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REVIEW

Neuro-Immune Interactions Focus

Microglia in Alzheimer's disease at single-cell level. Are there common patterns in humans and mice?

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Alzheimer's disease (AD) is characterized by extracellular aggregates of amyloid β peptides, intraneuronal tau aggregates, and neuronal death. This pathology triggers activation of microglia. Because variants of genes expressed in microglia correlate with AD risk, microglial response to pathology plausibly impacts disease course. In mouse AD models, single-cell RNA sequencing (scRNA-seq) analyses delineated this response as progressive conversion of homeostatic microglia into disease-associated microglia (DAM); additional reactive microglial populations have been reported in other models of neurodegeneration and neuroinflammation. We review all of these microglial signatures, highlighting four fundamental patterns: DAM, IFN–microglia, MHC-II microglia, and proliferating microglia. We propose that all reported microglia populations are either just one or a combination, depending on the clustering strategy applied and the disease model. We further review single-nucleus RNA sequencing (snRNA-seq) data from human AD specimens and discuss reasons for parallels and discrepancies between human and mouse transcriptional profiles. Finally, we outline future directions for delineating the microglial impact in AD pathogenesis.

Introduction

Alzheimer's disease (AD) is the most common form of dementia, affecting more than 5.5 million Americans. Age is the major risk factor; one third of patients are over 85 yr of age. AD is initiated by the cleavage of amyloid precursor protein (APP) into amyloid β (Aβ) peptides, which are prone to form extracellular aggregates; these promote intraneuronal tau hyperphosphorylation and aggregation, which subsequently lead to synaptic dysfunction and neuronal death (Kumar et al., 2015; Long and Holtzman, 2019). AD pathology elicits a secondary response by microglia, which expand and acquire unique transcriptional and functional features. Genetic studies of human AD have demonstrated that many risk genes are expressed in microglia (Bertram and Tanzi, 2009; Cuyvers and Sleegers, 2016; Kunkle et al., 2019), suggesting that their response can impact disease onset and/or progression.

Microglia are the major phagocytic population in the central nervous system (CNS; Prinz et al., 2019). They develop from yolk sac progenitors, migrate into the developing brain, and generate a population of brain-resident phagocytes that persist for life through self-renewal, with no influx of bone marrow–derived progenitors (Ginhoux et al., 2010). Microglia contribute to CNS development and establishment of functional neuronal circuits by clearing apoptotic cells (Sierra et al., 2013), pruning neuronal synapses via complement-mediated phagocytosis (Schafer and Stevens, 2015), and releasing growth factors, enzymes, cytokines, and lipoproteins that sustain neuronal development and metabolism (Vainchtein and Molofsky, 2020). Microglia also provide a defense mechanism against pathogens and sterile insults that cause tissue damage (Prinz et al., 2019). In neurodegenerative diseases, microglia restrain extracellular protein aggregates and clear damaged neurons (Song and Colonna, 2018). However, excessive activation of microglial proinflammatory functions may be detrimental and accelerate tissue damage (Hansen et al., 2018; Villacampa and Heneka, 2020).

The recent introduction of single-cell RNA sequencing (scRNA-seq) and single-nuclei RNA sequencing (snRNA-seq) analyses has greatly advanced our knowledge of microglia responses in AD and other neurodegenerative diseases, leading to the identification of special microglial subsets associated with neurodegeneration in both mouse models and human specimens. However, the heterogeneity of the microglial signatures in various studies, their designation with different acronyms, and the discrepancies between mouse and human data have

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made it difficult to achieve a consensus on the main features, pathways, and effects of microglial responses to neurodegeneration. Here, we present an overview of microglial features in AD emerging from available single-cell transcriptomic analyses and suggest questions and challenges that transcend transcriptome analyses.

