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REVIEW

Astrocytes and microglia: Models and tools

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Glial cells serve as fundamental regulators of the central nervous system in development, homeostasis, and disease. Discoveries into the function of these cells have fueled excitement in glial research, with enthusiastic researchers addressing fundamental questions about glial biology and producing new scientific tools for the community. Here, we outline the pros and cons of in vivo and in vitro techniques to study astrocytes and microglia with the goal of helping researchers quickly identify the best approach for a given research question in the context of glial biology. It is truly a great time to be a glial biologist.

Introduction

Glial cells are essential players in central nervous system (CNS) development, maintenance, and decline. They orchestrate CNS development and homeostasis, modulate neuronal communication, and participate in CNS degeneration and regeneration in the context of disease and injury (Barres, 2008). While our understanding of glial cell function has lagged behind that of neurons, contemporary glial biology is an exciting field with an array of tools designed to specifically study glia both in vivo and in vitro. This review provides a snapshot of currently available mouse models, cell type–specific markers, cell culture methods, and searchable online datasets for the study of astrocyte and microglial biology. We provide a short discussion of the relative benefits and utility of various reagents and applications and provide a simple flow diagram to help determine appropriate methods in specific contexts (Fig. 1).

Astrocytes

Astrocytes orchestrate neuronal development by secreting synaptogenic molecules and pruning excess synapses (Pfrieger and Barres, 1997; Mauch et al., 2001; Christopherson et al., 2005; Fuentes-Medel et al., 2009; Kucukdereli et al., 2011; Allen et al., 2012; Chung et al., 2013). They maintain CNS homeostasis and promote neuronal survival by shuttling metabolites, secreting trophic factors, and regulating blood flow (Meyer-Franke et al., 1995; Kornblum et al., 1998; Bélanger et al., 2011; MacVicar and Newman, 2015; Weber and Barros, 2015). They also respond to CNS injury and disease in a process called reactive astrogliosis, an activated state of glia cells that contributes to both inflammation and its resolution (Jacque et al., 1978; Liedtke et al., 1998; Bush et al., 1999; Bundesen et al., 2003; Gao et al., 2005; Lepore et al., 2008; Sofroniew, 2009; Zamanian et al., 2012; Kraft et al., 2013; Ren et al., 2013; Bloom, 2014; Cekanaviciute et al., 2014; Ben Haim et al., 2015; Heppner et al., 2015; Anderson et al., 2016; Liddelow et al., 2017; Liddelow and Barres, 2017; Rothhammer et al., 2018). While many astrocytic functions are known, there are countless discoveries still to be made. Fortunately, new and established tools to culture astrocytes in vitro and manipulate them in vivo have rapidly advanced research into astrocyte function (Table 1 and Fig. 1).

In vivo

With the advent of cell type–specific gene databases, the ability to target individual cell types in the CNS has exploded. Previously, in vivo studies of astrocyte biology were hampered by a lack of genetic lines to drive or knock out gene expression specifically in astrocytes while leaving neural progenitor cells (NPCs), neurons, and other glial cells unaffected. GFAP (glial fibrillary acidic protein; Eng et al., 1971) has long been accepted as the definitive astrocyte marker and has served as a basis for foundational work on the function of these cells. As with any marker, however, its limitations have become apparent over time. First, GFAP does not identify all astrocytes throughout the CNS, nor is *Gfap* expression sufficient to identify a cell as an astrocyte (Roessmann et al., 1980; Liu et al., 2010; Sofroniew and Vinters, 2010). Although *Gfap* is expressed in astrocytes across multiple brain regions and throughout development, expression levels of *Gfap* mRNA and GFAP protein levels are highly variable (Cahoy et al., 2008; Boisvert et al., 2018; Clarke et al., 2018; Table 2). Perhaps unsurprisingly given that astrocytes and neurons derive from the same pool of progenitor cells (Garcia et al., 2004; Bayraktar et al., 2014), *Gfap* is also expressed by NPCs, nascent neurons, and type 1 neural stem cells in the hippocampus (Steiner et al., 2006; Hodge et al., 2008; Liu et al., 2010). As a result, many studies

