

Patient-specific monocyte-derived microglia as a screening tool for neurodegenerative diseases

Hazel Quek^{1,2,*}, Anthony R. White^{1,2}

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Abstract

Microglia, the main driver of neuroinflammation, play a central role in the initiation and exacerbation of various neurodegenerative diseases and are now considered a promising therapeutic target. Previous studies on *in vitro* human microglia and *in vivo* rodent models lacked scalability, consistency, or physiological relevance, which deterred successful therapeutic outcomes for the past decade. Here we review human blood monocyte-derived microglia-like cells as a robust and consistent approach to generate a patient-specific microglia-like model that can be used in extensive cohort studies for drug testing. We will highlight the strength and applicability of human blood monocyte-derived microglia-like cells to increase translational outcomes by reviewing the advantages of human blood monocyte-derived microglia-like cells in addressing patient heterogeneity and stratification, the basis of personalized medicine.

Key Words: human *in vitro* microglia models; neurodegeneration; neuroinflammation; patient heterogeneity; patient stratification; peripheral blood monocyte-derived microglia-like cells; therapeutic target; transdifferentiation; translational outcomes

Introduction

Neuroinflammation is a critical process in all neurodegenerative diseases and is driven by microglia, the specialized innate immune cell of the brain. Microglia maintain homeostasis within the central nervous system (CNS) by regulating neuronal activity by pruning synapses, surveying the brain for cellular debris, and carrying out appropriate immune regulation. Alternatively, microglia in the diseased brain exacerbate disease progression and severity, where defects in microglial function can deter the clearance of pathological proteins, release neurotoxic cytokines and elicit cell death. Due to the multiple roles in promoting and alleviating disease progression, microglia have become a key therapeutic target in recent years. However, there are still no promising microglial therapeutics, mainly due to the lack of physiologically relevant models that can faithfully recapitulate disease pathology and preserve patient-specific heterogeneity within the CNS.

While cell lines, animal models, and post-mortem brain tissues have been invaluable in providing insights into neurodegenerative diseases and key concepts in microglial biology, these models have poor predictive power in identifying potential drug candidates for clinical therapies. One example of an unsuccessful drug candidate is minocycline, which despite showing potential in protecting against the toxic effects of β -amyloid by reducing microglial activation and inflammatory responses *in vitro* and in animal models of Alzheimer's disease, failed to show therapeutic benefits when trialed in Alzheimer's disease patients (Howard et al., 2020). This failure could be attributed to the difficulty in mimicking the complex aspects of neurodegenerative diseases driven by patient-specific heterogeneity in microglial function and the species-specific differences between humans and mice, especially concerning gene expression and function. Alternatively, microglia isolated from post-mortem brain tissues are of high physiological relevance compared to other model systems but lack reproducibility and scalability. These post-mortem brain tissues are often sampled in a small/selected brain region that does not reflect the natural heterogeneity within various brain regions (Tan et al., 2020).

Moreover, microglia are highly sensitive to their environment leading to the disparity in transcriptomic profiles between *ex vivo* and *in vitro* microglia, further altered due to delay with post-mortem tissue extraction, quality of the post-mortem tissue, and differences with isolation techniques (Gosselin et al., 2017; Cadiz et al., 2022). The advent of human patient-derived microglia

models obtained directly from patients has significantly bridged this gap, specifically with the recent advances in the generation of patient-specific microglia from induced pluripotent stem cells (iPSCs) and human peripheral blood monocytes. Importantly, accurate microglial models should have the ability to reflect age-related neurodegenerative disease features as well as patient-specific clinical manifestations to improve drug efficacies leading to successful therapeutic outcomes.

Search Strategy and Selection Criteria

Studies cited in the review were published between 2007–2022, and they were searched on the PubMed database using the following keywords: monocyte plasticity, monocyte infiltration, monocyte conversion, microglia-like cells, microglia repopulation, CCR2, iPSC derived hematopoietic stem cells, iPSC derived macrophage-like cells, iPSC derived microglia-like cells, neurodegeneration.

Monocyte-Derived Microglia: Are They Different from Monocytes or Brain Microglia?

Myeloid cells, including monocytes and microglia, are localized in their respective niche with their specialized tissue-specific function. However, under most pathological conditions where the blood-brain barrier is compromised, infiltrating monocytes are recruited into the CNS to aid dysfunctional or depleting microglia pools (Varvel et al., 2012; Stephenson et al., 2018). Within the CNS, this population of transient monocytes differentiates into human blood monocyte-derived microglia-like cells (MDMi), assuming a ramified morphology, expressing microglial genes such as *P2ry12* and *Tmem119*, and acquiring functional capabilities such as phagocytosis and eliciting a cytokine response similar to CNS resident microglia. These MDMi characteristics (morphology, gene expression, and functional changes) are different in monocytes, macrophages, and dendritic cells from the same individuals (Banerjee et al., 2021; Quek et al., 2022), and when treated with immunostimulatory stimuli such as lipopolysaccharide (Melief et al., 2016).