The disease-associated microglia (DAM) paradigm of microglia activation

The first scRNA-seq analysis of microglia in the 5xFAD mouse model of Aβ accumulation led to the discovery of a microglial subset distinct from homeostatic microglia named DAM (Keren-Shaul et al., 2017; Table 1). This subset featured reduced expression of genes for homeostatic markers (*Tmem119*, *P2ry12*, *P2ry13*, *Cx3cr1*, *Cst3*, *Cd33*, and *Csf1r*) and heightened expression of a vast array of genes for cell surface receptors (*Trem2*, *Tyrobp*, *Clec7a*, and *Lilrb4*), integrins (*Itgax*), tetraspanins (*Cd9* and *Cd63*), MHC-II (*Cd74*), cytokines (*Csf1*), chemokines (*Ccl6*), complement (*C3*), growth factors (*Igf1*), antimicrobial proteins (*Lyz2* and *Cybb*), molecules involved in lipid metabolism (*ApoE* and *Lpl*), iron metabolism (*Fth1*), lysosomal functions (*Cst7*, *Ctsb*, *Ctsd*, *Ctsl*, and *Ctsz*), phagocytosis (*Axl*), and tissue remodeling (*Gpnmb*, *Spp1*, *Timp2*; Table 1). Some of the DAM genes corresponded to risk genes for human AD, such as *APOE* and *TREM2*, corroborating that DAM may directly impact disease progression. Moreover, analysis of 5xFAD × *Trem2*$^{-/-}$ mice demonstrated that conversion of homeostatic microglia into DAM is a progressive change that occurs through a TREM2-independent stage (DAM1) followed by a TREM2-dependent stage (DAM2; Table 1). The DAM signature was also found in the SOD1-G93A model of amyotrophic lateral sclerosis, suggesting that DAM represent a general response to different neurodegenerative diseases although the amplitude of this response may vary (Deczkowska et al., 2018). Notably, the DAM signature was quite distinct from the conventional M1/M2 macrophage polarization profiles that had been widely used to define macrophage responses to pathology, thus establishing a new paradigm in macrophage biology.

A DAM-like signature of microglia was confirmed in four different disease models by bulk RNA sequencing (RNA-seq) of sorted FCRLS^{+} microglia (Krasemann et al., 2017; Table 1). This signature differed subtly from that identified by scRNA-seq for the down-regulation of additional homeostatic genes, which was attributed to reduced TGFβ-dependent signaling (Butovsky et al., 2014). Since this study relied on bulk RNA-seq of microglia sorted from whole brain, such a difference may reflect changes in brain microglia rather than the DAM subset alone. This study corroborated that the DAM signature is affected by TREM2 deficiency but also partially by APOE deficiency. It is likely that lack of APOE reduces Aβ plaque toxicity (Long and Holtzman, 2019; Chen et al., 2021) and affects microglia metabolism.

IFN-I and MHC-II microglia coexist with DAM

Two microglial subsets seemingly different from DAM, defined as type I IFN (IFN-I) and MHC-II, were identified by scRNA-seq in the CK-p25 mouse model of neurodegeneration (Mathys et al., 2017). CK-p25 mice express a tetracycline-controlled CDK5R1 protein in forebrain neurons. Upon removal of doxycycline, accumulation of CDK5R1 and its cleavage product, p25, cause neuronal loss and amyloidosis (Cruz et al., 2003). In contrast to most common amyloid models, neuronal loss occurs before amyloidosis in CK-p25 mice and is associated with tau hyperphosphorylation. This model revealed a microglia subset with a strong expression of MHC-II genes, whereas another subset expressed IFN-I–induced genes (Mathys et al., 2017; Table 1). Since many genes induced in CK-p25 microglia overlapped with DAM1 genes, it was proposed that IFN-I and MHC-II microglia may precede the final differentiation of DAM. IFN-I and MHC-II signatures were not unique to the CK-p25 model, as subsequent scRNA-seq studies identified them in other mouse models. One study found a microglia IFN-I signature in mouse models of Aβ accumulation (APP-PS1), tauopathy (P301S and P301L), amyotrophic lateral sclerosis, cuprizone-induced demyelination, ischemia, lipopolysaccharide, viral infection, and glioma (Friedman et al., 2018; Table 1). However, the IFN-I module was more evident in viral infection, lipopolysaccharide, and glioma than in neurodegenerative models, which, conversely, upregulated a set of "neurodegeneration-related genes" that largely overlapped with the DAM signature. Moreover, most of the entire neurodegeneration gene set was TREM2 dependent.

