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The influence of environment and origin on brain resident macrophages and implications for therapy

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Microglia are the tissue-resident macrophages of the brain and spinal cord. They are critical players in the development, normal function, and decline of the CNS. Unlike traditional monocyte-derived macrophages, microglia originate from primitive hematopoiesis in the embryonic yolk sac and self-renew throughout life. Microglia also have a unique genetic signature among tissue resident macrophages. Recent studies identify the contributions of both brain environment and developmental history to the transcriptomic identity of microglia. Here we review this emerging literature and discuss the potential implications of origin on microglial function, with particular focus on existing and future therapies using bone-marrow- or stem-cell-derived cells for the treatment of neurological diseases.

Microglia are the highly dynamic and process-bearing tissue resident macrophages of the brain and spinal cord, with increasingly recognized and central roles in health and disease. It is well established that during normal development and homeostasis, microglia constantly survey the local microenvironment, eliminate supernumerary synapses, and clear apoptotic cell corpses[1]. Microglia also rapidly respond to CNS injury and damage in myriad ways, including the secretion of cytokines, migration toward the site of an injury, and phagocytosis of debris[2,3].

Like other macrophages, microglia develop from hematopoietic progenitors, but they are the only member of the immune system to permanently reside in the brain tissue itself, making their identity the product of a unique combination of origin and environmental exposures. Here we first review a burgeoning literature on the developmental origins of microglia, focusing on the key factors responsible for their distinct transcriptomic identity and functions. We then describe the relative contributions of developmental origin and brain environmental signals to establishing and regulating microglial identity. Finally, we highlight the importance of macrophage origin to the pathogenesis of human diseases and describe the promise and challenges of creating new microglia-based therapies.

Microglia are unique CNS residents that arise from primitive hematopoiesis and that self-renew

Unlike astrocytes and oligodendrocytes, which share their developmental origin with neurons, microglia are derived from the hematopoietic system during embryogenesis. There are three 'waves' of hematopoiesis: (i) primitive hematopoiesis, which occurs in the extraembryonic yolk sac (YS) and gives rise to nucleated erythroblasts and some macrophages, (ii) a 'transient' wave in which erythromyeloid progenitor cells (EMPs) migrate from the YS to the fetal liver, and finally (iii) definitive hematopoiesis, during which hematopoietic stem cells (HSCs) give rise to all cell lineages[4]. Microglia are formed with the very first wave in the YS at the onset of the second embryonic week in rodents (Fig. 1). First hypothesized after the identification of F4/80$^+$ cells in the developing brain before the onset of definitive hematopoiesis, the unique origin of microglia was confirmed by genetic fate mapping using inducible *Runx1*^{Cre} mouse lines to trace the trajectory of YS-derived cells into the brain[5–7]. Since this discovery, fate labeling has revealed that most, if not all, tissue-resident macrophages initially arise from embryonic hematopoiesis[8–13]. YS-derived EMPs formed during the second wave migrate to the fetal liver, where they differentiate into either premacrophages or monocytes before seeding non-CNS tissues and replacing first-wave-derived macrophages[7,11,13,14]. Although microglia are thought to be wholly first-wave-derived, it remains somewhat controversial; a subset of microglia are labeled in a constitutive *Hoxb8*^{Cre} mouse line[15]. As labeled cells are rare in the YS but more abundant later in the fetal liver, it is hypothesized that a subset of microglia may derive from the second wave[16]. Conversely, however, microglia are not marked by a fetal monocyte driver line, *S100a4*^{Cre}[17], leaving the degree to which newcomers from the fetal liver contribute to the CNS pool unknown. The advent of more specific drivers and surface marker tools may help adjudicate the remaining controversies surrounding microglial origin (see Table 1 for existing approaches). Understanding the origin of tissue macrophages generally and microglia specifically is important not only to better characterize local immunity and network function, but also in consideration of potential surrogates for therapeutic replacement. Regardless of their initial origins, resident macrophages in most tissues other than the brain are progressively replaced by HSC-derived cells from definitive hematopoiesis[10,18]. Interestingly, adult zebrafish microglia are likewise replaced by *c-Myb*-dependent HSC-derived fetal monocytes[19]. This contrasts with mammalian microglia and a few other tissue macrophages, which are *Myb*-independent and are not replenished by circulating cells in the absence of injury or disease[17,20,21].

Given that microglia are not replaced by HSC-derived cells, how are microglia maintained? In the healthy brain, microglia are long-lived, with an estimated turnover rate of 8–41 months in postnatal mice, depending on brain region[22–24]; in contrast, estimates for circulating monocytes are on the order of hours (for 'classical' monocytes) to days (for 'patrolling' monocytes)[25]. Human microglia are likewise thought to be one of the most long-lived myeloid cells in

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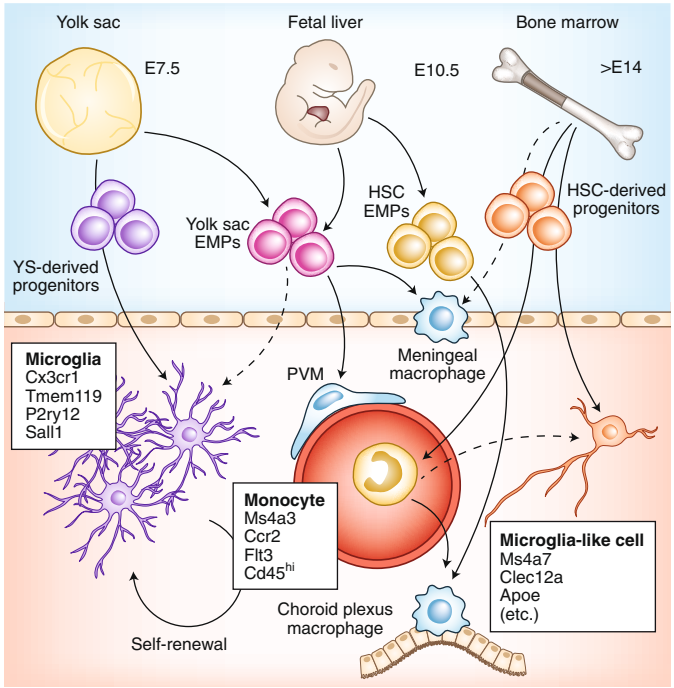

Fig. 1 | Origin of microglia and microglia-like cells. Microglia are derived from primitive hematopoiesis. YS progenitors migrate to the developing brain before embryonic day (E) 10.5. Microglia self-renew throughout life and are marked by high expression of Cx3cr1, Tmem119, P2ry12, and Sall1. Other brain-border-associated macrophages in the meninges and perivascular (PVMs) tissues may initially derive from YS progenitors (premacrophages, primitive macrophages), though their progenitors (EMPs) may also journey through the fetal liver; choroid plexus macrophages are HSC-derived and either derive from HSC-origin EMPs or blood monocytes as depicted. Blood monocytes are HSC-derived as they are marked by Flt3, as well as Ccr2, and have high expression of CD45. Transplantation studies reveal that HSC-derived microglia-like cells express unique molecular markers, including Clec12a and Ms4a7, as well as high levels of Apoe. Solid arrows depict well-established origins; dashed arrows show hypothesized or less well-established origins. See text for citations.

the body, with some microglia predicted to live for 20 years[26,27]. In addition, the discovery of Csf1r as an obligate microglial survival receptor[28] and the subsequent development of tools for microglia depletion provided clear evidence that microglia self-renew[20], serving as their own progenitors. Using small-molecule inhibitors, genetic ablation, whole body irradiation, or a combination thereof, up to 99% can be depleted within 2–14 days (Table 2). Following depletion, microglia rapidly repopulate the CNS and appear transcriptionally normal within weeks, even after depletion to less than 2% of normal numbers[29–32]. This repopulation occurs in proliferating clusters of cells and may require interleukin-1 receptor expression[31]. Using *Cx3cr1*^{CreER};*tdTomato*$^{loxP/loxP}$ mice to label microglia before acute depletion, a recent study showed that repopulating cells are derived from residual microglia[33]—a feat that highlights the incredible proliferative capacity of microglia. The ability of microglia to self-renew, paired with their long life span, suggest microglia live a distinct and somewhat isolated existence within the CNS.

