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Microglia in dementia with Lewy bodies

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ABSTRACT

Microglial activation (neuroinflammation) is often cited as a pathogenic factor in the development of neurodegenerative diseases. However, there are significant caveats associated with the idea that inflammation directly causes either α-synuclein pathology or neurofibrillary degeneration (NFD). We have performed immunohistochemical studies on microglial cells in five cases of dementia with Lewy bodies (DLB), median age 87, and nine cases of non-demented (ND) controls, median age 74, using tissue samples from the temporal lobe and the superior frontal gyrus. Three different antibodies known to label microglia and macrophages were employed: iba1, anti-CD68, and anti-ferritin. All DLB cases showed both α-synuclein pathology (Lewy bodies and neurites) and NFD ranging from Braak stage II to IV. In contrast, all controls were devoid of α-synuclein pathology but did show NFD ranging from Braak stage I to III. Using iba1 labeling, our current results show a notable absence of activated microglia in all cases with the exception of two controls that showed small focal areas of microglial activation and macrophage formation. Both iba1 and ferritin antibodies revealed a mixture of ramified and dystrophic microglial cells throughout the regions examined, and there were no measurable differences in the prevalence of dystrophic microglial cells between DLB and controls. Double-labeling for α-synuclein and iba1-positive microglia showed that cortical Lewy bodies were surrounded by both ramified and dystrophic microglial cells. We found an increase in CD68 expression in DLB cases relative to controls. Since microglial dystrophy has been linked to NFD and since it did not appear to be worse in DLB cases over controls, our findings support the idea that the additional Lewy body pathology in DLB is not the result of intensified microglial dystrophy. CD68 is likely associated with lipofuscin deposits in microglial cells which may be increased in DLB cases because of impaired proteostasis. Overall, we conclude that neurodegenerative changes in DLB are unlikely to result directly from activated microglia but rather from dysfunctional ones.

1. Introduction

Although microglial activation, which nowadays is often referred to by the neologism *neuroinflammation*, has long been implicated in the development of neurodegeneration in Alzheimer's (AD) and Parkinson's disease (PD), there remain a number of substantial caveats regarding a proposed causal link between CNS inflammation and neurodegeneration (Graeber, 2014; Streit, 2010). A common assumption has been that activated microglia are cells out of control (Gao and Hong, 2008), i.e. they represent neurotoxic immune effector cells that are damaging neurons. Such uncontrolled microglial neurotoxicity has only been shown using *in vitro* models and its perceived significance for neurodegenerative diseases is extrapolated from these models. It is unclear what kinds of events in humans could trigger an out-of-control chronic neuroinflammatory reaction so severe that it can end up destroying the brain and why it went unnoticed for decades by neuropathologists; prior to 1994, neurodegenerative diseases were never considered to have an inflammatory etiology (Akiyama, 1994; Streit et al., 2004a). Inflammatory reactions typically develop in response to various types of injury, infection, neoplastic or autoimmune processes and there is no evidence of such triggering events occurring in either AD, PD, or dementia with Lewy bodies (DLB). It is also unclear why in some individuals neuroinflammation would cause primarily neurofibrillary degeneration (NFD) characteristic of AD, while in others it produces alpha-synuclein pathology typical of PD, and in yet another group,

Abbreviations: AD, Alzheimer's disease; CNS, central nervous system; DLB, dementia with Lewy bodies; LB, Lewy body; MHC, major histocompatibility complex; LN, Lewy neurites; ND, non-demented; NFD, neurofibrillary degeneration; PD, Parkinson's disease.

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Table 1
Patient data and neuropathology of cases studied.

Case	Diagnosis	Age	Sex	Region	α-Synuclein (LB & LN)	Braak stag	Iba1	CD68	Cause of death
1	ND	80	M	SFG	Negative	II	0	+	Congestive heart failure
				TL	Negative		0	+	
2	ND	95	F	SFG	Negative	III	0	+	Congestive heart failure
				TL	Negative		0	+	
3	ND	80	M	SFG	Negative	III	++	++	Pancreatic cancer
				TL	Negative		0	++	
4	ND	93	M	SFG	Negative	I	0	++	Natural causes
				TL	Negative		+	+	
5	ND	74	M	SFG	Negative	I	0	++	Pancreatic cancer
				TL	Negative		0	++	
6	ND	68	F	SFG	Negative	III	0	+	Colon cancer
				TL	Negative		0	+	
7	ND	61	M	SFG	Negative	I	0	+	Lung cancer
				TL	Negative		0	++	
8	ND	59	F	SFG	Negative	I	0	++	Adrenocortical cancer
				TL	Negative		0	+	
9	ND	58	F	SFG	Negative	I	0	+	Non-Hodgkin's lymphoma
				TL	Negative		0	+	
10	DLB	87	M	SFG	Positive	II	0	++	Cardiopulmonary arrest
				TL	Positive		0	++	
11	DLB	87	F	SFG	Negative	IV	0	++	Pancreatic cancer
				TL	Positive		0	+++	
12	DLB	74	M	SFG	Positive	II	0	+++	Renal cancer
				TL	Positive		0	+++	
13	DLB	96	F	SFG	Positive	III	0	++	End-stage Alzheimer
				TL	Positive		0	++	
14	DLB	89	M	SFG	Negative	III	0	++	Pneumonia
				TL	Positive		0	++	

