ORIGINAL ARTICLE

Association of α-Synuclein Immunoreactivity With Inflammatory Activity in Multiple Sclerosis Lesions

Jian-Qiang Lu, MD, PhD, Yan Fan, MD, Alim P. Mitha, MD, Robert Bell, MD, Luanne Metz, MD, G. R. Wayne Moore, MD, and V. Wee Yong, PhD

Abstract

Multiple sclerosis (MS) has neurodegenerative features including neuronal and axonal loss and widespread atrophy of the brain and spinal cord. The cause of this neurodegeneration has been largely attributed to inflammation, but other mechanisms, including those associated with classic neurodegenerative diseases such as the α-synucleinopathies, might also be involved in MS pathogenesis. In this study, 96 brain lesions containing varying degrees of inflammatory activity and demyelinating activity from 12 autopsied MS cases were compared with corresponding regions from 6 neuropathologically normal controls; 2 cerebral biopsy lesions from an MS patient were also studied. We found α-synuclein immunoreactivity in the cytoplasm of cells in MS lesions with inflammatory activity but not in control samples. α-Synuclein–immunoreactive cells were identified in active (15/15 lesions in the brainstem, 9/13 in cerebral hemispheres) and chronic active (14/15 in the brainstem, 12/22 in cerebral hemispheres) lesions but were absent in chronic inactive lesions (0/31); the greater immunoreactivity in brainstem compared with cerebral hemisphere lesions was significant (p < 0.05). Double-immunofluorescence staining revealed localization of α-synuclein immunoreactivity mostly in neurons, microglia/macrophages, and oligodendrocytes, and only rarely in astrocytes. The results suggest that α-synuclein expression regulated by inflammatory signals may contribute to neurodegenerative processes in MS lesions.

Key Words: α-Synuclein, Immunoreactivity, Inflammation, Multiple sclerosis, Neurodegeneration.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease characterized by the formation of multifocal demyelinated lesions in the white and gray matter of the CNS (1). Multiple sclerosis–affected CNS tissues also show substantial neuronal and axonal loss (2, 3) that contributes to progressive atrophy of the cerebral cortex (1, 4), spinal cord (5, 6), subcortical white matter (1, 4), thalamus (3, 7), and caudate nucleus (8). Axonal injury and loss occur early (9, 10), including in clinically isolated syndromes, and continue over the course of the disease. This neurodegeneration likely contributes to progressive clinical disability in MS patients (3, 11).

Although inflammatory activity is an important component of the pathology of MS (12–14), other mechanisms may also contribute to its pathogenesis (15, 16). In particular, mechanisms involved in neurodegenerative diseases such as α-synucleinopathies may be relevant. α-Synuclein is a neuronal cytoplasmic protein localized predominantly to presynaptic terminals with little expression in cell bodies and dendrites (17). The physiological function of α-synuclein is unclear, but recent studies suggest that it may be a chaperone protein important for enhancement of synaptic activity and integrity (18, 19). Abnormal aggregation of α-synuclein in neurons or glia has been linked to neurodegeneration in Parkinson disease and other synucleinopathies (19). Moreover, the overexpression of α-synuclein in cultured dopaminergic neurons induces their apoptosis (20, 21). The long-term overexpression of human α-synuclein in the marmoset midbrain causes loss of dopaminergic neurons and oligodendrocytes (22). The increased expression of α-synuclein in these studies was diffuse and granular in the cytoplasm of cells and, therefore, unlike the intracellular inclusions seen in classic α-synucleinopathies (19–22). This may imply that diffuse overexpression rather than aggregation as intracellular inclusions of α-synuclein is sufficient to cause cell death and thus contribute to the neurodegenerative process.

A recent study reported that in an animal model of MS, experimental autoimmune encephalomyelitis, α-synuclein was substantially upregulated in neurons and glia of the spinal cord, particularly during exacerbations (23). The investigators conducted a preliminary analysis of MS tissues and reported that the cerebral lesions of 4 patients with secondary progressive MS also showed α-synuclein immunoreactivity. In view of the importance of identifying potential mediators of neurodegeneration in MS, we examined the expression of α-synuclein in 3 types of MS lesions with varying degrees of inflammatory and demyelinating activity. The results facilitate testing of the hypothesis that an initial expression of a toxic factor within neurons in MS leads to...
their demise, which then may initiate neuroinflammation in MS.