Another scRNA-seq study verified an IFN-I microglia subset distinct from an activated microglia subset in the APP$^{NL\text{-}G\text{-}F}$ knock-in model, in which human APP with the Swedish, Iberian, and Arctic mutations is driven by the endogenous *App* promoter (Sala Frigerio et al., 2019). In this model, microglia transitioned from a partially activated state marked by expression of MHC-II and ApoE to two terminally activated states, one with heightened expression of MHC-II and DAM genes and the other denoted by IFN-I–induced genes (Table 1). In snRNA-seq analysis of 5xFAD mice at different stages of pathology (Zhou et al., 2020), no MHC-II subset was formally distinguished from DAM (Table 1); however, MHC-II–related genes were increasingly upregulated during progression of the disease, paralleling a similar phenomenon in CK-p25 (Mathys et al., 2017) and APP$^{NL\text{-}G\text{-}F}$ mice (Sala Frigerio et al., 2019), which suggests that exacerbation of pathology may amplify the MHC-II signature. Furthermore, the DAM and MHC-II signatures were associated with enhanced expression of *Hif1a* and the IFN-I–inducible *Ch25h* (Zhou et al., 2020), which have been associated with aging and AD (Shibata et al., 2006; Ashok et al., 2017; Srinivasan et al., 2020). While more easily identifiable in disease models such as CK-p25 or P301S mice (Mathys et al., 2017; Wang et al., 2021), it is plausible that IFN-I and MHC-II signatures coexist with DAM in all models analyzed but are sometimes overshadowed by the DAM signature. If so, IFN-I and MHC-II microglia can be deconvoluted from the DAM population by high-resolution microglia subclustering, provided that enough cells are originally sequenced. Distinct MHC-II and DAM subsets were observed in the cuprizone model of demyelination (Masuda et al., 2019), suggesting that coexistence of DAM, MHC-II, and other microglial signatures occurs in demyelination.

Proliferating microglia: A parallel or converging trajectory?

In addition to the homeostatic, DAM, IFN-I, and MHC-II populations, two recent scRNA-seq studies in the 5xFAD model

Table 1. **Summary of microglial gene signatures reported in neurodegenerative mouse models**

Publication		Keren-Shaul et al., 2017			Zhou et al., 2020*	Krasemann et al., 2017	Sala Frigerio et al., 2019		Hammond et al., 2019a	Li et al., 2019	Safaiyan et al., 2021	Mathys et al., 2017		Friedman et al., 2018*	Wang et al., 2020 & Ellwanger et al., 2021*	
Method		scRNA-seq			snRNA-seq	RNA-seq	scRNA-seq		scRNA-seq	scRNA-seq	scRNA-seq	scRNA-seq		RNA-seq	scRNA-seq	
Disease model		5xFAD; hSOD1			5xFAD	APP/PS1; hSOD1	APP^{NL-G-F}		C57BL/6J	C57BL/6N	C57BL/6J; 5xFAD	CK-p25		PS2APP; 5XFAD; P301S/L	5xFAD + anti-TREM2	
Clusters / Signature	Genes	DAM (6 mo old)	DAM1	DAM2	DAM (15 mo old)	MGnD	ARM	IRM	ATM	PAM	WAM	IFN	MHC	IFN	MHC	CycM
Homeostatic	*Tmem119*															
	P2ry12															
	P2ry13															
	Csf1r															
	Hexb															
	Cst3															
	Cx3cr1															
	Siglech															
	Cd33															
	Tgfbr1															
	Sall1															
	Selplg															
	Mef2a															
	Jun															
	Ms4a6d															
	Bin1															
	Serinc3															
DAM-like	*Cd9*															
	ApoE															
	Trem2															
	Tyrobp															
	Clec7a															
	Cd63															
	Lgals3															
	Axl															
	Spp1															
	Cstb															
	Cstz															
	Cstl															
	Ctsd															
	Lpl															
	Itgax															
	B2m															
	Cst7															
	Csf1															
	Gpnmb															
	Igf1															
	Lilrb4															
	Irf8															
	Fth1															
	Lyz2															
	Ccl3															
	Ccl6															
	Timp2															
IFN	*Irf7*															
	Ifitm3															
	Ifit2															
	Ifit3															
	Cxcl10															
	Oasl2															
	Cd69															
	Isg15															
	Usp18															
MHC	*H2-D1*															
	H2-K1															
	H2-Eb1															
	H2-Aa															
	H2-Ab1															
	H2-DMa															
	Cd74															
Cyc-M	*Top2a*															
	Mki67															
	Cenpe															
	Mcm5															
	Birc5															
	H2afz															
	H2afv															

Dark blue, downregulated signature genes; light blue, additional downregulated genes; orange, upregulated signature genes; yellow, additional upregulated genes. *, these papers extend the profiles of defined clusters, or report a novel cluster. To show the difference between DAM1 and DAM2, only the top genes that were used for this characterization are listed. MGnD, microglial neurodegenerative phenotype; ARM, activated response microglia; IRM, IFN response microglia; WAM, white matter–associated microglia; IFN, IFN-I imprinted; MHC, MHC expressing; Cyc-M, (G)2/M phase–enriched cluster (proliferating microglia).

