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Rod-shaped microglia morphology is associated with aging in 2 human autopsy series

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ABSTRACT

A subtype of microglia is defined by the morphological appearance of the cells as rod shaped. Little is known about this intriguing cell type, as there are only a few case reports describing rod-shaped microglia in the neuropathological literature. Rod-shaped microglia were shown recently to account for a substantial proportion of the microglia cells in the hippocampus of both demented and cognitively intact aged individuals. We hypothesized that aging could be a defining feature in the occurrence of rod-shaped microglia. To test this hypothesis, 2 independent series of autopsy cases (total n = 168 cases), which covered the adult lifespan from 20 to 100+ years old, were included in the study. The presence or absence of rod-shaped microglia was scored on IBA1 immunohistochemically stained slides for the hippocampus and cortex. We found that age was one of the strongest determinants for the presence of rod-shaped microglia in the hippocampus and the cortex. We found no association with the presence of rod-shaped microglia and a self-reported history of a TBI. Alzheimer's disease–related pathology was found to influence the presence of rod-shaped microglia, but only in the parietal cortex and not in the hippocampus or temporal cortex. Future studies are warranted to determine the functional relevance of rod-shaped microglia in supporting the health of neurons in the aged brain, and the signaling processes that regulate the formation of rod-shaped microglia.

1. Introduction

Microglia are the resident tissue macrophage of the central nervous system. In the healthy central nervous system, microglia form a network of nearly uniformly distributed cells throughout the tissue, with highly thin-ramified cell processes. Changes in microglia morphology away from the ramified or 'surveying' type of cell are well described in the literature, but largely are centered around the hypertrophic or "activated" morphology. Despite recent studies defining a number of additional microglia morphologies (Bachstetter et al., 2015; Roth et al., 2014; Streit, 2006; Ziebell et al., 2012), little is known about the relevance of these morphological changes to human brain health and disease.

First described by Franz Nissl over 100 years ago (reviewed by [Graeber, 2010]), rod-shaped microglia are a particularly intriguing morphologically defined subtype. The modern literature describing rod-shaped microglia is sparse and is dominated by case reports, with the exception of a recent study that determined the relative amount of rod-shaped microglia in the hippocampus of different age-related neurodegenerative diseases (Bachstetter et al., 2015). Rod-shaped microglia were found in approximately 60% of the cases, including a subset of nondemented control cases, as well as in cases with different neurodegenerative disease (Bachstetter et al., 2015). The high prevalence of rod-shaped microglia in individuals of 65 years or older suggested that aging or an age-related degenerative process might be an important predictor for the presence of rod-shaped microglia.

In this study, we sought to determine if aging, Alzheimer's disease, or traumatic brain injury (TBI) could be a defining feature in the occurrence of rod-shaped microglia in the human brain. To this end, we used 2 independent series of cases. The first series included 61 cases that covered the adult lifespan from 20 to

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96 years of age, which were free of advanced neurodegenerative pathology. The second set of 107 cases was from an aged population–based series, with an age range of 77–100+ years, which included nondemented controls and cases with Alzheimer's disease. We found that older chronological age was a strong predictor for the presence of rod-shaped microglia, even when controlling for Alzheimer's disease pathology. Our data suggest that there may be an age-related change to neurons or microglia, which we are yet to define, that predisposes the aged brain to the presence of rod-shaped microglia.

2. Materials and methods

2.1. UK series: University of Kentucky human subjects and tissue processing

A set of 61 autopsy cases were collected from the University of Kentucky (UK) bio tissue repository (Table 1). The cases were selected to cover the adult lifespan from 20 to 96 years of age. Cases were selected by the investigators (J. H. N. and P. T. N.) to be free of advanced neurodegenerative pathology. Exclusion criteria included pathologically confirmed neurodegenerative disease: specifically, but not limited to, advance disease pathology associated with Alzheimer's disease, dementia with Lewy bodies, hippocampal sclerosis of aging, and vascular dementia. To identify rod-shaped microglia, brains were stained with the ionized calcium binding adapter molecule 1 (IBA1) antibody, which is used as a pan marker of macrophages/microglia in the brain. Paraffin-embedded tissue was processed, 8μm-thick sections were cut, and immunohistochemical staining was done using the primary antibody: IBA1 (rabbit polyclonal, 1:1000 immunohistochemical, Wako Pure Chemical Industries, Richmond, VA, USA). A biotinylated secondary antibody (Vector Laboratories) was amplified using avidin-biotin substrate (ABC solution, Vector Laboratories catalog no. PK-6100), followed by color development in Nova Red (Vector Laboratories). The Aperio ScanScope XT digital slide scanner was used to image the entire stained slide at 40× magnification to create a single high-resolution digital image. A tissue section from the hippocampus and the frontal cortex was analyzed by 2 observers blind to the experimental conditions (Y. H. and D. A.). The observers exhaustively inspected the gray matter of the entire slides for the presence of rod-shaped microglia at a minimum of 8× magnification. Presence of rod-shaped microglia was defined as at least 1 clearly defined rod-shaped microglia: Fig. 1 shows examples of cells defined as rod-shaped microglia.

