Journal of Neuroimmunology 312 (2017) 49–58

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Inflammatory responses in Multiple Sclerosis normal-appearing white matter and in non-immune mediated neurological conditions with wallerian axonal degeneration: A comparative study

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ARTICLE INFO

Keywords:
Multiple Sclerosis (MS)
Normal-appearing white matter (NAWM)
Microglia
Axon
Inflammation
VCAM-1
Sphingosine
S1P1
Wallerian degeneration

ABSTRACT

Inflammatory-like changes in the white matter (WM) are commonly observed in conditions of axonal degeneration by different etiologies. This study is a systematic comparison of the principal features of the inflammatory-like changes in the WM in different pathological conditions characterized by axonal damage/degeneration, focusing in particular on Multiple Sclerosis (MS) normal-appearing white matter (NAWM) compared to non immune-mediated disorders. The study was performed on sections of NAWM from 15 MS cases, 11 cases of non immune-mediated disorders with wallerian axonal degeneration (stroke, trauma, amyotrophic lateral sclerosis), 3 cases of viral encephalitis, 6 control cases.

Common features of the inflammatory-like changes observed in all of the conditions of WM pathology were diffuse endothelial expression of VCAM-1, microglial activation with expression of M2 markers, increased expression of sphingosine receptors. Inflammation in MS NAWM was characterized, compared to non immune-mediated conditions, by higher VCAM-1 expression, higher density of perivascular lymphocytes, focal perivascular inflammation with microglial expression of M1 markers, ongoing acute axonal damage correlating with VCAM-1 expression but not with microglia activation.

Inflammatory changes in MS NAWM share all the main features observed in the WM in non immune-mediated conditions with wallerian axonal degeneration (with differences to a large extent more quantitative than qualitative), but with superimposition of disease-specific perivascular inflammation and ongoing acute axonal damage.

1. Background

A complex relationship exists between axonal damage and inflammation in the CNS white matter (WM). Inflammation can induce axonal dysfunction and damage, through mechanisms well characterized in immune-mediated disorders, for example as observed in acute Multiple Sclerosis (MS) demyelinating lesions (Smith and McDonald, 2003). However, also axonal damage/degeneration due to any other pathological condition determines secondary inflammatory-like changes in the WM, through yet not fully known damage signals (Hussain et al., 2014; Russo and McGavern, 2015; Burda and Sofroniew, 2014; Amor et al., 2014). These inflammatory changes can be observed in experimental models of CNS lesions and can be found in the WM in any non-immune mediated neurological disorder with wallerian axonal degeneration (Griffin et al., 1992; Raivich et al., 1998; Graves et al., 2004; Griffiths et al., 2010; Palin et al., 2008). They are known to differ both quantitatively and qualitatively from "real" brain inflammation that is observed in immune-mediated or infectious neurological disorders (Estes and Mc Allister, 2014; Graeber, 2014). However, systematic studies evaluating differences and similarities between different conditions of WM pathology are lacking.

Widespread inflammatory changes in the normal-appearing white matter (NAWM), distant from focal demyelinating lesions, are a distinctive feature of MS (Kutzelnigg et al., 2005; Filippi and Rocca, 2005; Zeis et al., 2008; Frischer et al., 2009). Several studies have described the pathological changes in MS NAWM, but little data is available on the specificity of such findings and on comparisons with other conditions of WM pathology from other causes. Widespread wallerian axonal degeneration is known to occur in MS NAWM (Evangelou et al., 2000; Casanova et al., 2003; Dziedzic et al., 2010).

The aim of this study was to perform a systematic comparison of the principal features of the inflammatory-like changes observed in the WM, in several pathological conditions characterized by axonal damage/

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http://dx.doi.org/10.1016/j.jneuroim.2017.09.004
Received 13 July 2017; Received in revised form 28 August 2017; Accepted 6 September 2017
0165-5728/

Table 1
Results summary for immunohistochemistry.

	MS NAWM	WM in non immune-mediated neurological conditions with axonal degeneration	Control WM	Viral encephalitis WM
HLA-DR	56.85/mm^2 ± 32.93	41.70/mm^2 ± 22.79	0 (some intravascular leukocytes)	> 800/mm^2
VCAM-1 + vessels	68.62% ± 8.63**	39.77% ± 13.16**	0	> 90%
CD3 (T-lymphocytes)	2.54/mm^2 ± 0.76**	0.29/mm^2 ± 0.39**	0	> 200/mm^2
CD20 (B-lymphocytes)	0.33/mm^2 ± 0.20	0	0	> 50/mm^2
CD138 (plasma cells)	Very rare	0	0	+
CD83 (dendritic cells)	+	−	−	+ +
CD163	25.16/mm^2 ± 26.44*	30.40/mm^2 ± 28.86*	5.41/mm^2 ± 6.44*	> 500/mm^2
Mannose receptor	+ (perivascular macrophages)	+ (perivascular macrophages)	+ (perivascular macrophages)	+ + (perivascular macrophages)
S1P1	+ +	+ +	+/−	+ + +
S1P3	+/−	+/−	−	+ +
iNOS	+/−	−	−	+ + +
MMP-9	+/−	−	−	+ +
CD86	+/−	−	−	+ +
CD40	+/−	−	−	+ +
IgG	−	−	−	+ + +
Fibrinogen	−	−	−	+ + +

