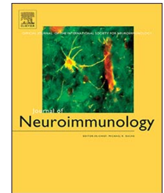
Journal of Neuroimmunology 310 (2017) 143–149

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim



Comparative morphology and phagocytic capacity of primary human adult microglia with time-lapse imaging

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ARTICLE INFO

Keywords:
Primary human adult microglia
Dendritic cells
E. coli bioparticles
Time lapse microscopy
Pseudopodic
Ramified
Amoeboid

ABSTRACT

Microglia provide immune surveillance within the brain and spinal cord. Various microglial morphologies include ramified, amoeboid, and pseudopodic. The link between form and function is not clear, especially for human adult microglia which are limited in availability for study. Here, we examined primary human microglia isolated from normal-appearing white matter. Pseudopodic and amoeboid microglia were effective phagocytes, taking up *E. coli* bioparticles using ruffled cell membrane sheets and retrograde transport. Pseudopodic and amoeboid microglia were more effective phagocytes as compared to ramified microglia or monocyte-derived dendritic cells. Thus, amoeboid and pseudopodic microglia may both be effective as brain scavengers.

1. Introduction

Microglia are the resident phagocytic cells of the CNS that actively scavenge and provide immune surveillance (Perry and Gordon, 1988; Glenn et al., 1992; Brown and Neher, 2014; Kettenmann et al., 2011). Seminal work by Pio del Rio-Hortega revealed that microglia have various morphological configurations that are accompanied by specialization of function. Ramified microglia have a small nucleus and cytoplasm with complex branched processes, amoeboid microglia have a large nucleus and are oval-shaped surrounded by ruffled membrane sheets, and pseudopodic microglia have oblong cell bodies with two protrusions containing fan-shaped ruffled membranes (Del Rio Hortega, 1920; Kernohan and Penfield, 1932; Morrison and Filosa, 2013; Karperien et al., 2015; Sivagnanam et al., 2010). Ramified microglia actively scan the environment, and upon activation may transform into amoeboid or pseudopodic morphologies to phagocytose particles (Kettenmann et al., 2011; Glenn et al., 1992). In multiple sclerosis, microglia can cause inflammation and injure neurons (González et al., 2014; Watanabe et al., 2016), but can also serve neuroprotective roles by cleaning up debris by phagocytosis and promoting cellular repair (Fu et al., 2014; Neumann et al., 2009). Microglia also protect the brain from intracerebral infections by phagocytosing bacteria into lysosomes (Schütze et al., 2014; Kaur et al., 2004; Ribes et al., 2009). Phagocytosis requires cytoskeletal network rearrangement and relies specifically on F-actin, a filamentous polymer of the cytoskeleton. The balance of actin polymerization and depolymerization enables cell motility, cytokinesis, and dynamic changes in cell shape. Particles that come in contact with transmembrane phagocytic receptors cause actin accumulation at the spot of contact, where the engulfment of the particle in a phagocytic cup follows membrane elongation (Gitik et al., 2010). Actin dynamics are often studied using fluorescent phalloidin staining (Miller et al., 2003). Studies on murine microglia suggest that impaired actin dynamics reduce the phagocytosis of bacteria particles (Uhlemann et al., 2016). The phagocytosis can be seen as membrane ruffling where F-actin accumulates in regions around the contact point with microspheres (Koizumi et al., 2007). Alteration to the F-actin filaments or to the directly-associated cytoskeletal proteins in microglia result in the increased probability of developing neurodegenerative diseases or

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http://dx.doi.org/10.1016/j.jneuroim.2017.05.012
Received 1 February 2017; Received in revised form 9 May 2017; Accepted 20 May 2017
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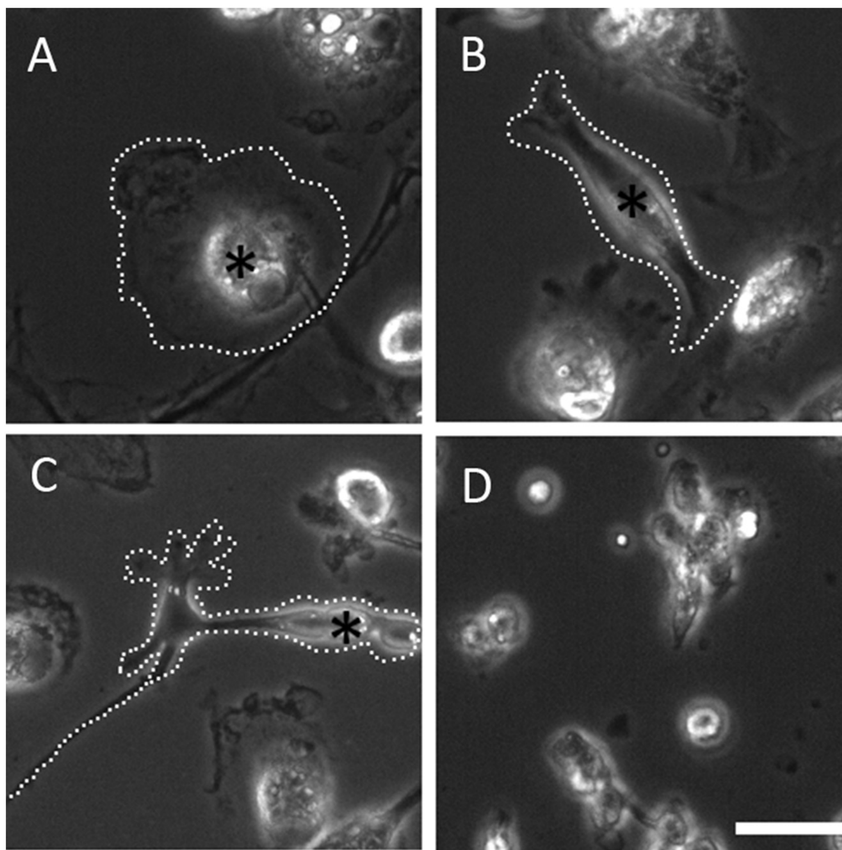