The rise of RNA-sequencing technologies and the recognition that microglia derive from the embryonic yolk sac led to widespread interest in understanding potential genetic differences between microglia and other macrophages[34–39]. Profiles of sorted cells showed that microglia express a unique complement of 'signature genes' not expressed by other tissue macrophages, including *Tmem119*, *P2ry12*, *Slc2a5*, *Olfml3*, and *Sall1*. Many microglia-specific or -enriched genes are developmentally upregulated, reaching mature levels by postnatal day 14 (P14) in mice, which corresponds with an overall normalization of gene expression and microglial maturity[13,39,40]. Importantly, most genes with orthologs are also microglia-specific in human, though some, such as *Hexb*, *Sparc*, and *Sall3*, are more specific in mouse[39,41,42]. Single-cell RNA sequencing of microglia throughout the lifespan further characterized the developmental changes of microglia and revealed increased heterogeneity during development that largely normalizes in the healthy adult, but reemerges during disease[43–47]. An example of this heterogeneity is the identification of a distinct, transient 'subtype' of microglia is axon-tract- or white matter-associated microglia that express high levels of *Spp1*, *Igf1*, *Lgals1*, *Lgals3*, and *Gpnmb*. These white matter-associated microglia appear between P4 and P7 and are localized to axon tracts destined for myelination[45,46]. Interestingly, these cells, which share genetic similarities with previously described Cd11c^{+} microglia that may promote myelination[48], appear to phagocytose newly formed oligodendrocytes[46]. Finally, several studies have recently revealed the global control of microglial identity by super-enhancers and epigenetic modifications[36,42,49]. Epigenetic changes also regulate microglial function, as illustrated by selective loss of repressive histone methylation: microglia-specific deletion of PRC2, which trimethylates histone H3K27, de-represses phagocytic gene programs within striatal and cortical microglia and leads to abnormal medium spiny neuron morphology and function[50]. Overall, microglial origin and identity are unique among macrophages, with important but incompletely understood implications for brain health and disease.

Both environment and origin influence microglial identity

The CNS is a distinct environment, guarded behind a tightly regulated barrier. To what extent is microglial identity dictated by this unique environment? Studies of microglia in culture reveal the profound influence of the CNS milieu. Isolated microglia dramatically lose their microglia-specific gene signature—as demonstrated by near complete loss of *Tmem119*, *Sparc*, and *P2ry12* expression and epigenetic remodeling in cultured microglia within hours[42,51]. Perhaps reflective of microglial responsiveness to brain injury, isolation methodology also has large effects on gene expression. For example, enzymatic dissociation and lengthy cell preparation steps result in steep upregulation of genes associated with activation, including *Tnf* and *Il1a*[39,42,45]. One hypothesis is that activation itself drives microglia signature gene loss. Indeed, expression of most microglial signature genes decreases after lipopolysaccharide (LPS)-induced inflammation[39,52] and in several disease models characterized by microglial activation[44,53]. It remains unclear how infiltrating macrophages might skew these profiles and the relationship between activation and identity. Incredibly, transplantation of cultured microglia into the CNS of *Csf1r*-knockout mice, which congenitally lack microglia, quickly and robustly re-induces microglial signature genes[51]. In addition, depletion and subsequent repopulation using two approaches—small-molecule inhibitors of Csf1r and genetic depletion in a *Cx3cr1*^{CreERT};*DT*$^{loxP/loxP}$ mouse—showed re-establishment of the unique morphology and density of microglia in the basal ganglia[54], further demonstrating possible region specific cues for programming microglia (Table 2). Together, these results suggest that microglial identity is in part dictated by CNS environment.

Despite downregulation of signature genes, cultured microglia, especially in the presence of Tgfb and the Csf1-receptor ligands Csf1 or IL34, remain genetically distinct from identically cultured bone-marrow-derived macrophages[35,51]. Should environment be the sole determinant of microglial identity, then one might expect myeloid lineage cells of different origin to 'become' microglia when introduced to a permissive CNS environment. Several approaches have

Table 1 | Existing methods for fate-mapping microglia and adjacent macrophages in mouse

Genetic or in vivo	Used to label	Induction age	Notes
$Cx3cr1^{GFP}$	MG & other tissue MPs, monocytes	E9.5 in YS; all MG from brain arrival	On in $Cx3cr1^{+}CD45^{+}$ $ckit^{-}$ ('A3-like' cells) & throughout MG lineage. Jax Stock #005582. Usage:[6,97,98]
$Cx3cr1^{CreERT2}$	MG & other tissue MPs, monocytes	With Tam after E9.5, ± time for monocytes to turn over	Some Tam-independent recombination[99]; caution in early development (constitutive Cre recombines neurons)[100]. Jax Stocks #021160, 020940. Usage:[25,101]
$Runx1^{MerCreMer}$	YS and HSC lineage cells	Tam at E7–7.5 labels MG; other MPs later	Good for tracing cells from yolk sac into the embryo. Usage:[6,102]
$Csf1r^{MerCreMer}$	YS EMPs, tissue MPs, MG	Tam at E8.5 labels $Csf1r^{+}AA4.1^{+}ckit^{+}CD45^{lo}$ YS cells	May also label endothelial cells; reported in neurons after injury; Jax Stock #019098. Usage:[10,103,104]
$Tie2^{Cre}$	HSCs & derivatives, endothelial cells; not MG	Induced E8.5–E11	Jax Stocks #008863, 004128. Usage:[10,105]
$Tnfsf11a^{Cre}$	Primitive MG, tissue resident MPs	By E10.5	$Rank^{Cre}$. Usage:[13,106]
Flk2/$Flt3^{Cre}$	HSC lineage, no MG or most tissue resident MPs	Transient expression by fetal HSC, expressed by progeny of adult HSCs	Used to label products of definitive (multipotent) but not primitive hematopoiesis. Usage:[8,107]
$Hoxb8^{Cre}$	Some MG, some sensory neurons	E8.5–E9.5	Usage:[15,16,108]
Vav^{Cre}	All hematopoietic cells, including MG	Unknown	From studies of *Vav* mRNA, would be only HSC progenitor. Usage:[65,109]
$Lyz2^{Cre}$, $Lyz2^{CreER}$	Myeloid cells: monocytes, neutrophils, some MPs; variable MG expression	Unknown	Reported expression in neurons; Jax Stock #004781. Usage:[65,110]
$Ms4a3^{Cre}$	Granulocytes, monocytes, not MG	Unknown	Usage:[66]
$Ccr2^{RFP}$,$Ccr2^{CreERT2}$	$Ly6C^{hi}$ monocytes, neutrophils, NK cells, DC cells; not MG	E8.5	Jax Stock #017586; Taconic (ER) #10471. Usage:[97,111,112]
$Tmem119^{CreERT2}$	MG, some leptomeningeal cells	Estimated < E17	Jax Stock #031820; and unpublished from authors. Usage:[113]
Parabiosis	Circulating cells of parabiont but not tissue-resident cells	Variable, conducted between adults	Connects blood supply of two mice, one of which can be GFP^{+} to label circulating but not tissue cells. Technically challenging. Usage:[20,17]
Surface markers	**Used to label:**	**Time of induction**	**Notes**
AA4.1/CD93	Some EMPs, fetal liver HSCs	E8.5 to ?	EMPs distinguished as $AA4.1^{+}CD45^{lo}Sca1^{+}kit^{+}$[12]
F4/80	MPs, MG included	E9.5–E10.5 onward	Tissue-resident MPs are generally $F4/80^{hi}$ versus $F4/80^{lo}$ circulating monocytes. Usage:[8,12]
Tmem119	Mature MG	P14 onward	mRNA is expressed from the time brain residence is established; protein expression begins later[39]
P2ry12	Mature MG, platelets	Postnatal	Target of clopidogrel, anti-platelet drug[114]
Fcrls	MG, monocytes	Unknown	No human ortholog[35]