Scoring of microglial responses (iba1): 0 ramified and/or dystrophic cells only; + activated (hypertrophic) cells and/or clustering; ++ mononuclear cell infiltration and/or rounded brain macrophages.
Scoring of CD68 staining intensity: + minimal staining; in isolated perivascular and/or microglial cells; ++ intermediate staining; in moderate numbers of microglial, perivascular, and/or infiltrating mononuclear cells; +++ intense staining; in numerous microglial, perivascular, and/or infiltrating mononuclear cells.

i.e. those with DLB, it may be responsible for both NFD and α-synuclein pathology. Furthermore, anti-inflammatory drugs do not slow the development or progression of neurodegeneration (Arvanitakis et al., 2008; Halliday et al., 2000; Martin et al., 2008), and activated microglia are absent around neurons undergoing neurodegenerative changes in human brain, including neurofibrillary tangles and Lewy bodies (Iseki et al., 2000; Rozemuller et al., 2000; Streit et al., 2009). Thus the suggestion that neuroinflammation causes neurodegeneration represents a real conundrum.

Although some authors have concluded that neuroinflammatory toxicity plays a pathogenic role in DLB (Mackenzie, 2000; Mrak and Griffin, 2007), we decided to take another look at the morphology and distribution of microglial cells in DLB brain using microglial markers not extensively studied specifically in the context of DLB. Our primary objective was to obtain further insights into the apparent conundrum above, and to this end we have utilized two well-known antibodies for microglia: one directed against the ionized calcium binding adaptor molecule 1 (iba1), and the other against macrosialin (anti-CD68), a lysosomal antigen found in macrophages. The iba1 antibody produces labeling of all microglial cells regardless of whether they are in an activated or non-activated state. It visualizes the cells' entire cytoplasm and therefore facilitates good assessment of the cells' activation state using morphological criteria, most characteristically the presence of cytoplasmic hypertrophy. The CD68 antigen on the other hand is a lysosome-associated, intracytoplasmic antigen expressed only in phagocytically active cells (Barrachina et al., 2006; Bauer et al., 1994; Dijkstra et al., 1985). Although it has been used in some studies to demonstrate presence of activated microglia, experimental studies in rats have shown that CD68 is not expressed by microglia that are activated but inactive phagocytes (Graeber et al., 1998), and thus its use for detecting activated microglia is limited to cells with CD68-positive lysosomes. Such lysosomes are associated with both auto- and heterophagocytosis (Barrachina et al., 2006; Brunk and Terman, 2002), and since expression of the CD68 antigen also increases with normal aging (Kullberg et al., 2001; Perry et al., 1993; Wong et al., 2005), it likely reflects aging-dependent lysosomal accumulations of lipofuscin within senescent microglial cells (Samorajski, 1976; Sekiryu et al., 2011; Streit and Xue, 2010; Xu et al., 2008). CD68 could therefore be a marker for either activated or senescent microglia depending on clinical and neuropathological circumstances, as well as on observer's bias and experience (Streit and Xue, 2009).

In addition to iba1 and CD68, we used an antibody against ferritin to further assess the status of microglial cells in DLB. Ferritin immunoreactivity has been associated largely with dystrophic microglia (Lopes et al., 2008; Simmons et al., 2007), which are abundant in the AD brain where they are preferentially co-localized with neurofibrillary tangles and neuritic plaques. Microglial dystrophy reflects senescent degeneration of these cells (Streit et al., 2004b) and we have hypothesized that such glial pathology may be linked to the development of neurofibrillary degeneration (Streit et al., 2009) in that neuronal degeneration occurs due to a loss of microglial support and neuroprotection. The spatial relationship of dystrophic microglia with Lewy bodies and Lewy neurites has not been explored and it is currently unknown if microglial dystrophy is more prevalent in brain areas marked by α-synuclein pathology. If that were the case, one might hypothesize that microglial degeneration/dysfunction may also be linked to development of α-synuclein pathology. Thus, a secondary objective of the current study was to investigate this possibility.

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