MATERIALS AND METHODS

Subjects

This study was approved by local Research Ethics Boards and performed on archival autopsy tissues from 12 MS and 6 control cases that were obtained from the Departments of Pathology of the University of Calgary and University of British Columbia. In addition, we studied an MS case with 2 biopsy specimens obtained at the same surgery from different lesions. We do not know the type of MS in 3 of the patients because the consent to review their clinical charts had not been obtained before their death (Table 1).

The diagnosis of MS was confirmed histopathologically by a neuropathologist. One patient (No. 12) had no clinical documentation of MS but met the histopathological criteria for a diagnosis of MS (24). The patient with biopsies was diagnosed as having MS based on histopathological findings and follow-up of subsequent clinical

TABLE 1. Characteristics of MS Patients and Lesions Studied

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age, years</th>
<th>Sex</th>
<th>Type</th>
<th>Cause of Death</th>
<th>No. Lesions Studied</th>
<th>Location of Lesions Studied</th>
</tr>
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<tr>
<td>1</td>
<td>32</td>
<td>F</td>
<td>Unknown*</td>
<td>Unknown</td>
<td>15</td>
<td>Subcortical and periventricular WM, BG, brainstem</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
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</tr>
<tr>
<td>3</td>
<td>35</td>
<td>F</td>
<td>RRMS</td>
<td>Cor pulmonale</td>
<td>13</td>
<td>Subcortical and periventricular WM, brainstem</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>F</td>
<td>RRMS</td>
<td>Pneumonia</td>
<td>2</td>
<td>Subcortical WM, BG, brainstem</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>M</td>
<td>RRMS</td>
<td>Liver failure</td>
<td>6</td>
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</tr>
<tr>
<td>6</td>
<td>60</td>
<td>M</td>
<td>RRMS</td>
<td>Cerebral hemorrhage</td>
<td>13</td>
<td>Subcortical and periventricular WM, brainstem</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>F</td>
<td>RRMS</td>
<td>Pneumonia</td>
<td>14</td>
<td>Subcortical WM, BG, brainstem</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>M</td>
<td>RRMS</td>
<td>Unknown</td>
<td>1</td>
<td>Periventricular WM</td>
</tr>
<tr>
<td>9</td>
<td>76</td>
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</tr>
<tr>
<td>10</td>
<td>76</td>
<td>M</td>
<td>PPMS</td>
<td>Pulmonary emboli</td>
<td>7</td>
<td>Subcortical WM, periventricular WM, internal capsule, brainstem</td>
</tr>
<tr>
<td>11</td>
<td>34</td>
<td>F</td>
<td>RRMS</td>
<td>N/A‡</td>
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<td>Occipital cortex and subcortical WM</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>F</td>
<td>Unknown†</td>
<td>Pulmonary emboli</td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>38</td>
<td>F</td>
<td>RRMS</td>
<td>N/A‡</td>
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<td></td>
</tr>
<tr>
<td>14-19</td>
<td>36-60</td>
<td>M2/F4</td>
<td>None</td>
<td>None</td>
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<td></td>
</tr>
</tbody>
</table>

*Consent to review clinical charts unavailable.
†No documentation of MS in the hospital chart but diagnosed by neuropathological examination.
‡Biopsied patient.

BG, basal ganglia; F, female; M, male; MS, multiple sclerosis; PPMS, primary progressive MS; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; WM, white matter.

FIGURE 1. α-Synuclein immunoreactivity is detected in cells in active multiple sclerosis lesions. An active lesion in the basis pontis of Patient 9 (Luxol fast blue [LFB]/hematoxylin and eosin; original magnification: [A] 200×; rectangles outline the areas sampled in adjacent sections in [insets B and C] shows decreased intensity of LFB staining in the lesion. α-Synuclein–immunoreactive cells are numerous in the lesion core (original magnification: [B] 400×) and edge (original magnification: [C] 400×). The activity of the lesion core in [A] is illustrated by the presence of numerous CD68-positive macrophages (original magnification: [D] 400×), CD3-positive T lymphocytes (original magnification: [E] 400×) and scattered amyloid precursor protein axonal immunostaining (original magnification: [F] 400×).
manifestations and magnetic resonance imaging scans over a year. The MS lesions are histopathologically classified using a modification of the Bo¨/Trapp staging system (2, 24). None of the MS patients were found to have concomitant neurodegenerative or CNS inflammatory disorders. Corresponding regions of 6 control subjects were also examined for comparison. The control subjects were free of neurological disease, and their CNS tissues were normal on neuropathological examination. The clinical data for the MS patients (mean age, 50.0 ± 17.5 years) and control subjects (mean age, 47.0 ± 16.7 years) are summarized in Table 1.