In addition, gene expression profiling of MDMi revealed a single nucleotide polymorphism for the Alzheimer's disease risk gene (PILRB). The same single nucleotide polymorphism was not observed in monocytes from the same

¹Mental Health and Neuroscience, QIMR Berghofer Medical Research Institute; ²School of Biomedical Science, The University of Queensland, Brisbane, QLD, Australia

*Correspondence to: Hazel Quek, PhD, hazel.quek@qimrberghofer.edu.au.
<https://orcid.org/0000-0001-5597-5372> (Quek Hazel)

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donor (Ryan et al., 2017), indicating that MDMi are distinct from monocytes. In line with this, transcriptomic analysis has shown that MDMi cluster closer to iPSC-derived microglia and infant brain microglia than monocytes and macrophages (Sellgren et al., 2017; Banerjee et al., 2021). It is clear from these studies that monocytes and MDMi, though are derived from the same hematopoietic origin and share many phenotypical and functional characteristics, behave differently from each other in a diseased context.

C-C chemokine receptor type 2 (CCR2), is a chemokine receptor typically found on infiltrating monocytes but not in microglia (Mizutani et al., 2012; Jara et al., 2019). Interestingly, MDMi retains the expression of CCR2 and express other microglial proteins such as CX3CR1 and P2RY12, suggesting that these cells resemble both infiltrating monocytes and microglia (Ohgidani et al., 2014; Banerjee et al., 2021). While the role of CCR2 in these cells is yet to be elucidated, it could be due to the use of interleukin-34 and granulocyte macrophage-colony stimulating factors in the differentiation of MDMi. This was shown in a study that converted infiltrating monocytes to macrophages by a cytokine-dependent process (Park et al., 2021; Mysore et al., 2022). Additionally, monocyte subsets, characterized by classical (CD14⁺CD16⁻), intermediate (CD14⁺CD16⁺), or non-classical (CD14⁺CD16⁺⁺) have varying CCR2 expressions and are correlated with poor clinical outcomes (Saederup et al., 2010). Whether MDMi transdifferentiated from the various monocyte subsets will present similar CCR2 expression warrants further investigation.

The origin, transcription, and function of monocytes and MDMi are distinct from parenchymal microglia (Yamasaki et al., 2014). As such, hematopoietic stem cells from the fetal liver or bone marrow give rise to blood monocytes, while microglia originate from embryonic yolk sac progenitors (Ginhoux et al., 2010). Microglia are also self-renewing and are typically maintained from the local CNS pool and not from the periphery (Ajami et al., 2007, 2011). Some questions remain regarding whether MDMi can replace parenchymal microglia in the long term and how long they could persist within the CNS. While this is still largely unclear, animal studies have demonstrated several events; where infiltrating monocytes are able to repopulate the microglia niche and become microglia-like, but remain distinct in phenotype and function (Varvel et al., 2012; Cronk et al., 2018; Lund et al., 2018; Shemer et al., 2018; Hohsfield et al., 2020; Feng et al., 2021), or, infiltrating monocytes that persist in inflammation do not replenish microglia niche (Ajami et al., 2011). These findings are largely dependent on experimental procedures used to deplete microglia to study the recruitment of myeloid cells. It is further postulated that the number of infiltrating monocytes or microglia-like cells is dependent on the type of disease (i.e. neurodegenerative, brain injury, and brain infection), disease severity, and the progression of disease (Yamasaki et al., 2014; Olah et al., 2020; Haage and De Jager, 2022).

Overall, MDMi can be utilized to further characterize the role of microglia-like cells to delineate the complex relationship between infiltrating monocytes and microglia, and their roles in disease progression.

Using Human Blood Monocyte-Derived Microglia-Like Cells to Identify Disease- and Patient-Specific Changes in Neurodegenerative Diseases

MDMi have significant advantages compared to other microglial models, such as hiPSC-derived microglia.

The ease of sample collection

Blood is sampled directly from living patient donors prior to peripheral blood mononuclear cell isolation. This method is less invasive, allows multiple testing for longitudinal studies, is straightforward for cell harvesting, is cost-efficient and most importantly, retains patient heterogeneity (clinical, disease-specific, patient-specific). Although only 5–10% of monocytes constitute the peripheral blood leukocytes, repeat sampling would ensure sufficient monocyte stocks for future usage.

