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Title

The involvement of microglia in Alzheimer's disease: a new dog in the fight.

Running title

The involvement of microglia in Alzheimer's disease

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Abstract

First described clinically in 1906, Alzheimer's disease (AD) is the most common neurodegenerative disease and form of dementia worldwide. Despite its prevalence, only five therapies are currently approved for AD, all dealing with the symptoms rather than the underlying causes of the disease. A multitude of experimental evidence has suggested that the once thought inconsequential process of neuroinflammation does, in fact, contribute to the AD pathogenesis. One such central nervous system (CNS) cell type critical to this process are microglia. Plastic in nature with varied roles, microglia are emerging as key contributors to AD pathology. This review will focus on the role of microglia in the neuroinflammatory response in AD, highlighting recent studies implicating aberrant changes in microglial function in the disease progression. Of critical note is that with these advances, a reconceptualization of the framework in which we view microglia is required.

Abbreviations

5XFAD	5X familial Alzheimer's disease mutation
AAV	Adeno associated virus
ABI3	ABI gene family member 3
ACH	Amyloid cascade hypothesis
AD	Alzheimer's disease
APOE	Apolipoprotein E
APP	Amyloid precursor protein
$APP_{SWE}/PS1_{\Delta E9}$	APP containing Swedish mutation and PS1 containing delta E9 mutation
CD	Cluster of differentiation
CD33	Cluster of differentiation 33/Siglec-3
CLU	Clusterin
CK-p25	Cyclin dependant kinase 5 protein 25
CR1	Complement receptor 1
DAMP	Damage associated molecular pattern
DAM	Damage associated microglia
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GWAS	Genome wide associated study
IBA1	Ionized calcium-binding adapter molecule 1
IL1RAP	Interleukin 1 receptor accessory protein
IRF	Interferon regulatory factor
iPSC	Induced pluripotent stem cell
LOAD	Late onset Alzheimer's disease
MCI	Mild cognitive impairment
MV	Microvesicle
PET	Positron emission tomography
PLCG2	Phospholipase C γ2

PS1, PS2	Presenilin 1, presenilin 2
RIPK1	Receptor-interacting serine/threonine-protein kinase 1
SP	Senile plaque
TLR	Toll-like receptor
TREM2	Triggering receptor expressed on myeloid cells 2

Alzheimer's disease

Pathologically, AD is characterised by death of pyramidal neurons and synapses in the cerebral cortex and certain subcortical regions, with classical hallmarks of senile plaque (SP) deposits and hyperphosphorylation of the microtubule associated protein tau. In particular, this loss occurs in the hippocampus resulting in gross atrophy of the brain and increased ventricular spaces. This presents clinically as a progressive pattern of worsening cognition and behavioural function. This pattern is divided into 3 stages: preclinical, mild cognitive impairment (MCI), and dementia (Jack et al., 2011). Despite identification of genetic mutations that confer significant risk factors for disease, the underlying cause of AD remains unproven and as such, raises difficulties in definitive diagnoses. Indeed, the MCI stage of AD, considered a prodromal stage of disease, is often attributed to normal ageing (Dubois & Albert, 2004).

The amyloid cascade hypothesis (ACH) was developed following the identification that SPs are composed of amyloid beta (Aβ), and that these deposits are unique to AD. The ACH posits that Aβ deposition is a critical event in AD pathology. First articulated in 1992 by Hardy and Higgins (1992), the ACH originally postulated that Aβ deposition is causative of AD pathology, though has now evolved to contend that Aβ deposition is a central event in AD pathology (Pimplikar, 2009). Regardless of the validity of the ACH, it has driven AD research and aided in our understanding of underlying biological mechanisms.

However, this has not translated into clinical trial results. All Aβ directed therapies have failed in clinical trials, many in multiple attempts (Cummings, Morstorf & Zhong, 2014). Similarly, the vast majority of non-Aβ directed therapies have also failed. This results in AD having one of the highest failure rates of any disease at 99.5-99.6% (Calcoen, Elias & Yu, 2015; Cummings, Morstorf & Zhong, 2014). Pfizer has even gone as so far to abandon the field entirely, citing money can be better spent elsewhere (Hawkes, 2018). This has not been unnoticed by those outside of the scientific sphere with individuals becoming disconcerted with the scientific process (Cummings, Morstorf & Zhong, 2014; Mannix, 2018).

To address this, it is clear that a re-evaluation of the precise role of the various pathologies seen in AD is required; one that accounts for the complex milieu observed. In concordance, our therapeutic approaches should also follow a re-evaluation. Once such revaluation involves investigating the role of neuroinflammation within AD.

Neuroinflammation

The term "neuroinflammation" refers to immune related responses; in particular the innate immune response that occurs within the CNS. Neuroinflammation is normally seen throughout the CNS with roles in both cellular and tissue homeostasis as well as proper neuronal functioning (Xanthos & Sandkuhler, 2014). Under normal physiological conditions this response is self-limiting. This acute form of neuroinflammation contrasts with the chronic form observed in AD that sees a failure of the immune clearance mechanism (Maderna & Godson, 2003; Shastri, Bonifati & Kishore, 2013).