Fig. 1. Distinct morphologies of microglia from adult temporal lobe cell cultures. Representative cropped images from time-lapse imaging depict distinctive morphologies coexisting in the same culture of primary human microglia. Representative cell in (A) amoeboid form, (B) pseudopodic form and in (C) ramified form. Dotted line tracings highlight outer edge of the microglia. Asterisk is placed on approximate center of cell body. D) For comparison, representative cells from monocyte-derived dendritic cells are shown at the same scale and magnification. Scale bar = 20 μm.

neuro-inflammatory disorders. Improper actin dynamics decrease the phagocytic ability of microglia, resulting in the accumulation of extracellular debris and disease development, as exemplified by the onset of Alzheimer's disease due to amyloid-β accumulation (Daria et al., 2016). Macrophages, being similar to microglia in function, display dysfunctional phagocytosis when the organization of F-actin filaments is inhibited (de Oliveira and Mantovani, 1988). Dendritic cells (DCs) have properties similar to microglia. DCs are found throughout body tissues where they capture, process, and present antigens to T cells via MHC molecules (Steinman and Cohn, 1973; Banchereau and Steinman, 1998; Savina and Amigorena, 2007; Segura and Villadangos, 2009; Merad et al., 2013; González et al., 2014). DC morphology is characterized by long cytoplasmic extensions of asymmetric shapes. Primary human adult microglia and fetal microglia were comparable to DCs in their capacity to stimulate T cells and phagocytose *E. coli* bioparticles (Lambert et al., 2008). *E. coli* bioparticles are killed *E. coli* bacteria that are prepared with a fluorescent dye to track phagocytosis. Human DCs derived from monocytes in vitro (mDCs) are functionally-competent, capable of cytokine production and phagocytosis (Durafourt et al., 2012). In the current study, we evaluated the morphology and capacity of primary human adult microglia and mDCs to phagocytose *E. coli* bioparticles, and quantified the relative uptake of the bioparticles in acidic compartments.

2. Methods

2.1. Cell cultures

Primary human microglia were obtained from normal-appearing white matter resected tissue of a temporal lobe surgery for non-tumor-related intractable epilepsy using previously published procedures (Yong and Antel, 1997; Williams et al., 1992; Williams et al., 1994). The study was approved by the McGill University institutional review board and samples were procured with informed consent. Cells were cultured in MEM with 5% FBS, penicillin, streptomycin, and L-glutamine in tissue culture-treated flasks. Oligodendrocytes were removed with shake-off. CD11c staining showed that these cultures were routinely > 90% pure microglia (Lambert et al., 2008). Cultures were maintained in culture for up to two weeks before using in the experiments. For time lapse imaging, microglia were trypsinized and plated onto glass-bottom round 35 mm culture dishes coated with extracellular matrix (Sigma-Aldrich, Mississauga, Canada) and allowed to adhere in the incubator. To obtain mDCs, monocytes were isolated from healthy human venous blood according to previous protocols (Tabatabaei Shafiei et al., 2014; Lambert et al., 2008). In brief, CD14 + selection of cells from venous blood (Miltenyi Biotec, Auburn, CA) yielded a 95% pure fraction as determined by flow cytometry. Cells were cultured for six days with recombinant granulocyte-macrophage colony-stimulating factor (50 ng/ml, PeproTech, Rocky Hill, NJ) and recombinant IL-4 (20 ng/ml, PeproTech). On the final day, 100 ng/ml of lipopolysaccharide (serotype 0127:B8, Sigma-Aldrich) was added for 24 h to induce DC maturation. Cells were trypsinized and plated onto glass-bottom round 35 mm culture dish coated with extracellular matrix (Sigma-Aldrich).

2.2. E. coli bioparticle exposure and microscopy

1×10^6 pHRodo acid-sensitive *E. coli* bioparticles were added to the imaging dish after being prepared according to manufacturer's instructions (P35361, Thermo Scientific). In brief, the particles were warmed up to room temperature and sonicated for 1 min to create an even suspension. The pHRodo dye-labelled *E. coli* becomes fluorescent upon entering acidic lysosomes or vesicles. Time-lapse imaging was performed for up to 200 min in a chamber with a temperature of 37 °C, 5% CO_2, and consistent humidification. A 20 × objective was used for time-lapse, with images taken automatically every 30 s using Northern Eclipse software (Empix Imaging, Mississauga, Canada). The focus was periodically checked for manual adjustment as needed. To measure the

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