Conversely, the generation of human embryonic stem cells (ESCs) or PSCs is not as straightforward. A skin biopsy has to be first expanded and reprogrammed to its pluripotent state. This reprogramming is often low in efficiency, can take months of validation and requires extensive manipulations, including cell sorting for enrichment. Another caveat in reprogramming involves the risk of insertional mutagenesis, leaky promoters, or off-targets, mainly through a genome integrating delivery method (i.e. lentivirus, adenovirus). Hence, loss of disease-relevant epigenetic traits due to extensive *in vitro* manipulations occurs within these cultures leading to the high propensity of developing genomic anomalies as observed by karyotyping. Further, reprogramming to pluripotency can hinder the study of age-related neurodegenerative diseases.

The ease by which we can generate patient-derived microglia-like cells

Blood monocytes can be isolated from peripheral blood mononuclear cells through CD14⁺ selection or plastic adherence (Cuní-López et al., 2022). Variations in these published protocols include the growth factors, days in

culture and extracellular matrix. The differences between MDMi generated from various methods are still unclear, but have shown to display similar microglia-like morphology, function, and key microglial genes (Banerjee et al., 2021).

The ease of generating MDMi have allowed the use of a patient cohort (> 10) to model neurological diseases such as Nasu-Hakola disease (Ohgidani et al., 2014), schizophrenia (Sellgren et al., 2019), and Huntington's disease (Rocha et al., 2021). Our group has recently demonstrated the feasibility of generating MDMi from amyotrophic lateral sclerosis patient cohort (> 30) (Quek et al., 2022), which would benefit patient stratification dependent on disease progression. More importantly, these amyotrophic lateral sclerosis MDMi display aberrant cytoplasmic TAR-DNA-binding protein-43 expression and/or phosphorylated TAR-DNA-binding protein-43 inclusion, a pathological hallmark similar to that observed in post-mortem brains in amyotrophic lateral sclerosis patients. Interestingly, aberrant phosphorylated TAR-DNA-binding protein-43 inclusions were heterogeneous in manner, where it was observed only in some MDMi cells within the same patient and is independent of the type of disease progression. These results demonstrate the applicability of MDMi to reflect disease-specific hallmarks and the heterogeneity within cells, which is key to understanding disease pathology.

The generation of myeloid cells from human ESC or PSCs requires a complex stepwise process that begins by patenting ESCs/PSCs into mesoderm progenitors. These cells are then differentiated into hematopoietic progenitors, responsible for forming all types of blood cells, followed by monocytes, which are then transdifferentiated into macrophage- or microglia-like cells. More recently, it has been shown that direct reprogramming (transdifferentiation/ forced expression) can be achieved by using lineage-specific transcription factors to direct somatic cells (i.e. fibroblast) to hematopoietic progenitors (Yanagimachi et al., 2013; Gomes et al., 2018), macrophage-like cells (Feng et al., 2008) or microglia-like cells (iMG) (Chen et al., 2021). Remarkably, direct reprogramming of iMG was shown to significantly reduce the time required for differentiation (from an average of > 40 days to > 10 days); however, no study has yet demonstrated this approach using patient-derived samples.

Overall, generating iMGs is expensive and time-consuming; hence it remains a challenge to sample/screen a large cohort of patients and generate homogeneous cultures of pure iMG (Speicher et al., 2019). The high variability in existing protocols to induce mature iMG, such as the co-culture of various cell types, fluorescence-activated cell sorting, and the use of serum in media can affect consistency in generating iMG, which may hinder disease-related pathology and/or patient-specific (genetic and epigenetic) outcomes. Therefore the simple and efficient methodology required for generating MDMi eliminates the above challenges and produces cells that retain epigenetic signatures essential for creating patient-specific disease models.

Comparative Studies Utilizing Human Blood Monocyte-Derived Microglia-Like Cells

MDMi model is able to simultaneously compare patient responses against a large cohort of healthy people of matching age and sex, thereby eliminating experimental inconsistencies such as reagent batch variability and increasing the consistency of results. The utility of a larger sample size increases the likelihood of heterogeneity where factors such as diet and medications are considered. This inherent heterogeneity is necessary for personalized drug therapy, which is now viewed as a promising therapeutic strategy in multi-systemic diseases such as cancer and neurodegeneration. In this manner, MDMi can be used as a pre-clinical screening platform using characteristics such as disease severity, genetic aberrations, cytokine secretion, and phagocytic capability. In a broader context, blood-based biomarkers can be correlated alongside MDMi to better understand drug efficiency. These results may improve therapeutic outcomes and provide a platform for personalized treatment regimes, as shown in **Figure 1**. Moreover, with an increasing number of studies integrating bulk RNA-seq in their experimental design, it is imperative to have a large enough patient cohort to identify differentially expressed biologically meaningful genes.