Neuroinflammation is a complex process that involves a number of cell-cell interactions within the CNS (Skaper, Facci, Zusso & Giusti, 2018).The inflammatory cascade is principally initiated through two glial cell types: microglia and astrocytes (Perry, Nicoll & Holmes, 2010). Upon recognition of an initial stimulus, these cells secrete a number of hallmark pro-inflammatory cytokines including tumor necrosis factor alpha (TNFα), interleukin 1 beta (IL1β) and interleukin 6 (IL6), as well as a number of chemokines that recruit further glial cells. These pro-inflammatory mediators can then facilitate the neurodegeneration of otherwise healthy neighbouring neuronal populations, with secreted cellular contents and damage associated molecular patterns (DAMPs) produced by these dying neurons further contributing to the inflammatory milieu.

Neuroinflammation in AD is considered to be a chronic and detrimental process (Eikelenboom, Veerhuis, Scheper, Rozemuller, Van Gool & Hoozemans, 2006). Human post mortem examination of AD individuals consistently reports enhanced levels of microgliosis

and astrogliosis surrounding Aβ plaques (Heneka, Kummer & Latz, 2014a). This is in concordance with increased levels of inflammatory cytokines and chemokines (Heneka, Kummer & Latz, 2014a). Furthermore, levels of neuroinflammation have been shown to correlate with disease progression and impairments in cognition (Heneka, Kummer & Latz, 2014b; Morris, Clark & Vissel, 2014). Recent studies suggest that microglia are the critical cell type that contributes to this neuroinflammatory related pathology (Sarlus & Heneka, 2017).

Microglia

Microglia are a class of innate immune cells within the CNS. Unlike other CNS glial cell types, they share a myeloid lineage with their peripheral counterparts macrophages (Ginhoux, Lim, Hoeffel, Low & Huber, 2013). Critically, microglia are formed from primitive macrophage progenitors that infiltrate the brain during development (Ginhoux et al., 2010). These microglia have a defined core gene set that distinguishes them from related peripheral cells (Butovsky & Weiner, 2018). The median lifespan for a single microglial cell is 15 months, over half the lifetime of a mouse (Fuger et al., 2017). However, when genetically depleted microglia can repopulate to normal cell levels within 5 days (Bruttger et al., 2015). Under homeostatic conditions, microglial repopulation is shown to be exclusively from CNS residing progenitor cells (Butovsky & Weiner, 2018). However, it is now appreciated that under certain conditions infiltration of peripheral myeloid cells can indeed differentiate into microglia (Cronk et al., 2018; Lund et al., 2018). These cells do express a subset of the unique microglial gene signature, but have differential functional roles (Butovsky & Weiner, 2018). Microglial proliferation is increased in a number of neurodegenerative conditions in mice, including Alzheimer's disease (Gomez-Nicola, Fransen, Suzzi & Perry, 2013).

Microglia exhibit a number of cell surface markers allowing for their identification and distinction from other immune cell types. Both microglia and peripheral macrophages are macrophage-1 antigen and cluster of differentiation (CD) 11b (CD11b) positive, whereas they can be distinguished based on being $CD45^{low}$ and $CD45^{high}$ respectively (D'Mello, Le &

Swain, 2009). Ionized calcium binding adaptor molecule 1 (IBA1) is also used as a microglial marker, and has historically been used as a marker for microglial "activation" (Ito, Imai, Ohsawa, Nakajima, Fukuuchi & Kohsaka, 1998). IBA1 has been shown to be involved in actin remodelling and subsequently phagocytosis, a key functional role that microglia are involved in (Ohsawa, Imai, Sasaki & Kohsaka, 2004). CD68 expression has also been used with IBA1 to identify "phagocytic" microglia (Boche, Perry & Nicoll, 2013).

Microglia fulfil a number of varied roles within the CNS including the immune response, maintenance of homeostasis, extracellular signalling, phagocytosis, antigen presentation and synaptic pruning (Kettenmann, Hanisch, Noda & Verkhratsky, 2011; Walker et al., 2014). The varied roles microglia may be attributed to their limited self-renewal under normal physiological conditions (Lawson, Perry & Gordon, 1992).

It follows that microglial populations are heterogeneous in nature; populations isolated from different regions within the brain under different conditions will ultimately differ in phenotype, associated markers and function (Wes, Sayed, Bard & Gan, 2016). In an effort to characterise microglia, the M1/M2 dichotomy was adopted from their peripheral macrophage counterparts, representing "classical" and "alternative" activation states. Whilst popular for some time, this is now considered an oversimplification of the vast array of phenotypes microglia can adopt. Ransohoff (2016) provides the most compelling argument for this. Briefly, these microglial subtypes are based on specific cytokines that induce each phenotype as described *in vitro*, which fails to account for the complex milieu seen in the CNS. Furthermore, the suggested spectrum view of microglia with "M1 like" and "M2 like" phenotypes as bookends also fails, with differing disease states' failing to identify an axis on which polarization occurs. This is further compounded with the original notion of M1/M2 macrophages also under debate (Martinez & Gordon, 2014). Extending this, describing microglia as either "activated" or "resting/quiescent" further hinders our understanding of these cells and conclusions we subsequently draw. A more appropriate view of microglia (Figure 1) shifts away from such pigeonholing, and rather views them as an extensive array of distinct, though overlapping phenotypes with corresponding functions. This is not to

entirely dismiss previous research. Rather, a more holistic view is required when drawing inferences about microglial phenotype and subsequent function.

