



Human Microglia Seize the Chance to be Different

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Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry

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Microglia, the specialized innate immune cells of the central nervous system, play crucial roles in neural development and function. Different phenotypes and functions have been ascribed to rodent microglia, but little is known about human microglia (huMG) heterogeneity. Difficulties in procuring huMG and their susceptibility to cryopreservation damage have limited large-scale studies. Here we applied multiplexed mass cytometry for a comprehensive characterization of postmortem huMG (103-104 cells). We determined expression levels of 57 markers on huMG isolated from up to 5 different brain regions of 9 donors. We identified the phenotypic signature of huMG, which was distinct from peripheral myeloid cells but was comparable to fresh huMG. We detected microglia regional heterogeneity using a hybrid workflow combining Cytobank and R/Bioconductor for multidimensional data analysis. Together, these methodologies allowed us to perform high-dimensional, large-scale Immunophenotyping of huMG at the single-cell level, which facilitates their unambiguous profiling in health and disease.

Commentary

Microglia are macrophages that reside exclusively in the central nervous system and actively participate in maintaining neural network homeostasis and regulating immune responses. Microglial cells are highly dynamic and can quickly change their functional status in response to signals that indicate tissue damage, pathogen invasion, or altered neural activity.^{1,2} Functional alterations are accompanied by morphological changes from highly ramified cells, which take on physiological and homeostatic roles, to amoeboid cells which are often linked to inflammatory and phagocytic phenotypes. In the healthy brain, microglia promote neuronal survival, guide angiogenesis, phagocytose apoptotic neurons, and remove excessive synaptic elements to support the establishment of functional neural circuitries.¹ Under pathological conditions, microglia typically release cytokines, which can promote or control inflammation, and phagocytose dead/dying neurons.² In addition, microglia engage in pathological complement-mediated synaptic pruning in neurodegenerative disorders,³ a phenomenon that may also occur in epilepsy.⁴

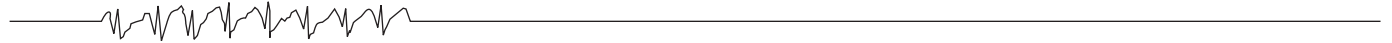
Recent studies support the presence of a heterologous population of microglia with a myriad of morphologies, cytokine profiles, and phagocytic receptors in epilepsy.⁵⁻⁸ Evidence from preclinical experimental models suggest that these phenotypes may evolve with different spatiotemporal profiles.⁹⁻¹¹ The heterogeneity of microglial functions is governed by a

variety of surface receptors which help detect and react to disturbances in the brain parenchyma.² Because this evidence comes mainly from animals housed in unique pathogen-free environments, it has been argued that the microglial profiles in healthy and diseased states described in the literature may not accurately recapitulate those of the human. Thus, in recent years, leading cutting-edge technologies such as multiplexed single-cell mass cytometry (CyTOF), fluorescence-assisted cell sorting, and flow cytometry combined with transcriptomics, among others, have been applied to characterize human microglial biological profiles across different brain regions and ages.^{5,6,12-14} Importantly, these studies often include brain tissue samples surgically resected from patients with refractory epilepsy because epilepsy is one of few disorders in which fresh brain biopsies can be obtained, thus making the findings particularly relevant to the epilepsy community.

In the *Nature Neuroscience* study by Böttcher et al, CyTOF was used to characterize the immune phenotype of isolated human microglial cells obtained from different brain regions from postmortem autopsies (nonepileptic) and fresh biopsies (temporal lobe epilepsy) and compared them with peripheral immune cells from blood or cerebrospinal fluid. The CyTOF uses antibodies tagged with heavy metal ions instead of fluorochromes, which results in an unbiased and more specific characterization of multiple immune markers. First, this study provides evidence further supporting that microglial cells have



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unique immune signatures compared to peripheral immune cells including higher levels of the purinergic receptor P2Y₁₂, the phagocytic receptor Trem2, and the transmembrane marker TMEM119. Further, microglia derived from the epileptic tissues showed higher signal intensities for the interferon regulatory factor 8, P2Y₁₂, the lysosomal/endosomal-associated glycoprotein CD68, and the Major histocompatibility complex (MHCII) class II cell surface receptor Human Leukocyte Antigen –DR isotype than the brain autopsy samples. In parallel, higher frequencies of IR8^{hi}P2Y₁₂ cells were evident in the epileptic samples, thereby suggesting a prominent presence of a population of reactive phagocytes in human epilepsy. Similar profiles were reported after transcriptomic analyses of single microglial cells¹³ and whole tissue homogenates⁶ from human epilepsy. For instance, transcriptomic profiling of single microglia from refractory epilepsy revealed that TMEM119, P2Y₁₂, Trem2, the fractalkine receptor CX3CR1, and complement molecules such as C3 and C1qA-C were highly abundant in these cells.¹³ These findings are significant because they are supported by observations in experimental models^{8,9,15} and suggest roles for proteins such as P2Y₁₂, Trem2, C1q, and C3 in epilepsy.

