Exploring iPSCs-derived microglia in psychiatry: methods, implementations, and challenges.

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Summary

Microglia, the principal immune cells of the central nervous system (CNS), have been implicated in playing a key role in psychiatry. These cells are involved in maintaining and protecting the brain against pathogens through mechanisms that promote neuroinflammation. While neuroinflammation is essential for achieving brain homeostasis, its chronic activation can lead to impaired brain function, disrupted neuronal development, and altered synaptic activity. Research into psychiatric disorders such as schizophrenia (SCZ) and autism spectrum disorders (ASD) has shown increased microglia activity and density, although most studies rely on post-mortem tissue or positron emission tomography (PET) imaging. Recent advances in induced pluripotent stem cells (iPSCs)-derived microglia (iMGL) offer new opportunities to study psychiatry disorders at the molecular and cellular levels, potentially leading to a better understanding of these disorders and their underlying mechanisms, as well as contributing to the development of effective biomarkers and treatments. Despite this potential, the application of iMGLs in psychiatric research remains limited. This review discusses the formation and function of microglia in both health and psychiatric conditions. Additionally, outlines various protocols for generating iMGLs, evaluates their current research applications, and addresses the limitations and associated challenges**.**

Plain Language Summary

Microglia are important cells found in the brain. They are the primary cells responsible for maintaining the brain's health and protecting it from abnormalities. Microglia are constantly monitoring their surroundings, and when something unusual occurs, they move to the affected area. Depending on the problem, they change shape and release various particles known as cytokines, which help address the issue through neuroinflammation. Once the problem is resolved, microglia clean up any waste through a process called phagocytosis, where they engulf and eliminate unnecessary materials, such as non-functional neurons. They also support other cells, like neurons, to develop and grow properly and communicate effectively, ensuring the brain functions well. However, when microglia are activated for extended periods, they start to release excessive amounts of cytokines, and the brain neuroinflammation produced disrupts other mechanisms essential for brain maintenance, such as neuronal communication, which can be detrimental to health and lead to various psychiatric disorders.

These psychiatric disorders are unique because they vary greatly among individuals, and their origins are not well understood. However, many people suffer from these conditions,

and it is crucial to understand them to develop effective treatments. Scientists have recently developed a type of cell known as induced pluripotent stem cells (iPSCs), which are special because they can be derived from a patient's skin or blood and programmed to become any type of cell, including microglia. Different laboratories have established protocols to generate these microglia-like cells (iMGLs) from patient iPSCs, based on the biological origin and formation of microglia. By adding specific factors as the iMGL develops in culture, it is possible to produce cells that closely resemble real human microglia. This approach has enabled the study of microglia derived directly from patients with schizophrenia (SCZ), autism spectrum disorder (ASD), and others. However, much more research is needed to fully understand how microglia are involved in these disorders.

So far, iMGLs derived from patients have been studied either on their own or in combination with other brain cells, such as neurons. At the same time, other models can replicate a human brain environment, for example, the brain organoids also from patient iPSCs. These organoids are small structures that can mimic the organization and function of a brain on a smaller scale, allowing for the study of microglial interactions with other cell types or mechanisms. There are also transplantation models, where iMGLs or organoids are implanted into the brains of mice by surgery, and they form direct connections with the mouse brain elements, providing deeper insights into the role of iMGLs in a real brain context.

In this review, we explain how microglia are created and their function in both health and psychiatry. We also present various protocols for generating microglia from patient iPSCs and their differences. Additionally, we explore various methods of using microglia, either in isolation or in combination with other cell types or structures. Finally, we discuss the challenges involved in their use in psychiatric research. This review aims to advance the understanding of psychiatric disorders and microglia implications, with the potential for rapid diagnosis and the development of possible treatments in the future.

Introduction

Microglia are the resident immune cells of the central nervous system (CNS), playing a crucial role in maintaining homeostasis within the brain (Magni, Riboldi & Ceruti., 2024). They regulate neuronal network formation and synaptic pruning during development and are involved in the maintenance and protection of the CNS, as well as in synaptic plasticity (Eggen et al., 2019). Microglia are key players in neuroinflammation, responding to pathogens or other abnormalities in the brain (Li & Barres., 2018). This role has made them a focal point in

psychiatric research, as prolonged neuroinflammation is implicated in these disorders (Heider et al., 2021; Rahimian et al., 2021; Shemer et al., 2015).

The study of psychiatric disorders has been limited by significant heterogeneity among patients and the overlapping molecular signatures throughout different disorders (Brennand, 2022), which complicates clinical translation. Despite these challenges, studies using animal models (Baker et al., 2020), post-mortem tissue from patients (Ai et al., 2023), and positron emission tomography (PET) imaging (Meyer et al., 2020) have consistently identified neuroinflammation as a prominent feature in psychiatric disorders, such as schizophrenia (SCZ), autism spectrum disorder (ASD), bipolar disorder (BD), and others. These studies have also demonstrated increased microglia density and activation suggesting that neuroinflammation driven by microglia activation may play an important role in our understanding of the psychiatry field. However, these models are limited in fully understanding the implications of microglia in psychiatry due to a lack of dynamic monitoring of microglia activity at cellular levels, as well as the species-specific differences observed between humans and mice (Hong., et al 2023)

Since the publication of the first protocol to generate microglia from patient-derived induced pluripotent stem cells (iPSCs) (Muffat et al., 2016), new opportunities have emerged for psychiatric research. iPSCs can be derived from patients and reprogrammed to a pluripotent state, enabling differentiation into diverse cell types carrying a specific genetic background (Hong et al., 2023). To date, several protocols have been developed to generate iPSCs-derived microglia (iMGL) to enhance the study of different neurological and neurodegenerative diseases (Wurm et al., 2021), and implement them in different models such as mono- or co-culture, cerebral organoids or transplantations into mice brains (Haenseler & Rajendran., 2019). Although, the application of these approaches in psychiatry remains limited, largely due to the significant complexity inherent in these disorders and the difficulties associated with defining microglia (Quadrato, Brown & Arlotta., 2016; Brennand et al., 2022; Paolicelli et al., 2022).