An additional patient’s brain samples (n = 7) with cerebral infarct-induced Wallerian degeneration (CI-WD) were studied. The patient’s samples were selected based on the following criteria: the presence of pathologically confirmed cerebral infarcts with resultant WD of the corticospinal tracts and exclusion of comorbid disorders, as for the MS group; 5 neuropathologically normal patient samples were used as controls for this comparison.

Neuropathology and Immunohistochemistry

The postmortem delay was less than 24 hours in all the cases. All brain tissues were fixed in 10% formalin for at least 2 weeks. Neuropathological examination was performed on 1-cm-thick slices of both cerebral hemispheres and 0.5-cm-thick slices of the brainstem and cerebrum. The tissue blocks containing MS lesions and control areas were sampled from various brain regions and were embedded in paraffin and sectioned for histological and immunohistochemical examination.

![Image of brain samples](https://example.com/image)

**FIGURE 2.** α-Synuclein is present in chronic active multiple sclerosis (MS) lesions, particularly at the edge. A lesion in the subcortical white matter of Patient 11 (with primary progressive MS) exhibits a hypocellular core with diminishing Luxol fast blue (LFB) stain (left upper, original magnification: [A] 200×) and an inflammatory edge with scattered CD68-positive macrophages (original magnification: [B] 200×) and CD3-positive T lymphocytes (original magnification: [C] 400×). α-Synuclein-immunoreactive cells are scattered along the edge (original magnification: [D] 400×, with a higher magnification in the inset) and only occasionally in the core (original magnification: [E] and [F] 2,400×). The midbrain of Patient 5 (with relapsing-remitting MS) (Luxol fast blue [LFB]/hematoxylin and eosin; original magnification: [G] 200×, with a higher magnification in the inset) shows decreased intensity of LFB staining in the lesion. ([H] demonstrates frequent CD68-positive macrophages at the edge of the lesion (CD68 immunostaining; original magnification: 200×, with a higher magnification in the inset). A corresponding area at the edge of lesion shows frequent α-synuclein profiles (original magnification: [I] 200×, with a higher magnification in the inset).

| TABLE 2. Frequency of Cellular Immunoreactivity for α-Synuclein in Multiple Sclerosis Lesions |
|-----------------------------------------------|----------------|----------------|----------------|----------------|---------------|---------------|
| Active | Chronic Active | Chronic Inactive | |
| | Core | Edge | Core | Edge | Core | Edge |
| +++ | 5 | 7 | 1 | 9 | 0 | 0 |
| ++ | 7 | 10 | 4 | 9 | 0 | 0 |
| + | 9 | 7 | 7 | 8 | 0 | 0 |
| -/+ | 0 | 1 | 5 | 1 | 0 | 4 |
| - | 7 | 3 | 20 | 10 | 31 | 27 |
| Total lesions sampled | 28 | 28 | 37 | 37 | 31 | 31 |

*Edge is defined as the area within 0.2 cm (1 medium-power field inward and outward) along the boundary of distinct Luxol fast blue staining; core is defined as the central area of the multiple sclerosis lesion inside the edge. α-Synuclein immunoreactivity is semiquantitatively assessed as follows: –, none; –/+, 1 to 3; +, 4 to 9; ++, 10 to 19; ++++, 20 or more positive cells per high-power field (original magnification: 200×).*

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paraffin. The samples were cut into 6-μm-thick tissue sections, deparaffinized, and stained with hematoxylin and eosin and Luxol fast blue (LFB) stains to identify lesions. The nature of the plaques was morphologically assessed and confirmed by immunohistochemistry. For the latter, deparaffinized sections were first subjected to antigen retrieval by boiling in sodium citrate (pH 6.5) for 10 minutes. After leaving sections at room temperature for 20 minutes, endogenous peroxidase activity was eliminated by incubating the sections for 10 minutes in 3% H₂O₂ in methanol. Triton X-100 (0.25%) was then added for 15 minutes; sections were blocked for nonspecific binding, and the primary antibody was applied overnight.