identified a fifth microglial population enriched for cells in growth (G)2/mitotic (M) phase with a proliferation module (Cyc-M cluster) featured by the expression of *Top2a*, *Mki67*, *Cenpe*, *Mcm5*, *Birc5*, etc. (Wang et al., 2020; Ellwanger et al., 2021; Table 1). The identification of proliferating microglia corroborated previous reports of a cluster of microglia expressing the

proliferation marker Ki67 around Aβ plaques in 5xFAD mice (Wang et al., 2016) and of a limited increase of mitotic genes in neurodegenerative models (Friedman et al., 2018). In one of these scRNA-seq studies, a trajectory analysis further addressed the relationships among all microglial populations (Ellwanger et al., 2021), showing that homeostatic microglia differentiate through a continuum of progressively activated states, which ultimately branch into four separate trajectories: DAM, IFN-I, MHC (expressing both MHC-II and MHC-I genes), and proliferating (Cyc-M). Further studies will be necessary to verify whether any of these trajectories convert into another at some point. The representation of all four terminal fates was reduced in 5xFAD × *Trem2*$^{-/-}$ mice, demonstrating a general requirement of TREM2 for microglia activation. However, a single injection of anti-TREM2 antibody in adult 5xFAD mice with advanced Aβ aggregation selectively expanded proliferating microglia and had little effect on other differentiation fates (Wang et al., 2020; Ellwanger et al., 2021). Since *Trem2*$^{-/-}$ mice have a constitutive microglial defect that precedes the onset of Aβ aggregation, the limited impact of antibody-mediated TREM2 activation on microglia may be dictated by the late and transient timing of this intervention. Early and/or sustained TREM2 engagement may be necessary to induce the DAM, IFN-I, and MHC-II microglial populations.

DAM-like signatures in development and aging

Microglia responses to AD pathology recapitulate, at least in part, microglial function during CNS development, such as phagocytosis of apoptotic cells and damaged myelin. Thus, it is not surprising that the "DAM" paradigm has been confirmed by scRNA-seq during CNS development. One example is the axon tract-associated microglia, which appear in postnatal day 4 (P4)/P5 mice but are absent in embryonic day 14.5 mouse embryos and P30-P100 adult mice (Hammond et al., 2019; Table 1 and Fig. 1). These microglia, which were found along axon tracts that become heavily myelinated and were shown to prune synapses in a transient time window of brain development (Schafer and Stevens, 2015), exhibited a DAM-like expression profile. Similar DAM-like microglia were reported to be present in P7 mice in association with postnatal myelination and scarce in adult mice (Li et al., 2019; Table 1 and Fig. 1). Remarkably, this study showed that a lack of TREM2 or APOE, both of which affect DAM differentiation in AD pathology, has no impact on DAM-like microglia during development. It is possible that myelin and synapse debris trigger pathways of activation partially different from those elicited by Aβ aggregates. Moreover, other glial cells or neuronal cells may produce soluble factors during development that can compensate for TREM2 and/or APOE deficiency (Li and Barres, 2018). Another report showed that activated microglia with a DAM signature are present in the mouse embryonic brain but diminish postnatally, allowing another microglia population expressing *Cst3* and *Sparc* to populate the postnatal brain (Masuda et al., 2019). Altogether, these studies concur that waves of activated microglia with a DAM-like profile differentiate during critical stages of CNS development to facilitate recycling of lipid components resulting from remodeling of neuronal synapses and myelin (Fig. 1).

Physiological aging is associated with an accumulation of degenerative features in the CNS, which also elicit the differentiation of DAM, although with delayed kinetics compared with AD pathology (Fig. 1). Accordingly, a recent study identified a microglia population within the white matter that develops with aging, partially overlaps with DAM, and may eventually progress into DAM with further accumulation of tissue damage (Safaiyan et al., 2021; Table 1). This signature was affected by TREM2 deficiency, but not by APOE deficiency, unless the aged mice also carried the APP/PS1 transgene mediating Aβ accumulation (Safaiyan et al., 2021), suggesting that the impact of APOE on the DAM signature during aging is minimal unless AD pathology is present (Krasemann et al., 2017; Wang et al., 2021).