This review provided an overview of the use of iMGL in psychiatric research. We first describe the origin and function of microglia in both health and disease, emphasizing the importance of understanding these aspects to effectively follow the proposed protocols and their relevance in psychiatry. Next, we summarize a few established protocols for iMGL generation, highlighting differences in culture models, the starting point of differentiation

relative to microglia ontogeny, and growth factors used for iMGL differentiation. Moreover, we discuss iMGLs molecular markers, assays employed, and iMGL response to LPS. Subsequently, we examine the experimental use of iMGLs in psychiatric research, focusing on patient-derived iMGLs in monoculture and co-culture studies, and the application of transplantations and three-dimensional (3D) models to recreate brain-like environments. Additionally, we discussed the challenges associated with applying iMGL in psychiatric research. Thus, advancing our understanding of these complex disorders could facilitate the development of specific biomarkers for early diagnosis and pave the way for the creation of potential therapeutic interventions.

1. The role of microglia in the brain

1.1. Microglia formation and development

Before exploring the functions of microglia, it is essential to understand their origin and development. The origin of microglia has been debated for many years, but it is now widely accepted that microglia derive from primitive yolk sac macrophages (Csatári, Wiendl & Pawlowski., 2024; Dermitzakis et al., 2023; Eggen et al., 2019). During early embryonic development, microglia are formed in the yolk sac, where the process of primitive hematopoiesis begins from early erythromyeloid progenitors (EMPs). These progenitors give rise to primitive myeloid cells, which are characterized by the expression of the transcription factor PU.1, which guides their differentiation into microglia (Mendes & Majewska., 2020; Paolicelli et al., 2022).

These yolk sac-derived progenitors migrate to the developing central nervous system (CNS), where they can pass without restriction since the blood-brain-barrier (BBB) is not yet fully formed (Mendes & Majewska., 2020; Eggen et al., 2019). Upon entering the CNS, the yolk sac-derived progenitors begin to differentiate into microglia (Figure 1), driven by signaling molecules such as colony stimulated factor 1 (CSF-1), transforming growth factor-beta (TGF-b), and Interleukin-34 (IL34). These factors promote the survival, proliferation, and differentiation of the myeloid cells into microglia (Paolicelli et al., 2022; Spittau, Dokalis & Prinz., 2020).

During the differentiation, the expression of microglia-specific genes and the characteristic morphology of microglia begin to emerge. This differentiation process is regulated at both, epigenetic and transcriptional levels (Csatári, Wiendl & Pawlowski., 2024). Key factors in this regulation include the transcription factor PU.1, IRF8 and Sall1, Sall3, as well as MEIS3 and

MAFB, and proteins such as IBA1, P2YR12, TMEM119, CSF-1, which are crucial for establishing and maintaining microglia during development (Paolicelli et al., 2022). After differentiation, microglia start to proliferate and spread throughout the brain, promoting the differentiation of different CNS populations (Mendes & Majewska., 2020), and establishing a self-renewing property, essential for their maintenance into adulthood (Paolicelli et al., 2022; Eggen et al., 2019).

1.2. Microglia roles in health and psychiatry

Microglia are recognized as the primary immune cells in the brain, playing a crucial role in the immune defense mechanism of the central nervous system (CNS). These cells are essential for protecting the brain from both endogenous and exogenous pathogens (Michalski & Wen., 2023). Microglia perform immune surveillance within the CNS, continuously monitoring the environment to detect any disruption in the brain homeostasis. Upon detecting disturbances, microglia become activated and migrate to the site of the issue, where they engage in phagocytosis to eliminate pathogens, damaged cells, or other abnormalities (Li & Barres., 2018). Additionally, activated microglia release cytokines, which are small proteins critical for immune cell signaling in initiating and resolving immune response within the brain and supporting neighboring cells (Eyo & Dailey., 2013). This activation process involves changes in their functional and morphological state (Magni, Riboldi & Ceruti., 2024). Depending on their type, cytokines secreted by microglia can either promote inflammation (e.g., IL-1β, IL-6, TNF-α) to address the problem or reduce inflammation and aid in tissue repair (e.g., TGF-β, IL-4, IL-10) (Eggen et al., 2019). An illustrative scheme of microglia formation and functionality is presented in Figure 1.

Recent research has highlighted the involvement of microglia in the progression of neurodegenerative and psychiatric disorders, suggesting that persistent microglia activation can lead to sustained neuroinflammation in the brain (Heider et al., 2021). Neuroinflammation arises when microglia are exposed to pathogens, such as bacterial lipopolysaccharides (LPS) or viral infections which trigger the activation and production of inflammatory cytokines, resulting in inflammation of the CNS (Paolicelli et al., 2022). While this inflammatory response is critical for maintaining brain homeostasis and neuroprotection, chronic neuroinflammation is believed to contribute to the progression of various neuropsychiatric disorders (Heider et al., 2021; Rahimian et al., 2021).

Neuropsychiatric disorders are complex diseases, with their origins and implications remaining unclear (Reis de Assis et al., 2021). These disorders are characterized by high heterogeneity and are challenging to study due to the complexity of brain connections. Additionally, the lack of clear biomarkers and available data makes it difficult to identify the underlying pathophysiology and assess treatment efficacy (Brennand., 2022). Nonetheless, current studies support the hypothesis that prolonged microglial activation leads to aberrant cytokine production, disrupting normal protein secretion and synaptic pruning processes, thereby affecting the appropriate elimination of synaptic connections, neuronal integrity, and synaptic plasticity (Heider et al., 2021; Hong et al., 2016; Mondelli et al., 2017). For instance, microglia have been shown to express several risk genes for neuropsychiatric diseases, such as C4, which is associated with SCZ. This gene is involved in synaptic pruning mediated by the phagocytic activity of microglia (Prinz, Jung & Priller., 2019; Reis de Assis et al., 2021).

The previous statement is based on the notion that microglia are in constant interaction with neurons, assisting appropriate neuronal activity during development and maturation, and contributing to proper connectivity between neurons (Eyo & Dailey., 2013). Studies of post-mortem brain tissue from schizophrenia (SCZ) patients revealed a reduction in prefrontal cortical volume, along with decreased synaptic density, which is presumed to be associated with high microglial density and activation (Breitmeyer et al., 2023; Ai et al., 2023). However, positron emission tomography (PET) imaging showed lower microglial activation in SCZ (Meyer et al., 2020), but confirmed elevated activation in bipolar disorder (BD) and autism spectrum disorder (ASD) (Haarman et al., 2014). Moreover, animal models of psychiatric research have demonstrated elevated cytokine levels linked to microglia activation, synaptic dysfunction, and accelerated inflammation. These findings are observed in various models, including maternal immune activation (MIA) or Knockout mice (Andoh, Ikegaya & Koyama., 2019). For example, Rogers et al., (2011) showed that genetic mutations in microglia-encoded genes, such as *CX3CR1* or *CX3CL1*, can induce SCZ-like phenotype. Thus, emphasizing the need for further research to unrevealed the precise contribution of microglia to psychiatric disorders.