E, embryonic day. P, postnatal day. MG, microglia. MP, macrophage. Tam, tamoxifen.

been used to test this. Bone marrow transplantation (BMT) using lethal irradiation and alkylating agents promotes CNS engraftment of peripheral cells, with varying degrees of engraftment across different colonies[31,39,55]. Interestingly, this induces partial expression of some microglia-enriched genes in CNS engrafted bone-marrow-derived macrophages[31,55]. Even 6 months after BMT, however, HSC-origin cells lack expression of most microglia-specific genes[39]. In contrast to traditional BMT paradigms, transplantation of HSC-origin cells into mice lacking microglia, such as in *Csf1r*-knockout mice or following chronic microglial depletion, promotes reliable, rapid, and widespread engraftment of donor-derived macrophages (Table 2). Remarkably, engrafted cells express *Tmem119*, *Hexb*, and many other microglia-specific genes, pointing again to the dramatic reprogramming of myeloid lineage cells induced by CNS environment[56–58]. These findings are similar to those in other tissues, most notably the lung, in which transplantation of HSC-origin macrophages in *Csf2rb*-knockout mice lacking resident YS-derived alveolar macrophages results in adoption of alveolar macrophage identity[59]. The mechanism by which microglia-depletion models allow robust engraftment and reprogramming of HSC-derived cells is unknown, though one might hypothesize that loss of microglia induces unidentified permissive cues for engraftment, while traditional conditioning paradigms have limited effect on endogenous microglial numbers[60,61].

Despite the robust influence of brain environment on microglial identity, HSC-origin macrophages engrafted in the CNS express hundreds of genes differently than microglia. Several genes are expressed in an origin-specific manner, including *Sall1* in YS-derived microglia and *Ms4a7* and *Clec12a* in HSC-origin microglia-like cells (MLCs; Fig. 1)[55–58]. MLCs also express higher levels of *Apoe*

Table 2 | Common microglia depletion methods with citations

Genetic models

Genetics	Induction with:	Time course	Depletion	Comments
$Cx3cr1^{CreERT/+}$ $Csf1r^{loxP/loxP}$	Tamoxifen chow	Daily for 1–12 weeks	25%[57]	As with all inducible methods, strong repopulation occurs without tamoxifen
	Head-shielded body irradiation and BMT, then tamoxifen chow	12 weeks, then 8 weeks of control	52%[57]	
$Cx3cr1^{CreERT/+}$ $DT^{loxSTOPlox}$	Tamoxifen gavage	2× over 3 days	>99%, 9 days later[32]	In retina, via $Iba1^+$ IHC
	4-OH tamoxifen	3 days IP	>99%*; 2 days later[54]	In basal ganglia, $eYFP^+$ cells from Cre.*raw numbers not reported; plot estimate.
	Tamoxifen	3 days SC	>95%; 7 days later[58]	GFP^+ FACS & $P2RY12^+$ or $eYFP^+$ from Cre IHC
$Cx3cr1^{CreER/+}$ $DTR^{loxSTOPlox}$	Tamoxifen, then DT	Tamoxifen IP × 1 at P14; DT 1× at 8 weeks	80%, 3 days later[31]	FACS and $Iba1^+$ IHC
$CD11b^{HSVTK}$	Ganciclovir	2 weeks ICV	>90%[29]	In neocortex $Iba1^+$ IHC
$Csf1r^{KO}$	None	Developmental loss	100%[28,56]	Mice are quite ill
$Csf1r^{\Delta FIRE/FIRE}$	None	Developmental loss	100%[115]	In brain $Iba1^+$ IHC, gene expression.

Receptor tyrosine kinase inhibitors

Drug	Treatment	Depletion	Comments
PLX5622 chow	1 week	>99%*[54]	In basal ganglia, GFP^+ histology
	21 days	>99%[33]	In somatosensory cortex, GFP^+ histology
BLZ945	5 days, 169mg/kg oral	>99% (ref. [130])	Measured by Iba1+ IHC in cortex
PLX3397 chow	21 days	>99%[30]	In hippocampus, $Iba1^+$ IHC.
PLX3397 intranasal	1 week, 12 h	92%[116]	GFP^+ FACs and C1q RNA
GW2580	3 months	17%[26]	PU.1 IHC. May block proliferation rather than kill.
PLX5622 chow	2 weeks	>90%*[38]	GFP^+, $Iba1^+$ IHC

Of note, colony and laboratory differences in the degree of peripheral cell engraftment using identical BMT methods have been noted at many scientific meetings and throughout the literature. Understanding this nuance is of great scientific and therapeutic interest. DT, diphtheria toxin; DTR, diphtheria toxin receptor; ICV, intracerebroventricular injection; IHC, immunohistochemistry; IP, intraperitoneal; SC, subcutaneous injection; FACS, fluorescence-activated cell sorting.

and lower levels of microglia signature genes, and they are phenotypically less process-bearing, as compared with transplanted microglia[56]. These findings further highlight the importance of both cell origin and environment in determining microglial transcriptomic identity.

Does origin matter?

While microglia have unique origins and transcriptomics, several lines of evidence suggest that origin is also important for microglia function. Host microglia and HSC-derived MLCs in two different models display distinct transcriptional and epigenetic responses to systemic LPS-induced inflammation[55,58]. While more experimental work is required to demonstrate the extent of differences between identically engrafted microglia and HSC-derived MLCs, several studies in which microglia and HSC-derived macrophages inhabit the brain side-by-side suggest divergent functions. First, in models of white matter disease, infiltrating monocytes, but not endogenous microglia, contribute to pathology. Ajami and colleagues induced experimental autoimmune encephalitis (EAE), a model of demyelinating disease which recapitulates features of multiple sclerosis, in wild-type mice and in mice with impaired infiltration of peripheral monocytes into the CNS (*Ccr2*-knockout). They found less-severe clinical disease in mice with reduced monocyte infiltration, suggesting that peripherally derived monocytes but not microglia precipitate or worsen EAE[62]. In another example, depletion of microglia in $Cx3cr1^{CreERT2};DT^{loxP/loxP}$ mice promotes CNS engraftment of peripherally derived cells. Loss of *Tgfbr2* in these HSC-origin MLCs causes progressive and profound neurodegenerative disease. By contrast, demyelination and neuron cell death is slower or absent in mice in which *Tgfbr2* is deleted in microglia without infiltration from the periphery[63,64]. Finally, Wong et al. showed that loss of *Nrros*, which encodes a myeloid cell-specific protein that regulates reactive oxygen species production, leads to depletion of endogenous microglia and their replacement by perivascular macrophage-like cells (PV-MLCs) in the CNS. PV-MLCs express high levels of CD206 but lack canonical microglia genes; their presence in *Nrros*-knockout mice leads to neurodegeneration. Interestingly, disease occurs with developmental loss of *Nrros* in all hematopoietic cells (including microglia) using Vav^{Cre}, but not peripheral myeloid cells using $Lyz2^{Cre}$-targeted loss. Wong and colleagues found that *Nrros* loss affects microglial differentiation and infiltration of PV-MLCs and that this, rather than inherent loss of microglial *Nrros*, promotes neurodegeneration[65]. These studies demonstrate that exposure of the CNS to HSC-origin cells can be pathogenic, which has important implications for the use of BMT (the intentional engraftment of HSC-origins cells into the CNS). Importantly, all three examples involve potentially vulnerable brains and damage-associated recruitment signals due to autoimmune demyelination, microglial cell death, or loss of a critical immune regulator. These confounders are unavoidable, however, as existing methods to study origin–environment interaction involve some sort of conditioning (Table 2), and loss of microglial identity and inflammation seem inextricably linked.

