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REVIEW

Advancing basic and translational research to deepen understanding of the molecular immune-mediated mechanisms regulating long-term persistence of HIV-1 in microglia in the adult human brain

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Abstract

Knowledge about the diversity microglia (MG) type and function in the rodent and human brain has advanced significantly in the last few years. Nevertheless, we have known for 40 years that MG, monocytes, and macrophages in the brain play crucial roles in the pathogenesis of the HIV-1 in all tissues. HIV enters and spreads in the brain early, long before the initiation of antiviral therapy. As a result, many people with HIV continue to experience neurologic and neuropsychiatric comorbid conditions collectively known as HIV-associated neurocognitive disorder (HAND). HIV pathogenic sequelae in the CNS pose a challenge for cure strategies. Detailed understanding at a mechanistic level of how low-level and latent HIV-1 infection in MG negatively impacts neuroglial function has remained somewhat elusive. Direct rigorous in vivo experimental validation that the virus can integrate into MG and assume a latent but reactivatable state has remained constrained. However, there is much excitement that human in vitro models for MG can now help close the gap. This review will provide a brief background to place the role of MG in the ongoing neurologic complications of HIV infection of the CNS, then focus on the use and refinement of human postmitotic monocyte-derived MG-like cells and how they are being applied to advance research on HIV persistence and proinflammatory signaling in the CNS. Critically, an understanding of myeloid plasticity and heterogeneity and rigorous attention to all aspects of cell handling is essential for reproducibility.

Summary Sentence: This review focuses on human postmitotic monocyte-derived microglia-like cells as tools to advance research on HIV persistence and neuroinflammatory signaling.

KEYWORDS

monocytes, innate immunity, inflammation, central nervous system, bone marrow progenitors, myeloid, phagocytosis

Abbreviations: BBBblood–brain barrier, blood–brain barrier; CSF, cerebrospinal fluid; hMMGs, human monocyte-derived microglia-like cells; HSC, hematopoietic stem cell; iPSCs, induced pluripotent stem cells; MG, microglia; NOG, NOD.Cg-Prkdcscidil2gtmlSug/Jic mouse strain; TIC, TGF-beta, IL-34, cholesterol in DMEM/F12 base tissue culture media.

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KEY CONCEPTS

Myeloid plasticity: The phenotype and function of myeloid cells (monocytes, macrophages, and microglia) are shaped and regulated by intercellular signals. These include cytokines, chemokines, and other cues from neighboring cells in the tissue microenvironment. In addition, paracrine and autocrine signals under the homeostatic state are altered with injury, stress, infection, or chronic disease conditions. In responding to these cues, myeloid cells undergo some or all of the following: morphologic changes, altered metabolism, variation of components released into the extracellular matrix, increased migration, cytokine/chemokine production, and phagocytosis.

Human monocyte-derived microglia (hMMGS): Purified subpopulations of bone marrow-derived monocytes incubated in highly defined in vitro culture conditions that support the development of microglia-like cells. Their phenotype closely resembles primary cells (e.g., TMEM119, CXC3R1, P2YR12, PU.1, IRF8), and functionally, hMMGs are phagocytic and possess synaptic pruning and calcium signaling activity.

1 | INTRODUCTION

1.1 | Human microglia as reservoirs for HIV in the CNS

Forty years ago, an aggressive dementia-like syndrome accompanied by movement disorders and a general failure to thrive or wasting was the first defining differential diagnosis for AIDS.[1] These clinical findings pointed to the brain, spinal cord, and immune system as targets for the retrovirus that would become known as the HIV-1. Examination of human postmortem brain tissue showed a regional congregation of microglia (MG) forming walled-off barriers around a central core (named microglial nodules), the massive proliferation of astrocytes (called astrocytosis), and multinucleated giant cells, the products of macrophage-to-macrophage cell fusion.[1–5] These neuropathologic hallmarks provided the most substantial evidence at the time that HIV-1 enters and replicates in brain macrophages and MG.[1–5] With techniques like laser capture microdissection, HIV-1 proviral DNA was detected in human brain astrocytes.[6] HIV-1 infects astrocytes via CD4-independent alternative entry pathways resulting in limited viral replication.[7–9] However, under conditions of inflammation or drugs of abuse that are known to potentiate HIV replication, infected astrocytes could be a significant source of early gene viral proteins.[10,11] Using sensitive sandwich ELISA detection methods, the HIV transcriptional activator protein Tat can be readily found in cerebrospinal fluid (CSF).[12] A recent study found a positive correlation between levels of Tat and soluble insulin receptor in the CSF of cognitively impaired persons with HIV infection.[13] HIV Tat alters cellular pathways controlling calcium regulation that causes neurons to die by apoptosis.[14–16]