Although post-mortem and imaging studies provide insights into the functioning and affection of patient brains, it is challenging to thoroughly investigate the role of microglia in psychiatry. As previously mentioned, iPSCs present a promising approach to studying complex diseases (Reis de Assis et al., 2021). In psychiatry, iPSCs have been used to model disorders such as SCZ or ASD, thereby recapitulating molecular mechanisms underlying the

disorders *in vitro* (Magni, Riboldi & Ceruti et al., 2024). This innovative technology provides a valuable *in vitro* model to address the challenges associated with understanding the role of microglia in psychiatry (Heider et al., 2021; Koskuvi et al., 2024). Nevertheless, studies focusing on the generation of iPSC-derived microglia (iMGL) and their influence in psychiatry remain limited.

2. Established protocols to differentiate iPSC-derived microglia (iMLG)

Microglia have increasingly been recognized for their pivotal role in the initiation and progression of diseases affecting the central nervous system (CNS) (Magni, Riboldi & Ceruti., 2024). The creation of induced pluripotent stem cells (iPSCs) as a versatile tool for generating various cell types from a renewable source (Speicher et al., 2019) has promoted extensive research into the generation of iPSC-derived neurons or astrocytes (Haenseler & Rajendran et al., 2019). However, it was not until Muffat et al. (2016) that the first protocol for generating microglia-like cells (iMGLs) from iPSCs was introduced. Since then, multiple protocols have emerged, which have been comprehensively reviewed elsewhere (Speicher et al., 2019; Wurm et al., 2021).

These emerging protocols can be broadly categorized depending on different perspectives of microglia ontogeny (Figure 2). The first category is based on the notion that microglia originate from myeloid progenitors in the yolk sac, and then migrate to the developing brain to complete their formation (Csatári, Wiendl & Pawlowski., 2024). Protocols following this notion begin with the differentiation of iPSCs into embryoid bodies (EBs), followed by the generation of yolk sac-like embryoid bodies (YS-EBs) to produce microglia-like precursors (Muffat et al.), or they proceed directly from EBs to embryonic macrophage precursors (Haenseler et al., 2017) before obtaining fully formed iMGL.

Conversely, the second category of protocols employs a two-step approach to recapitulate microglia ontogeny. This approach begins with the differentiation of iPSCs into hematopoietic progenitor cells (iHPCs), which are crucial intermediates in microglia development (Mendes & Majewska., 2020; Figure 2). Following the generation of iHPCs, a protocol and its adaptation initiate microglia differentiation, culminating in a maturation phase that includes the addition of specific growth factors (Table1), to produce iMGLs (Abud et al., 2017; McQuade et al., 2018). Recently, a different protocol has been proposed that bypasses the maturation phase; it includes a cryopreservation step that allows the direct generation of iMGLs from iHPCs (Lanfer et al., 2022). Additionally, Douvaras et al. (2017) utilized a two step-approach where

iPSCs were differentiated into myeloid progenitors instead of iHPCs, subsequently giving rise to microglia progenitors, which then develop into iMGLs.

Furthermore, two different protocols highlight the importance of microglia interaction with other brain cell types during their development. These co-culture models aim to mimic the natural cellular environment of the CNS, promoting more physiologically relevant microglial phenotype and function. Following EB differentiation, Haenseler et al. (2017) suggest that co-culturing embryonic macrophage precursors with cortical neurons, also derived from iPSCs, is essential for generating iMGLs. Similarly, Pandya et al. (2017) employed a co-culture model using astrocytes instead of neurons. In this protocol, iHPCs are co-cultured with astrocytes after their formation, and once microglia are generated, the MGLs are isolated from the culture (Pandya et al., 2017). In both protocols, iMGL expresses key microglia-specific markers and functionality, and compared to monoculture models, these co-culture systems enhance homeostatic functions and promote a more balanced cytokine response, favoring anti-inflammatory and pro-remodeling activities. These suggest that co-culture models may better replicate authentic microglial physiology (Haenseler et al. 2017).

Besides 2D cultures, various protocols have been proposed for co-culturing iMGLs with 3D cerebral organoids (Zhang et al., 2023). This integration of iMGL has been shown to promote both organoid maturation (Park et al., 2023) and neuronal maturation by showing synapse pruning and more mature electrophysiological properties (Popova et al., 2021; Michalski & Wen., 2023). Specifically, Popova et al. (2021) demonstrated that the addition of exogenous iMGL to cerebral organoids in culture led to the preservation of cytokines and chemokine gene expression in a human-like brain environment. This approach can also be extended to *in vivo* contexts through xenotransplantation into mouse brains. This suggests that brain-specific conditions provide essential cues for microglia development and preservation in culture for longer periods, which further influence the development of other cell types and enable the observation of mature microglia phenotypes. (Popova et al., 2021; Quadrato, Brown & Arlotta., 2016). Figure 2 provides a summarized illustrative scheme presenting various models used to study iMGLs.

Overall, all these protocols yield microglia-like cells with morphological characteristics similar to native microglia in the CNS, expressing microglia-specific markers for immunocytochemistry such as IBA1, CX3CR1, TREM2, or CD45. In addition, most protocols used flow cytometry to characterize microglia cell populations with markers such as CD11b,

CD11c, and P2y12, and RNA-sequencing (RNA-seq) to analyze the transcriptional profile of the resultant iMGL. Moreover, these iMGLs exhibit functional characteristics typical of active microglia, including cytokine production and phagocytosis in response to environmental stimuli. iMGL functionality is frequently validated by stimulating the cells with LPS, which induces the secretion of pro-inflammatory cytokines such as IL-6, CXCL10, or TNFα (Michalski & Wen., 2023). However, the specific methods, differentiation timelines, growth factors, and markers differ between the protocols. Table 1 offers a clear and concise overview of each protocol, emphasizing their unique features, including the culture models used, differentiation starting points considering EBs or iHPCs, duration *in vitro* from iPSCs, growth factors included in iMGLs differentiation, iMGLs molecular markers and inflammatory response to LPS.