Table 1
Case series characteristics

Characteristics	UK series	AI-ACT series
Number of cases	61	107
Age		
20–29	6 (9.8%)	0 (0%)
30–39	12 (19.7%)	0 (0%)
40–49	7 (11.5%)	0 (0%)
50–59	6 (9.8%)	0 (0%)
60–69	4 (6.6%)	0 (0%)
70–79	5 (8.2%)	12 (11.2%)
80–89	16 (26.2%)	45 (42.1%)
90–99	5 (8.2%)	43 (40.2%)
100+	0 (0%)	7 (7.5%)
Sex		
Male	27 (43.3%)	63 (58.9%)
Female	31 (50.8%)	44 (41.1%)
Dementia status		
No dementia	61 (100%)	57 (53.3%)
Dementia	0 (0%)	50 (46.7%)
Braak NFT stage		
0/I/II	61 (100%)	28 (26.2%)
III/IV	0 (0%)	44 (41.1%)
V/VI	0 (0%)	32 (29.9%)
CERAD rating		
None	61 (100%)	24 (22.4%)
Sparse	0 (0%)	33 (30.8%)
Moderate	0 (0%)	25 (23.4%)
Frequent	0 (0%)	25 (23.4%)

Number of cases and percent of total cases for the series are shown in the table. University of Kentucky bio tissue repository series of cases (UK series). The Aging, Dementia and Traumatic Brain Injury Study series (2016 Allen Institute for Brain Science. Aging, Dementia and TBI. Available from: http://aging.brain-map.org/overview/explore) (AI-ACT series). Braak neurofibrillary tangle (NFT) Stage. Consortium to Establish a Registry for Alzheimer's Disease (CERAD).
Key: ACT, Adult Changes in Thought; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; NFT, neurofibrillary tangle.

2.2. AI-ACT series: aging, dementia, and TBI study of the Allen Institute (AI) for Brain Science

The Aging, Dementia and Traumatic Brain Injury Study is a series of 107 autopsy cases with and without a history of TBI drawn from the Adult Changes in Thought (ACT) study (AI-ACT; Table 1). Data used for the current project are publically available through the AI website (2016 Allen Institute for Brain Science. Aging, Dementia and TBI. Available from: http://aging.brain-map.org/overview/explore). High-magnification digital histopathological images of the entire physical slides are available to download for 3 brain regions—hippocampus, temporal cortex, and parietal cortex—for a number of neuropathological stains. Additional information is available in the AI-ACT database, including results from extensive biochemical assays, and clinical information about the cases. To determine how often rod-shaped microglia were found in this sample population, the digital histopathological images of IBA1 for the hippocampus, temporal cortex, and parietal cortex were downloaded (all regions were not available for all cases) and were independently scored by 3 observers blind to the experimental conditions (Y. H., D. A., and A. D. B.) for the presence or absence of rod-shaped microglia in the gray matter. Presence of rod-shaped microglia was defined as at least 1 clearly defined rod-shaped microglia; Fig. 1 shows examples of cells defined as rod-shaped microglia.

2.3. Statistics

JMP Pro Software version 12.0 or SAS 9.4 (SAS Institute, Inc.; Cary, NC, USA) was used for statistical analysis. Statistical significance was set at 0.05. Contingency tables of categorical variables (presence of rod-shaped microglia, sex, age group, history of TBI) were compared using the Pearson χ^2 test or Fisher's exact test. The Cochran-Armitage Test was used to assess for a linear trend in the association between age and frequency of rod-shaped microglia. Logistic regression was used to assess the variable of interest (age) and potential confounders (sex, dementia status, Braak neurofibrillary tangle (NFT) stage, and Consortium to Establish a Registry for Alzheimer's Disease [CERAD] neuritic plaque rating) and to estimate odds ratios (ORs) for the presence of rod-shaped microglia. Age groups were generated by a median split into 20–69 and 70 years or above for the UK series, and 70–89 and 90 years or above for the AI-ACT series. Availability of confounder variables differed in the UK and AI-ACT cohorts. For UK, only sex (coded male = 1, female = 2) was available for all the cases. For the AI-ACT series, additional variables were available: sex was coded as male = 1, female = 2, Braak NFT stage was coded as low (stage 0/I/II), moderate (III/IV), or high (V/VI); CERAD neuritic plaque rating was coded as none, sparse, moderate, or frequent; dementia status was coded as no dementia versus dementia (DSM-IV criteria). Multivariable regression models to adjust clinicopathological associations for potential confounding factors have been