Microglia transcriptomic changes in AD

A number of efforts have been made to accurately characterise microglia primarily through transcriptomic approaches. Though the first microglial specific characterisation is less than five years old, this approach has now expanded and aided in understanding the complexity that is microglial phenotype (Orre et al., 2014). Utilising direct RNA sequencing (RNAseq), the microglial "sensome" has been identified, a distinct group of transcripts encoding for ligand sensing proteins that changes in ageing (Hickman et al., 2013). In the cyclin dependant kinase 5 protein 25 (CK-p25) inducible mouse model of neurodegeneration, which shows similar transcript profiles to the 5X familial Alzheimer's disease mutation (5XFAD) AD model, Mathys et al. (2017) utilized single cell RNAseq to characterise microglial heterogeneity (Hargis & Blalock, 2017). Clustering of transcription profiles revealed a number of disease specific microglial states that were not observed in wild type controls. In a separate study in the 5XFAD AD model, single cell RNAseq was used to profile all immune cell types within the brain, after which a cluster of microglial cells termed the damage associated microglia (DAM) was identified. These cells were plaque associated, involved the downregulation of inhibitory checkpoints, and were conserved in humans (Keren-Shaul et al., 2017). Evidence has shown that both triggering receptor expressed on myeloid cells 2 (TREM2) and apolipoprotein E (APOE), known AD risk factors, act as regulators for the phenotypic switching of microglia (Krasemann et al., 2017). Utilizing the double transgenic AD mouse model containing amyloid precursor protein (APP) with the Swedish mutation and presenilin (PS) 1 with the delta E9 mutation ($APP_{SWE}/PS1_{\Delta E9}$), in parallel to models of multiple sclerosis and amyotrophic lateral sclerosis, mRNA counts using Nanostring technology identified a molecular signature of DAM dependant on both TREM2 and APOE signalling.

Ageing alone is known to alter microglial phenotype deleteriously, yet its exact contribution to disease remains unknown (Olah et al., 2018; Silvin & Ginhoux, 2018). This includes observations of microglial priming, in where aged microglial populations exhibit an increased pro-inflammatory response upon insult (Norden & Godbout, 2013; Rawji, Mishra, Michaels, Rivest, Stys & Yong, 2016). In aged mice, peripheral injections of LPS resulted in increased levels of pro-inflammatory cytokine levels when compared to young mice (Godbout et al., 2005; Henry, Huang, Wynne & Godbout, 2009). In addition, *ex vivo* microglia isolated from adult mice show greater secretion of both IL6 and TNFα (Njie, Boelen, Stassen, Steinbusch, Borchelt & Streit, 2012). Gene ontology analysis on transcriptomic data from young and aged mice reveals that these changes are largely interferon (IFN) dependant (Deczkowska et al., 2017). These changes are accompanied by morphological alterations, most notably with observations of hypertrophy (Conde & Streit, 2006). In addition to altered immune related functions phagocytotic functions are also altered, with the ability to phagocytose $A\beta_{1\text{-}42}$ decreasing with age (Floden & Combs, 2011). Whether this is indeed *bona fide* senescence however is undetermined (Baker & Petersen, 2018). Furthermore, issues arise when attempts are made to distinguish the individual contributions that either AD or normal ageing make on observed dysfunction.

The extent that these findings translate to humans is unclear. Whilst whole brain transcriptional profiles for humans and rodents in ageing alone are concordant, when AD humans and AD mouse models are examined concordance is not observed (Hargis & Blalock, 2017). Different rodent models of AD recapitulate different features and states of disease. Critically, all are based on familial disease states. Additionally, whilst similar profiles are seen within matched AD individuals, separate rodent models result in separate transcriptomic profiles (Hargis & Blalock, 2017). Furthermore, examination of whole tissue or entire cell populations has the potential to mask distinct populations. In an AD model containing APP_{SWE} and the N141I mutation in PS2, genes that were altered in whole brain tissue were found to be highly enriched in microglia and astrocytes, however only a fraction of these genes were in fact up-regulated when examined in these cell types as a specific population. Of these, microglia accounted for 87% (Kim, Kwon, Kim, Do, Lee & Han,

2016). Single cell RNAseq continually reports multiple glial cell populations, even in wild type mice (Zeisel et al., 2015). However, the extent that individual microglial populations translate across both models and species is yet to be fully explored. Epigenomic signals do however appear to be conserved between mice and humans. In the CK-p25 AD-like model, conserved transcriptional changes were shown to be associated with immune related genes, as well as conserved chromatin dynamics (Gjoneska et al., 2015).

Once such technique to overcome this is the use of induced pluripotent stem cells (IPSCs) from humans. Though a recent technique, these cells are indeed microglial-like and have been validated as a viable research tool (Abud et al., 2017; Pocock & Piers, 2018). Generation of microglial-like iPSCS from APOE4 carriers show transcriptomic changes alongside impaired phagocytosis of Aβ (Lin et al., 2018). Microglial iPSCs have recently been used to investigate specific missense mutations in TREM2, a newly identified AD risk factor (Garcia-Reitboeck et al., 2018).