Binding of the HIV envelope (env) protein with chemokine receptors such as CCR5 and CXCR4 on cultured neurons induces death signals.[17–21] Indeed, the first rodent models for HAND were generated by constitutive or regulated expression of HIV env driven by the astrocyte-specific promoter for glial acidic fibrillary protein.[22] In the absence of anti-HIV therapy, the action of cytokines, chemokines, and metabolic pathways, which under normal conditions serve to protect and maintain neural function, instead become chronically active and pathologic. The clinical syndrome described earlier, known as HIV-associated neurocognitive disorder (HAND), though significantly decreased in severity with current antiretroviral therapy, remains a debilitating comorbidity for many people with HIV.[23,24]

Today, HIV replication and person-to-person transmission are effectively controlled by strict adherence to an antiretroviral drug regimen that targets 2-to-3 different phases of the viral life cycle. Unfortunately, by ultrasensitive assays, we know that long-term treatment does not effectively increase the clearance of infected T-cells, macrophages, and MG in tissues nor completely block early viral gene transcription.[25] Another early gene, the harmful effector protein, Nef, interferes with immune recognition mechanisms that help target infected cells for elimination.[26–29] Additionally, these viral proteins, perhaps carried in extracellular vesicles[13,30–32] and low-level virus replication in tissues, stimulate immune activation and inflammatory signaling in a chronic fashion. The comorbid conditions, including cardiovascular disease, metabolic disorders, and neurologic and neuropsychiatric complications that affect many aging with HIV, are exacerbated in a proinflammatory milieu.[33–35] In the brain, while MG are the primary resident immune cells, they also collaborate with astrocytes and neurons in regulating homeostasis in the CNS.

To fully realize a cure for HIV, we need to understand whether the molecular mechanisms of the viral life cycle and potential for latent infection in macrophages and MG are similar or not to those in T-cells.

Given their central roles in neuroinflammation and neurodegeneration, researchers working in these fields, and more broadly in neurovirology, have renewed interest in reproducible, physiologically relevant, in vitro postmitotic human MGl culture systems for mechanistic studies. Several recent excellent reviews on induced pluripotent stem cell-derived (iPSC) MG have been published.[36–38] A comprehensive comparison between in vitro models to study HIV-microglial biology using cultured primary human MG, microglial cell lines, human postmitotic monocyte-derived microglia-like cells (hMMGs), iPSC-MG, and 3D organoids was published most recently.[39] Each model has advantages and limitations in that they recapitulate aspects of primary brain MG morphology, gene expression, function, and capacity to support HIV replication.[39] Importantly, all agree that these model systems provide a starting point from which continuous cycles of assessment and refinement are expected. This review will focus on hMMG models, recent experimental findings using this technology, and how these cells will be employed in humanized mouse models for HIV neuropathogenesis to gain translational insights.

1.2 | Early postmitotic microglial culture models and microglial cell lines

The precursor to modern-day human brain organoids was dissociated fetal or adult brain cell cultures plated on plastic surfaces in which MG were established as the targets of infection by HIV.[3,40] These cultures had a mixture of astrocytes (70–90%), neurons (10–30%), and MG (1–5%) in similar proportions to the 3D brain.[40] Long-term productive infection of brain human fetal MG cultured with GM-CSF alone or with LPS was used to bring more reproducibility to these model systems.[41] Out of these landmark studies, CD4 and the chemokine receptors CXCR4, CCR3, and CCR5 were confirmed as the molecules allowing HIV entry and replication in human MG.[40] Electron microscopy visualized the accumulation of immature viral particles in fetal MG.[42] Analyses of HIV replication kinetics in MG showed that the virus grew at lower titers than monocyte-derived macrophages but could form syncytia formation.[42] Cultures maintained for 3–4 weeks showed low-level viral gene expression in long-term surviving MG 70 days after infection.[3,41]

