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Full Length Article

Characterization and comparative analysis of a new mouse microglial cell model for studying neuroinflammatory mechanisms during neurotoxic insults

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ABSTRACT

Microglia are the first responders of the central nervous system, acting as the key modulators of neuroinflammation observed during neurotoxic insults as well as in the pathophysiology of several neurodegenerative disorders including Alzheimer's (AD), Parkinson's (PD), and Huntington's diseases (HD). The number of publications on microglia has increased steadily throughout the past decade because of immense interests in the neuroinflammation that precedes the neurodegenerative process. To study microglial biology and its role in modulating neuroinflammation, immortalized microglial cell lines derived from mice, rats, and humans have been developed. Among these, the BV2 mouse microglial cell line is the most well characterized and widely used cell culture model. However, even unstimulated BV2 cells exhibit an amoeboid, hypertrophied morphology, indicating a highly activated and inflammatory state compared to primary microglia, thus making them less than ideal for studying the low-dose effects of toxicants on microglial activation. Therefore, we performed an in-depth characterization of a recently developed mouse microglial cell (MMC) line, which we compared with primary mouse microglia (PMG) and BV2s to identify which cell line was best suited for studying the microglial response to neurotoxicants. Comparative analyses reveal that MMCs are strikingly more similar to PMGs in basal activity, morphology, and sensitivity, than are BV2s. Furthermore, basal nitrite and inflammatory cytokine levels are significantly higher in BV2s compared to MMCs. BV2 cells are also less reactive to the inflammagen LPS compared to MMCs, due to the higher basal activation state of BV2s. Collectively, our in-depth analyses of morphology, basal activity, and responsivity to two different stimuli (LPS, aggregated α-synuclein) demonstrate that MMCs closely mimic neonatal PMGs, and are discernibly more suitable than BV2s for studying the neuroinflammatory mechanisms of neurotoxicants.

1. Introduction

Microglia are the first immune responders in the brain. Persistently active and unregulated microglia are key modulators of chronic neuroinflammation observed in several neurodegenerative disorders. Activated microglia are distinct from resting microglia both morphologically as well as functionally with respect to the production and secretion of pro-inflammatory factors (Glass et al., 2010; Panicker et al., 2015). Lipopolysaccharide (LPS)-activated microglia undergo morphological changes, in general, from a ramified (surveillant) type to a hypertrophic, amoeboid type that is typically observed releasing ROS, nitrite, and various other pro-inflammatory mediators and cytokines that can be toxic over time. Like macrophages, microglia can also undergo an alternative activation, which leads to the production of anti-inflammatory factors. For example, IL-4 can induce this alternative anti-inflammatory phenotype in microglial cells (Nakagawa and Chiba, 2014). Walker and Lue (2015) pondered the probable existence of a third microglial phenotype, known as M3. Such a polarization may be induced via CSF-1 or IL-34 signaling, either of which can activate CSF-1R, which could tightly regulate microglial cell division; however, the evidence is still scant. Thus, microglial activation is complex, and the molecular footprints underlying the various phenotypes are the focus of much research.

Several lines of evidence, from cell culture to animal models to

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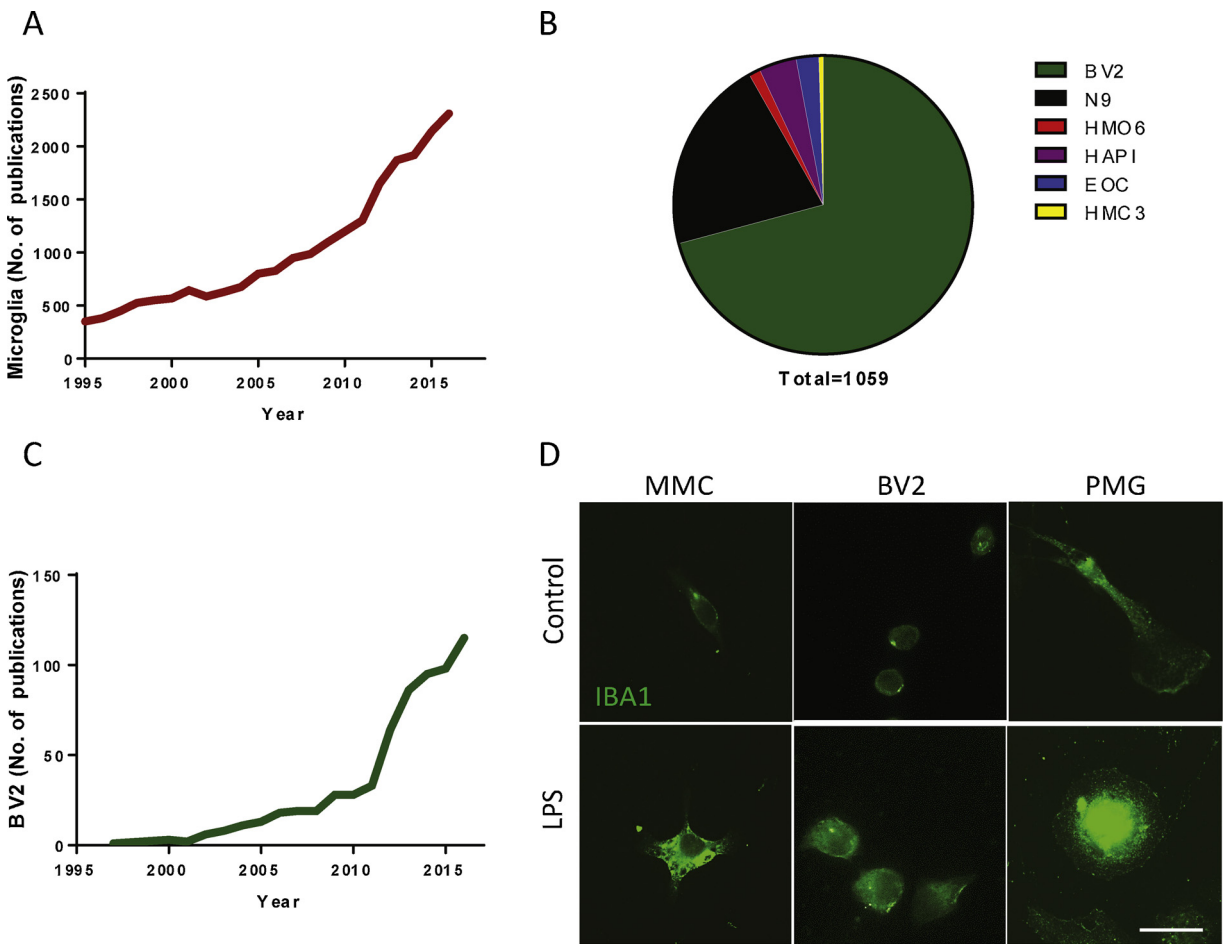