Indeed, a second line of evidence about the importance of origin in brain myeloid function is the relationship between identity and inflammation. As discussed, loss of YS-identity-specific *Sall1* downregulates microglia signature gene expression and increases inflammatory gene expression. *Sall1*-knockout mice have fewer

proliferating doublecortin-positive neuroblasts in the hippocampal subgranular zone, a sign of decreased neurogenesis. A single injection of LPS, which induces microglial activation, results in a similar decrease in neuroblasts, suggesting that microglial activation phenocopies the loss of microglial identity[38]. Similarly to *Sall1*, loss of *Tgfb1* results in downregulation of microglia signature genes[35] and, as with *Tgfbr2* loss[64], neurodegeneration. That loss of microglial identity recapitulates an 'activated' or 'proinflammatory' phenotype is of particular interest given the recent identification of disease-associated microglia (DAMs). Revealed by single-cell RNA sequencing of microglia from mouse models of neurodegenerative disease, this genetically distinct population expresses lower levels of microglial signature genes and higher levels of Alzheimer disease (AD)-risk associated genes, including *Apoe*, *Ctsd*, *Trem2*, and *Tyrobp*. While the precise functions of DAMs are unclear, these cells are also associated with AD plaques and may be more phagocytic based on their expression of phagocytosis-associated genes[53]. All these cells—those lacking identity genes such as *Sall1* or *Tgfb1*/*Tgfbr2* signaling, as well as DAMs—share features with HSC-derived MLCs that similarly express higher levels of *Apoe* and lower levels of signature genes. In addition, several genes enriched in MLCs, such as *Ms4a7* (and the recently identified monocyte marker *Ms4a3*[66]) and *Clec12*a, are part of gene families that are overexpressed by microglia in AD. The MS4A gene cluster was recently identified as an AD susceptibility locus[67]. In addition, blockade of Clec12a using antibodies delays EAE onset and limits severity by limiting myeloid cell infiltration[68]. While these findings fall short of elaborating the extent of functional differences between 'true' microglia and HSC-derived MLCs, together they strongly suggest that dyshomeostatic microglia, disease-associated microglia, and microglia-like cells share overlapping genetic profiles, which at best respond differently to and at worst potentiate CNS pathology.

Why origin matters: beyond scientific intrigue

Functional differences between microglia and HSC-derived surrogates are of paramount importance given the growing interest in microglia as therapeutic targets for human disease, as well as the existing use of hematopoietic transplantation to treat neurological disease by engrafting donor HSC-origin MLCs in the brain (Table 3). Recent studies have strengthened causal relationships between microglial dysfunction and disease. *Trem2*, or triggering receptor expressed on myeloid cells 2, is exclusively expressed by microglia and other myeloid-lineage cells. Large genome-wide association studies revealed a relationship between eight linkage regions in *Trem2* and late-onset AD[69], with null mutations overrepresented in patients with AD[70]. Complete loss of function mutations in *Trem2* cause polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL or Nasu–Hakola disease; see Table 3 for microglia-related diseases), which leads to presenile dementia and bone cyst formation[71]. While more work is needed to elucidate the mechanisms by which *Trem2* mutations promote disease, mutant mice recapitulate some of the classical features of neurodegeneration by disrupting the ability of microglia to sense lipids which interact with pathogenic amyloid-β[72]. In addition, heterozygous loss of function mutations in *Csf1r* cause a rare but well-described neurodegenerative syndrome, adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP). Homozygous mutations in a recently reported pediatric patient led to near absence of microglia, with associated developmental structural pathologies closely resembling those in *Csf1r*-knockout mice: agenesis of the corpus callosum, hydrocephalus, and neuronal loss[28,73]. In a separate study, compound heterozygous patients with biallelic *Csf1r* mutations had a variety of neurological and skeletal symptoms[74]. Together these results point to a critical role for microglia, and *Csf1r*, during normal human brain development and function.

Another stunning example of microglial dysfunction in human disease is Langerhans cell histiocytosis (LCH), a multisystem disorder characterized by infiltrative myeloid lesions in multiple organs but particularly the bone and skin, along with late-onset dementia. Pioneering work by Mass and colleagues found that mosaic expression of *BRAF* mutations in EMPs, the YS progenitor intermediates of microglia, leads to clonal expansion of mutant microglia that is associated with astrogliosis, demyelination, and neurodegeneration. Neuronal loss was found to be worst in areas of greater clonal expansion, and *BRAF*-mutant microglia showed biochemical signs of ERK activation in both mouse and human[75].

These studies represent just a few recent examples of a direct link between microglial dysfunction and disease, and although the precise mechanisms require further study, one might posit that either the loss of normal microglial homeostasis or the acquisition of pathogenic functions would be detrimental. In one mechanistic example, peripheral LPS and nerve injury lead to microglia-mediated astrocyte reactivity via secretion of IL1a, TNF, and C1q. Reactive astrocytes promote the death of neurons by an unidentified mediator. They are also less able to promote synapse formation and elimination[76]. Inhibiting the effect of microglia on astrocytes with neutralizing antibodies against or full deletion of the microglia-secreted molecules prevents astrocyte reactivity and is neuroprotective against injury and in mouse models of AD and Parkinson disease[77]. The extent to which this microglia-mediated astrocyte–neuron cross-talk underlies other neurologic diseases requires additional study, but points to possible therapeutic in-roads targeting signals to and from microglia.

The link between microglial dysfunction and disease suggests that targeting microglial pathways or replacing them with healthy donor cells is a promising therapeutic option for neurological diseases (Fig. 2 and Table 3). As a form of 'microglia replacement therapy' where donor HSC-derived macrophages enter the CNS, classical bone marrow or hematopoietic stem cell transplant (BMT and HSCT, respectively) have some efficacy in the treatment of the leukodystrophies, a group of monogenic neurodegenerative diseases characterized by relatively normal development followed by progressive demyelination and severe impairment. Indeed, BMT or HSCT are one of only a few effective therapies for X-linked adrenoleukodystrophy (ALD). If performed early in ALD, transplant can temporarily halt demyelination, and it works best when performed on presymptomatic patients[78–80]. While this raises a challenge in terms of identifying potential patients early (Box 1), the results are clear: transplantation is not curative, though it delays progression. The benefit of transplant for both ALD and metachromatic leukodystrophy is thought to be replacement of endogenous microglia with peripheral surrogates, which either replace defective microglia or provide a critical function or enzyme[81,82]. Similar strategies have been proposed or trialed for other neurological diseases, especially metabolic diseases, including globoid cell leukodystrophy[83] and mucopolysaccharidosis type I[84]. In mouse models of Rett syndrome, microglia are likely dysfunctional and treatment with transplant has also shown promise[85,86]. The results vary, but clinical trials for the neurometabolic diseases largely show delay of symptoms, with cure thought to be limited by time-to-transplant, preclinical pathology, limited cell engraftment, and/or morbidity of the transplant itself. An understanding of microglial physiology, targeting, and replacement holds great promise for advancing the treatment of these and many other neurological diseases.