The availability of cell lines that faithfully recapitulate aspects of biology/physiology remains a vital research tool with an understanding of the need to validate research results in primary cells and in vivo. As highlighted in a recent review by Timmerman et al.,[43] 2 cell lines derived from the adult human brain telencephalon or cortex were immortalized with SV40 T antigen and the human telomerase reverse transcriptase gene (Huμglia) or with the v-myc oncogene (HMO6).[44,45] Clonal Huμglia lines containing integrated single-round-fluorescently tagged reporters were developed to study the mechanism of latency.[46] Prototypical proinflammatory molecules reactivated HIV gene expression of Huμglia clone HC69.5 to different extents, with TNF-a being the most effective at stimulating 94% of cells, IL-1b, 78%, and LPS, 67%.[44] The Huμglia displayed a wide range of cytokine/chemokine secretion activity upon stimulation, had a gene expression profile similar to primary human MG, could clear dead neurons in culture through phagocytosis, and also exhibited a gene expression profile migratory ability demonstrating these expected MG functions are intact.[44] While there is some variability in the expression of CD4 and HIV coreceptors CCR5 and CXCR4 with time in culture, infection with replication-competent HIV is possible. DNA mimics activating TLR 3 signaling released the MG clones from a latent state via a mechanism dependent on IRF3.[46] For TLRs 1, 2, 4, and 6, reactivation from latency was driven via NFκ-b activation.[46] The HC69 Huμglia clone demonstrated its utility for identifying the possible role (s) of cortical and dopaminergic neurons in mechanisms of HIV entry and exit from latency,[46] testing the ability of novel eradication strategies to cross in vitro blood–brain barriers (BBBs) to disrupt the formation and maintenance of latent HIV infection in MG[47,48] and repopulation of the brain in humanized mice.[49]

1.3 | Postmitotic monocyte-derived microglial culture models

The myeloid field is currently in the midst of an exciting inflection phase. The fuller realization of myeloid tissue cells' functional and phenotypic heterogeneity and their critical roles in health and disease is rapidly emerging. We thought that all tissue myeloid cells originated from progenitor cells residing in the bone marrow until 11 years ago. With a better understanding of the critical transcription factors and promoters that drive lineage-specific differentiation, landmark studies using genetically tagged mice convincingly demonstrated that during development, MG arise in the mesodermal layer from erythro-myeloid progenitors in the yolk sac. Under homeostatic conditions, bone marrow-derived stem cells do not contribute significantly to this pool.[50–52] Single-cell RNA (sc-RNA) sequencing studies of mouse brain MG isolated across several time points in embryogenesis to the postnatal stage demonstrate the vast heterogeneity of MG phenotypes in health and neurodegenerative disease.[53–57] In the mouse brain, canonical MG subpopulations segregate with Tmem119, Fcrls, P2yr12, Cx3cr1, Trem2, and Clqa.[53,58,59,60] In mouse models of multiple sclerosis, 4 significant MG subpopulations were found. All expressed low levels of Tmem119, P2yr12, and either Apoe, Cxcl10, and Tnfa, or Ccl5 as unique genes in their respective signatures.[60] Four MG populations were found in the adult human brain without disease Like mice, the human canonical MG MG1, MG2, and MG3 expressed TMEM119, P2YR12, and CX3CR1, while the.[61] Instead, the human MG4 population was defined by the expression of CCL2, CCL4, EGR2, and EGR3.[61] Like in the diseased mouse brain, the canonical markers TMEM119, P2YR12, and CX3CR1 decrease in multiple sclerosis and Alzheimer's disease.[59] There is an understanding that these new insights are viewed in the context of the current knowledge that the phenotypic and functional plasticity of myeloid cells

TABLE 1 Comparison of culture methods for postmitotic monocyte-derived microglia-like cells