DAM-like signatures in microglia repopulation

Microglia development and maintenance is dependent on engagement of the CSF1 receptor by IL-34 secreted by neurons and CSF1 released by astrocytes and microglia themselves (Chitu et al., 2016). Administration of small molecules that inhibit CSF1R, such as PLX5622, induce a profound depletion of microglia, which is reversed upon suspension of treatment; residual microglia proliferate and repopulate the CNS (Bruttger et al., 2015; Huang et al., 2018), although the origin of the proliferating cells is a matter of debate (Elmore et al., 2014; Jäkel and Dimou, 2017; Askew et al., 2017). A recent scRNA-seq analysis of microglia 2 d after removal of PLX5622 divulged a decline in the homeostatic signature, paralleled by the appearance of three distinct signatures: one enriched in mitosis markers, a second expressing MHC-II, and a third DAM-like signature with elevated expression of chemokines (Zhan et al., 2020). All subsets expressed Galectin-3 (MAC2 or Lgals3), which has been reported to be a ligand for TREM2 (Boza-Serrano et al., 2019). Why repopulating microglia acquire MHC-II and DAM-like characteristics rather than homeostatic features is not clear. Given that CSF1R inhibitors are being tested as potential therapeutic agents in the treatment of AD and neurodegenerative diseases (Waisman et al., 2015; Shi et al., 2019; Spangenberg et al., 2019; Casali et al., 2020; Green et al., 2020), it will be important to establish whether and how repopulating microglia modulate pathology.

Discrepancies between human and mouse microglia signatures in neurodegeneration

Microglial signatures obtained from snRNA-seq of human AD brain specimens evince considerable heterogeneity and seem to differ from mouse signatures. In an initial study, AD-enriched modules mainly included genes for myelination and neuronal survival, whereas no DAM signature was observed (Mathys et al., 2019; Table 2). Major gene expression changes included (1) up-regulation of heat shock protein chaperones, perhaps indicative of a reported unfolded protein response to tau aggregation (Ballatore et al., 2007; Ittner and Götz, 2011); (2) up-regulation of the neuron apoptosis regulator *LINGO1*; (3) down-regulation of the neuron regeneration genes *NEGR1*, *BEX1*, and *NTING1*; and (4) sex bias for some AD-related changes, including a more drastic reduction of inhibitory neurons in females than in

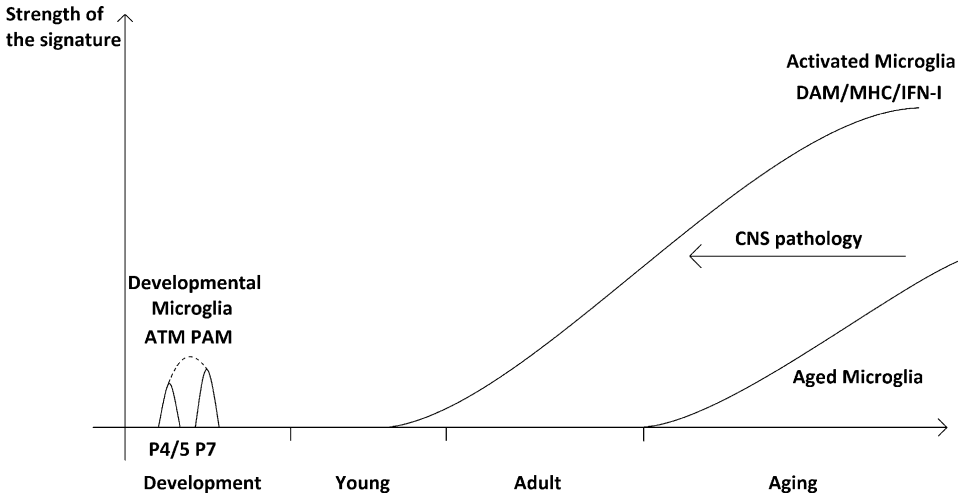

Figure 1. **Temporal appearance of microglial subsets with activated signatures in mouse life span.** Signatures of proliferative region–associated microglia (PAM) and axon tract–associated microglia (ATM) transiently appear in early stages of postnatal development. The dashed line above ATM and PAM indicates the possibility that ATM and PAM may belong to the same wave of microglia activation. Moreover, microglia with DAM/MHC/IFN-I activation signatures slowly increase with aging, a process that is accelerated in models of neurodegeneration.

males. Only a few microglia genes, including *APOE* and *SPP1*, were upregulated (Table 2). A subsequent study in which more nuclei/specimens were sequenced identified an incomplete DAM pattern with up-regulation of *TREM2*, *APOE*, *CD68*, and *HLA-DRA* (Zhou et al., 2020; Table 2). Other key DAM genes were either undetected (*CST7*, *GPNMB*, and *LPL*) or downregulated (*SPP1*), while expression of some homeostatic signature genes was heightened (*TMEM119*, *P2RY12*, and *CX3CR1*). Enhanced expression of genes not reported in mouse models of AD was also observed. These included *IRF8*, a regulator of reactive microglia (Kierdorf et al., 2013); *SORL1*, an AD risk factor involved in APOE uptake and APP trafficking (Rogaeva et al., 2007; Li et al., 2008); the chitinase-like protein *CHI3L1*, a CSF biomarker for preclinical AD; and *Alpha-2-macroglobulin*. Subsequent -omics analyses of human AD also reported an incomplete DAM signature. Few DAM signatures genes (*ApoE* and *HLA-DRA*), together with other genes such as *CD163* and *HIF1A*, were also captured by another snRNA-seq study (Grubman et al., 2019; Table 2). In one study of prefrontal cortex specimens, a small population of AD-specific microglia showed up-regulation of genes for complement (*C1QA*, *C1QB*, and *C1QC*) and cytokine receptors (*IL4R* and *IL1-RAP*; Lau et al., 2020; Table 2). In another study of patients carrying PSEN1 mutations causing familial AD, the top five differentially expressed microglial genes included *EEF1A1*, *GLULL*, *KIAA1217*, *LDLRAD3*, and *SPP1* (Del-Aguila et al., 2019), together with ∼20 DAM genes (Table 2).