While there is great promise with these approaches, there is still more work to do to overcome transplant-associated morbidities, while maximizing the therapeutic benefit of cell-based therapies. An ideal microglia-based therapy for brain disease would be timely, have low treatment morbidity, and lead to high brain engraftment with functionally competent microglial surrogates. Candidate diseases would be those where microglia have demonstrable

Table 3 | Microgliopathies or diseases for which microglial replacement is a potential candidate, showing presumed mechanism of disease, basic clinical features, and current therapies

Mechanism	Phenotype	Treatment
Gaucher disease (*Gba*)		
Beta-glucocerebrosidase loss → glucocerebroside accumulation	Three types; for neuronopathic: onset in infancy (II) or first decade of life (III). CNS manifestations: hypotonia, spasticity, gaze abnormalities, dysphagia, seizures, DR. Systemic features: HSM, bone fractures, anemia, coagulopathy[117]	HSCT, ERT
GBA-related Parkinson disease (*Gba*)		
Unknown	GBA variants in 5–10% of PD patients; Gaucher type I: 20× increased risk of PD, onset 5 years earlier than sporadic PD[118]	Symptomatic
Mucopolysaccharidosis I; Hurler syndrome (*Idua*)		
α-L-iduronidase deficiency → GAG accumulation	CNS: DD, DR, hydrocephalus, coarse features, corneal clouding, macroglossia, valvular heart disease, respiratory failure, HSM, skeletal abnormalities[119]	HSCT, ERT
Mucopolysaccharidosis II; Hunter syndrome (*Ids*)		
Iduronate-2-sulfatase enzyme deficiency → GAG accumulation	Onset: 2–4 years of age. Symptoms: coarse facial features; severe form with developmental regression by age 6–8 years. Other features: retinopathy, valvular heart disease, HSM, skeletal abnormalities[120]	HSCT, ERT
Globoid cell leukodystrophy; Krabbe disease (*Galc*)		
Galactosylceramidase loss → psychosine & other toxic accumulation	Onset: infantile & later-onset. CNS: demyelination with irritability, feeding difficulties, axial hypotonia with extremity spasticity, vision loss, seizures, peripheral neuropathy[121]	HSCT
Niemann–Pick disease type C (*Npc1*, *Npc2*)		
Lipid trafficking defect → cholesterol & toxic lipid accumulation	Onset: varies. Neonatal: ascites and liver disease, respiratory failure, hypotonia, DD. Child: ataxia, eye movement abnormalities, seizure, dystonia. Adults: dementia or psychiatric disease. All: progressive neurological decline[122]	Symptomatic
X-linked adrenoleukodystrophy; XALD (*Abcd1*)		
Loss of peroxisome ABCD1 protein → toxic very-long-chain fatty acid accumulation	Childhood cerebral: ADD/ADHD, progressive neurological decline. Adrenomyeloneuropathy form: patients age 20–50 progressive weakness, sphincter and sexual, adrenocortical dysfunction. In women, 20% display spastic paresis by age 40–60[123]	HSCT
Metachromatic leukodystrophy; MLD (*Arsa*)		
Arylsulfatase A deficiency → sulfatide accumulation	Onset: variable. CNS: progressive demyelination, neurological deterioration. Adults can have psychiatric type[124]	HSCT
Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia; ALSP or HDLS (*Csf1r*)		
Csf1r loss → microglia death and dysfunction → white matter disease	Onset: age 40–50 years. Features: progressive cognitive decline, personality changes, frontal lobe syndrome, motor impairments[125]	Symptomatic
***Mecp2*-related disorders; Rett syndrome, variant Rett (*Mecp2*)**		
Mecp2 loss → complex neurodevelopmental dysregulation	Onset: 6–18 months with DR, stereotypies, ataxia, apneas, tremors, seizure, progressive neurological decline[126]	Symptomatic
Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy; PLOSL or Nasu–Hakola (*Trem2*)		
Loss of *Trem2* → microglia dysfunction → cerebral and basal ganglia atrophy	Normal development, then progressive bony changes (in the third decade of life) before progressive insidious personality changes, cognitive decline[127]	Symptomatic
Alzheimer disease; AD (*Trem2*, *Apoe*, others)		
Multigenic, with amyloid beta plaques and tau accumulation	Onset: variable in adulthood. CNS: progressive cortical atrophy, dementia	Symptomatic
Aicardi–Goutières syndrome (*Adar*, *Rnaseh2a-c*, *Samhd1*, *Trex1*, *Ifih1l*)		
Increased IFN levels, leukocytosis → CNS calcification, vascular disease, inflammation	Early, progressive encephalopathy with spasticity, dystonia, axial hypotonia, hyperekplexia, hepatomegaly, eczema, thrombocytopenia[128]	Symptomatic

ADD/ADHD, attention deficit (hyperactivity) disorder; DD, developmental delay; DR, developmental regression; ERT, enzyme replacement therapy; GAG, glycosaminoglycan; HSCT, hematopoietic stem cell transplant; HSM, hepatosplenomegaly; IFN, interferon; PD, Parkinson disease.

dysfunctional roles or where they might serve to produce defective gene targets (Table 3), though it is important to note that several of these diseases have systemic features that may not be helped by brain-specific targeting. Morbidity of existing transplant methods is often secondary to toxic conditioning paradigms such as radiation or chemotherapy, or due to the side effects of immunosuppression to reduce graft–host interactions. Newly developed transplant methods using antibody-mediated bone-marrow suppression promise to limit cytotoxicity[87]. Viral transduction with nucleases or other DNA-editing machinery (i.e. CRISPR–Cas9) to specifically correct single- or short-nucleotide gene defects theoretically enables autologous transplantation (to limit graft versus host disease and donor availability) or in situ targeting to circumvent cell transplantation, but is dependent on high efficiency and high specificity of infection. Unfortunately, microglia are both radioresistant and largely impervious to viral transduction. Replacement, with few exceptions[55,88],

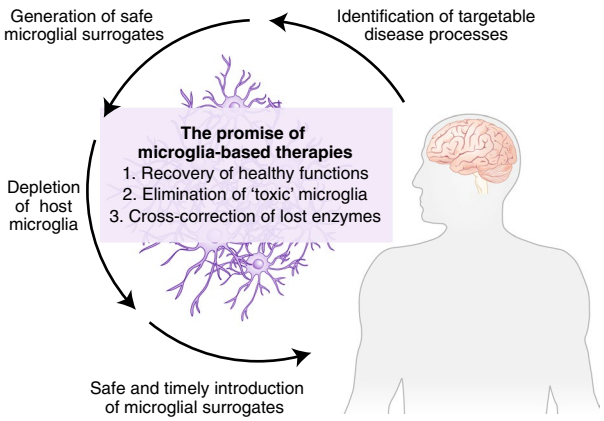

Fig. 2 | The promise of microglia-based therapies. Microglia are critical for homeostatic functions of the brain and spinal cord, and their dysfunction has been implicated in many diseases. They are also highly replaceable. They are thus suited for use as a cell-based therapy. The promise of microglia-based therapies for the treatment of neurological disease hinges on several factors: (i) the identification of diseases that are potentially targetable, in which replacement of microglia would either reverse an ongoing disease process or prevent its progression; (ii) the generation of safe microglial surrogates, from either iPSC technologies or blood-derived substitutes; (iii) the safe depletion of host microglia through the use of small molecule inhibitor or genetic targeting; and (iv) the safe introduction of 'healthy' surrogates into a diseased brain. Each phase of this pipeline requires further scientific study and the collaboration of physicians, scientists, and regulatory bodies.

Box 1 | Early identification of potentially transplantable neurological diseases

Currently, ALD and other leukodystrophy patients are often identified by testing after symptom onset, or by an older affected relative and subsequent testing. Partly due to patient-driven legislation, in 2013, ALD was added to the Recommended Uniform Screening Panel in the United States, meaning that it now appears on the Newborn Screen in eleven states with more likely by the end of 2019. As BMT and HSCT are still non-curative, the move to screen all newborns for ALD (and other high-benefit/high-risk diseases such as metachromatic leukodystrophy or Krabbe) is controversial[129] and highlights the importance of research in developing tractable cures.

first requires targeted depletion of endogenous microglia for widespread engraftment (Fig. 2). Modification of existing methods for microglial depletion (Table 2) or the development of new ones is a promising, albeit early-stage, alternative to BMT or HSCT in the treatment of neurological disease.