3. Application of iPSC-derived microglia in psychiatric studies

Schizophrenia (SCZ) and autism spectrum disorder (ASD) are among the most thoroughly investigated psychiatric conditions due to their association with genetic risk factors (Singh et al., 2022; Nóbrega et al., 2024). Based on the protocols earlier discussed, current research has focused on evaluating the functionality and characteristics of iMGLs from individuals affected with these disorders. This approach aims to elucidate the role of microglia in the pathophysiology of psychiatric conditions, particularly concerning microglia activation and its potential disruption (Hong et al., 2023; Hanger et al., 2020).

Koskuvi et al., (2024) conducted a study focused on examining the transcriptional and functional differences between iMGLs from SCZ patients compared to healthy controls. Their findings revealed dysregulation in homeostatic and inflammatory gene expression in SCZ-iMGL, including upregulation of IBA1 and downregulation of TREM2, both key microglia markers. Furthermore, they observed aberrant expression of inflammatory genes, such as the upregulation of the pro-inflammatory cytokine IL-1β, indicative of impaired microglia activation and neuroinflammation. These results suggest a strong genetic component in SCZ microglia that contributes to the neuroinflammatory processes associated with the disorder. However, these findings are based on a monoculture model, which may be insufficient to fully understanding the role of microglia (Haenseler & Rajendran et al., 2019), especially given the complexity involved in psychiatric disorders (Brennand., 2022).

Other authors believe that co-culture models are essential to understanding and improving the approach to microglia in psychiatry, as impaired interactions between microglia and other

glial cells or neurons are linked to ASD or SCZ (Ormel et al., 2018). Co-culture systems allow for more accurate phenotypic insights by facilitating microglia interaction with other CNS cell types. This setup provides a clearer understanding of the onset, progression, and severity of psychiatric symptoms (Heider, 2021; Haenseler et al., 2017). An established co-culture experiment demonstrated synaptic dysregulation in healthy-derived neurons when co-culture with SCZ-derived iMGL, specifically in the synaptic formation and increased synaptic pruning (Breitmeyer et al., 2023). These results align with findings from Sellgren et al., (2019), which reported increased synaptic elimination and engulfment in SCZ-iMGL in culture with neurons. Importantly, this last investigation did not use iPSC-derived microglia but still reported that the SCZ-associated risk variant in the C4 component increased microglia phagocytosis of synapses only when co-culture with neurons, with no effect when C4 was present only in microglia (Sellgren et al., 2019).

Similarly, Bose et al., (2014) showed that deletion of the ASD-associated gene *NRXN1* altered the expression of its isoform *NRXN1α*. This change led to increased secretion of pro-inflammatory interleukin IL-6 in iMGLs, which is associated with ASD. Additionally, multielectrode array (MEA) analysis revealed that this deletion impaired the capacity of iMGLs to support the formation of functional neuronal networks, resulting in aberrant neuronal circuits (Bose et al., 2014). These findings suggest that genetic background in both neurons and microglia contributes to the altered crosstalk seen in psychiatric disorders (Michalski & Wen., 2023). Park et al., (2020) further demonstrated that conditioned media from activated microglia reduced respiratory activity in cortical interneurons from both healthy controls and SCZ patients, suggesting metabolic remodeling. Despite these findings, more recent work showed minimal effects on neuronal activity in co-culture systems involving SZC-derived iMGL compared to healthy-derived iMGL (Koskuvi et al., 2024), emphasizing the need for further research due to heterogeneity in the results.

Previously, we discussed that co-culture systems involving iMGL and cerebral organoids represent promising platforms for the study of complex disorders, including those within the psychiatric domain. Organoids are generated from iPSCs by first forming embryoid bodies, which can develop into progenitors from the endoderm, ectoderm, and mesoderm, the three germinal layers. Then, through specific media and growth factors, these progenitors are directed to form CNS-specific tissues (Ormel et al., 2018). It has been demonstrated that cerebral organoids derived from SCZ patients recapitulate disorder-related mechanisms, such as altered progenitor survival and disrupted neurogenesis (Notaras et al., 2021). Wu et al.,

(2014) investigated the role of the ASD-associated gene *SCN2A* in human cerebral organoids populated with iMGLs. These cerebral organoids carried an SCN2A protein-truncation mutation found in children with ASD. They showed increased synaptic disruption and higher post-synaptic elimination in iMGLs within the organoids carrying the mutation. These results indicate that microglia's role in synaptic pruning is important for regulating synaptic transmission and modulating synapse density in ASD models associated with *SCN2A* deficiency (Wu et al., 2014)

Although iPSCs have been used to elucidate the cellular and molecular mechanisms underlying CNS diseases, the complex, dynamic interactions within the brain remain challenging to fully replicate *in vitro* (Magni, Riboldi & Ceruti, 2024). To overcome this limitation, researchers have proposed the transplantation of iMGL into neonatal mouse brains. This approach allows microglia to assume phenotypes and gene expression signatures similar to those observed in the human brain (Svoboda et al., 2019). Building on this, Schafer et al. (2023) developed an *in vivo* xenotransplantation approach to study human-specific microglia phenotypes in ASD. They generated human brain organoids from both ASD and unaffected control-derived iPSCs, populated these organoids with iMGL from the same sources, and further transplanted the resultant organoids into mice to achieve physiological conditions *in vivo*. Overall, iMGL from healthy control exhibited normal morphological and functional features, including brain surveillance and response to abnormalities. Conversely, ASD-derived iMGL in both unaffected and ASD brain organoids transplanted displayed phenotypes associated with active microglia, characterized by morphological changes linked to the ASD-associated brain environment (Schafer et al., 2023). However, cytokine and chemokine signatures associated with the human brain environment were downregulated in iMGLs transplanted into mouse brains (Popova et al., 2021). Therefore, significant differences persist between iMGLs and primary microglia transplanted into mice, which can be attributed to intercellular interaction (Zhang et al., 2023).