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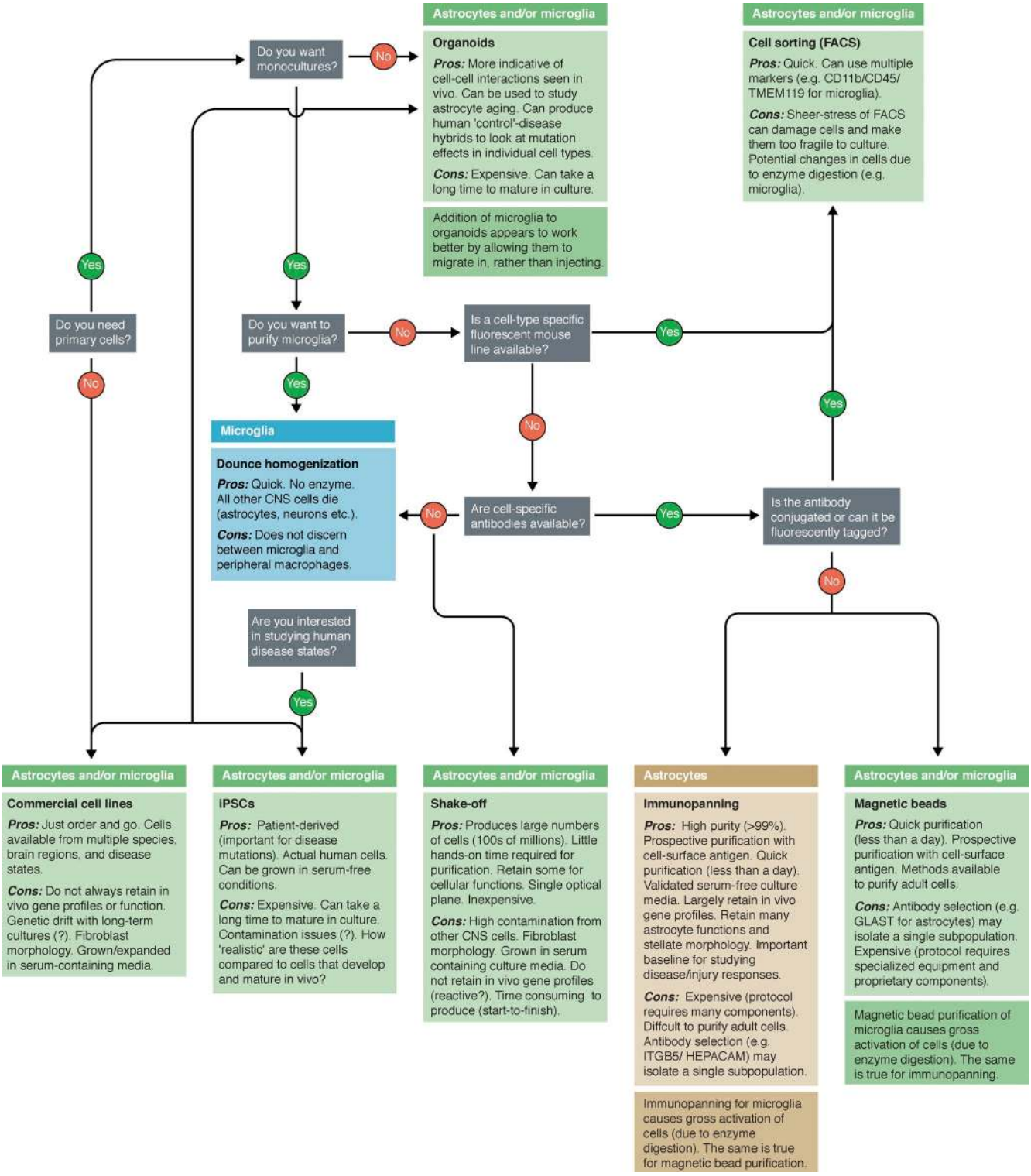

Figure 1. **Purification flow chart.** Methods for purification of astrocytes and microglia. Reasons for selection will vary depending on antibody availability, species required, and disease states of interest. iPSC, iPS cell.

that use human (Zhuo et al., 2001; Ganat et al., 2006) or mouse (Brenner et al., 1994) *Gfap*-Cre lines to drive or knock out gene expression in astrocytes might also manipulate neurons (Su et al., 2004; Fujita et al., 2014). As with *Gfap*, other Cre lines thought to be astrocyte specific also show off-target effects in neurons, including *Scl1a3* (GLAST), *Gjb6* (CX30), *Slc6a13* (GAT2), and *S100b* (S100B; Table 1; Slezak et al., 2007; Srinivasan et al., 2016).

While in some instances unintended effects on a subset of neurons may not prove problematic, off-target effects become extremely important when studying genes that are highly ex-

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Table 1. **Common astrocyte markers and reagents**

Gene (protein)	Labeled cells (CNS)	Genetic lines	Antibodies	Notes	Reference
Gfap (GFAP)	Astrocytes + NPCs	Fluorescent reporter, Cre, CreERT2	Y	Upregulated in some reactive astrocytes	Brenner et al., 1994; Zhuo et al., 2001; Su et al., 2004; Ganat et al., 2006; Liu et al., 2010
Aldh1l1 (ALDH1L1)	Astrocytes	Fluorescent reporter, CreERT2 (new)	Y		Cahoy et al., 2008; Srinivasan et al., 2016; Winchenbach et al., 2016
Slc1a3 (GLAST)	Astrocytes + NPCs	Fluorescent reporter, CreERT	Y	Developed by Jeremy Nathans (Mouse Genome Informatics)	Regan et al., 2007; Kang et al., 2010; de Melo et al., 2012; Wang et al., 2012
Slc1a2 (GLT1)	Astrocytes + NPCs	Fluorescent reporter	Y		Regan et al., 2007; Yang et al., 2011
S100b (S100B)	Astrocytes + OL lineage	Fluorescent reporter, CreERT2	Y		Zuo et al., 2004; McMahon et al., 2008; Harding et al., 2011
Gjb6 (CX30)	Astrocytes + NPCs	CreERT2	Y		Slezak et al., 2007; Srinivasan et al., 2016
Slc6a11 (GAT3)	Astrocytes + NPCs	CreERT2	Y		Srinivasan et al., 2016
Nes (NESTIN)	Astrocytes + NPCs	Fluorescent reporter, Cre, CreERT2	Y	Upregulated in some reactive astrocytes	Betz et al., 1996; Tronche et al., 1999; Battiste et al., 2007; Lagace et al., 2007
Vim (VIMENTIN)	Astrocytes + NPCs	Fluorescent reporter, LacZ	Y	Upregulated in some reactive astrocytes	Colucci-Guyon et al., 1994
C3	Astrocyte + certain Cx3cr1^{+} cells	Fluorescent reporter (not finalized)	Y (human)	Upregulated in A1 reactive astrocytes; in situ hybridization required for murine tissue	Liddelow et al., 2017