Primary antibodies consisted of mouse immunoglobulin to α-synuclein (used at 1:100 dilution, from Zymed Laboratories, San Francisco, CA, Catalog No. 18-0215), rabbit polyclonal α-synuclein (1:1000, Chemicon, Pittsburgh, PA, Catalog No. AB5038p), mouse anti-NeuN to detect neurons (1:100, Chemicon, Catalog No. MAB377X), mouse anti-human CD68 (KP-1) to identify macrophages (Ventana, Tucson, AZ, Catalog No. 790-2931), rabbit anti-Iba1 to identify macrophage/microglia (1:500, WAKO, Richmond, VA, Catalog No. 019-19741), mouse anti-CD3ζ to identify T cells (Santa Cruz Biotechnology, Santa Cruz, CA, Catalog No. SC-1239), rabbit anti-glial fibrillary acidic protein to identify astrocytes (1:500, DAKO, Glostrup, Denmark, Catalog No. Zo334), rabbit anti-Nogo-A to identify oligodendrocytes (25) (1:100, Chemicon, Catalog No. AS664P), and mouse anti-β amyloid precursor protein ([β-APP] 1:150, Calbiochem, San Diego, CA, Catalog No. 171537). For subsequent development using diaminobenzidine, the secondary antibody was biotinylated anti-rabbit or mouse immunoglobulin G, directed against the species in which the primary antibody was raised, and staining was visualized with ABC kit (Vector Laboratories, Burlingame, CA) using diaminobenzidine as the substrate.

Double-immunofluorescence analyses were used for localization of α-synuclein immunostaining in particular cell types. After the tissue processing steps previously mentioned, the α-synuclein antibody raised in rabbit or mouse was applied, followed by goat anti-rabbit Cy3 (Jackson Immuno Research, West Grove, PA, 1:300, Catalog No. 111-045) or goat anti-mouse Alexa 488 (Invitrogen, Carlsbad, CA, 1:300, Catalog No. A11008), respectively.

FIGURE 3. α-Synuclein in multiple sclerosis (MS) lesions is associated with widespread neurodegeneration. The occipital lobe (Luxol fast blue [LFB]/hematoxylin and eosin [H&E]; original magnification: [A] 1×, with rectangles indicating the areas of [D], [E], and [F] in adjacent sections) of Patient 1 exhibits an active lesion (arrowhead) and chronic active lesion (arrow), and widespread neurodegeneration with atrophic cortex, sulcal enlargement, and thinning of the gyri and subcortical WM (Vent, lateral ventricle). The chronic active lesion contains numerous CD68-positive macrophages (original magnification: [B] 400×) and scattered intraparenchymal CD3-positive T lymphocytes (original magnification: [C] 400×) at the edge. α-Synuclein-immunoreactive cells are present at the edge (original magnification: [D] 400×) and core (original magnifications: [E] and [F] 400×) of this chronic active lesion. An active lesion in the basal ganglia shows numerous macrophages (LFB/H&E; original magnification: [G] 400×); neuron-like cells are immunoreactive for α-synuclein (original magnification: [H] 800×) at the edge; and macrophage-like cells contain α-synuclein-immunoreactive aggregates (original magnification: [I] 800×) in the core.
was then followed by the primary antibody to cell-type specific marker previously described for diaminobenzidine visualization, ensuring that this primary antibody was raised in a different species from that of the α-synuclein antibody. The appropriate secondary antibody was then applied, and sections were analyzed using fluorescence microscopy.

We characterized the nature of plaques by defining active lesions as actively demyelinating, with inflammatory activity throughout the lesion, and containing CD3-immunoreactive T cells, CD68-immunoreactive macrophages, and acutely damaged axons immunoreactive for APP. Chronic active lesions had hypocellular centers but were hypercellular along the edges that contained inflammatory activity. Chronic inactive lesions were defined as having no evidence of ongoing demyelination, although they contained infrequent CD68-immunoreactive macrophages.