In summary, these data suggest that heightened sensitivity to inflammatory stimuli is linked to a psychiatric genetic background, which simultaneously increases intrinsic inflammatory activation (Michalski & When., 2023). Despite these findings, there are relatively few studies involving iPSC-derived microglia from psychiatric patients across various models. Further research is therefore needed to establish the relevance, significance, and broader integration of iMGL as a tool for advancing molecular insights into the psychiatric domain, and gaining a

deeper understanding of the role of microglia in these disorders. Figure 3 presents a summary of the key features and alterations identified in these studies.

4. Challenges in current research

Despite advancements in the generation and utilization of iMGL, their application in psychiatry remains challenging. From a technical perspective, as previously described, various protocols involve differing timeframes, materials, growth factors, and models (Speicher et al., 2019). Research groups often select and modify specific protocols according to their research objective, sample characteristics, and factors such as efficiency, reproducibility, and yield of iMGLs required (Hong et al., 2023). This variability complicates the development of a unified and efficient protocol for generating iMGL, making it difficult to standardize experiments across studies (Wurm et al., 2021).

Moreover, while most protocols yield iMGL cells that exhibit microglia characteristics, achieving maturation and a phenotype that accurately reflects adult brain microglia remains challenging. iMGLs more closely resemble fetal microglia, displaying an immature phenotype compared to primary microglia, which limits their application in certain contexts (Zhang et al., 2023). Concurrently, the absence of standardized markers and assays hinders the ability to achieve consistency and comparability in research on microglia-derived cells. Furthermore, psychiatric disorders are complex and multifaceted, involving long-term changes in brain function. However, maintaining iMGLs in culture for extended periods is a significant challenge, which limits the utility of *in vitro* studies to capture the essential characteristics of these conditions (Speicher et al., 2019; Brennand et al., 2022). Therefore, studying iMGL in isolation may not fully elucidate the complexities of these disorders (Heider et al., 2021). Researchers have addressed this challenge by developing co-culture systems and 3D cerebral organoids (Hong et al., 2023, Quadrato, Brown & Arlotta., 2016) or transplantation of the iMGL cerebral organoids into mouse brains (Popova., et al 2021). However, further optimizations are needed to fully integrate iMGLs into these models.

It has been reported that microglia densities vary between different organoids, suggesting that isolating microglia from diverse brain areas and utilizing organoids that resemble different brain niches could offer valuable insights (Quadrato, Brown & Arlotta., 2016). However, the protocols available to generate cerebral organoids and integrate iMGL in the culture are still limited. Zhang et al., 2022 underscored the importance of robust brain organoid models to achieve reproducible results in microglia research. Concurrently, some of these protocols

remain poorly defined or time-consuming, suggesting a need for novel methodologies for generating these organoids. Recent advancements, such as the protocol proposed by Mark van der Kroeg et al., (2024), offer promising alternatives, as they allow organoids to be maintained in culture for extended periods, enabling more advanced maturation stages. This underscores the potential for future studies to implement these novel protocols, as they may facilitate the investigation of iMGL function and interactions across developmental stages.

The origins and genetics of psychiatric diseases vary significantly between disorders and patients due to their high complexity (Brennand., 2022). While iPSCs have proven to be highly useful over the years for approaching and understanding various diseases, their generation and maintenance involve substantial costs and time, making them less accessible. Additionally, finding suitable healthy control samples for these disorders has been a major challenge due to the significant variability between patients (Michalski & Wen., 2023), which limits their use. To address this issue, some groups have implemented a more rapid and less complex approach by generating microglia-like cells directly from peripheral blood mononuclear cells (Sellgren et al., 2019; Ormel et al., 2020). It has been proved that these microglia-like cells in terms of morphology and functionality are quite similar to the microglia-like cells derived from iPSCs (Bsibsi et al., 2023; Sheridan et al., 2024), suggesting that alternative techniques for generating microglia-like cells may be considered depending on the specific research project.

Furthermore, some of the studies mentioned employed various assays, including inflammatory response assays to LPS or IFN-γ stimuli, phagocytosis assays, and transcriptional profiling via RNA sequencing or microarrays (Speicher et al., 2019). However, as previously noted, there are few investigations utilizing iMGLs in psychiatry, with most research focusing on neurodegenerative diseases such as Alzheimer's and multiple sclerosis (Michalski & Wen., 2023). These neurodegenerative conditions feature a well-established role for microglia, including interactions with disease-specific proteins and the availability of standardized biomarkers, which facilitate experimental reproducibility (Hasselmann & Blurton-Jones., 2019; Haenseler & Rajendran., 2019). Consequently, the assays developed for studying iMGLs in neurodegeneration are often more comprehensive, incorporating proteomics, toxicity assays, drug screening, and CRISPR-Cas9 technologies, among others (Jäntti et al., 2024; Nikel, Talbot & Vahsen et al., 2024). These techniques allow for the identification of microglia-related mechanisms and interactions in neuroinflammation and disease. This suggests that a broad array of assays could be applied to iMGLs and integrated into psychiatric research in future studies.

In conclusion, advances in technology, improved differentiation protocols, and better integration of iMGL with other model systems are essential for overcoming these challenges and advancing our understanding of psychiatric disorders.

5. Discussion

The generation of iMGLs from iPSCs represents a powerful tool for studying human microglia, as they closely resemble microglial in functionality and morphology during differentiation and maturation (Wurm et al., 2021). However, their use in psychiatry has been limited, making it challenging to establish a standardization model for studying and collecting data on microglia influence in psychiatric disorders. Microglia are highly complex cells, with morphology and transcriptional patterns changing in response to environmental cues (Zhang, 2022). While some studies describe resting microglia as highly ramified and thin, and activated microglia as adopting thicker, short-branched forms (Kettenman et al., 2011), it is still uncertain whether these morphological changes reflect the underlying molecular and functional alterations that microglia undergo during pathology, due to the complexity and inconsistency observed when comparing pathological states with healthy conditions (Prinz, Jung & Priller., 2019). In psychiatry, no definitive conclusions have been drawn about the role of microglia in specific disorders, as optimal models are still being developed.