OL, oligodendrocyte; Y, yes.

pressed by neurons or when neuronal/synaptic dysfunction is the primary phenotypic readout (Sloan and Barres, 2014). For instance, knocking out a gene involved in astrocytic phagocytosis using a *Gfap-Cre* is unlikely to cause major problems, as the neuronal cells that might also be affected are largely nonphagocytic neural progenitor cells (Morizawa et al., 2017). However, when studying phenomena more broadly relevant to many CNS cell types using behavior, electrophysiology, or other indicators of neuronal function, it can be difficult to separate effects in astrocytes from off-target effects in neurons. While we now appreciate that single markers cannot definitively label all astrocytes (often two markers with different profiles are needed, such as GFAP and S100β), these Cre lines remain enormously valuable. And although each has weaknesses, the relevant genes are often more or less specific to astrocytes in different brain areas or during different stages of development. Careful validation of specificity is therefore best practice when choosing reagents with which to manipulate astrocyte gene expression (Song and Palmiter, 2018).

New genetic lines based on the astrocyte-specific enzyme ALDH1L1 come closer to achieving complete and specific astrocyte targeting. The *Aldh1l1*-eGFP line, in which enhanced GFP is expressed in all astrocytes, has been used for isolation by FACS and for investigation of transcriptomic or proteomic responses to disease, injury, and other experimental conditions (Cahoy et al., 2008; Yang et al., 2011; Zhang et al., 2014). Recently developed *Aldh1l1-Cre/Aldh1l1-CreERT* lines (Srinivasan et al., 2016; Winchenbach et al., 2016) allow for inducible and temporal control of astrocyte gene expression. However, *Aldh1l1*-based lines still have caveats; for example, *Aldh1l1* is expressed by cells in several peripheral organs, including lung, liver, kidney, and small intestine (Winchenbach et al., 2016), which might act as a confounder. Further, purification of astrocytes from these lines relies on enzymatic digestion to achieve single-cell suspensions, a manipulation that induces transcriptional changes in astrocytes (Wu et al., 2017). An alternate approach is bacterial artificial chromosome (BAC) translating ribosome affinity purification (TRAP), by which ribosomes from genetically accessible cell populations are isolated, allowing for sequencing of mRNAs that are actively undergoing translation (Doyle et al., 2008; Heiman et al., 2008, 2014). The *Aldh1l1-eGFP-L10a BAC-TRAP* mouse has been used to investigate how the astrocyte transcriptome changes with age (Boisvert et al., 2018; Clarke et al., 2018) and in the context of diseases such as amyotrophic lateral sclerosis (Sun et al., 2015). Importantly, BAC-TRAP lines can still be contaminated by highly expressed mRNAs from nontargeted cell types such as neurons (Boisvert et al., 2018; Clarke et al., 2018). Expression of these transcripts may be due to nonspecific pulldown of ribosomes or unintended off-target effects of the chosen promoter (Foo and Dougherty, 2013). Thus, findings from both ribosome pulldown techniques and FACS studies should always be validated with complementary methods such as in situ hybridization.

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Table 2. **Common transcriptome resources**

Website	Laboratory	Reference	Focus
http://igc1.salk.edu:3838/astrocyte_aging_transcriptome/	Allen	Boisvert et al., 2018	Aging mouse astrocytes, multiple brain areas
http://www.brainrnaseq.org/	Barres	Zhang et al., 2014, 2016; Bennett et al., 2016; Clarke et al., 2018	Glial cell specific in mouse and human; mouse microglia throughout development; aging mouse astrocytes, multiple brain areas
http://bioinf.nl:8080/GOAD2/	Boddeke	Holtman et al., 2015	Repository of multiple other published glia sequencing datasets
http://shiny.maths.usyd.edu.au/Ellis/MicrogliaPlots	Bradshaw		Aged human microglia
http://astrocyternaseq.org/	Khakh	Srinivasan et al., 2016; Chai et al., 2017	Adult mouse brain regional differences in astrocytes
http://www.mousebrain.org/	Linnarsson	Zeisel et al., 2018	Single-cell analysis of many cell types from different brain regions and developmental stages of the mouse
http://www.dropviz.org/	McCarroll	Saunders et al., 2018	Single-cell analysis of many cell types from different mouse brain regions
https://astrocyte.rnaseq.sofroniewlab.neurobio.ucla.edu/	Sofroniew	Anderson et al., 2016	Mouse astrocyte reactivity in spinal cord injury and inflammation
http://www.microgliasinglecell.com/	Stevens/ McCarroll	Hammond et al., 2018	Single cell microglia during age, by sex, and in demyelinating disease model

Additional datasets for non-glial CNS cells are reviewed in Keil et al., 2018.