**Semiquantitative Analysis of α-Synuclein Immunostaining**

The α-synuclein immunostaining was assessed semiquantitatively by counting the number of α-synuclein-immunoreactive cells per high power field (200×). Scores were assigned as “−” if there were no positive cells, “−/+” if there were 1 to 3 positive cells, “+/+” for 4 to 9 positive cells, “+++” for 10 to 19 cells; and “++++” for 20 or more positive cells per high power field. Positive cells were defined as those with visible diffuse or granular immunoreactivity within the cytoplasm.

Because the edge and core of each lesion had different inflammatory activity especially in the chronic active lesions, these areas were separately assessed. The edge was defined as the area within 0.2 cm (1 medium-power field inward and outward) along the boundary of distinct LFB staining, and core was the central area of the MS lesions inside the edge.

**Statistical Analysis**

The Fisher exact test was used to assess the difference in frequency between groups of lesional categories and of brain regions. Values of $p < 0.05$ were regarded as significant.

**RESULTS**

**Identification of MS Lesions**

The clinical features and location of lesions analyzed for each patient are shown in Table 1. Ninety-six lesions from the autopsied brains of 12 MS patients (mean age, 50.0 ± 17.5 years) were identified based on the presence of discrete focal regions of decreased intensity of LFB staining for myelin (Fig. 1A) and extensive analysis of immunostains including CD68 for macrophages, CD3 for T lymphocytes, and β-APP labeling for acute axonal damage (Figs. 1D–F). These lesions were distributed in various brain regions (Table 1). Two biopsied lesions from Patient 13 were assessed separately for comparison.

**Immunoreactivity for α-Synuclein in Cells Within and Surrounding MS Lesions**

α-Synuclein immunoreactivity was observed in diffuse or granular patterns in the cytoplasm of cells within the cores and along the edge of MS lesions. The patterns were similar and within the same cell types whether the mouse monoclonal or the rabbit polyclonal antibody to α-synuclein

![Image](https://academic.oup.com/jnen/article-abstract/68/2/179/2917112)
was used. α-Synuclein–immunoreactive cells were morphologically most suggestive of macrophages and neurons, although other cell types could not be excluded.

In active MS lesions as identified by LFB–hematoxylin and eosin stain (Fig. 1A), α-synuclein immunoreactivity was present in most of the cores (21/28; Fig. 1B) and edges (24/28; Fig. 1C); all active lesions contained numerous CD68-positive macrophages (B), CD3-positive T cells (C), and acutely damaged axons (D, amyloid precursor protein staining) in the lesion. α-Synuclein–positive cells and profiles are present in the lesion core (E) and edge (F). Some α-synuclein–immunoreactive structures without apparent nuclei may be axonal spheroids (original magnification: [A–D] 200×; [E and F] 400×).

Of the chronic active lesions (Fig. 2), α-synuclein immunoreactivity was observed in 12 of 37 of the cores and 26 of 37 of the edges (Table 2). The difference in detection in the cores and edges is significant (p < 0.01). The chronic active lesion in a case with primary progressive MS (Figs. 2A–F) contained α-synuclein–immunoreactive cells in a pattern similar to that in chronic active lesions from patients with relapsing-remitting MS (Figs. 2G–I). In atrophic brains with neurodegenerative features (Patients 1 and 2), the lesions showed α-synuclein immunoreactivity patterns similar to those in nonatrophic brains (Fig. 3). α-Synuclein immunoreactivity was absent in all 31 chronic inactive lesions (Figs. 4A–C).

The semiquantitative assessment for the extent of α-synuclein immunoreactivity across the different lesional activities in autopsied specimens is summarized in Table 2.

### TABLE 3. Cellular Immunoreactivity for α-Synuclein in Multiple Sclerosis Lesions

<table>
<thead>
<tr>
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<th>Active</th>
<th>Chronic Active</th>
<th>Chronic</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Core</td>
<td>Edge</td>
<td>Overall</td>
</tr>
<tr>
<td>Cerebral hemispheres</td>
<td>7/13</td>
<td>9/13</td>
<td>9/13</td>
</tr>
<tr>
<td>Brainstem</td>
<td>14/15*</td>
<td>15/15*</td>
<td>15/15*</td>
</tr>
</tbody>
</table>

*Edge is defined as the area within 0.2 cm (1 medium-power field inward and outward of the lesion) along the boundary of distinct Luxol fast blue staining; core is defined as the central area of multiple sclerosis lesions inside the edge.*

*The numerator represents the number of the lesions with positive (+ to ++++) cells; the denominator represents the number of lesions studied.*

*Statistical significance is expressed as *, p < 0.05 using the Fisher exact test, compared with the cerebral hemispheres.*

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Overall, for the autopsy cases, α-synuclein immunoreactivity was more frequently identified in the cores of active lesions (21/28) than chronic active (12/37; p < 0.001) ones, and was absent in chronic inactive lesions (Tables 2 and 3) and in specimens of control brains (data not shown). α-Synuclein immunoreactivity along the edges of active lesions (24/28) was slightly more frequent in chronic active lesions (26/37; no statistical significance).