A novel snRNA-seq approach allowing for increased numbers of nuclei sequenced/specimen characterized two microglial populations; dystrophic microglia expressed genes for iron metabolism (*FTL* and *FTH1*), and amyloid-responsive microglia expressed *CD163*, *BIN1*, *MS4A6A*, and *CELF1* (Nguyen et al., 2020; Table 2). Although *CD163* expression correlated with AD-associated *APOE* (*APOE4*) and *TREM2* ($TREM2^{R47H}$) polymorphisms, the amyloid-responsive microglia evoked no DAM signature except for a slight up-regulation of *APOE*, while other DAM genes were downregulated. Conversely, the highest expression of *TREM2*, *APOC1*, *APOE*, *HLA-DRA*, and *HLA-DRB1* was noted in the dystrophic cluster. The relationship between these microglial populations remains to be defined. The limited overlap between human and mouse AD microglial signatures was corroborated by proteomics of human AD specimens, which revealed an AD-associated astrocyte-microglial metabolism module characterized by high expression of CD44, PRDX1, and DDAH2, while only few of the proteins in this module mirrored the mouse DAM phenotype (Johnson et al., 2020). scRNA-seq profiles of surgically dissected human brain specimens from patients with multiple sclerosis revealed various microglia populations expressing DAM genes, although multiple sclerosis and AD are quite different diseases (Masuda et al., 2019); this implies that at least some of the DAM genes may reflect a conserved pattern of microglial responses to different CNS diseases, despite the heterogeneity of brain pathology.

Biological and technical bases for microglial profiles heterogeneities in AD

One biological reason for the differences between human and mouse microglial signatures is that human specimens represent a terminal stage of AD with amyloid and tau pathology, as well as extensive neuronal cell death (Kumar et al., 2015; Long and Holtzman, 2019); mouse specimens, however, often encapsulate either earlier stages of the disease characterized most prominently by Aβ accumulation or frontotemporal dementia–like tauopathy without amyloidosis (Götz et al., 2018). To determine how human microglia respond to progressive stages of AD with either amyloid pathology or tauopathy, a recent study performed snRNA-seq after separating the occipital cortex (Gerrits et al., 2021), which mainly develops amyloid pathology, from the occipitotemporal cortex, which accumulates both amyloid aggregates and neuronal fibrillary tangles (Jucker and Walker, 2013, 2018). This approach led to the identification of

Table 2. **Summary of human microglial gene signatures**

Publication		Mathys et al., 2019	Zhou et al., 2020	Grubman et al., 2019	Lau et al., 2020	Del-Aguila et al., 2019	Masuda et al., 2019	Olah et al., 2020	Sobue et al., 2021	Nguyen et al., 2020		Gerrits et al., 2021
Method		snRNA-seq	snRNA-seq	snRNA-seq	snRNA-seq	snRNA-seq	scRNA-seq	scRNA-seq	snRNA-seq	snRNA-seq		snRNA-seq
Microglia #		955 (AD) 965 (C)	919 (AD) 1547 (C)	449 (Total)	~7966 (Total)	79 (LOAD) 05 (ADAD)	422 (MS) 1180 (C)	8543 (AD)	N.A.	1441 (Aβ) 2226 (Aβ&Tau) 315 (C)		95587 (AD) 51655 (C)
Clusters / Signature	Genes		DAM(Partial)		C1q&Cyto			DAM/IFN		CD163	Dystrophic	GRID2
Homeostatic	*TMEM119*											
	P2RY12											
	P2RY13											
	CX3CR1											
	SELPLG											
	BIN1											
DAM-like	*CD9*											
	APOE											
	TREM2											
	TYROBP											
	CD63											
	CD68											
	AXL											
	SPP1											
	CTSB											
	CTSD											
	LPL											
	ITGAX											
	CST7											
	CSF1											
	GPNMB											
	IRF8											
	FTH1											
IFN	*IFITM3*											
	IFIT3											
	CXCL10											
	STAT1											
	IRF7											
	ISG15											
MHC	*HLA-A/E*											
	HLA-DRB1											
	HLA-DPB1											
	CD74											
	HLA-DRA											
	CD83											
	CD81											
Other	*A2M*											
	CHI3L1											
	SORL1											
	SOCS6											
	SLC11A1											
	S100A8											
	S100A9											
	HAMP											
	C1QA											
	C1QB											
	C1QC											
	IL4R											
	IL1RAP											
	EEF1A1											
	GLU1L											
	KIAA1217											
	LDLRAD3											
	APOC1											
	LGALS1											
	CCL2											
	CCL4											
	EGR3											
	FTL											
	INPP5D											
	ST6GAL											
	CSF3R											
	MS4A6A											
	CD163											
	GRID2											
	HSPA1A											
	GAPDH											
	CRYAB											
	HSPB1											
	HSP90AA1											
	HSPA8											