In vitro

Studying astrocytes in culture is another powerful way to understand their function. The most widely used technique for purifying and culturing primary rodent astrocytes was developed by Ken McCarthy and Jean de Vellis and involves producing a mixed cell suspension from rodent brains via enzymatic digestion and dissociation (McCarthy and de Vellis, 1980). When the cell mixture is plated in a flask, astrocytes adhere tightly, whereas oligodendrocytes and microglia adhere more loosely or remain suspended. Astrocytes are then obtained by shaking the culture to remove overlying cells. The resulting astrocytes (commonly referred to as MD astrocytes after the pioneering development by McCarthy and de Vellis) are highly mitotic and are maintained in serum-containing media. This revolutionary culture technique led to many discoveries into fundamental aspects of glial biology, for example the identification of astrocyte-derived synapse modulating cues (Mauch et al., 2001; Christopherson et al., 2005; Allen et al., 2012). It remains a powerful culture system by which to investigate astrocyte function, with benefits such as low cost and high cell yield, and it is particularly useful for studies requiring large numbers of cells, dividing cells, or large amounts of protein. However, the system also has limitations. First, although cells isolated by this method are largely astrocytic, there is contamination by neurons, microglia, and oligodendrocytes. Second, MD astrocytes behave more like astrocyte precursors than mature astrocytes, with high rates of mitosis and expression of transcripts not seen in mature, postmitotic cells. Another limitation is the requirement for serum to culture these cells, which creates a nonphysiological environment given that steady state astrocytes are normally shielded from blood/serum in vivo by the blood–brain barrier (BBB) except following CNS injury or in disease. Serum exposure accordingly induces a reactive state in astrocytes that is reminiscent of that seen during injury or disease.

Due to small percentages of contaminating cells in MD astrocyte cultures, it can be difficult to determine if an effect is truly cell autonomous. For instance, stimulating MD astrocytes with a TLR4 agonist might seem to result in astrocytic changes, but rodent (unlike human) astrocytes appear not to contain the necessary receptors (e.g., TLR4) or downstream signaling proteins and adaptor proteins (e.g., MYD88 and TRAM) to respond to TLR4 agonists (Cahoy et al., 2008; Zhang et al., 2014; Anderson et al., 2016; Srinivasan et al., 2016; Chai et al., 2017). In fact, small percentages of contaminating microglia or macrophages can respond dramatically to TLR4 agonists and release sufficient cytokines to induce secondary changes in astrocytic populations. In addition to issues of contamination, it can be difficult to study responses to disease or injury in MD astrocytes, as these cultures are highly reactive at baseline due to serum exposure (Foo et al., 2011; Zamanian et al., 2012).

New serum-free isolation methods have been developed that use antibodies conjugated to magnetic beads (magnetic-activated cell sorting) or Petri dishes (immunopanning; Foo et al., 2011; Scholze et al., 2014) to achieve astrocytes of very high purity from both human and rodent brain tissue (Zhang et al., 2016). Astrocytes cultured by serum-free methods are minimally mitotic, morphologically more similar to in vivo astrocytes, and less activated (Foo et al., 2011; Zamanian et al., 2012; Anderson et al., 2016; Liddelow et al., 2017). Although these techniques produce highly pure populations of cells that retain in vivo gene profiles, they are considerably more expensive and have lower yields compared with MD cultures. Further, unpublished results in our laboratory suggest that highly mitotic MD astrocytes are easier

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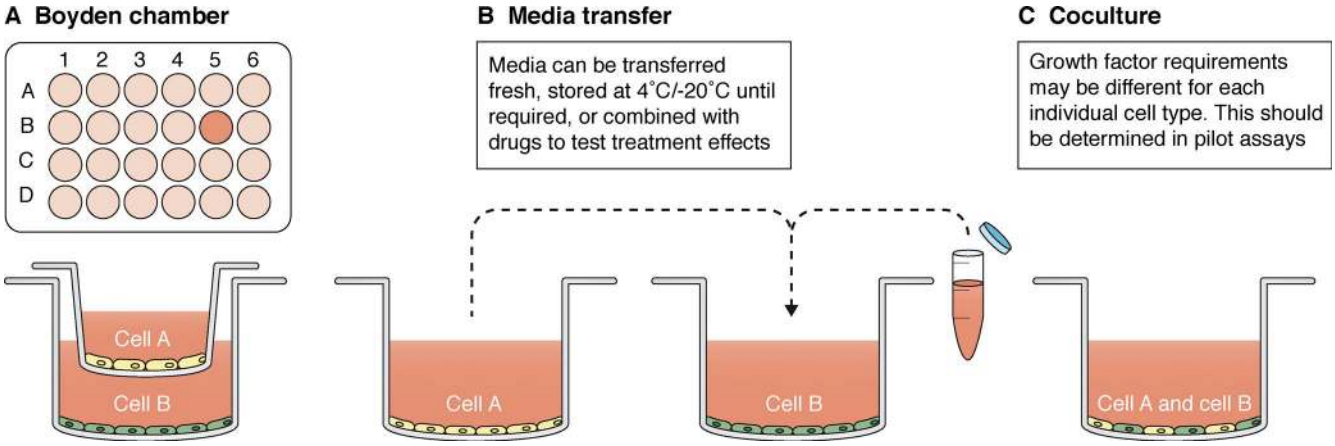