Microglia morphology changes in AD

A key constituent of the plastic nature of microglia is the capacity for rapid morphological transformations. It has been widely observed that under normal physiological conditions, “quiescent” microglia adopt a ramified morphology, and “activated” microglia appear ameboid in shape (Boche, Perry & Nicoll, 2013). Furthermore, this transition is stepwise in nature (Stence et al., 2001). Multiple morphological measurements available can be used to compare separate microglia. Under normal conditions, microglia exhibit an altered morphology that is region dependant (Lawson, Perry, Dri & Gordon, 1990). LPS administered mice were shown to differ in measurements of cell perimeter, roundness and soma size (Kongsui, Johnson, Graham, Nilsson & Walker, 2015). In the dual Indiana and Swedish APP mutation AD model, plaque associated microglia show alterations in branch length, area and ramification when compared to wild type controls (Plescher, Seifert, Hansen, Bedner, Steinhauser & Halle, 2018). Examination of human AD autopsy samples also reveals distinct morphological microglial populations. In a human cohort, 5 distinct morphological

types were identified in AD and cognitively normal controls. Differences were seen in various regions of the hippocampus (Bachstetter et al., 2015). However, further work is required to elucidate the link between morphology and phenotype. Microglia that exhibit similar morphologies can indeed differ in underlying phenotype (Wes, Sayed, Bard & Gan, 2016). Though a recent technique, these studies provide evidence for novel inferential analysis of microglial function through morphology.

Microglial function in AD

Functional alterations in microglia are indeed observed contiguous to phenotypic changes, though the translational effect of these changes is varied (Figure 2). The initial sensing of the neuroinflammatory trigger by the Aβ peptide and its aggregates via microglia is varied. This sensing occurs through a number of receptors, including toll-like receptors (TLRs), NOD-like receptors, receptors for advanced glycation endproducts , formyl peptide receptors, scavenger receptors, pentraxins and the complement cascade. All of which are involved in the initiation of a neuroinflammatory response through a number of signalling pathways (Salminen, Ojala, Kauppinen, Kaarniranta & Suuronen, 2009). This sensing can also occur through the NOD, leucine-rich-containing family, pyrin domain-containing-3 inflammasome, multiprotein complexes involved in the maturation and secretion of pro-inflammatory cytokines IL1β and IL18 (Halle et al., 2008). The most studied of the receptors involved in this sensing are the TLRs. TLRs belong to a superfamily of pattern recognition receptors recognizing both DAMPs and pathogen associated molecular patterns. Of these, the TLR2 and TLR4 subtypes are considered critical in Aβ recognition. Microglial TLR2 recognition of Aβ is considered the principal method that triggers the neuroinflammatory response (Liu et al., 2012). Downstream TLR signalling through NFκB, activator protein 1, and interferon regulatory factor (IRF) pathways lead to pro-inflammatory gene transcription (Miyake, 2007).

Microglia are also involved in the clearance of Aβ through endocytosis. This process is dependent on whether Aβ is in a fibrillar or soluble oligomeric state, resulting in phagocytic or macropinocytic processes, respectively. Fibrillar Aβ is recognised through an assembly of

receptors to form a cell surface receptor complex, consisting of class A scavenger receptor, class B scavenger receptor, α6β1 integrin, CD14, CD36, CD47, TLR2, TLR4, TLR6 and TLR9 (Bamberger, Harris, McDonald, Husemann & Landreth, 2003; Lee & Landreth, 2010). Of these receptors, the TLRs appear to be the most crucial, with TLR activation increasing fibrillar Aβ clearance through phagocytosis (Tahara, Kim, Jin, Maxwell, Li & Fukuchi, 2006). Furthermore, TLR2 and TLR4 ablation results in a failure of the cell surface receptor complex to recognise Aβ and subsequently initiate phagocytosis (Reed-Geaghan, Savage, Hise & Landreth, 2009). Soluble Aβ species are cleared by microglia through a process known as fluid phase macropinocytosis. Soluble Aβ is brought into the cell through an invagination of the cellular membrane, which then closes to form a vesicle. This uptake also occurs in astrocytes, although not as effectively (Mandrekar, Jiang, Lee, Koenigsknecht-Talboo, Holtzman & Landreth, 2009). Excessive Aβ deposition and neurofibrillary tangles have been shown to impair the Aβ related clearance abilities of recruited microglia and astrocytes, compounding the issue (Yamanaka, Ishikawa, Griep, Axt, Kummer & Heneka, 2012).

Microglia also perform varied functions through the release of extracellular microvesicles (MVs) through exocytosis. MVs have been shown to be potent modulators of inflammation and immunity, and released MVs can stimulate synaptic activity both *in vitro* and *in vivo* (Antonucci et al., 2012; Sadallah, Eken & Schifferli, 2011) Critically, MVs of likely microglial origin are elevated within AD individuals. Examination of these MVs show toxicity towards to neurons and promote the formation of Aβ (Joshi et al., 2014).