Light blue, downregulated genes; yellow, upregulated genes; gray, not detected; orange, used in signature definition. Genes present in Table 1 but absent in Table 2 were not differentially expressed in human samples. The table includes a partial list of the main differentially expressed genes discovered in the referenced papers. C1q&Cyto, C1q and cytokines; C, control; NA, not available.

three populations: (1) homeostatic microglia expressing *P2RY12* and *CX3CR1*; (2) amyloid-associated microglia expressing DAM genes; and (3) tauopathy-associated microglia expressing the gene for the glutamate receptor *GRID2*, which was highly enriched around neuritic plaques but may not be functional (Gerrits et al., 2021; Table 2). Future investigation of microglial genes in mouse models of tauopathy are warranted to further advance the notion of tauopathy-associated microglia. Other biological reasons that may explain the heterogeneous detection of DAM signature genes in human AD are: (1) the different brain regions from which the specimens originate; (2) the genetic diversity of AD patients; and (3) the disparate ethnic origins of the various cohorts analyzed, which may impact both the genetic background and environmental factors regulating AD.

There are also several technical reasons that can contribute to human–human and human–mouse discrepancies in the AD profiles. While DAM were mainly identified by scRNA-seq, almost all microglial profiles in human AD were determined by snRNA-seq. The number of microglia nuclei sequenced may be insufficient to identify a DAM cluster (Table 2); indeed, DAM and IFN-I signatures were detected in AD patients by bulk RNA-seq, which provides more depth (Sobue et al., 2021), and SPP1 was detected in two studies in which large numbers of nuclei were sequenced (Mathys et al., 2019; Del-Aguila et al., 2019). Additionally, mRNAs may be less abundant in the nucleus vs. cytosol; accordingly, mRNA of key DAM markers was less abundant in nuclei than in whole cells obtained from the same individuals by neocortical resection (Thrupp et al., 2020). Finally, the overall quality of human RNA samples may be poorer than mouse samples due to postmortem intervals preceding human sample collection and processing. Indeed, a study showed that human postmortem samples lost the expression of certain mRNA, including SPP1 and SOCS3 (Dachet et al., 2021). Moreover, this study showed an up-regulation of CD68 in microglia with postponed fixation for tissue staining. Studies of human samples promptly collected after neurosurgery or autopsy and analyzed by scRNA-seq have reported microglial profiles more similar to those identified in mouse models (Masuda et al., 2019; Olah et al., 2020). Thus, differences in tissue collection and processing may contribute to heterogeneity of human samples and human–mouse discrepancies.

Innovative approaches for investigating human microglia in AD

Beyond high-resolution transcriptional profiling, several additional approaches have been developed to investigate human microglia in AD and understand the impact of AD risk variants in their functions. Microglia have been generated from human induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs; Douvaras et al., 2017; Abud et al., 2017; Haenseler and Rajendran, 2019). DAM-like signatures were observed in microglia from human ESCs in which familial AD-associated mutations were introduced in APP and PSEN1 genes (Liu et al., 2020). TREM2 dependency of DAM was reproduced in TREM2-sufficient and TREM2-deficient human iPSCs (Reich et al., 2021). Since culture conditions may not fully reproduce the complexity of CNS microenvironment (Gosselin et al., 2017), iPSC- and ESC-derived microglia have been transplanted into the mouse CNS (Hasselmann et al., 2019; Mancuso et al., 2019; McQuade et al., 2020). Transplanted human ESC–derived microglia not only retained a transcriptome profile distinct from that of endogenous mouse microglia for 2 mo after intracranial injection (Hasselmann et al., 2019) but also recapitulated some key microglia patterns observed in human AD samples when injected together with oligomeric Aβ (Mancuso et al., 2019). Moreover, microglia from human iPSCs with or without the *TREM2* gene were injected into the brain of humanized 5xFAD × MITRG xenotransplantation-compatible mice (*CSF1*, *CSF2*, *TPO*, *Rag2* knockout, and *Il2rg*- knockout); comparison of the two revealed TREM2-dependent DAM- and MHC-II–related signatures (Hasselmann et al., 2019; McQuade et al., 2020).