It is known that psychiatric disorders are inherently complex, with limited investigation at the cellular and molecular levels (Rahimian et al., 2021). To date, schizophrenia (SCZ) has been the most extensively studied disorder, particularly after the identification of several associated genes a few years ago, which significantly advanced its molecular research (Singh et al., 2022). Research on microglia using iMGL has generally shown evidence of microglia activation and synaptic reduction (Sellgren et al., 2019), consistent with observations from Post-mortem and PET studies (Ai et al., 2023; Meyer et al., 2020). Similar findings have been reported in models of ASD and BD (Schafer et al., 2023; Haarman et al., 2014), suggesting that neuroinflammation may be a common feature across psychiatric conditions. Nonetheless, determining the precise role of microglia in each disorder remains a challenge for scientific research.

To improve the understanding of microglia functionality, iMGLs are believed to achieve better functional maturation when co-culture with other CNS cells (Michalski & Wen, 2023). Therefore, co-culture models can be established in 2D, combining iMGL with neurons or astrocytes, or using more advanced 3D models such as cerebral or through transplantations of

iMGL and organoids into mouse brains (Won et al., 2023; Magni, Riboldi & Ceruti, 2024). Currently, several protocols have been developed to co-culture microglia with cerebral organoids (Zhang et al., 2022), indicating that the aggregation of iMGLs supports both organoid formation and maturation, while simultaneously promoting microglia development. This brain-like environment offers critical mechanisms for proper CNS development.

However, given the variety of available protocols, future studies need to adapt their experimental models to their specific research questions. For example, combining brain organoids with transplantation into mice brains provides an *in-vivo* environment that enhances the native microglial state and promotes brain organoid maturation, while neurons integrate into the mouse neuronal circuit (Zhang et al., 2022; Wong et al., 2023). Additionally, microglia do not emerge robustly in organoids compared to other cell types, such as neurons or astrocytes, but their presence can be improved and reconstituted through transplantation (Ormel et al., 2018) and they can express microglia-specific genes that are typically lost *in vitro* (Bennet et al., 2021). This combination represents a promising tool for mimicking human brain conditions, promoting a better approach to studying the influence and interaction of iMGLs with other structures.

Furthermore, the importance of microglia in a co-culture environment is underscored by their key role in neuronal formation and maturation. Microglia strongly influence neurogenesis, particularly in the production and integration of new neurons into neuronal circuits, by inducing neurotrophic factors and pruning weak synapses (Pérez et al., 2021). Increased microglia-mediated pruning of synapses has been observed early in ASD (Hong et al., 2016) and synaptic elimination in SCZ (Sellgren et al., 2019). However, synaptic pruning and synaptic plasticity are complex mechanisms to study *in vitro*, as iMGLs and neurons generally represent early developmental stages, whereas these processes are more relevant in mature states. Therefore, incorporating iMGLs into developing organoids may enable the modeling of these abnormal events related to psychiatric disorders (Quadrato, Brown & Arlotta, 2016).

Although iPSC-derived microglia can effectively model many aspects of microglia transcriptomic identity and provide reproducibility for experimental studies, they often do not fully capture the complex states associated with disease (Michalski & Wen., 2023). Therefore, some studies adopted the direct isolation of microglia rather than deriving them from iPSCs. The strategy involves generating microglia directly from peripheral blood mononuclear cells without prior reprogramming into iPSCs, obtaining similar characteristics in the resultant

iMGL (Sellgren et al., 2019, Ormel et al., 2020). This direct isolation allows for the rapid culture and subsequent re-induction of specific gene expression through *in vivo* engraftment (Bohlen et al., 2017). This approach is generally more cost-effective and technically less complex compared to iPSC reprogramming, but also allows for the rapid generation of microglia from multiple donors, which is challenging with iPSCs (Michalski & Wen., 2023). The selection of the appropriate method should therefore be guided by the specific aims and research questions of the study.

In conclusion, this review discusses the origin of microglia, from their formation in the yolk sac to their migration to the CNS, and their role in the development of other cell types, particularly neurons. It also addresses the detrimental effects of chronic active microglia in psychiatry and their involvement in neuroinflammation, although these features can vary, making it difficult to draw definitive conclusions. Additionally, the review highlights how iMGLs derived from patient iPSCs offer a valuable tool for studying disease-specific molecular and cellular signatures. While various protocols for iMGL differentiation exist, standardization remains a challenge, and only a limited number of studies have applied iMGLs in psychiatric research. It is important to note that different assays have been employed to study iMGLs, but their application in psychiatric research remains largely unexplored. Advancing the study of psychiatric disorders is crucial, as they affect a significant portion of the population (Brennand., 2022). Their complexity has limited our understanding of their origin, progression, and the factors influencing their severity. iMGLs offer a promising avenue for studying these disorders, but further investigative progress and consistency are needed to unlock their full potential for future applications and clinical translation.

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Table 1. Summary of different protocols for generating microglia-like cells (iMGs) from iPSCs.

Protocol	Culture Model	Differentiation starting point	DIV from iPSCs	Growth factor for iMGL differentiation	iMGLs molecular markers	Inflamatory response to LPS
Muffat et al., 2016	Mono-culture	EBs	74	CSF1 Il-34	**ICC:** TMEM119, P2Y12, IBA1, C45	↑CXCL10, ↑IL-6 ↑MIP1a, ↑TNFα
Haenseler et al., 2017	Co-culture (microglia+neurons)	EBs	70	Il-3 CSF1	**FCM:** CD11b, CD11c, CD14, CD45	↑MIP-1β, ↑IL-8 ↑CXL10, ↑IL-6
Abud et al., 2017 McQuade et al., 2018	Mono-culture	iHPCs	38	CSF1 Il-34 TGF-β Maturation +CD200, +CXCL1	ICC: PU.1, CX3CR1, TREM2, P2Y12, TFGBR1, PROS1, MERKT, ITGB5 FCM: CD11b,CD45	↑IL-6 ↑CXCL10 ↑IL-8 ↑IL-1α ↑CCL2 ↑TNFα ↑CCL4
Pandya et al., 2017	Co-culture (microglia+Astrocytes)	iHPCs	29	IL-3 CSF1 CSF2	**FCM:**CD11b, IBA1, CD45, CX3R1, HLA-DR	↑TNF-α
Douvaras et al., 2017	Mono-culture	iHPCs	60	CSF1 CSF2 IL-34	**ICC:** CD11c, IBA1, P2Y12, TMEM119	
Lanfer et al., 2022	Mono-culture	iHPCs	28	GM-CSF Il-34	**ICC:** IBA1, TREM2 **FCM:** IBA1	↑IL-1β ↑IL-6 ↑TNF-α

