biol Psych 2017
rs2718058	NME8	cytoskeletal and axonal transport	Liu et al. Oncotarget 2016
rs3851179 rs541458	PICALM	endocytosis	Zhao et al. Nat Neurosci 2016
rs72824905	PLCG2	calcium signaling	Conway et al. Molecular Neurodegener 2018
rs145999145	PLD3	APP processing	Satoh et al. Alzheimers Res Ther
rs3848140	PPP1R37	phosphotase activity	Han er al. PLoS One 2017
rs2058716	PRKD3	inflammatory signaling	Baker et al. PLoS One 2018
rs28834970	PTK2B	inflammation	Beck et al. Oncoscience 2014
rs2301275	PVR	immune activation	Stamm et al. Oncogene 2018
rs10402271 rs1871047	PVRL2	chlosterol metabolism	Lin et al. J Hu Genetics 2016
rs2376866 rs117612135	RELB	immune migration	Dohler et al. Front Immunol 2017
rs10498633	SLC24H4-RIN3	cardiocascular function	Giri et al. Clin Interv Aging 2016
rs12285364	SORL1	lipoprotein receptor	Holstege et al. Eur J Hum Genet 2017
rs760136 rs10524523	TOMM40	chlosterol metabolism	Lin et al. J Hu Genetics 2016
rs28367893	TRAPPC6A	protein transport	Chang et al. Oncotarget 2015
rs75932628	TREM2	phagocytosis, migration, activation	Gratuze et al. Mol Neurodegen 2018

SNP ID	Proposed gene affected	Function	Citation
rs9381040	TREML2	immune activation, phagocytosis	Zheng et al. Neurobiol Aging 2017
rs1476679	ZCWPW1	histone modification	Gao et al. Oncotarget 2016