Figure 2. **Cell–cell interactions in a culture dish.** Several methods available for investigating interactions between cell types. **(A)** Boyden chamber: Two cell types grown in the same well but separated via semipermeable membrane. This retains bidirectional cell–cell communication via secreted cues. **(B)** Media transfer: Individual cells grown in isolation with exchanged media containing secreted factors. Benefits include ability to produce and store conditioned medium in bulk (if factors are stable at storage temperatures) and amenity to neutralizing antibodies or drugs. **(C)** Coculture experiments: Two (or more) types of cells in the same culture well, allowing for communication by secreted factors and direct cell–cell contact.

to manipulate via traditional CRISPR (clustered regularly interspaced short palindromic repeats) knockout techniques than largely postmitotic immunopanned astrocytes (likely due to the fact that Cas9 cuts DNA more efficiently in dividing cells). Both in vitro and in vivo, manipulating postmitotic astrocytes may prove easier using newly developed CRISPR interference-based gene inactivation techniques (Zheng et al., 2018).

Given the variety of new tools available to study astrocytes in vitro, it is important to remember to choose the method that is best suited to the scientific question. For instance, many researchers are attempting to disentangle the interplay between various CNS cells—for example, recent work has shown that neuronal neurexins interact with astrocytic neuroligins to influence astrocyte morphology and function (Stogsdill et al., 2017), and that neuronal fibroblast growth factor can dictate astrocyte morphogenesis (Stork et al., 2014). These experiments, by definition, require cocultures of distinct cell types. Cocultures can be used to study direct or indirect interactions, via growing two cell types in the same culture (mixed culture method), separating individual cell types by use of a Boyden chamber, or by transferring conditioned media from one cell onto another (Fig. 2). We recently used coculture methods using activated microglia/macrophages to characterize factors that induce astrocyte reactivity during neuroinflammation (Liddelow et al., 2017). Such studies highlight the need for more complex, multicellular culture systems that maintain the physiological behavior of glial cells.

Existing culture methods are also hampered by age and location restrictions of the tissue from which healthy quiescent astrocytes can be derived. Dissociating CNS tissue into single-cell suspensions is traumatic, and astrocytes are easiest to obtain before extensive myelination occurs (Foo et al., 2011), a process that begins around day 5 after birth in rats (Bayraktar et al., 2014). As such, large numbers of quiescent astrocytes are most efficiently obtained from early postnatal rodent pups in which astrogenesis has begun but myelination is limited. Culturing astrocytes from highly myelinated adult tissue without altering their gene expression profiles remains difficult with current methods. Improved techniques for culturing mature mouse astrocytes will provide a powerful way to couple mouse genetics with the ease of in vitro experiments. New dissociation kits such as the Miltenyi Adult Brain Dissociation Kit are purported to achieve higher yield, single-cell suspensions that are less reactive. Antibodies have also been identified that facilitate rapid isolation of relatively pure populations of astrocytes from single-cell suspensions, such as those targeting ACSA-2 (ATP1B2; Batiuk et al., 2017; Kantzer et al., 2017). These techniques may prove instrumental in allowing researchers to bypass the difficulties associated with culturing primary glia from old or diseased tissue.

Finally, there has been a great deal of effort devoted to inducing the differentiation of stem cells or stem-like cells into astrocytes. One benefit of this approach is that astrocytes can be differentiated from patient-derived induced pluripotent stem (iPS) cells to better understand how astrocytes function in human disease. However, these techniques are subject to the same limitations as those that apply to primary purified astrocytes including issues of cell purity and reactivity. For instance, many astrocyte differentiation protocols use reagents that can induce astrocyte reactivity, and thus it is important to consider the potential contribution of reactive changes (Gupta et al., 2013; Krencik and Ullian, 2013; Magistri et al., 2016). Further, both in single-cell layer cultures and in organoids, astrocytes continue to mature, even after more than a year in culture, meaning the maturity of the cells might impact experimental outcomes (Paşca et al., 2015; Sloan et al., 2017). This slow maturation can be viewed as a strength of these culture methods rather than a limitation, as we now know that astrocytes undergo prolonged transcriptomic changes during normal development and aging in vivo (Boisvert et al., 2018; Clarke et al., 2018), and aging cultures can be used as tools to dissect such changes.