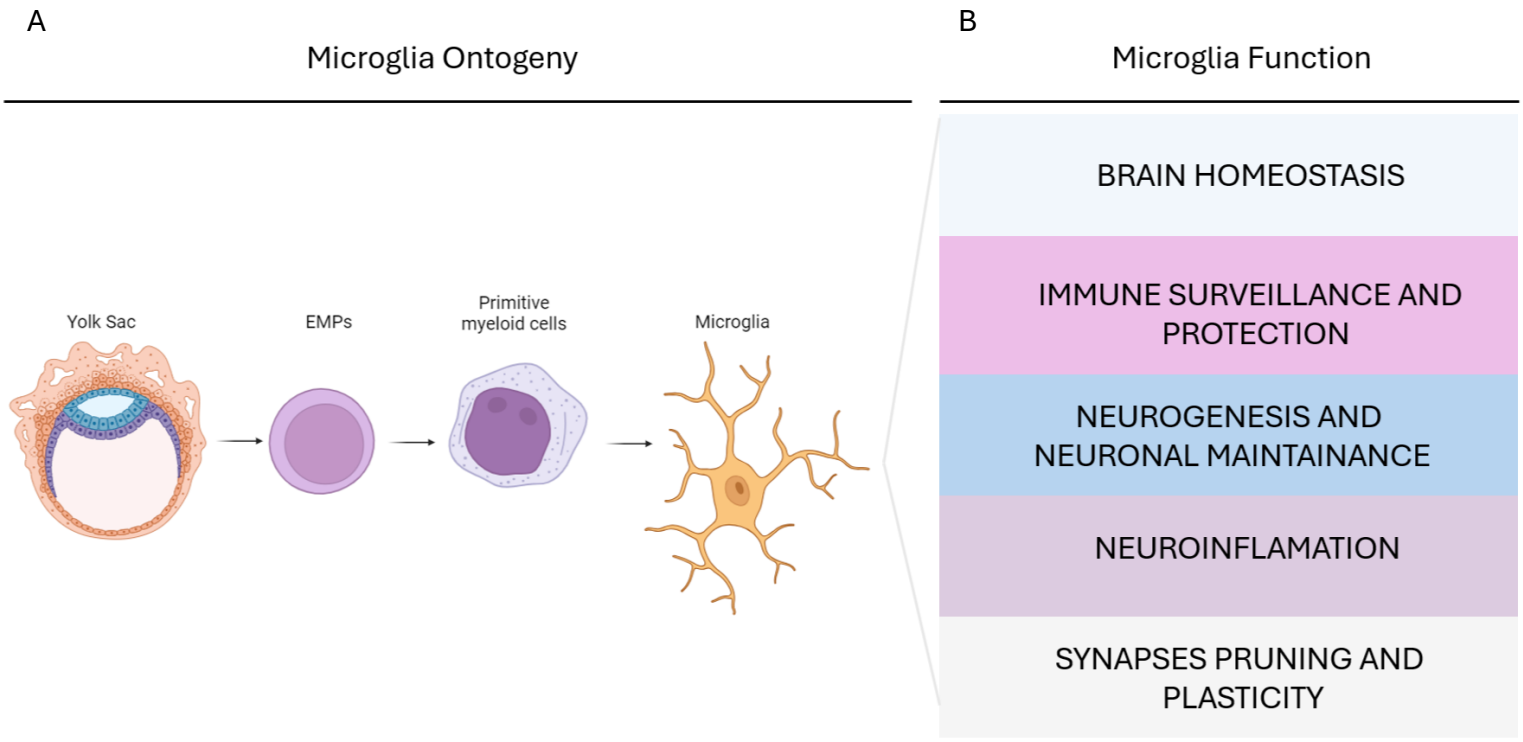

Figure 1. Microglia development and function in the CNS. (A) Schematic representation of microglia formation, beginning in the yolk sac, where they first develop into erythromyeloid progenitors (EMPs). These progenitors then differentiate into primitive myeloid cells, which later mature into microglia. (B) Characteristics features of microglia function when established in the central nervous system (CNS). Key functional characteristics of microglia within the central nervous system (CNS). Microglia perform essential roles, including maintaining brain homeostasis, conducting immune surveillance, and defending against pathogens. Additionally, microglia secrete pro-inflammatory cytokines, which can lead to neuroinflammation, while also supporting neurogenesis, promoting neuronal development, synaptic pruning, and contributing to synaptic plasticity.