Reference (differentiation time)	Isolation method	Base medium	Serum	M-CSF (ng/ml)	GM-CSF (ng/ml)	Human embryonic astrocyte conditioned medium	IL-34 (ng/ml)	CCL2 (ng/ml)	NGF (ng/ml)	TGF-β (ng/ml)
Leone et al., 2006 (10 days)	Elutriation	DMEM, Pen (100 U/ml)/Strep (100 μg/ml)	10% FCS	10	1	25% (with the indicated cytokines for 8 days)	None	None	None	None
Bertin et al., 2012 (14 days)	Period of adherence	RPMI-1640	10% FCS	10	1	25–35%	None	None	None	None
Ohgidani et al., 2014 (14 days)	Overnight adherence	RPMI1640 GlutaMAX™	10% FCS	10	10	None	100	None	None	None
Ohgidani et al., 2014 (14 days)	Overnight adherence	RPMI1640 GlutaMAX™	10% FCS	None	10	None	None	None	None	None
Lannes et al., 2017 (7–10 days)	PBMC, 2 h adherence	RPMI1640 GlutaMAX™	None	10 ng/ml	10 ng/ml	None	None	50	10	None
Bohlen et al., 2018	Immunopanning (rodent microglia)	DMEM/F12 1 μg/ml oleic acid, 0.001 μg/ml gondoic acid, 5 μg/ml N-acetyl cysteine, 5 μg/ml insulin, 100 μg/ml Apo-transferrin, 0.1 μg/ml sodium selenite, 2 ng/ml hTGF-ß2,100 ng/ml hIL-34, and 1.5 μg/ml cholesterol	None	None	None	None	100	None	None	2

All humidified atmosphere, 5% CO_2, 37°C; Pen, penicillin; Strep, streptomycin.

is profoundly shaped and influenced by cytokine/chemokine signals from neighboring support cells and between other myeloid-to-cell specific interactions particular to each tissue microenvironment.[62,63,64] Regarding the brain, these cells include astrocytes, neuronal subtypes, endothelial/epithelial cells of the BBB and blood–CSF barrier, the lymphatics, meninges, blood vessels, and other discrete anatomical structures that interface with immune cells in the peripheral circulation. Knowledge of myeloid plasticity and their tissue heterogeneity can be harnessed to fill the gaps in understanding MG–pathogen molecular mechanisms.

For example, inhibition of M-CSF receptor signaling with pharmacologic agents induces the rapid death and depletion of brain MG[65] demonstrating the critical role that the ligands M-CSF and IL-34 plays in the trophic support for these cells.[66,67] These studies revealed that newly divided MG arise in 2 weeks from a brain progenitor population. Under conditions of stress or injury to the brain, local proliferation and HSC progenitors infiltrating the brain from the periphery contribute to repopulation.[65,68–70] Although the bone marrow-derived MG were less dense processes than their yolk-sac counterparts,[71] these studies demonstrate decisively the potential for blood myeloid postmitotic progenitors to cross blood–brain barriers, enter the brain parenchyma and differentiate into MG. Many researchers have investigated the possibility of human postmitotic monocytes developing into MG. One of the first was Leone et al., who used elutriated monocytes incubated on plastic flasks in DMEM with 10% heat-inactivated fetal calf serum, M-CSF, and GM-CSF (see Table 1). However, there have been significant recent advances in culturing physiologic human MG. A recent study by Rai et al., comparing MG cell lines (C20, HMC3), iPSC-derived MG, and hMMG found ∼78% conservation among 780 MG-enriched genes.[72] After 14 days of HIV_{Bal} infection, the iPSC-MG, in contrast to hMMG, showed significantly increased apoptotic death.[72] Overall, they found that the hMMGs and iPSC-derived MG were more similar to human primary MG than the C20 and HMC3 cell lines examined.[72] We have been able to successfully adapt a protocol by Bohlen et al.,[73] to reproducibly, and efficiently induce human postmitotic monocytes into MG-like cells that express TMEM119,[71,74] P2YR12, and CX3CR1, markers of resting MG[75] (Figure 1). A cautionary tale and comprehensive investigation of the historic development and subsequent usage of the HMC3 microglial cell line (known by different names) in laboratories worldwide that the ATCC recently validated were recently published.[76] We direct interested readers to another recent review of culture methods for human embryonic or PSCs-derived MG, which require 30–180 days to develop.[43,77,78] Several groups are beginning to successfully apply these culture systems to ask key mechanistic questions about microglial function.[79,80] Their findings show that hMMGs provide a pathway to gaining molecular insights into HIV-MG pathogenesis. A key advantage of hMMGs is that they can be readily derived from adult human donor blood monocytes providing an opportunity to understand the contribution of biologic sex and somatic gene expression on phenotype and function.