Microglia

Microglia follow a unique developmental path into the CNS. Unlike neurons, astrocytes, and oligodendrocytes that derive from neural crest epithelium, microglia originate from yolk sac mac-

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Table 3. **Common microglia markers and reagents**

Gene (protein)	Labeled cells	Genetic lines	Antibodies	Notes	Reference
Cx3cr1 (CX3CR1)	Microglia and other myeloid lineage cells	Fluor. reporter, Cre, CreERT	Y	Quadruple-colored PrismPlus lines available	Jung et al., 2000; Parkhurst et al., 2013; Yona et al., 2013; Tay et al., 2017
Aif1 (IBA1)	Microglia and other myeloid lineage cells	Fluor. reporter	Y	Increase in IBA1 staining often used to suggest activation	Hirasawa et al., 2005
Ptprc (CD45)	Microglia and other myeloid lineage cells	Fluor. reporter, Cre	Y		Yang et al., 2008
Itgam (CD11b)	Microglia and other myeloid lineage cells	DTR/GFP line, Cre	Y		Ferron and Vacher, 2005; Stoneman et al., 2007
Tmem119 (TMEM119)	Microglia	N	Y	Protein does not label young microglia	Bennett et al., 2016
Sall1 (SALL1)	Microglia	CreERT	Y		Takasato et al., 2004; Inoue et al., 2010; Buttgereit et al., 2016
Fcrls (FCRLS)	Microglia	N	Y		
P2ry12 (P2RY12)	Microglia	N	Y		
Adgre1 (F4/80)	Microglia and other myeloid lineage cells	Cre	Y		Schaller et al., 2002
Cd68 (CD68)	Microglia and other myeloid lineage cells	Fluor. reporter, rtTA, CreERT2	Y	Often used as a marker of microglial activation	Pillai et al., 2009; Franke et al., 2013; Iqbal et al., 2014
Cd40 (CD40)	Microglia and other myeloid lineage cells	N	Y		
Csf1r (CSF1R)	Microglia and other myeloid lineage cells	Fluor. reporter, Cre		Required for microglial survival	Sasmono et al., 2003; Deng et al., 2010; Schreiber et al., 2013; Loschko et al., 2016

DTR, diphtheria toxin receptor; Fluor. reporter, fluorescent reporter; N, no; Y, yes.

rophage progenitors that migrate into brain early during embryonic development (Alliot et al., 1999; Ginhoux et al., 2010; Schulz et al., 2012; Aguzzi et al., 2013; Gomez Perdiguero et al., 2015; Li and Barres, 2018). Because microglia function as professional CNS phagocytes and are related to myeloid cells, they have long been studied using tools originally created by immunologists to study peripheral cells. It is now apparent that various peripheral immune cells infiltrate the BBB and reside in the leptomeningeal, ventricular, and perivascular spaces during normal brain physiology; these cells also breach the inner glial-limitans and choroid plexus–cerebrospinal fluid barrier in pathological conditions (Kivisäkk et al., 2003; Agrawal et al., 2006; Engelhardt and Ransohoff, 2012). Although similar to microglia, these nonparenchymal CNS macrophages comprise a separate population with unique phenotypic and genotypic markers and are subject to distinct transcriptional regulation during development (Goldmann et al., 2016). Many tools used to study microglia do not distinguish between microglia and CNS macrophages, and this limitation has prompted a new wave of innovation in tools to study microglia in vivo and in vitro (Table 3).

In vivo

Microglia are highly dynamic cells, and based on their similarity to peripheral immune cells and nonparenchymal macrophages, a discrete set of markers that definitively identifies microglia in all contexts and conditions has not yet been identified (Bennett et al., 2016; Segal and Giger, 2016). Many studies rely on unbiased clustering of whole transcriptome data, as analysis of a large group of genes expressed at high or low levels is required to clearly define a cell as being highly microglia-like (Table 3). Comparisons become even more complex when assaying the heterogeneous microglial responses to disease (Keren-Shaul et al., 2017).

The most common cell line used to manipulate microglia is based on *Cx3cr1* (encoding fractalkine receptor), a gene classically associated with leukocyte adhesion (Combadiere et al., 1998). *Cx3cr1-eGFP* lines are used for visualizing microglia (Jung et al., 2000), whereas *Cx3cr1*-Cre (Parkhurst et al., 2013) and *Cx3cr1*-CreER (Littman, 2013; Yona et al., 2013) lines are used to manipulate microglial gene expression. While *Cx3cr1* is predominantly expressed by microglia in the CNS, it is also expressed by leptomeningeal macrophages and various peripheral cells including lymphocytes, natural killer cells, and peritoneal macrophages, among others (Jung et al., 2000). Because these peripheral cells infiltrate the CNS and interact with local cells both in the healthy brain and during disease or injury, deficits seen in the brain parenchyma after manipulating gene expression using *Cx3cr1* lines could potentially involve nonmicroglial cells (Yona et al., 2013). As with astrocytes, the likelihood of this depends largely on the question being studied. Given that most CX3CR1$^+$ cells in healthy brain are microglia, studies of microglial phago-

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cytosis during development are less subject to potential off-target effects of *Cx3cr1*-based targeting (Schafer et al., 2012). By contrast, studies focused on microglial phagocytosis at sites of acute injury could be complicated by an influx of peripheral CX3CR1^{+} myeloid cells (Perry et al., 1987). Further, some microglial functions such as synaptic pruning depend on CX3CR1 (Paolicelli et al., 2011; Schafer et al., 2012), so special consideration needs to be taken when using knockin genetic lines such as the *Cx3cr1-eGFP* line as each eGFP allele knocks out an endogenous allele of *Cx3cr1* (Wolf et al., 2013; Jobling et al., 2018).