Microglia activity within AD can also affect tau and drive tau-related pathology (Maphis et al., 2015). Analysis of human brain samples has demonstrated a senescent morphological microglial phenotype that is associated with tau (Streit, Braak, Xue & Bechmann, 2009). In addition, microglial mediated inflammation has been linked to tau aggregation and phosphorylation. In wild type mice, LPS induced inflammation has been shown to induce tau aggregation with this further enhanced in transgenic mice lacking the microglial fractalkine

receptor CX3C chemokine receptor 1. (Bhaskar, Konerth, Kokiko-Cochran, Cardona, Ransohoff & Lamb, 2010)

It is known that microglia are involved in synaptic pruning, a process critical not only in development but also in the mature brain (Hong, Dissing-Olesen & Stevens, 2016; Paolicelli et al., 2011). Initially thought to engulf whole synapses, recent evidence questions this with the process of "trogocytosis" observed, where partial phagocytosis has been linked to the selective pruning (Weinhard et al., 2018). Aberrant engulfment of synapses by microglia can lead to dysfunction. In lupus, a microglial mediated synapse loss has been observed which was rescued upon promoting an anti-inflammatory phenotype (Bialas et al., 2017). This is now being investigated within AD, where it is suggested that microglia may indeed be involved in such a process (Hong, Dissing-Olesen & Stevens, 2016). The genome wide associated study (GWAS) identified risk gene, clusterin (CLU), which encodes for the receptor complement component 3b, has been shown to be critical in the maturation of developing neural circuits by microglia (Schafer et al., 2012).

Similarly, it has recently been observed that microglia can execute neuronal death through phagocytosis on stressed but otherwise viable neurons, a process termed phagoptosis (Brown & Neher, 2014). This is due to an increase in various "eat me" signals (phosphatidylserine, complement component 1q, calreticulin, de-sialylated glycoprotein) and/or loss of "don't eat me" signals (glycoprotein, CD47, neuraminidase) presented on neuronal surfaces. In an inflammatory setting, both microglia and astrocytes release milk fat globule EGF factor 8 that is able to bind to exposed phosphatidylserine, opsonising the neuron. In excessive neuroinflammation, this process is skewed where microglia are able to phagocytose healthy neurons (Brown & Neher, 2012). In neuronal-glial co-cultures stimulated with either LPS or Aβ, a lack of discrimination occurs between stressed and healthy neurons targeted for phagoptosis by microglia (Neniskyte, Neher & Brown, 2011)

GWAS microglia

Genetic mutations in either APP or PS1/2 remain the only identified causative agents of early onset AD, with genetic determinants for late onset AD (LOAD) less characterised. Recent GWAS have revealed a number of genetic mutations that confer an increased risk of LOAD. Interestingly, a number of these SNPs reside in genes that directly relate to innate inflammatory function. As such, collections of these variants have the potential to drive pathological neuroinflammation and contribute to the progression and exacerbation of LOAD. These genes include APOE, major histocampaitibility complex class II, complement receptor 1 (CR1), CLU, membrane spanning 4 domain subfamily A, and ATP-binding cassette sub-family A member 7 (ABCA7) (Villegas-Llerena, Phillips, Garcia-Reitboeck, Hardy & Pocock, 2016). Of the set of inflammatory related genes identified by GWAS, a subset has also been shown to play additional roles in microglial function. The genes CD33 (Siglec 3), TREM2, CR1, phospholipase C γ2 (PLCG2), ABI gene family member 3 (ABI3) and interleukin 1 receptor accessory protein (IL1RAP) have been shown to be involved in regulation of phenotypic switching and Aβ phagocytosis (Ramanan et al., 2015a). Of these, the contributions of CD33 and TREM2 to AD pathology have been explored in a rodent model.

CD33 is a type-I transmembrane receptor and member of the sialic acid-binding immunoglobulin-type lectins (Siglec) family and is expressed exclusively on immune cells. CD33 has been shown to mediate cell-cell interactions that inhibit or restrict immune responses, although no precise role for CD33 has been described in the CNS (Crocker, McMillan & Richards, 2012). Carriers of CD33 SNPs have been shown to have higher Aβ deposition as measured through Aβ deposition in both cortical and hippocampal regions in addition to decreased soluble Aβ1-42 levels. Furthermore, primary microglia isolated from these knockout mice showed an increased ability to phagocytose Aβ1-42, whilst CD33 over expression in the BV-2 murine microglial cell line inhibited phagocytosis (Griciuc et al., 2013). Functioning as a type-I transmembrane glycoprotein, TREM2 is predominantly expressed on CNS-residing microglia. Post mortem examination of AD affected individuals reported increased levels of TREM2 within microglia surrounding Aβ plaques (Lue, Schmitz & Walker, 2014). The rs75932628 SNP identified by GWAS leads to a loss of function