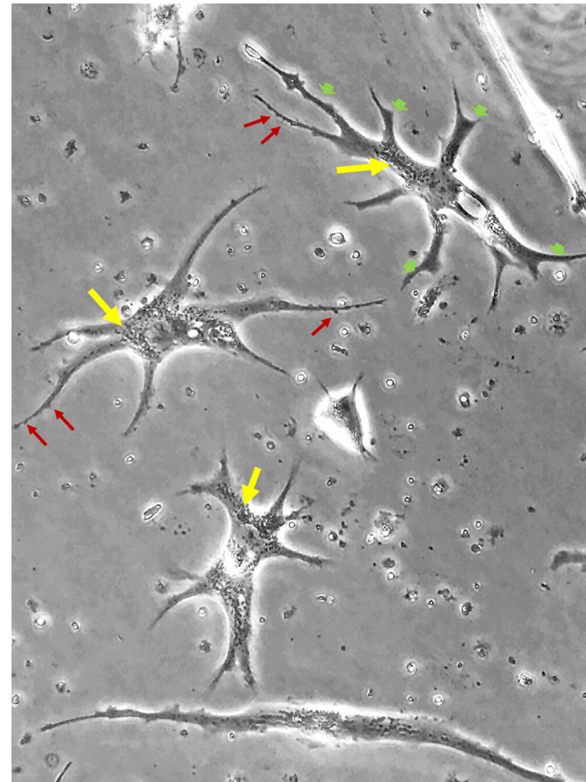

FIGURE 1 Human monocyte-derived microglia-like cells 28 days after differentiation. Negatively selected pan-monocyte bead-purified human monocytes incubated in serum-free DMEM/F12 base medium with 2 ng/ml TGF-β and 100 ng/ml IL-34 (method of Bohlen et al.[73]) at 28 days after plating. Cells assume different intricate morphologies with extensive processes (green arrowheads), spine-like structures (burgundy arrows), and intracellular vesicles (yellow arrows)

1.4 | Future translational potential

Novel approaches using humanized mice have allowed researchers to take more rigorous experimental methods to demonstrate that myeloid cells can serve as sources of HIV replication under antiviral therapy.[81–83] Most recently, Mathews et al.[84] showed that NOG-mice brains expressing human IL-34 could be engrafted with fetal cord blood stem cells that mature into hMG. The hMG showed dense complex processes, expressed microglial signature genes, and was susceptible to HIV infection.[84] In ongoing studies, we plan to use NOG-IL-34 mice to determine whether hMMGs delivered to the neonatal brain differentiate into mature MG-like cells and support HIV replication. If successful, this approach could help ease the challenges and expense of procuring cord blood needed to generate humanized mice. iPSCs often retain a gene expression pattern that is embryonic.

In contrast, with hMMGs originating from postmitotic cells, the gene expression signatures are expected to represent more differentiated adult cells. Furthermore, while a blood draw is required, this is a safe, relatively quick procedure conducted by an expert in an outpatient setting. Therefore, the hMMG approach could expand experimental