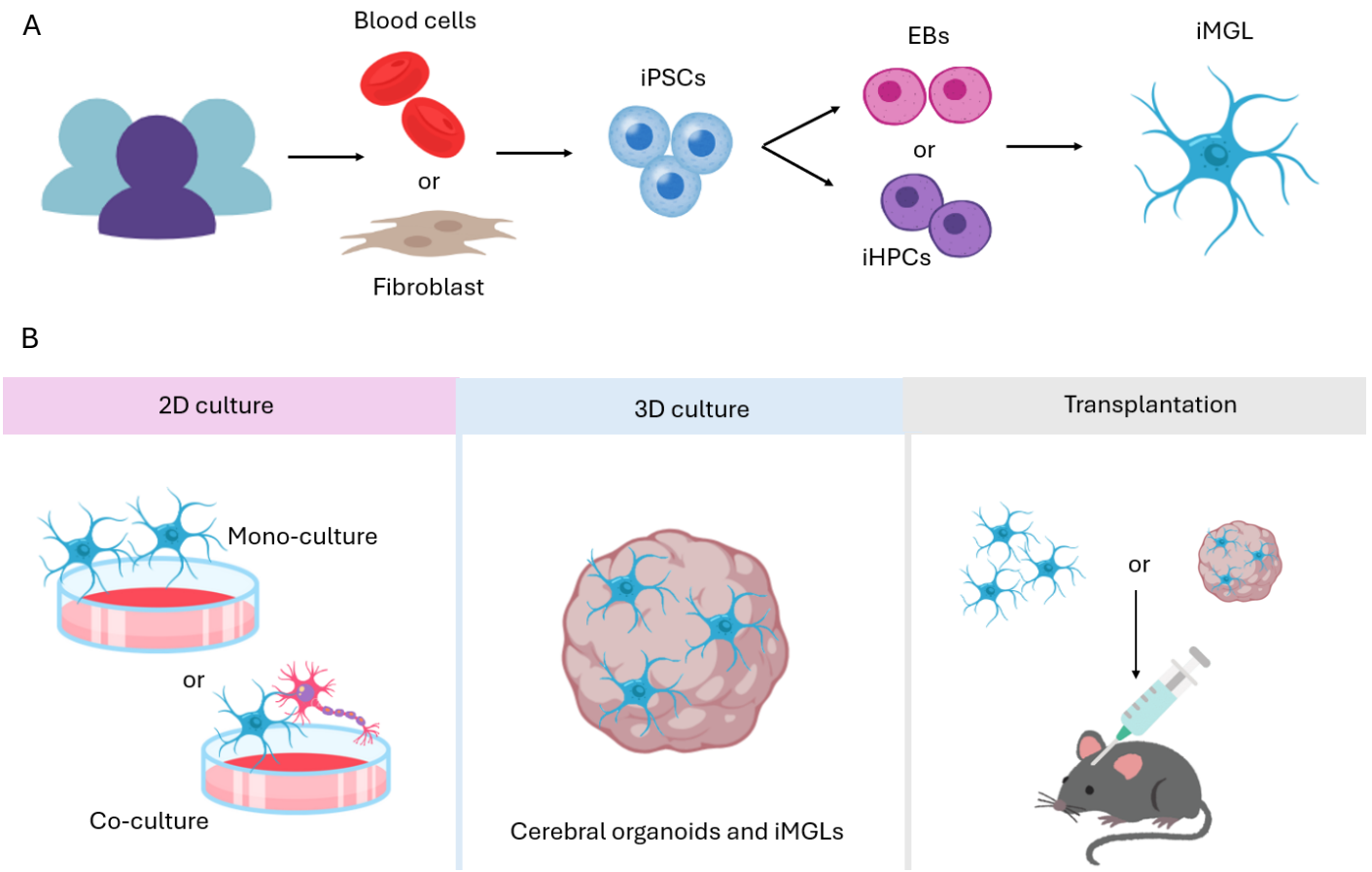

Figure 2. iMGL Microglia Differentiation and Implementation Models. (A) Schematic representation of iMGL differentiation. Induced pluripotent stem cells (iPSCs) can be derived from the blood or fibroblasts of patients with psychiatric disorders. These iPSCs are reprogrammed into embryoid bodies (EBs) or hematopoietic progenitor cells (iHPCs), which subsequently differentiate into microglia-like cells (iMGLs). (B) iMGLs have been applied in psychiatric research across various platforms for disease modeling. These include 2D models, where monoculture systems are used to study microglial functionality in isolation, and co-culture models that examine the interactions between microglia and other cell types, such as neurons. Additionally, brain organoids can be generated from patient-derived iPSCs and co-cultured with iMGLs to create a brain-like environment. Finally, iMGLs and cerebral organoids can be transplanted into mouse brains, allowing them to integrate and interact with the host brain, thus mimicking a more realistic *in-vivo* environment.

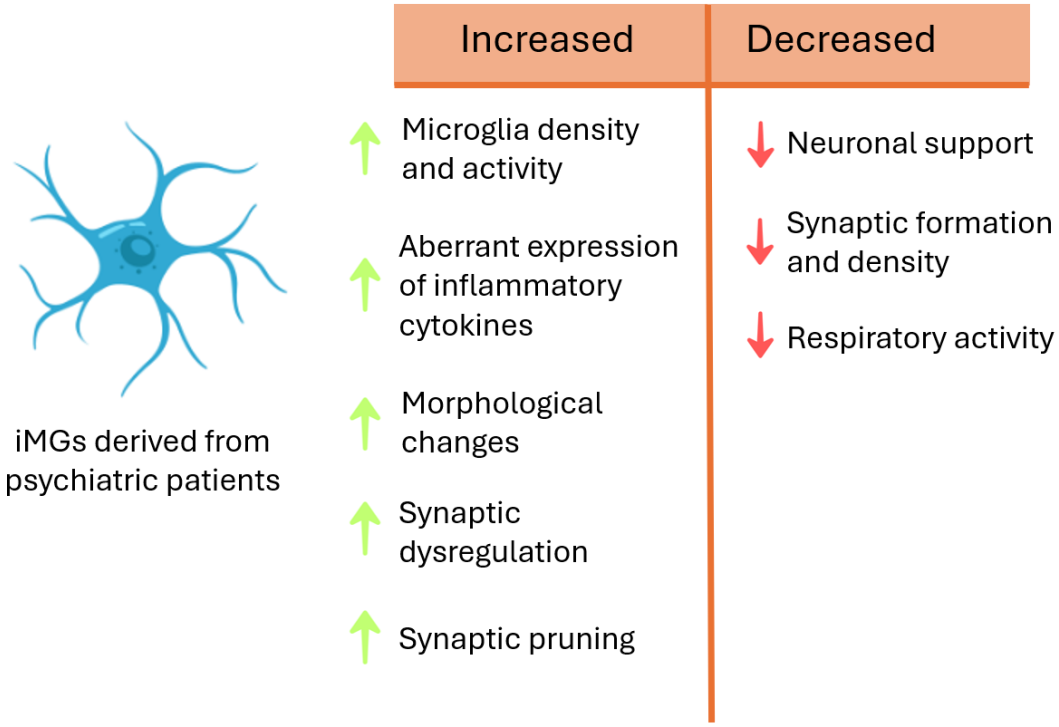

Figure 3. Summary of alterations and involvement of iMGs generated from iPSCs derived from psychiatric patients. This figure provides an overview of the observed changes across various studies utilizing iMGs derived from psychiatric patients. Overall, these studies reported increases in microglial activity and density, accompanied by heightened secretion of pro-inflammatory cytokines. Additionally, some studies identified morphological changes and disruptions in synaptic regulation, including diminished synaptic pruning and reduced synapse formation, which impact neuronal support and network development.

Generative AI statement

For this project I used ChatGPT for looking to appropriate words and synonyms by asking “Can you give me a synonym of [specific word]; Can you show me appropriate words to refer to [phrase]”, and Grammarly to provide feedback on my writing style.

Grammarly. (n.d). Grammarly [Software]. https://app.grammarly.com/

OpenAI. (2024). *ChatGPT* (GPT-4) [Large multimodal model]. https://chatgpt.com/