mutation, with $APP_{SWE}/PS1_{\Delta E9}$ mice overexpressing this mutation demonstrating increased hippocampal Aβ deposition (Ulrich et al., 2014). These mice also exhibited impaired recruitment of microglia to Aβ plaques. Knockout of TREM2 in both $APP_{SWE}/PS1_{\Delta E9}$ and 5xFAD mice leads to increased Aβ deposition and attenuated microglial activity. It was concluded that this effect was due to the infiltration of peripheral macrophages lacking TREM2 enhancing the pathology (Wang et al., 2015). However, a second study investigating TREM2 knockout in 5xFAD mice found that reduced TREM2 expression conferred an increased Aβ load despite a similarly observed attenuated microglial function (Jay et al., 2015). Within the same 5xFAD mice, a recent study has demonstrated that gene dosage increases of TREM2 alters microglia morphology and function. Critically, increases were seen in phagocytosis related genes and alterations in plaque types (Lee et al., 2018). The contrasting nature of these two studies is thought to be attributable to the differences in Aβ deposition rates between mouse models (Leavy, 2015). Despite this, both studies show a crucial role for TREM2 in the production of the pro-inflammatory cytokines TNFα and IL1β.

APOE4 is the most commonly identified gene by GWAS undertaken, with this allele conferring the major genetic risk for LOAD. Examination of 42 families with a pedigree of LOAD were analysed for the E4 allele variant and individuals homozygous for this allele succumbed to LOAD in over 90% of examined cases (Corder et al., 1993). This gene dosage effect of APOE4 has been replicated in a number of subsequent studies. Indeed, a key comparison made in GWAS and identification of SNPs is the APO allelic variant individuals possess. Utilizing CRISPR/Cas9 editing, Lin et al. (2018) recently generated isogenic induced pluripotent stem cell lines containing either APOE3 or APOE4, which were then differentiated to microglial-like cells. APOE4 containing cells were shown to have enhanced inflammatory transcriptomes and impairment in the uptake of $A\beta_{1-42}$.

A recently completed GWAS was coupled with longitudinal positron emission tomography (PET) scans identified IL1RAP as a potential risk factor. IL1RAP is involved in phenotypic switching of microglia. Individuals with the rs12053868-G SNP had a higher chance of progressing from MCI to AD as well as higher deposition rates of Aβ, with further PET scans

revealing lower rates of microglial mediated inflammation (Ramanan et al., 2015b). This data highlights the intricate link between neuroinflammation and the immune system with Alzheimer's. An extensive study examining 85,133 independent samples from both control and LOAD individuals identified 2 novel rare coding variants in PLCG2 and ABI3, in addition to a new variant in TREM2 (Sims et al., 2017). PLCG2 encodes the protein PLCγ2, a phospholipase that hydrolyzes inositol 1,4,5-biphosphate to generate inositol 1,4,5-trisphosphate and DAG. Interestingly, this variant is protective in AD, with an odds ratio of 0.68. PLCG2 is primarily expressed in microglia, with PLCG2 variants leading to PCLG2 associated antibody deficiency and immune dysregulation disorders (Milner, 2015). ABI3 is suggested to have roles in the innate immune response, in particular through IFN mediated pathways (Sims et al., 2017). The CR1 encodes complement receptor 1, a protein involved in the regulation of the complement system in immunity. Critically, it is involved in the enhancement of phagocytosis of particles that have been opszonized (Fonseca et al., 2016). There are currently 4 identified SNPs in CR1 that confer increased AD risk (Zhu, Yu, Jiang, Wang, Cao & Tan, 2015). Critically, the aforementioned genes as well as the continued number of identified genes need to be further explored to confirm an altered functional role in microglia.

Targeting microglia

A considered approach is needed when targeting microglia therapeutically. As the CNS is composed of heterogenous microglial populations, of which a number are involved in homeostatic roles, broad eliminations of microglial function are inappropriate. Rather, a more appropriate therapeutic strategy is one that results in a net shift of populations to a more protective phenotype. Disease state is also critical in the therapeutic management of microglia. Heightened microglial activity may indeed be beneficial during early stages of disease, yet detrimental during later stages (Butovsky & Weiner, 2018). This is of critical note as clinical trials in AD are moving further towards prodromal stages of disease, aiming to prevent rather cure disease. Furthermore, issues arise when selecting what specifically to target. Certainly, targeting the associated proteins of immune related risk genes is a novel

personalised medicine approach for those that possess these mutations. However, for the vast majority of individuals this is not feasible. The precise biological mechanisms by which these genes alter AD risk remains unknown.

Currently, there are no approved selective therapeutics directed towards microglia. A number of clinical trials are however underway that work through modulating microglia. Receptor-interacting serine/threonine-protein kinase 1 (RIPK1), an enzyme downstream of TNFα signalling, has been shown to be mediate microglial responses in AD (Ofengeim et al., 2017). Denali Therapeutics purportedly has a RIPK1 inhibitor that has now entered phase 1 trials (Mullard, 2018). GliaCure has a small molecule that claims to promote microglial phagocytosis through binding of the microglial purinergic P2Y6 receptor. It is currently in phase 1, examining safety for both Alzheimer's and healthy individuals (GliaCure, 2014; GliaCure, 2015). Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to reduce amyloid burden and increase microglial numbers in AD mice (Boyd et al., 2010). Sargramostim, a recombinant form of GM-CSF, is currently in phase 2 (University of Colorado & Foundation, 2011). CSP-1103, a small molecule that binds to the APP intracellular binding domain, has been shown to modulate microglial phenotype both *in vitro* and *in vivo* (Porrini et al., 2015). It is currently in phase 3. A number of major pharmaceutical companies are indeed working with microglia and have made significant efforts to increase internal microglial research (Mullard, 2018)