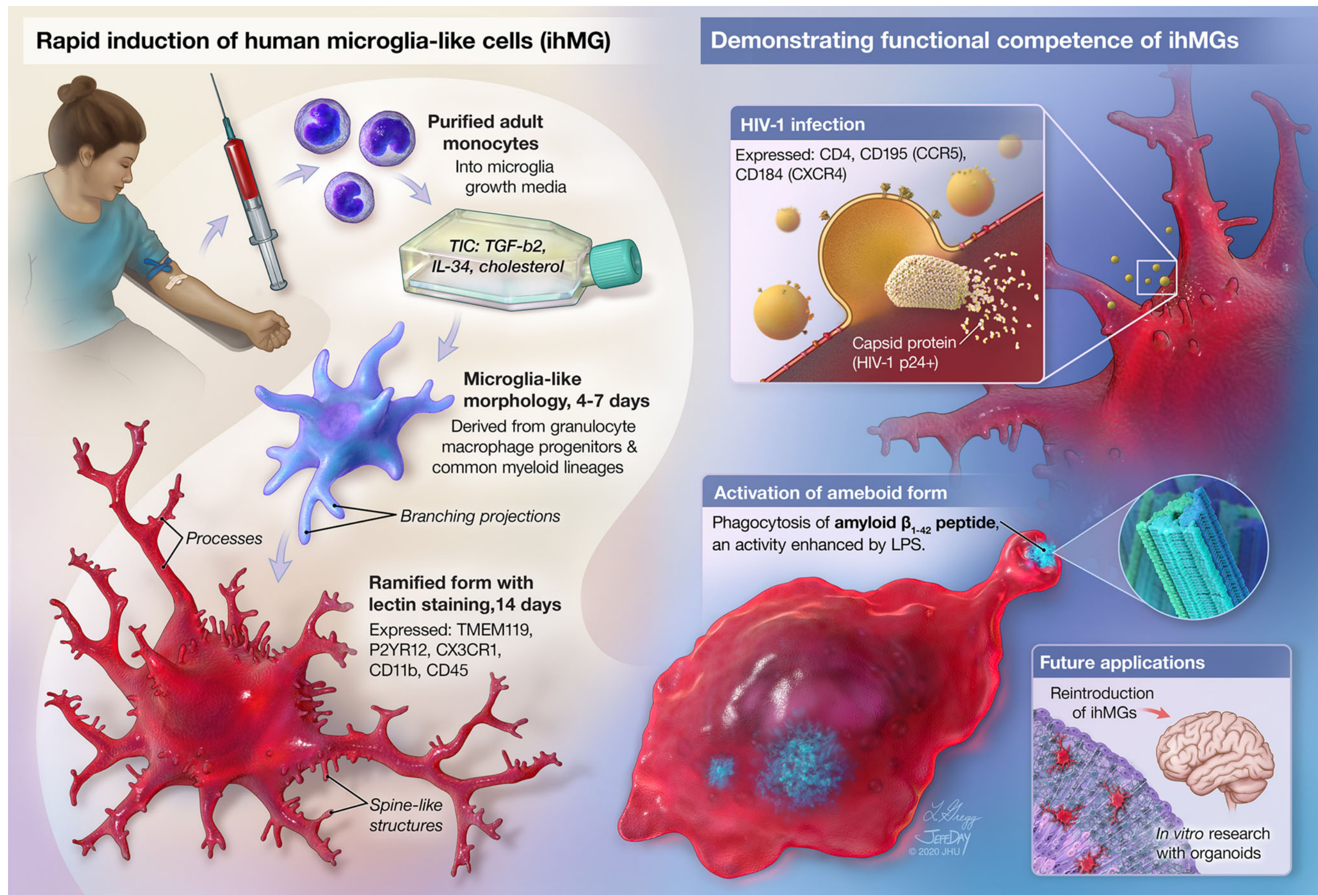

FIGURE 2 Use of postmitotic adult human monocytes to generate microglia-like cells (hMMGs) and future basic and clinical translational potential. Monocytes purified from leukopaks obtained from healthy blood donors were, after gradient centrifugation, negatively selected with magnetic beads and plated in a serum-free medium containing cholesterol, IL-34 and TGF-β2 (TIC media) described by Bohlen et al.[73] for primary mouse microglia. Within 4—7 days, the differentiated cells assumed a microglia-like phenotype with numerous branched projections. Immunochemistry and flow cytometric analyses are used to confirm the expression of microglia defining surface proteins, TMEM119, P2YR12, CX3CR1, CD11b, CD45 as well as CD4 and the coreceptors, CCR5 and CXCR4 used by HIV-1. The functional competence of hMMGs is demonstrated by productive infection with HIV and phagocytosis of toxic amyloid beta1–42 peptide. With the ability to rapidly produce functional human microglia-like cells having an adult phenotype in vitro, such cells are now in use in 2D-, 3D-, and in vivo rodent models of increasing complexity. The end goals are to develop more relevant screening assays that better reflect the genetic architecture and physiology of adult microglia. Illustration created by Lydia Gregg and Jeff Day © 2020 Johns Hopkins University

findings' translational potential and relevance. The feasibility of this approach is also encouraged based on recent successful studies using human iPSC-derived MG implanted in mouse brains.[85–87]

2 | CONCLUDING REMARKS

As artfully illustrated by Ohgidani et al. and others, with continued refinement and attention to the rigor of the postmitotic hMMG approach combined with advances in the range of technologies, 3D in vitro brain models and humanized mice to safely translate the findings to better the lives of those suffering from HAND and other neurologic and neuropsychiatric disorders will be possible in the long term (Figure 2).[88–91] Moreover, these investigations will continue to advance our understanding of novel regulatory mechanisms at the juxtaposition of the brain and peripheral immune systems.

AUTHORSHIP

T. B. and A. M. B. developed and refined methods for hMMG culturing; S. L. conducted studies with hMMGs; A. M. B. wrote and edited the manuscript; T. B. and S. L. reviewed the manuscript.

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DISCLOSURE

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