Which cells should be used to replace microglia? In light of recent developments in our understanding of microglia origin-environment relationships, it remains unclear the degree to which bone-marrow-derived cells are viable surrogates or whether cells more similar to microglia would be preferable. Induced pluripotent stem cells (iPSCs) may offer an abundant and autologous source of suitable donor cells. Numerous protocols now exist to generate MLCs from murine and human iPSCs[89–92], with cells expressing some microglia-specific genes. Methods to more closely recapitulate the developmental origin of microglia are now available[93]. These cells quickly lived up to their promise as microglial surrogates; iPSC-derived MLCs were recently used to demonstrate that microglia from patients with schizophrenia phagocytose synapses more quickly than those from healthy controls[94]. In addition, iPSC-derived MLCs xenograft into mouse brains and transcriptionally resemble mature microglia[95]. While incredibly promising, the utility of iPSCs, and indeed all microglial surrogates, remains somewhat constrained by gaps in our current understanding of how to induce and maintain microglial identity in vitro, as well as lack of clarity about how similar surrogates must be to recapitulate helpful microglial functions[96]. Furthermore, these models and approaches, beyond teaching us about the potential for microglial-based therapies, promise to more globally expand our understanding of the molecular and cellular processes underlying human neurological disease.

Conclusions

Microglia are central players in CNS health and disease. Their ability to integrate into neural circuitry during embryogenesis and after repopulation makes them uniquely poised therapeutic targets. Although they are similar to other macrophages in some ways, they are also distinct, for reasons that include not only brain environmental signals but also intrinsic properties whose genesis and consequences we do not fully understand, especially in the setting of neurological disease. These properties require primitive hematopoietic origin, are encoded in the epigenome, and likely reflect nuanced programming through layered cellular interactions over time. Understanding the molecular nature of these interactions, their step-wise consequences, and their ultimate effects on microglial function holds the potential to unlock a new class of therapies for a broad array of CNS diseases.

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References

1. Li, Q. & Barres, B. A. Microglia and macrophages in brain homeostasis and disease. *Nat. Rev. Immunol.* **18**, 225–242 (2018).
2. Hickman, S., Izzy, S., Sen, P., Morsett, L. & El Khoury, J. Microglia in neurodegeneration. *Nat. Neurosci.* **21**, 1359–1369 (2018).
3. Hammond, T. R., Robinton, D. & Stevens, B. Microglia and the brain: complementary partners in development and disease. *Annu. Rev. Cell Dev. Biol.* **34**, 523–544 (2018).
4. Dzierzak, E. & Bigas, A. Blood development: hematopoietic stem cell dependence and independence. *Cell Stem Cell* **22**, 639–651 (2018).
5. Alliot, F., Godin, I. & Pessac, B. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res. Dev. Brain Res.* **117**, 145–152 (1999).
6. Ginhoux, F. et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841–845 (2010).
This study focused new attention on the origin of microglia and tissue-resident macrophages and set the stage for subsequent studies using fate-mapping to identity the nuances of macrophage origin.
7. Stremmel, C. et al. Yolk sac macrophage progenitors traffic to the embryo during defined stages of development. *Nat. Commun.* **9**, 75 (2018).
8. Schulz, C. et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* **336**, 86–90 (2012).
9. Kierdorf, K. et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat. Neurosci.* **16**, 273–280 (2013).
10. Gomez Perdiguero, E. et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* **518**, 547–551 (2015).
11. Hoeffel, G. et al. C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* **42**, 665–678 (2015).
12. Sheng, J., Ruedl, C. & Karjalainen, K. Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. *Immunity* **43**, 382–393 (2015).
13. Mass, E. et al. Specification of tissue-resident macrophages during organogenesis. *Science* **353**, aaf4238 (2016).
14. Hoeffel, G. et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J. Exp. Med.* **209**, 1167–1181 (2012).
15. Chen, S.-K. et al. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell* **141**, 775–785 (2010).