** Difference MS NAWM vs WM in non immune-mediated neurological conditions with axonal degeneration, $p < 0.0001$.
* Difference MS NAWM vs WM in non immune-mediated neurological conditions with axonal degeneration vs control WN, $p < 0.0001$.

degeneration. Another aim was also to add to current data the notion of what is actually specific to MS NAWM and what are instead more aspecific features of WM undergoing axonal damage.

The NAWM in MS was compared with the WM in non immune-mediated neurological conditions with axonal degeneration (NINCs) (such as wallerian degeneration in stroke or traumatic brain injury, or neurodegeneration of corticospinal tracts in ALS), and with the WM in infectious CNS disorders (with inflammation as an appropriate response to CNS infection, such as in viral encephalitis). We specifically addressed microglial activation, markers of M1/M2 macrophage differentiation, expression of vascular adhesion molecules, markers of lymphocytes and dendritic cells, expression of effectors of tissue damage, expression of sphingosine receptor and markers of acute axonal damage.

2. Materials and methods

This study was performed on autoptic formalin-fixed, paraffin-embedded brains, assessing the following pathological conditions:

- MS NAWM, distant from focal demyelinating lesions (14 MS cases; 6 relapsing-remitting (RR) MS, 7 secondary progressive (SP) MS, 1 hyperacute MS)
- pure wallerian degeneration (pontine and spinal corticospinal tracts degeneration after a middle cerebral artery ischemic stroke: 1 case)
- wallerian degeneration and focal axonal damage (diffuse WM injury after brain trauma resulting in vegetative state: 4 cases)
- neurodegenerative disorders with axonal degeneration (corticospinal tracts degeneration in amyotrophic lateral sclerosis (ALS): 6 cases)
- infectious CNS disorders, with WM inflammation as an appropriate response to CNS infection (HSV-1 viral encephalitis in immunocompetent host: 3 cases).

Samples from 6 brains of patients without neurological disorders were used as controls.

For each case, tissue blocks were selected including the corticospinal tracts in the cervical spinal cord and in the pons; the lateral portion of the temporal lobe was selected for the viral encephalitis cases. In MS brains only NAWM areas were selected, defined by absence of demyelination (normal Luxol staining and MBP immunostaining), at least 1 cm distant from focal demyelinating lesions.

WM areas in diffuse traumatic brain injury were selected excluding areas of focal necrosis or haemorrage. In viral encephalitis cases, temporal lobe subcortical WM was selected excluding areas of focal necrosis or haemorrage.

Tissue was obtained from the archives of the University of Turin and the University of Genoa. Post-mortem interval was < 36 h in all cases and controls.

Mean age of death in MS cases was 50.2 years (range 27–66 years); mean duration of disease course was 15.1 years (range 6 months–30 years). Mean age of death was 59.4 years (range 52–65 years) in control cases, 57.2 years (range 42–72 years) in the cases of non immune-mediated neurological conditions with axonal degeneration. Disease course in MS cases (RR or SP MS) was retrospectively defined from hospital records. The clinical features of all cases are summarized in Supplementary Table 1.

2.1. Histology and immunohistochemistry

Consecutive 5 μm sections were obtained. Standard hematoxylin/eosin, Luxol and Bodian stainings were performed for each section. Immunohistochemistry was performed with the antibodies listed in Supplementary Table 2 (HLA-DR, CD68, CD163, mannose receptor, iNOS, MMP-9, MPO, CD40, CD3, CD20, CD138, CD83, CD86, fibrinogen, IgG, VCAM-1, ICAM-1, S1P1, S1P3, APP, MBP). After deparaffinization, sections were treated with 3% H_2O_2 for 10 min and then processed for antigen retrieval; details on antigen retrieval are listed in Supplementary Table 2. The sections were incubated with 10% normal serum for 30 min; they were later incubated overnight with the primary antibodies. After washing with TBS, the sections were incubated at room temperature for 30 min with the Envision complex (Dako, Glostrup, Denmark). Peroxidase labeling was visualized with 10% 3,3-diaminobenzidine. Sections were counterstained with hematoxylin. Double immunohistochemistry was performed as needed to confirm colocalization of antigens. Immunohistochemistry was performed as described above. First, peroxidase labeling for the first antibody was visualized with 10% 3,3-diaminobenzidine, then immunohistochemistry for the second antibody was performed after blocking with normal serum, and peroxidase labeling was visualized with VIP red (Vector Laboratories, Burlingame, USA).

2.2. Image acquisition

The sections were examined using either a Zeiss Axiophot