In 2 MS biopsy samples from the junctions of gray and white matter of the occipital lobe in a patient during a relapse of MS (Patient No. 13 in Table 1), there were immunoreactivity patterns similar to those in active lesions from autopsies (Fig. 5). These included numerous CD68-positive macrophages, focally frequent CD3-positive T lymphocytes, acutely damaged axons immunoreactive for APP, and scattered α-synuclein immunoreactivity in the lesion core (Fig. 5E) and edges (Fig. 5F).

Preferential Expression of α-Synuclein in the Brainstem

α-Synuclein immunoreactivity was seen in 9 of 13 active lesions within the cerebral hemispheres and 15 of 15 within the brainstem (p < 0.05, compared with the cerebral hemispheres) (Table 3). α-Synuclein was detected in 12 of 22 chronic active lesions within the cerebral hemispheres and 14 of 15 within the brainstem (p = 0.01, compared with the cerebral hemispheres), suggesting preferential expression in brainstem lesions.

Identification of α-Synuclein–Immunoreactive Cells Using Double-Immunofluorescence Microscopy

Using antibodies directed against cell-type specific markers, we found that α-synuclein–immunoreactive cells

![Figure 6](https://example.com/figure6.png)
colocalized with some NeuN-labeled neurons (Figs. 6A–C), CD68-positive macrophages (Figs. 6D–F), Nogo-A–positive oligodendrocytes (Figs. 6J–L), and, where some of the cells had the morphology of ramified microglia, with Iba1-labeled macrophages/microglia (Figs. 6G–I). Notably, α-synuclein was rarely present in glial fibrillary acidic protein (GFAP)-positive astrocytes (Figs. 6M–O). Thus, α-synuclein immunoreactivity is detected in multiple cell types other than astrocytes within MS and/or along MS lesion edges.

FIGURE 7. α-Synuclein immunoreactivity in cerebral infarct-induced Wallerian degeneration. Wallerian degeneration of the corticospinal tracts in the basis pedunculi of the midbrain (A–C) and in the medullary pyramid (D, E) is demonstrated by a focally decreased density of Luxol Fast blue stain (original magnification: [A] 1×; the rectangle outlines the area shown [B and C]). There are numerous associated CD68-immunoreactive macrophages ([B] in the basis pedunculi; [D] in the medullary pyramid) and frequent α-synuclein-immunoreactive cells ([C] in the basis pedunculi; [E] in the medullary pyramid). Original magnification: (B–E) 100×; insets show higher magnifications.

α-Synuclein Immunoreactivity in Non-MS Degenerative and Inflammatory Lesions

To compare the α-synuclein immunoreactivity results with those in another degenerative and inflammatory process, samples from patients with CI-WD of the corticospinal tracts were examined (Fig. 7A). This analysis revealed the presence of numerous CD68-immunoreactive macrophages (Figs. 7B, D) and α-synuclein–immunoreactive cells (Figs. 7C, E) in the basis pedunculi of the midbrain and in the medullary pyramid. Table 4 summarizes the clinical characteristics of the 7 CI-WD patients (mean age, 65.6 ± 9.3 years) and 5 control subjects (mean age, 70.4 ± 8.0 years; p > 0.01 by Student t-test vs CI-WD patients) and corresponding α-synuclein immunoreactivity in the basis pontis and medullary pyramid samples in this analysis.

DISCUSSION

In addition to its traditional definition as an inflammatory/demyelinating disease, MS is now widely recognized to have neurodegenerative pathological features including loss of axons and neurons (1–3, 5, 6, 11, 26). Because the markers of axonal damage and activated inflammatory cells are colocalized in MS lesions, these neurodegenerative changes have been attributed mostly to inflammatory mechanisms (2, 12, 14, 27, 28). Moreover, inflammatory cells including macrophages and T cells can produce molecules that may injure neurons and axons in vitro (29–31).