Traditional immunohistological markers of microglia have similarly suffered from an inability to delineate between microglia and peripheral immune cells. Like many macrophages, microglia express *Cd11b* (ITGAM), *Aif1* (IBA1), *Adgre1* (F4/80), *Cd45* (CD45), *Spi1* (PU.1), and *Cd115* (CSF1R). Historically, microglia were often distinguished from other macrophages based on their relatively low expression of *Cd45*, but defining microglia as CD11b^{+}CD45^{low} is most useful in the context of FACS (Ford et al., 1995). Expression of many of these common markers can increase or decrease in the context of injury or disease, when separating the influence of infiltrating peripheral cells is especially critical (Keren-Shaul et al., 2017). For instance, activated microglia up-regulate expression of *Aif1* as they undergo hypertrophy and divide; however, following injury, peripheral immune cells expressing high levels of *Aif1* (IBA1) often flood into the injured site where they can exhibit altered morphology, making them difficult to distinguish from activated microglia. On the other hand, there are research questions that might not necessitate separating the effects of microglia and peripheral immune cells; for instance, a study focused on identifying cytokines that activate CNS cells following injury might not necessitate identification of the specific cellular source of the cytokines.

Many experimental approaches have been developed to circumvent problems associated with shared gene expression between microglia and related cells. *Cx3cr1*-CreER mice allow for inducible manipulation of gene expression in microglia and other peripheral cells, with peripheral cells eventually being replaced by nonrecombined cells from the bone marrow, thus distinguishing them from CreER-expressing microglia (Goldmann et al., 2013). While this approach may not work for all developmental studies given the time required for peripheral cells to be completely replaced, it is a creative way to make *Cx3Cr1*-based manipulations more microglia-specific. The combination of *Cx3cr1*-Cre mice with R26R-Confetti mice creates a mouse in which one of four fluorescent proteins is stochastically and permanently expressed in each individual microglia in a tamoxifen-inducible fashion (Tay et al., 2017). The resulting "microfetti" mouse facilitates visual tracking of microglial proliferation and expansion throughout development.

In addition to creative uses of *Cx3cr1*, new markers and genetic lines have been developed to study microglia more unambiguously. Expression of *Sall1* is highly microglial specific in the CNS (although this gene is also expressed in peripheral organs such as developing kidney), and *Sall1*-based GFP and CreERT lines allow for specific labeling and manipulation of microglia (Takasato et al., 2004; Inoue et al., 2010; Buttgereit et al., 2016). *Tmem119* was also identified as a novel microglia-specific marker that is not expressed by peripheral myeloid cells (Bennett et al., 2016). Antibodies to TMEM119 label microglia in tissue sections and can be used to isolate microglia via FACS in both mice and humans. While *Tmem119* is expressed in all microglia, it is developmentally regulated, and TMEM119 protein is not expressed in all microglia before postnatal day 14. Efforts are underway to develop inducible lines that use the *Tmem119* promoter. Finally, a recent study of peripheral immune cells that infiltrate and populate the CNS in both mouse and humans has led to the identification of several markers that are expressed by cells in the CNS that also express traditional microglial markers, but derive from the periphery (blood and bone marrow); this should help to determine the contribution of infiltrating cells in the context of various diseases (Bennett et al., 2018).

In addition to manipulating gene expression in microglia, debate has arisen about how to eliminate microglia from the CNS, often with an eye toward replacing them with genetically modified microglia or peripheral immune cells (Capotondo et al., 2012). Elimination approaches include the use of diphtheria toxin receptor (Parkhurst et al., 2013; Bruttger et al., 2015) or herpes simplex virus 1 thymidine kinase (HSV-1-tk; Heppner et al., 2005; Varvel et al., 2012) driven by a microglia-specific promoter. Another approach is to eliminate receptors that microglia and other macrophages require for survival. For instance, global deletion of *Csf1r* prevents microglia from populating the CNS during development (Ginhoux et al., 2010), and inhibitors of CSF1R can induce large-scale microglial apoptosis (Elmore et al., 2014).