Fig. 1. Morphological comparison between the mouse microglial cell (MMC) line, the BV2 cell line, and primary microglia (PMG). A) Number of yearly publications on microglia in PubMed. B) Distribution of the number of PubMed-indexed publications on various microglial cell lines. C) Number of yearly publications on BV2 microglia in PubMed. D) Immunostaining for IBA1 on BV2, MMC, and PMG cells after a 6-h LPS (1 μg/mL) exposure. Scale bar is 10 μm.

postmortem tissue analyses to genetic linkage analyses, conclusively demonstrate that sustained neuroinflammation contributes to neuronal dysfunction and death (Block et al., 2007; Gordon et al., 2016; Kirkley et al., 2017; Panicker et al., 2015; Sarkar et al., 2017b). Microglial activation leading to neurodegeneration has been well documented in AD, PD, and other neurodegenerative diseases (Block et al., 2007; Glass et al., 2010; Solito and Sastre, 2012). Misfolded proteins, including aggregated α-synuclein (αSyn_{Agg}) and amyloid-β, the respective hallmarks of PD and AD pathologies, can hyper-activate microglia, leading to chronic inflammation and loss of neurons (Halle et al., 2008; Heneka et al., 2013).

The number of publications on microglial cells in the last decade has increased steadily with every passing year (Fig. 1A), especially the importance of microglia in AD, PD, multiple sclerosis (MS), and other neurodegenerative disorders. To study microglial biology and its role in modulating neuroinflammation, several immortalized microglial cell lines have been developed (Fig. 1B), including BV2, N9, and EOC (mouse microglial cell lines); HAPI (rat microglial cell line); and the HMC3 and HMO6 (human microglial cell lines). Among the six prevalent cell lines, BV2 cell lines have been used in ~75% of publications (Fig. 1B). Since 2010, the number of publications reporting on BV2s has substantially increased (Fig. 1C). Though BV2 microglial cell lines are not identical to primary microglia in morphology or activation state, the prevalent use of BV2s may be attributed to the following: i) lack of good, easily obtainable cell lines; ii) cost of primary culture; iii) low yield of primary microglial cells; and iv) difficulty in isolating primary cells. Blasi et al. (1990) created the BV2 cells via the J2 retrovirus carrying the v-myc/v-raf genes from C57BL/6 primary mouse microglia. In this study, we performed a more in-depth characterization of a newly developed microglial cell line that was also immortalized with the J2 retrovirus from C57BL/6 primary mouse microglia (Halle et al., 2008). We compared this new mouse microglial cell line (MMC) with the predominantly used BV2 microglia. Our results demonstrate that the basal inflammatory level of BV2 microglia is higher than that of MMCs, and that the responsiveness of MMCs to LPS and αSyn_{Agg} stimulation far exceeds that of BV2s, suggesting that MMCs more closely mimic PMGs than do BV2s.

2. Material and methods

2.1. Chemicals, reagents and instruments used

Dulbecco's modified Eagle's medium (DMEM), DMEM-F12, fetal bovine serum (FBS), L-glutamine (Q), penicillin/streptomycin (P/S), Sodium Pyruvate (SP), and Non-Essential Amino Acids (NEAA) were obtained from Invitrogen (Carlsbad, CA). CellTiter Glo Luminescent Cell Viability Assay kit was obtained from Promega (Madison, WI). The CD11b magnetic separation kit was purchased from Stem Cell Technologies (Vancouver, Canada). All standards used for the Luminex multiplex cytokine assay were purchased from PeproTech, Inc (Rocky Hill, NJ). Streptavidin-Biotin and biotinylated antibodies used for Luminex were purchased from eBioSciences (San Diego, CA). Phagocytosis assay kit was purchased from Cayman Chemicals (Ann Arbor, MI).