Other potential causes of neurodegeneration in MS, including ionic disturbances, energy failure, glutamate excitotoxicity, and free radical generation have also been proposed (12, 15, 16). Although these mediators of injury may act independently to produce neurodegenerative changes, it is difficult to separate them from inflammatory components of MS lesions; they might mediate injury before, concordant with, or as a result of inflammation.

We have explored other possible causes of the neurodegenerative changes in MS, and we have been guided by findings in α-synucleinopathies. The present results indicate diffuse α-synuclein immunoreactivity in the cytoplasm of neurons, oligodendrocytes, and microglia/macrophages within the core and at the edge of plaques with inflammatory demyelinating activity. These results extend the findings of a previous study that focused on α-synuclein expression in experimental autoimmune encephalomyelitis (23), but add preliminary observations of α-synuclein in neurons and glial-like cells in MS lesions. Therefore, we provide novel data indicating that α-synuclein is commonly observed in active lesions, less so in chronic active lesions, and that it is not detected in chronic inactive MS lesions or in specimens from normal controls. In addition, we demonstrate the more frequent expression of α-synuclein in MS lesions in the brainstem than in the cerebral hemispheres and suggest some potential cellular sources of α-synuclein. Taken together, the present results suggest a role for α-synuclein in neurodegenerative processes in MS.

In addition to α-synuclein, a recent report on the presence of abnormally phosphorylated tau in the brains of patients who died with secondary progressive MS highlights...
the possibility that multiple proteins that are associated with classical neurodegenerative diseases may be implicated in MS pathogenesis (32). The precise functions of α-synuclein remain uncertain, but 1 potential role seems to be as a chaperone protein in the presynaptic terminal of neurons that enhances synaptic activity and integrity (18) and synaptic vesicle recycling (33). It has been shown that mutant α-synuclein and abnormal accumulation of α-synuclein result in neurodegeneration in α-synucleinopathies (19, 23, 34). In these conditions, α-synuclein deposits as aggregates such as in Lewy bodies found in Parkinson disease. Diffuse expression of α-synuclein in the neuronal cytoplasm has also been shown to induce apoptosis of neurons in vitro (20, 21, 35) and to cause neurodegeneration in rodents (36) and nonhuman primates (22). The α-synuclein expression in cells in our study tended to be diffusely spread across the cytoplasm (Figs. 1–5) rather than as prominent aggregates.

The mechanisms by which α-synuclein might damage neurons are unclear, but oxidative stress has been implicated (23, 37, 38); there is potentially a positive feedback loop because oxidative stress has also been found to cause an increase in α-synuclein levels (37). Oxidative stress has been suggested to play a pathogenic role in α-synucleinopathies (19, 38, 39) as well as in MS (40). Thus, the abnormal α-synuclein accumulation in neuronal cytoplasm might relate to increased oxidative stress and contribute to neuronal death in MS. This mechanism might also occur in oligodendrocytes and contribute to their loss in MS. We are uncertain as to the significance of α-synuclein immunoreactivity in microglia and macrophages.

This analysis of autopsied specimens makes it difficult to determine whether α-synuclein expression is an early or late occurrence in MS lesions. We think that the α-synuclein immunoreactivity demonstrated is likely secondary to inflammation because its expression in macrophages in tissue culture studies is increased by stimulation with interleukin-1β (41) and because the injection of inflammagen into the substantial nigra is associated with accumulation of insoluble α-synuclein, subsequently causing dopaminergic neuronal death in α-synuclein–genetically engineered mice (42). An association of α-synuclein with inflammation is suggested in the present study because α-synuclein was more frequently detected in active than in chronic active lesions, its increased frequency at the edges of chronic active lesions compared with cores, and its absence in chronic inactive lesions. Whatever its origin, however, the presence of α-synuclein suggests involvement in the neurodegenerative processes occurring in MS lesions.