Another interesting finding shown in Böttcher et al is that huMG have gene profiles that are brain region-specific and may potentially result in different functions that are neural circuit dependent. For instance, microglia in the subventricular zone (SVZ) are characterized by high expression of Ki-67 and cyclins A and B1 when compared to at least 4 different brain regions from the same individuals (autopsy). Note that these molecules regulate cell cycle and proliferation mechanisms, thereby suggesting that microglia may be highly active and in constant proliferation in the SVZ. Regional heterogeneity of microglial morphological and biochemical phenotypes were also described in mesial temporal lobe epilepsy with hippocampal sclerosis.⁶ Morin-Brureau et al showed populations of microglia with different morphologies throughout the hippocampus, with CA1 and CA3 containing larger numbers of amoeboid microglia. These microglia also expressed higher levels of MHCII and CD68 and correlated with areas of low neuronal densities.⁶ Furthermore, the correlation between amoeboid CD68-positive microglia and areas of neuronal and dendritic loss overlaps with cortical areas of high interictal spiking in pediatric patients with refractory epilepsy.⁵ While it is not definitively known how the different properties of these cells contribute to the neuropathology and pathophysiology of drug-resistant epilepsy, the expression of phagocytic markers such as CD68 and Trem2 suggests that microglia may have a highly phagocytic phenotype. It is possible that these hungry microglia are contributing to exacerbated synaptic pruning and/or unwanted elimination of stressed but healthy neurons (phagoptosis) in the epileptic brain. These are novel roles recently described for microglia that are yet to be comprehensively investigated in epilepsy.

In summary, the use of advanced and innovative approaches has produced strong evidence of microglial heterogeneity in the human brain. Importantly, the microglial biological profiles

obtained from tissues surgically resected from patients with epilepsy can provide insights into the potential roles of microglia in the pathophysiology of epilepsy. A limitation of this study is that it seems to utilize temporal lobe epilepsy samples as a means to get brain biopsy tissue, but was not really investigating differences in epileptic versus nonepileptic tissues. Although distinct microglial profiles were found between the biopsy (epilepsy) and autopsy tissues, it is difficult to distinguish whether the differences are a cause or result of seizures. Note that it is also possible that the differences in the microglial biological profiles may be due to differences in live/biopsy tissue versus postmortem tissue, not to epilepsy. Furthermore, the microglia were extracted only from tissues adjacent to the epileptogenic focus that were deemed nonpathological. Yet, a recent study showed that the numbers of microglia were similar in the epileptogenic centers and margins,¹² suggesting that the microglia in these areas may have some comparable properties. Taken together, these findings suggest that huMG have unique biological profiles that may be governed by the intrinsic properties of the neural circuitries in which they reside. Thus, the biological complexity of these phagocytes in human epilepsy may be more challenging to understand than previously thought.

By Amy L. Brewster

References

1. Kierdorf K, Prinz M. Microglia in steady state. *J Clin Invest*. 2017;127(9):3201-3209.
2. Sierra A, Abiega O, Shahraz A, Neumann H. Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. *Front Cell Neurosci*. 2013;7:6.
3. Salter MW, Stevens B. Microglia emerge as central players in brain disease. *Nat Med*. 2017;23(9):1018-1027.
4. Koenig J, Dulla C. Complements to the chef: are microglia eating neurons in the epileptic brain? *Epilepsy Curr*. 2018;18(2):128-130.
5. Dachtel F, Bagla S, Keren-Aviram G, et al. Predicting novel histopathological microlesions in human epileptic brain through transcriptional clustering. *Brain*. 2015;138(pt 2):356-370.
6. Morin-Brureau M, Miliot G, Royer J, et al. Microglial phenotypes in the human epileptic temporal lobe. *Brain*. 2018;141(12):3343-3360.
7. Wyatt SK, Witt T, Barbaro NM, Cohen-Gadol AA, Brewster AL. Enhanced classical complement pathway activation and altered phagocytosis signaling molecules in human epilepsy. *Exp Neurol*. 2017;295:184-193.
8. Abiega O, Beccari S, Diaz-Aparicio I, et al. Neuronal hyperactivity disturbs ATP microgradients, impairs microglial motility, and reduces phagocytic receptor expression triggering apoptosis/microglial phagocytosis uncoupling. *PLoS Biol*. 2016;14(5):e1002466.
9. Schartz ND, Wyatt-Johnson SK, Price LR, Colin SA, Brewster AL. Status epilepticus triggers long-lasting activation of complement C1q-C3 signaling in the hippocampus that correlates with



- seizure frequency in experimental epilepsy. *Neurobiol Dis.* 2018; 109(Pt A):163-173.
10. Wyatt-Johnson SK, Herr SA, Brewster AL. Status epilepticus triggers time-dependent alterations in microglia abundance and morphological phenotypes in the hippocampus. *Front Neurol.* 2017;8:700.
 11. Schartz ND, Herr SA, Madsen L, et al. Spatiotemporal profile of Map2 and microglial changes in the hippocampal CA1 region following pilocarpine-induced status epilepticus. *Sci Rep.* 2016; 6:24988.
 12. Xu D, Robinson AP, Ishii T, et al. Peripherally derived T regulatory and gammadelta T cells have opposing roles in the pathogenesis of intractable pediatric epilepsy. *J Exp Med.* 2018; 215(4):1169-1186.
 13. Gosselin D, Skola D, Coufal NG, et al. An environment-dependent transcriptional network specifies human microglia identity. *Science.* 2017;356(6344).
 14. Galatro TF, Holtman IR, Lerario AM, et al. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. *Nat Neurosci.* 2017;20(8):1162-1171.
 15. Eyo UB, Peng J, Swiatkowski P, Mukherjee A, Bispo A, Wu LJ. Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. *J Neurosci.* 2014;34(32):10528-10540.