16. De, S. et al. Two distinct ontogenies confer heterogeneity to mouse brain microglia. *Development* **145**, dev152306 (2018).
17. Hashimoto, D. et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* **38**, 792–804 (2013).
18. Bain, C. C. et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat. Immunol.* **15**, 929–937 (2014).
19. Ferrero, G. et al. Embryonic microglia derive from primitive macrophages and are replaced by cmyb-dependent definitive microglia in zebrafish. *Cell Rep.* **24**, 130–141 (2018).
20. Ajami, B., Bennett, J. L., Krieger, C., Tetzlaff, W. & Rossi, F. M. V. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat. Neurosci.* **10**, 1538–1543 (2007).
21. Goldmann, T. et al. Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat. Immunol.* **17**, 797–805 (2016).
22. Lawson, L. J., Perry, V. H. & Gordon, S. Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* **48**, 405–415 (1992).
23. Füger, P. et al. Microglia turnover with aging and in an Alzheimer's model via long-term in vivo single-cell imaging. *Nat. Neurosci.* **20**, 1371–1376 (2017).
24. Tay, T. L. et al. A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nat. Neurosci.* **20**, 793–803 (2017).
25. Yona, S. et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* **38**, 79–91 (2013).
26. Askew, K. et al. Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. *Cell Rep.* **18**, 391–405 (2017).
27. Réu, P. et al. The lifespan and turnover of microglia in the human brain. *Cell Rep.* **20**, 779–784 (2017).
28. Dai, X.-M. et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood* **99**, 111–120 (2002).
29. Varvel, N. H. et al. Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. *Proc. Natl Acad. Sci. USA* **109**, 18150–18155 (2012).
30. Elmore, M. R. P. et al. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron* **82**, 380–397 (2014).
31. Bruttger, J. et al. Genetic cell ablation reveals clusters of local self-renewing microglia in the mammalian central nervous system. *Immunity* **43**, 92–106 (2015).
32. Zhang, Y. et al. Repopulating retinal microglia restore endogenous organization and function under CX3CL1-CX3CR1. *Regul. Sci. Adv.* **4**, p8492 (2018).
33. Huang, Y. et al. Repopulated microglia are solely derived from the proliferation of residual microglia after acute depletion. *Nat. Neurosci.* **21**, 530–540 (2018).
34. Hickman, S. E. et al. The microglial sensome revealed by direct RNA sequencing. *Nat. Neurosci.* **16**, 1896–1905 (2013).
35. Butovsky, O. et al. Identification of a unique TGF-β-dependent molecular and functional signature in microglia. *Nat. Neurosci.* **17**, 131–143 (2014).
36. Gosselin, D. et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* **159, 1327–1340 (2014). This study and the following study by Lavin et al. crystallized the powerful programming effects of environment on tissue macrophages**.
37. Lavin, Y. et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* **159**, 1312–1326 (2014).
This study and the previous study by Gosselin et al. crystallized the powerful programming effects of environment on tissue macrophages.
38. Buttgereit, A. et al. Sall1 is a transcriptional regulator defining microglia identity and function. *Nat. Immunol.* **17**, 1397–1406 (2016).
39. Bennett, M. L. et al. New tools for studying microglia in the mouse and human CNS. *Proc. Natl. Acad. Sci. USA* **113**, E1738–E1746 (2016).
40. Matcovitch-Natan, O. et al. Microglia development follows a stepwise program to regulate brain homeostasis. *Science* **353**, aad8670 (2016).
41. Zhang, Y. et al. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. *Neuron* **89**, 37–53 (2016).
42. Gosselin, D. et al. An environment-dependent transcriptional network specifies human microglia identity. *Science* **356**, eaal3222 (2017).
43. O'Koren, E. G. et al. Microglial function is distinct in different anatomical locations during retinal homeostasis and degeneration. *Immunity* **50**, 723–737.e7 (2019).
44. Jordão, M. J. C. et al. Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. *Science* **363**, eaat7554 (2019).
45. Hammond, T. R. et al. Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. *Immunity* **50**, 253–271.e6 (2019).
46. Li, Q. et al. Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing. *Neuron* **101**, 207–223. e10 (2019).
47. Van Hove, H. et al. A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. *Nat. Neurosci.* **22**, 1021–1035 (2019).
48. Wlodarczyk, A. et al. A novel microglial subset plays a key role in myelinogenesis in developing brain. *EMBO J.* **36**, 3292–3308 (2017).
49. Datta, M. et al. Histone deacetylases 1 and 2 regulate microglia function during development, homeostasis, and neurodegeneration in a context-dependent manner. *Immunity* **48**, 514–529.e6 (2018).
50. Ayata, P. et al. Epigenetic regulation of brain region-specific microglia clearance activity. *Nat. Neurosci.* **21**, 1049–1060 (2018).
51. Bohlen, C. J. et al. Diverse requirements for microglial survival, specification, and function revealed by defined-medium cultures. *Neuron* **94**, 759–773.e8 (2017).
52. Friedman, B. A. et al. Diverse brain myeloid expression profiles reveal distinct microglial activation states and aspects of Alzheimer's disease not evident in mouse models. *Cell Rep.* **22**, 832–847 (2018).
53. Keren-Shaul, H. et al. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* **169**, 1276–1290.e17 (2017).
54. De Biase, L. M. et al. Local cues establish and maintain region-specific phenotypes of basal ganglia microglia. *Neuron* **95**, 341–356.e6 (2017).
55. Shemer, A. et al. Engrafted parenchymal brain macrophages differ from microglia in transcriptome, chromatin landscape and response to challenge. *Nat. Commun.* **9**, 5206 (2018).
This study and the following three studies by Bennett et al., Cronk et al. and Lund et al. showed in short order that microglial transcriptomic identity is garnered by both origin and environment.
56. Bennett, F. C. et al. A combination of ontogeny and CNS environment establishes microglial identity. *Neuron* **98**, 1170–1183.e8 (2018).
This study and the three studies by Shemer et al., Cronk et al. and Lund et al. showed in short order that microglial transcriptomic identity is garnered by both origin and environment.
57. Cronk, J. C. et al. Peripherally derived macrophages can engraft the brain independent of irradiation and maintain an identity distinct from microglia. *J. Exp. Med.* **215**, 1627–1647 (2018).
This study and the three studies by Shemer et al., Bennett et al. and Lund et al. showed in short order that microglial transcriptomic identity is garnered by both origin and environment.
58. Lund, H. et al. Competitive repopulation of an empty microglial niche yields functionally distinct subsets of microglia-like cells. *Nat. Commun.* **9**, 4845 (2018).
This study and the previous three studies by Shemer et al., Bennett et al. and Cronk et al. showed in short order that microglial transcriptomic identity is garnered by both origin and environment.
59. van de Laar, L. et al. Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages. *Immunity* **44**, 755–768 (2016).
60. Gibson, E. M. et al. Methotrexate chemotherapy induces persistent tri-glial dysregulation that underlies chemotherapy-related cognitive impairment. *Cell* **176**, 43–55.e13 (2019).
61. Mildner, A. et al. Microglia in the adult brain arise from Ly-6ChiCCR2^{+} monocytes only under defined host conditions. *Nat. Neurosci.* **10**, 1544–1553 (2007).
62. Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M. & Rossi, F. M. V. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat. Neurosci.* **14**, 1142–1149 (2011).
63. Arnold, T. D. et al. Impaired αVβ8 and TGFβ signaling lead to microglial dysmaturation and neuromotor dysfunction. *J. Exp. Med.* **216**, 900–915 (2019).
64. Lund, H. et al. Fatal demyelinating disease is induced by monocyte-derived macrophages in the absence of TGF-β signaling. *Nat. Immunol.* **19**, 1–7 (2018).
65. Wong, K. et al. Mice deficient in NRROS show abnormal microglial development and neurological disorders. *Nat. Immunol.* **18**, 633–641 (2017).
66. Liu, Z. et al. Fate mapping via Ms4a3-expression history traces monocyte-derived cells. *Cell* **178**, 1509–1525.e19 (2019).
67. Hollingworth, P. et al. Common variants at *ABCA7*, *MS4A6A*/*MS4A4E*, *EPHA1*, *CD33* and *CD2AP* are associated with Alzheimer's disease. *Nat. Genet.* **43**, 429–435 (2011).
68. Sagar, D. et al. Antibody blockade of CLEC12A delays EAE onset and attenuates disease severity by impairing myeloid cell CNS infiltration and restoring positive immunity. *Sci. Rep.* **7**, 2707 (2017).
69. Jonsson, T. et al. Variant of TREM2 associated with the risk of Alzheimer's disease. *N. Engl. J. Med.* **368**, 107–116 (2013).
70. Guerreiro, R. et al. TREM2 variants in Alzheimer's disease. *N. Engl. J. Med.* **368**, 117–127 (2013).