The neurodegeneration in MS is also partially attributed to WD secondary to axonal destruction in plaques (1). Wallerian degeneration leads to dysfunction of mitochondria, degradation of neurofilaments and their axons, with production of neural fragments that can be removed by macrophages (43, 44). Wallerian degeneration may be regarded in part as the inflammatory response of the nervous system to axonal injury, primarily attributable to the production of cytokines, the mediator molecules of inflammation (45). In the present study, we also found α-synuclein immunoreactivity within the CI-WD in the brainstem, indicating that α-synuclein could be involved not only in WD secondary to multifocal demyelination but also in non-MS degenerative and inflammatory processes.

Because multiple system atrophy (MSA) represents the typical white matter synucleinopathy, we considered using cases of MSA as controls. Multiple system atrophy would not, however, be ideal as a control for this study because immunoreactivity for α-synuclein in MSA would not help interpret the findings of α-synuclein in MS and because the extent of neuroinflammation in MSA is considerably less than that in MS.

We encountered more than 20 α-synuclein–immunoreactive cells per high-power field in active lesions

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**TABLE 4. Cerebral Infarct–Induced Wallerian Degeneration Patient Clinical Characteristics and Immunoreactivity for α-Synuclein**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, years</th>
<th>Sex</th>
<th>Infarct Location</th>
<th>Infarct Age, month*</th>
<th>α-Synuclein in the Basis Pedunculi (midbrain)</th>
<th>α-Synuclein in the Pyramid (medulla)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>M</td>
<td>None</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>M</td>
<td>None</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>M</td>
<td>None</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>F</td>
<td>None</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>79</td>
<td>F</td>
<td>None</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>F</td>
<td>Rt frontal lobe</td>
<td>120</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>79</td>
<td>F</td>
<td>Rt frontal lobe, IC</td>
<td>11</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>M</td>
<td>Lt frontal lobe</td>
<td>108</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>72</td>
<td>M</td>
<td>Lt frontal lobe</td>
<td>1</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>65</td>
<td>M</td>
<td>Lt basis pontis</td>
<td>12</td>
<td>N/A</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>69</td>
<td>F</td>
<td>Rt frontal lobe</td>
<td>12</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>51</td>
<td>M</td>
<td>Lt IC, caudate nucleus</td>
<td>14</td>
<td>⬤</td>
<td>+++</td>
</tr>
</tbody>
</table>

α-Synuclein immunoreactivity is semiquantitatively assessed as follows: −, none; −/−, 1 to 3; −, 4 to 9; ++, 10 to 19; ++++, 20 or more positive cells per high-power field (original magnification: 200×).

*Age of the infarcts is determined by both the pathological features and clinical history.

†The patient had another 6-week-old infarct in the left medulla; there is no section from the midbrain, but sections of the corticospinal tract in the pons were scored as +++. IC, internal capsule; Lt, left; N/A, not applicable; Rt, right.
of MS (Table 2). Because MS is a lifelong disease and it is possible that an α-synuclein–immunoreactive cell has a relatively short life span given the potential toxicity of accumulated α-synuclein, the frequency of α-synuclein–positive cells found in this study seems high. Analysis of autopsied specimens does not allow ascertainment of whether α-synuclein immunoreactivity is caused by reactive de novo production of α-synuclein within neurons, and accumulation in the cell body because of disrupted axonal transport cannot be excluded. Moreover, α-synuclein immunoreactivity in macrophages may be caused by either the synthesis of α-synuclein within these cells and/or secondary acquisition of α-synuclein by phagocytosis or pinocytosis (46, 47). Furthermore, studies of autopsied samples cannot assess possible mechanisms of α-synuclein–mediated neurodegeneration.

A high expression of α-synuclein immunoreactivity can be found in the ventral portion of the midbrain, basis pontis, inferior olive of the medulla, and hippocampus in the normal CNS (48, 49); a distribution of that may be important in the clinical manifestations and pathogenesis of α-synucleinopathies. Similarly, in the present study, α-synuclein immunoreactivity in active and chronic active MS lesions was more frequent in the brainstem than in the cerebral hemispheres. This regional preference in α-synuclein immunoreactivity may reflect the normal distribution of α-synuclein in the brain.

In conclusion, our study emphasizes that there is prominent α-synuclein immunoreactivity in MS lesions in association with inflammmatory activity. A proportion of α-synuclein–expressing cells are neurons. Whereas inflammatory molecules and other processes may help drive the neurodegenerative process in MS, the increase of α-synuclein suggests another pathway by which neurons and axons may be compromised in MS.

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REFERENCES