Studies that have used these methods to eliminate microglia have been instrumental in our evolving understanding of microglial biology, but certain caveats should be considered when selecting a method. First, as discussed earlier, most of these techniques also target peripheral macrophages. This lack of specificity might not pose a problem in studies of CNS functions that have very little peripheral involvement, but in general, studies in which microglia are eliminated should incorporate controls for off-target effects on peripheral cells. Another consideration is the effect of microglial elimination on nearby cells. For example, inducing large-scale microglial death triggers an inflammatory response that can induce changes in surrounding cells (Bruttger et al., 2015). It is also important to stress that no technique appears to reliably achieve complete elimination of all microglia. For some functions, such as synaptic pruning, a substantial reduction is sufficient to induce a phenotype. However, in the context of immune responses, which often involve amplifying signaling cascades, even a small percentage of remaining microglia can induce an inflammatory response (Liddelow et al., 2017). Incomplete elimination also typically results in the rapid repopulation of the CNS by the remaining microglia within 1–3 d (Elmore et al., 2014) due to the ability of microglia to sustain themselves in perpetuity (Huang et al., 2018). Finally, in the absence of microglia, peripheral myeloid cells can infiltrate the CNS and differentiate into cells morphologically resembling microglia (Bennett et al., 2018). Because this occurs at a very low rate in the healthy brain, many elimination studies are coupled with treatments such as radiation that create a niche for peripheral cell engraftment. However, radiation also induces BBB breakdown, inflammation,

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and other systemic changes that must be controlled for when considering effects of peripheral infiltration.

In vitro

Many mechanistic insights have come from studies of microglia in culture (Stansley et al., 2012). However, as for astrocytes, techniques for culturing microglia have largely relied on serum to maintain cell viability, and few techniques allow for isolation of extremely pure populations of primary microglia. Given that microglia are innate immune cells, they are highly tuned to the health of the brain parenchyma, and serum exposure results in activation and conversion to an ameboid morphology that differs from the highly process-bearing morphology of microglia under steady state conditions (Stansley et al., 2012). The use of serum-exposed cultures thus poses problems for studying the function of microglial in the uninjured CNS. To avoid these issues, many studies use ex vivo brain slices, which retain important cell–cell interactions, and this approach has provided the basis for many fundamental studies in microglial biology (Brockhaus et al., 1996; Petersen and Dailey, 2004). That said, preparation of ex vivo brain slices exerts trauma (especially neuronal axotomy), resulting in many of the same pathological changes in microglia that occur with serum exposure (Haynes et al., 2006; Masuch et al., 2016). Thus, methods that better reflect in vivo microglia in their physiological environment would be invaluable to better understand the full repertoire of microglial functions.

One of many attempts to develop new culture methods for microglia was based on the ability of astrocyte-conditioned medium to maintain cell survival and induce morphological changes in microglia as well as the observation that CNS microglia lack cholesterol synthesis machinery (Zhang et al., 2014; Bohlen et al., 2017). This led to development of serum-free medium supplemented with cholesterol, CSF1/Il-34, and TGFβ, allowing microglia to be cultured in a somewhat quiescent and process-bearing state with low expression of injury/disease response genes (Salimi et al., 2003; Butovsky et al., 2014; Bohlen et al., 2017). Although this method has some advantages over previous culture systems, microglia cultured in this system still lose expression of many microglia-specific genes including *Tmem119* and *Sall1* (Bennett et al., 2016; Bohlen et al., 2017), a phenomenon that also occurs in human microglia purified from postmortem samples (Gosselin et al., 2017). As we are still discovering new markers and behaviors that define microglia in a nondiseased state, it is difficult to determine how successfully new methods model endogenous microglial behavior. Researchers must therefore determine which in vitro systems most accurately reproduce the in vivo physiology of interest, and findings should be validated in vivo.

Microglia derived from human iPS cells provide an exciting alternative to traditional primary cultures. Considerable effort has been devoted to the generation of microglia from human iPS cells, especially given that microglia express many genes associated with neurological diseases, raising the possibility that intrinsic changes in microglia might underlie some of these diseases (Muffat et al., 2016; Abud et al., 2017; Douvaras et al., 2017; Haenseler et al., 2017). Despite extraordinary innovation in stem cell biology, our inability to perfectly define microglia has made it difficult to fully evaluate the success of these efforts. Given that genuine primary microglia turn off expression of key microglial genes when removed from the CNS, it is still unclear what minimum set of genes or in vitro functions might represent a gold standard for successful generation of microglia from iPS cells. Although no iPS cell–derived microglia recapitulate all aspects of microglial function, each provides a good model of a subset of microglia characteristics, such as cytokine secretion, phagocytosis, etc. This is a rapidly evolving field, and thus an exhaustive discussion of published reports on iPS cell–derived microglia is beyond the scope of this review. As with all reductionist approaches, however, it is important to choose the system that best recapitulates the function of interest and to validate results in vivo where possible.

Conclusions

For the first time, we are on the verge of being able to specifically manipulate individual glial cell types, and culture systems are improving in their accuracy and complexity with regard to the ability to maintain glial cell survival without fundamentally altering their function. Historically, the ability to specifically manipulate cell types has provided the foundation for mechanistic studies of cell biology, but the plethora of tools with which to study glial cells has led to confusion about the best models to use. The diversity of techniques will only grow as single-cell sequencing provides unbiased, high-throughput data on the physiological and pathological functions of heterogeneous glial cells. As we learn more about the complexity of glia at the single-cell level, we are building new methods to dissect their intricate interactions. These new tools in conjunction with those already in use will enable us to continue to unravel the mystery and magic of these important cells.

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