While genetic studies have identified many risk variants for human AD potentially affecting microglia, most of the studies have focused on coding variants of *APOE* and *TREM2*. However, many AD risk variants are present in noncoding regions of the human genome, affecting enhancers regulating gene expression in microglia (Nott et al., 2019). The introduction of genome-wide analyses of chromatin-accessible regions and histone modifications coupled with single-cell analyses has significantly advanced our understanding of the microglia regulome and how it is affected by AD risk variants (Gosselin et al., 2014, 2017). For example, although the bridging integrator 1 (*BIN1*) gene is expressed in both neurons and microglia, it has been shown that the AD risk variant of *BIN1* affects an enhancer active in microglia, but not in neurons, suggesting that this variant predisposes to AD by affecting microglial rather than neuronal functions (Nott et al., 2019). The precise definition of promoter–enhancer regulomes in humans and mice may also explain some of the discrepancies between human AD and mouse models.

Finally, because requiring disruption of tissues into cell or nuclei suspensions, scRNA-seq and snRNA-seq analyses cannot define the precise location of microglia subsets and signatures within CNS niches and potential interactions with other cells. However, novel technologies have been developed to overcome this limitation; for example, spatial transcriptomics has been applied to study $App^{NL\text{-}G\text{-}F}$ mice, which confirmed the association between DAM and amyloid plaques (Chen et al., 2020b). Strategies that combine multiplexed fluorescence in situ hybridization with sequencing, known as seqFISH and MERFISH, have also been developed (Moffitt et al., 2018; Eng et al., 2019). For these techniques, RNAs are imprinted with oligo-conjugated barcodes that are measured through sequential rounds of hybridization and super-resolution imaging. Application of such technologies to neurodegeneration will provide information on individual cells within their environment of surrounding cells and AD pathology.

Conclusions

Overall, the studies in mouse models discussed here have shown fundamental and consistent patterns of microglial activation in response to AD pathology: DAM, IFN-I microglia, MHC-II microglia, and proliferating (Cyc-M) microglia. We conclude that all currently characterized microglia subsets correspond to either one of them or a combination, depending in part on the clustering strategy applied to the analysis, as well as the AD model being assessed (Table 1). The DAM, MHC-II, and IFN-I signatures appear not only in models of neurodegeneration but also during development and in aged mice, although to a lesser

extent (Fig. 1). On the contrary, human microglia are more heterogeneous (Table 2). Beyond a detailed description of different microglia subsets, it will be essential to investigate the biological functions of these populations and determine whether they have a beneficial or detrimental impact in AD progression. DAM genes encode TREM2 and phagocytic molecules that may slow down the progression of disease. However, other molecules, such as inflammatory cytokines and chemokines, have not been thoroughly studied. MHC-II expressed by microglia in AD may present antigens. Studies have shown a strong correlation between expression of MHC-II and ApoE in the cuprizone model of demyelination (Olah et al., 2012; Poliani et al., 2015; Masuda et al., 2019). Moreover, microglial secretion of ApoE may contribute to capture of lipoprotein antigens that can be processed and presented (Bonacina et al., 2018). Thus, it is possible that $ApoE^{+}MHC\text{-}II^{+}$ microglia present lipoprotein antigens during aging and neurodegenerative diseases.

Although the studies reviewed here mainly focus on microglia, high-resolution single-cell and single-nuclei analyses in neurodegenerative and neuroinflammatory diseases are currently being extended to other brain macrophages and $CCR2^{+}$ monocytes, which might infiltrate the CNS and convert into microglia-like cells in certain contexts (Mrdjen et al., 2018; Chen et al., 2020a). A wealth of transcriptome single-cell data are also becoming available for other glial cells, such as astrocytes and oligodendrocytes, as well as neurons (Mathys et al., 2019; Mundt et al., 2019; Jordão et al., 2019; Zhou et al., 2020; Habib et al., 2020; Leng et al., 2021). Integration of these analyses with microglia data will provide a global view of cellular responses to AD pathology in humans and mice.

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