Other microglial targeting therapeutics have however failed. Receptor for advanced glycation endproducts, is involved in both microglial Aβ recognition and inflammation (Burstein, Sabbagh, Andrews, Valcarce, Dunn & Altstiel, 2018). A small molecule antagonist against this receptor recently failed in phase 3 after failing to meet primary outcomes. Minocycline, an anti-inflammatory antibiotic which has classically been described as a putative microglial inhibitor has shown to be ineffective in modifying disease (Huntington Medical Research, 2012). Indeed, this historical classification of minocycline has shown to be incorrect, with evidence demonstrating that is indeed not a *bona fide* microglial inhibitor (Moller et al., 2016). As AD is indeed a heterogenous disease, reducing it to a single therapeutic target may

indeed be an incorrect approach. Future treatment strategies will most likely be combinatorial, addressing multiple aetiologies. In addition, novel microglial targets have been identified. Homing peptides directed towards microglia that contained siRNA for IRF5 resulted in a decrease in neuropathic pain (Terashima et al., 2018). Injection of microglial precursor cells transduced with TREM2 *ex vivo* in an EAE model of multiple sclerosis facilitated clearance of cellular debris and ameliorated disease (Takahashi, Prinz, Stagi, Chechneva & Neumann, 2007).

Shifting microglial phenotypes can also be achieved by modulation of the overarching process of neuroinflammation by targeting so called "master regulators". One such regulator are the type-I IFNs, which have been recently but consistently reported to be involved in microglial function. One observed role of type-I IFNs is the ability to act as master regulators of the peripheral immune response, with evidence also supporting this role within the CNS (Akira, Uematsu & Takeuchi, 2006). C57BL/6 mice lacking type-I IFN signalling exhibit an immunocompetent phenotype, but importantly fail to produce a robust neuroinflammatory response in models of traumatic brain injury, Parkinson's disease and AD (Karve et al., 2016; Main et al., 2016; Taylor, Minter, Newman, Zhang, Adlard & Crack, 2014). Microglial type-I IFN signalling has shown to be critical to microglial pathology (McDonough, Lee & Weinstein, 2017). In a number of recent studies aiming to characterise microglial phenotypes, type-I IFN signalling has been shown to be crucial for specific populations (Hickman et al., 2013; Keren-Shaul et al., 2017; Mathys et al., 2017). Blocking type-I IFN signalling in lupus prevents microglial mediated synapse loss (Bialas et al., 2017). The most attractive evidence though is a meta-analysis of microglial transcripts from 69 individual disease states including AD. Critically, across all datasets a co-regulated interferon gene set was observed (Friedman et al., 2018).

Other "master regulators" have also been identified. Removal or inhibition of iNOS in $APP_{SWE}/PS1_{\Delta E9}$ mice shifts microglial phenotype that results in decreased levels of Aβ. Functionally, this was shown to rescue cognition as measured through the radial arm maze behavioural test (Kummer et al., 2011). Deletion of TNF type 1 death receptor, the

endogenous receptor for TNFα, in APP23 mice leads to decreased levels of Aβ as well as decreased numbers of CD11b+ microglia in the entorhinal cortex (He et al., 2007). Hippocampal injections of an adeno associated virus (AAV) expressing IL1β into $APP_{SWE}/PS1_{\Delta E9}$ mice leads to increased numbers of arginase-1 positive microglia, which are associated with clearance of Aβ plaques (Cherry, Olschowka & O'Banion, 2015). Reductions in microgliosis and improvements in cognition have been reported following AAV delivery of IL4 or IL10 to hippocampal regions of $APP_{SWE}/PS1_{\Delta E9}$ mice (Kiyota, Ingraham, Swan, Jacobsen, Andrews & Ikezu, 2012; Kiyota, Okuyama, Swan, Jacobsen, Gendelman & Ikezu, 2010). In APP transgenic mice expressing astrocytic TGFβ, decreased Aβ levels in parallel with increases in overall microgliosis was reported (Wyss-Coray et al., 2001). This emerging body of work demonstrates that, at least as a proof of principle, targeting microglia does in fact ameliorate AD pathology.

Discussion

Current therapies for AD are symptomatic and for the most part ineffective. This is exacerbated by the notion that we are indeed unaware of the underlying and causative disease mechanisms in AD. There is a clear need for therapeutics that are able to target downstream effects of hallmark pathologies, and as such manage disease exacerbation and progression. One such downstream effect is neuroinflammation in which a chronic form is seen in AD. The neuroinflammatory process is complex and multifaceted, with a number of considerations required regarding its management and modulation. Critical, however, are the class of resident CNS immune cells, microglia. It is only recently that research has begun to understand and appreciate the multitude of roles they play within AD and how this contributes to the pathology. The emerging literature suggests that targeting these cells presents as a novel therapeutic area for the management of AD.