71. Saitoh, B.-Y. et al. A case of hereditary diffuse leukoencephalopathy with axonal spheroids caused by a de novo mutation in *CSF1R* masquerading as primary progressive multiple sclerosis. *Mult. Scler.* **19**, 1367–1370 (2013).
72. Wang, Y. et al. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. *Cell* **160**, 1061–1071 (2015).
73. Oosterhof, N. et al. Homozygous mutations in *CSF1R* cause a pediatric-onset leukoencephalopathy and can result in congenital absence of microglia. *Am. J. Hum. Genet.* **104**, 936–947 (2019).
74. Guo, L. et al. Bi-allelic *CSF1R* mutations cause skeletal dysplasia of dysosteosclerosis-Pyle disease spectrum and degenerative encephalopathy with brain malformation. *Am. J. Hum. Genet.* **104**, 925–935 (2019).
75. Mass, E. et al. A somatic mutation in erythro-myeloid progenitors causes neurodegenerative disease. *Nature* **549**, 389–393 (2017).
This paper showed a stunning example of how cell of origin can influence disease expression.
76. Liddelow, S. A. et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* **541**, 481–487 (2017).
77. Yun, S. P. et al. Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat. Med.* **24**, 931–938 (2018).
78. Shapiro, E. et al. Long-term effect of bone-marrow transplantation for childhood-onset cerebral X-linked adrenoleukodystrophy. *Lancet* **356**, 713–718 (2000).
79. Peters, C. et al. Cerebral X-linked adrenoleukodystrophy: the international hematopoietic cell transplantation experience from 1982 to 1999. *Blood* **104**, 881–888 (2004).
80. Mahmood, A., Raymond, G. V., Dubey, P., Peters, C. & Moser, H. W. Survival analysis of haematopoietic cell transplantation for childhood cerebral X-linked adrenoleukodystrophy: a comparison study. *Lancet Neurol.* **6**, 687–692 (2007).
81. Biffi, A. et al. Gene therapy of metachromatic leukodystrophy reverses neurological damage and deficits in mice. *J. Clin. Invest.* **116**, 3070–3082 (2006).
82. Cartier, N. et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science* **326**, 818–823 (2009).
83. Allewelt, H. et al. Long-term functional outcomes after hematopoietic stem cell transplant for early infantile Krabbe disease. *Biol. Blood Marrow Transplant.* **24**, 2233–2238 (2018).
84. Boelens, J. J. et al. Outcomes of transplantation using various hematopoietic cell sources in children with Hurler syndrome after myeloablative conditioning. *Blood* **121**, 3981–3987 (2013).
85. Derecki, N. C. et al. Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* **484**, 105–109 (2012).
86. Schafer, D. P. et al. Microglia contribute to circuit defects in Mecp2 null mice independent of microglia-specific loss of Mecp2 expression. *eLife* **5**, e15224 (2016).
87. Kwon, H.-S. et al. Anti-human CD117 antibody-mediated bone marrow niche clearance in non-human primates and humanized NSG mice. *Blood* **133**, 2104–2108 (2019).
88. Capotondo, A. et al. Brain conditioning is instrumental for successful microglia reconstitution following hematopoietic stem cell transplantation. *Proc. Natl. Acad. Sci. USA* **109**, 15018–15023 (2012).
89. Muffat, J. et al. Efficient derivation of microglia-like cells from human pluripotent stem cells. *Nat. Med.* **22**, 1358–1367 (2016).
90. Abud, E. M. et al. iPSC-derived human microglia-like cells to study neurological diseases. *Neuron* **94**, 278–293.e9 (2017).
91. Pandya, H. et al. Differentiation of human and murine induced pluripotent stem cells to microglia-like cells. *Nat. Neurosci.* **20**, 753–759 (2017).
92. Ormel, P. R. et al. Microglia innately develop within cerebral organoids. *Nat. Commun.* **9**, 4167 (2018).
93. Takata, K. et al. Induced-pluripotent-stem-cell-derived primitive macrophages provide a platform for modeling tissue-resident macrophage differentiation and function. *Immunity* **47**, 183–198.e6 (2017).
94. Sellgren, C. M. et al. Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. *Nat. Neurosci.* **22**, 374–385 (2019).
95. Hasselmann, J. et al. Development of a chimeric model to study and manipulate human microglia in vivo. *Neuron* **103**, 1016–1033.e10 (2019).
96. Pocock, J. M. & Piers, T. M. Modelling microglial function with induced pluripotent stem cells: an update. *Nat. Rev. Neurosci.* **19**, 445–452 (2018).
97. Mizutani, M. et al. The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. *J. Immunol.* **188**, 29–36 (2012).
98. Cardona, A. E. et al. Control of microglial neurotoxicity by the fractalkine receptor. *Nat. Neurosci.* **9**, 917–924 (2006).
99. Fonseca, M. I. et al. Cell-specific deletion of *C1qa* identifies microglia as the dominant source of C1q in mouse brain. *J. Neuroinflammation* **14**, 48 (2017).
100. Haimon, Z. et al. Re-evaluating microglia expression profiles using RiboTag and cell isolation strategies. *Nat. Immunol.* **19**, 636–644 (2018).
101. Parkhurst, C. N. et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* **155**, 1596–1609 (2013).
102. Samokhvalov, I. M., Samokhvalova, N. I. & Nishikawa, S. Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature* **446**, 1056–1061 (2007).
103. Luo, J. et al. Colony-stimulating factor 1 receptor (CSF1R) signaling in injured neurons facilitates protection and survival. *J. Exp. Med.* **210**, 157–172 (2013).
104. Plein, A., Fantin, A., Denti, L., Pollard, J. W. & Ruhrberg, C. Erythro-myeloid progenitors contribute endothelial cells to blood vessels. *Nature* **562**, 223–228 (2018).
105. Tang, Y., Harrington, A., Yang, X., Friesel, R. E. & Liaw, L. The contribution of the $Tie2^+$ lineage to primitive and definitive hematopoietic cells. *Genesis* **48**, 563–567 (2010).
106. Maeda, K. et al. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat. Med.* **18**, 405–412 (2012).
107. Boyer, S. W., Schroeder, A. V., Smith-Berdan, S. & Forsberg, E. C. All hematopoietic cells develop from hematopoietic stem cells through Flk2/Flt3-positive progenitor cells. *Cell Stem Cell* **9**, 64–73 (2011).
108. Witschi, R. et al. Hoxb8-Cre mice: A tool for brain-sparing conditional gene deletion. *Genesis* **48**, 596–602 (2010).
109. Georgiades, P. et al. *vavCre* transgenic mice: a tool for mutagenesis in hematopoietic and endothelial lineages. *Genesis* **34**, 251–256 (2002).
110. Orthgiess, J. et al. Neurons exhibit *Lyz2* promoter activity in vivo: implications for using LysM-Cre mice in myeloid cell research. *Eur. J. Immunol.* **46**, 1529–1532 (2016).
111. Saederup, N. et al. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS One* **5**, e13693 (2010).
112. Croxford, A. L. et al. The cytokine GM-CSF drives the inflammatory signature of $CCR2^+$ monocytes and licenses autoimmunity. *Immunity* **43**, 502–514 (2015).
113. Kaiser, T. & Feng, G. Tmem119-EGFP and Tmem119-CreERT2 transgenic mice for labeling and manipulating microglia. *eNeuro* **6**, ENEURO.0448-18.2019 (2019).
114. Haynes, S. E. et al. The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nat. Neurosci.* **9**, 1512–1519 (2006).
115. Rojo, R. et al. Deletion of a Csf1r enhancer selectively impacts *CSF1R* expression and development of tissue macrophage populations. *Nat. Commun.* **10**, 3215 (2019).
116. Villa, A. et al. Sex-specific features of microglia from adult mice. *Cell Rep.* **23**, 3501–3511 (2018).
117. Pastores, G.M. & Hughes, D.A. Gaucher disease. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1269/ (University of Washington, Seattle, 2000).
118. Aflaki, E., Westbroek, W. & Sidransky, E. The complicated relationship between Gaucher disease and Parkinsonism: insights from a rare disease. *Neuron* **93**, 737–746 (2017).
119. Clarke, L.A. Mucopolysaccharidosis type I. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1162/ (University of Washington, Seattle, 2002).
120. Scarpa, M. Mucopolysaccharidosis type II. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1274/ (University of Washington, Seattle, 2007).
121. Orsini, J.J., Escolar, M.L., Wasserstein, M.P. & Caggana, M. Krabbe disease. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1238/ (University of Washington, Seattle, 2000).
122. Patterson, M. Niemann-Pick disease type C. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1296/ (University of Washington, Seattle, 2000).
123. Raymond, G.V., Moser, A.B. & Fatemi, A. X–linked adrenoleukodystrophy. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1315/ (University of Washington, Seattle, 1999).
124. Gomez-Ospina, N. Arylsulfatase A deficiency. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1130/ (University of Washington, Seattle, 2006).
125. Konno, T. et al. Diagnostic criteria for adult-onset leukoencephalopathy with axonal spheroids and pigmented glia due to CSF1R mutation. *Eur. J. Neurol.* **25**, 142–147 (2018).
126. Christodoulou, J. & Ho, G. MECP2 disorders. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1497 (University of Washington, Seattle, 2001).
127. Paloneva, J., Autti, T., Hakola, P. & Haltia, M.J. Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL). in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1197/ (University of Washington, Seattle, 2002).

128. Crow, Y.J. Aicardi-Goutières syndrome. in *GeneReviews* (eds Adam, M. P. et al.) https://www.ncbi.nlm.nih.gov/books/NBK1475/ (University of Washington, Seattle, 2005).
129. Kelly, N., Makarem, D. C. & Wasserstein, M. P. Screening of newborns for disorders with high benefit-risk ratios should be mandatory. *J. Law Med. Ethics* **44**, 231–240 (2016).
130. Beckmann, N. et al. Brain region-specific enhancement of remyelination and prevention of demyelination by the CSF1R kinase inhibitor BLZ945. *Acta Neuropathol. Commun.* **6**, 9 (2018).

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M.L.B. and F.C.B. were equal contributors to the conception and writing of this review.

Competing interests

M.L.B and F.C.B are co-inventors on a pending patent filed by The Board of Trustees of The Leland Stanford Junior University (application 16/566,675) related to methods of microglia replacement.

Additional information

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