Summary and Future Perspective

With the rising prevalence of neurodegenerative diseases, there is an urgent need to develop a microglial platform capable of recapitulating a complete disease pathology and providing accurate, efficient, and consistent microglial-targeted therapies. While there is no current consensus on the best method for generating *in vitro* microglia models, both iPSC and monocyte-derived microglia currently represent a relevant human primary cell model for disease modeling. Notably, both iMG and MDMi cells resemble fetal brain microglia as opposed to adult post-mortem microglia, suggesting that both models are still at their early stages (not fully matured). The question remains whether advanced modeling of iMG and MDMi in 3D co-culture or brain organoids would enhance their microglial phenotype. Better refinement of these model systems will be crucial for translational studies and drug screening platforms for treating various neurodegenerative diseases.

Neurodegenerative diseases

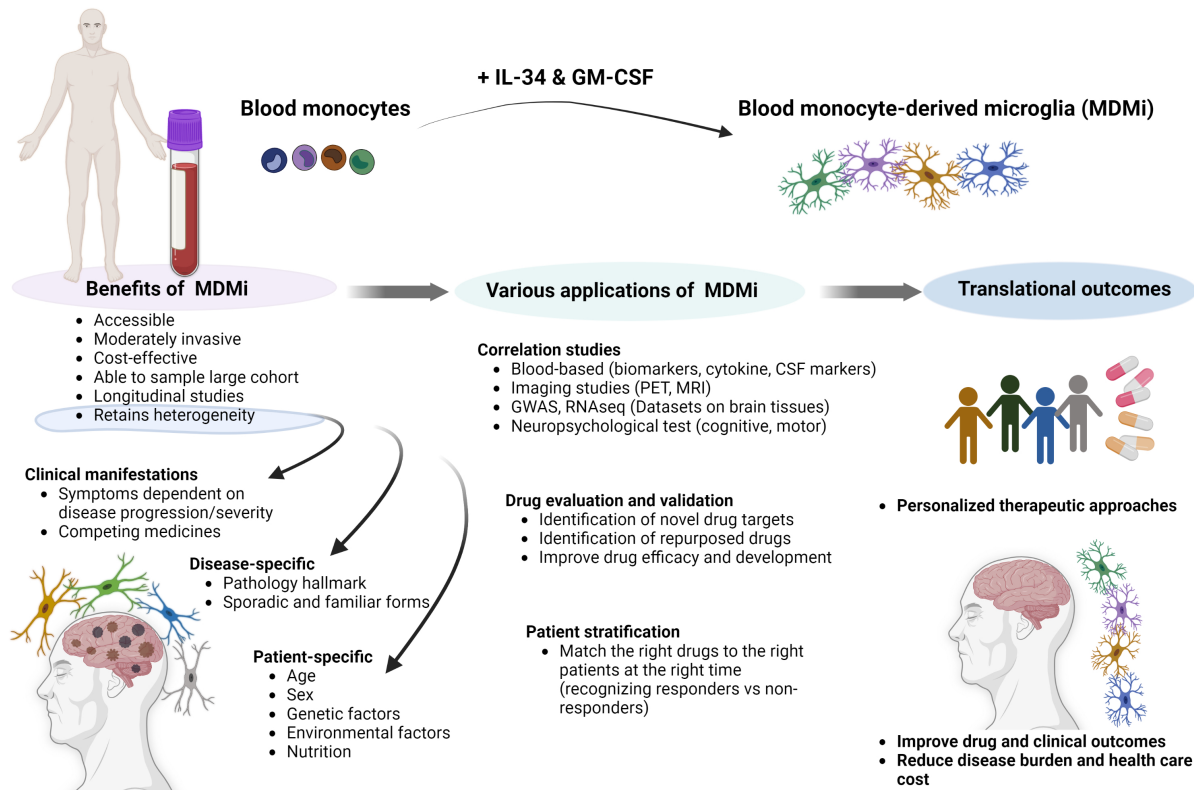


Figure 1 | Current applications of patient-derived MDMi to model neurodegenerative diseases.

MDMi is a promising tool for disease modeling and can recapitulate disease pathology and reflect disease- and patient-specific heterogeneity. Data generated using the MDMi model enables correlation studies that provide pre-clinical stratification, identification of biomarkers, and improved therapeutic regimens. Patient stratification will improve the efficiency of therapeutic outcomes for personalized medicine, thereby increasing the success rate of clinical trials and reducing the disease burden on patients and healthcare costs. CSF: Cerebrospinal fluid; GWAS: genome-wide association study; MDMi: human blood monocyte-derived microglia-like cell; MRI: magnetic resonance imaging; PET: positron emission tomography; RNAseq: RNA-sequencing.

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