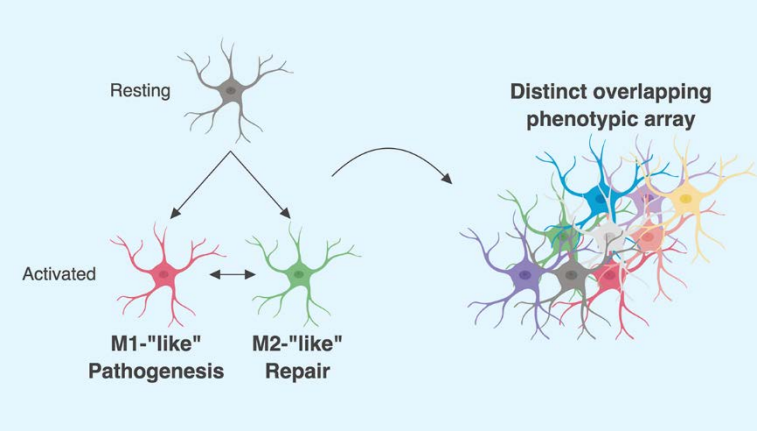

Figure 1: A proposed new framework for microglia characterization. The M1/M2 paradigm for microglial characterization is now recognized as a vast oversimplification of microglial phenotypes and functions. Rather than pigeonholing these cells as either “good” or “bad”, a context dependant label, a more appropriate view is to recognise them as a distinct array of overlapping phenotypes. Microglia can in fact have altered expression levels of both classical M1 and M2 markers. This framework also includes shifting away from notions of resting/quiescent and active microglia. Whilst this may seem an issue of semantics, not only does it not dismiss these phenotypes entirely as previously done in the past, it allows for a more holistic view when interpreting data. For simplicity, morphological differences have not been illustrated. Created with BioRender.

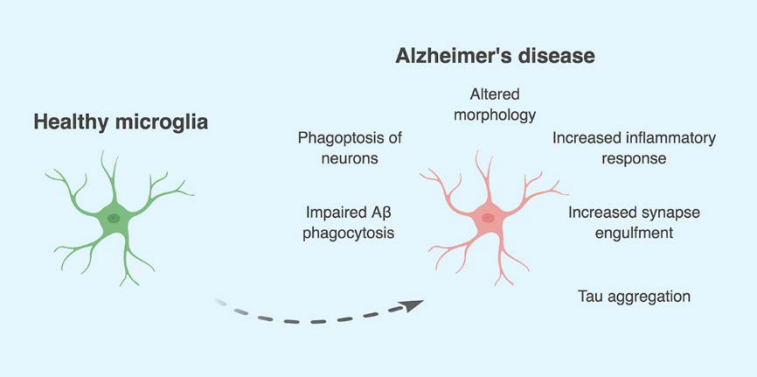

Figure 2: Alterations in microglial phenotype and function as seen in AD. A multitude of experimental evidence has now demonstrated that these changes are indeed deleterious and may in fact contribute to disease progression. Created with BioRender.

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Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY Harding et al. (2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 Alexander et al. (2017).

Conflicts of interest statement

The authors declare they have no competing interests

Authorship contribution statement

ZM, JMT and PC all contributed to the writing of this manuscript

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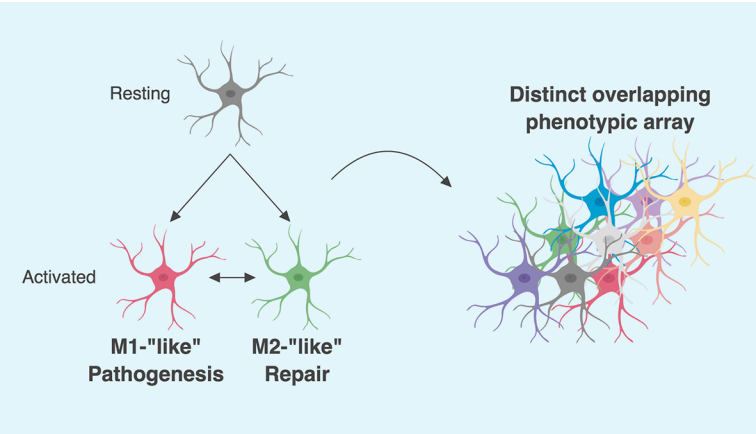

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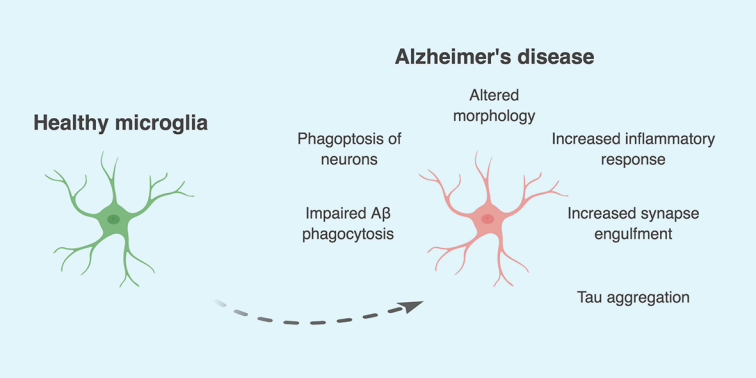

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