Myelin Degeneration in Multiple System Atrophy Detected by Unique Antibodies

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A rabbit antiserum (anti-EP), induced against a synthetic peptide corresponding to residues 68 to 86 of guinea pig myelin basic protein, powerfully immunostained abnormal-appearing oligodendrocytic processes and cell bodies in demyelinating areas associated with multiple system atrophy (MSA). However, as we reported previously, the antiserum, which is highly specific for the sequence QDENPVV corresponding to human myelin basic protein residues 82 to 88, failed to recognize any structures in normal human brain. QD-9, a mouse monoclonal antibody raised against human myelin basic protein residues 69 to 88, which also recognizes specifically the epitope QDENPVV, gave the same results as did anti-EP. The unusual epitope recognized by anti-EP/QD-9 antibodies appears to be accessible in areas of myelin degeneration, and the antibodies have been shown to detect such areas in multiple sclerosis and infarcted brains. These antibodies detect myelin degeneration more widely than previous conventional methods. The present study emphasizes the importance of myelin degeneration in the pathogenesis of multiple system atrophy. (Am J Pathol 1998, 153:735–744)

Multiple system atrophy (MSA) is an entity that includes striatonigral degeneration, olivopontocerebellar atrophy, and the Shy-Drager syndrome. This nosological entity was initially described by Graham and Oppenheimer in 1969.¹ Despite intensive research, the precise etiology of MSA remains to be established. Pathological changes in white matter, including demyelination and glial cytoplasmic inclusions (GCIs), are among the most prominent features observed in MSA brain.^{2,3} After the first report of GCIs by Papp et al,² the importance in MSA of pathological changes in oligodendroglia, as well as neuronal changes, has been recognized.^{4–9} However, there have been few reports focusing on the changes in myelin.

As we reported previously, we raised a unique antibody (anti-EP) that recognizes the synthetic peptide

QDENPVV, corresponding to human (h) myelin basic protein (MBP) residues 82 to 88. The anti-EP antibody can specifically detect demyelinating lesions in brains with multiple sclerosis, as well as infarcted brains.¹⁰ The anti-EP antibody is, therefore, a very useful tool for detecting demyelination. Furthermore, we have raised a new mouse monoclonal antibody (QD-9) that also recognizes QDENPVV and degenerating myelin in multiple sclerosis.¹¹ Neither anti-EP nor QD-9 stains myelin in normal brain.^{10,11}

To investigate oligodendroglial changes in MSA, we examined MSA brains by using anti-EP and QD-9 antibodies as markers of degenerating myelin.

Materials and Methods

The Production of Anti-EP/QD-9 Antibodies

The production of anti-EP antiserum and its characterization were reported previously.¹⁰ The monoclonal antibody QD-9 was made against a synthetic peptide corresponding to residues 69 to 88 of hMBP by the known method of Kohler and Milstein.^{12,13} In brief, BALB/c mice were immunized by a conjugate of the synthetic peptide with hemocyanin from keyhole limpet. Spleens were obtained from the immunized mice. Spleen cells were suspended in RPMI 1640 culture medium. The spleen cells and SP-2 myeloma cells were hybridized in 50% polyethylene glycol 1500 (Sigma Chemical Co., St. Louis, MO). The hybridomas were screened by enzyme-linked immunosorbent assay using the QDENPVV peptide. Ascites fluid was produced in mice primed with pristane by injecting 5×10^5 hybrid cells.

Immunohistochemical Procedures

Nine brains from MSA patients were examined and compared with six brains from cases without neurological disease. Details concerning age, sex, source of the

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Table 1. Cases Used in this Study*

Case	Diagnosis (predominant type) [†]	Sex	Age (years)	Postmortem interval (hours)
M1	MSA (OPCA)	M	78	7
M2	MSA (SND > OPCA) [‡]	F	53	12
M3	MSA (SDS)	F	60	11
M4	MSA (OPCA = SND) [§]	F	72	2
M5	MSA (OPCA)	F	75	6
M6	MSA (OPCA)	F	71	8
M7	MSA (OPCA)	F	78	2
M8	MSA (OPCA)	F	72	10
M9	MSA (OPCA)	M	76	11
C1	NNC (HCC)	M	73	8
C2	NNC (unknown)	M	69	5
C3	NNC (AMI)	M	69	9
C4	NNC (LC)	M	69	12
C5	NNC (unknown)	M	84	8
C6	NNC (AMI)	F	82	7

*All brains were obtained from the Kyoto University Neuropathology Brain Bank.

[†]SND, striatonigral degeneration; OPCA, olivopontocerebellar atrophy; SDS, Shy-Drager syndrome; NNC, nonneurological case; AMI, acute myocardial infarction; HCC, hepatocellular carcinoma; LC, lung cancer.

[‡]SND > OPCA, SND was predominant, but OPCA was also significant.

[§]OPCA = SND, both OPCA and SND were equally observed.

brains, and postmortem interval are given in Table 1. For all of the brains, fresh tissue was fixed in 4% paraformaldehyde, pH 7.4, for 2 to 3 days before being transferred to a maintenance solution of 20% sucrose in 0.1 mol/L phosphate-buffered saline (PBS). Sections were cut on a freezing microtome at a 30- μ m thickness. Some sections were directly stained by the Klüver-Barrera or Bielschowsky method to confirm the diagnosis of MSA or of neurological normality. The MSA cases were diagnosed by clinical records and postmortem pathological features such as GCIs and neuronal loss. Other sections, used for immunohistochemical staining, were rinsed for several hours in 0.01 mol/L PBS (pH 7.4) containing 0.3% Triton X-100 (PBS-T). They were pretreated with 0.5% H₂O₂ for 1 hour to reduce endogenous peroxidase, washed in PBS-T, and blocked at room temperature for 2 hours with PBS-T containing 5% skim milk. They were then incubated for 48 to 72 hours at 4°C with one of the primary antibodies. The anti-EP (rabbit polyclonal antibody, 1:10,000) and QD-9 (mouse monoclonal antibody, 1:30,000) were used to visualize degenerating myelin, and anti-ubiquitin antibody (rabbit polyclonal antibody, 1:1,000; Sigma) was used to detect GCIs. Anti-leukocyte common antigen (mouse monoclonal antibody, 1:1000;

Figure 1. A: Specificity of anti-EP and QD-9 antibodies examined by Western blot. Lane a, anti-EP antibody; lane b, clone QD-9. The two antibodies detect a major 18.5-kd band (arrow) and a weaker 7.2-kd band (arrowhead) in extracts of MSA brain homogenates. B and C: Comparison between anti-EP and QD-9 immunohistochemistry in MSA brains. Anti-EP (B) and QD-9 (C) recognized identical structures in MSA brains. B and C are nearby sections from the putamen of an MSA patient (case M4). D: No immunoreactivity is detected in control brains by anti-EP or QD-9. D shows a control cerebellum stained by anti-EP (case C4). Bars, 50 μ m (B to D).

Figure 2. Double staining of specific markers and EP/QD-9 in MSA cases. The distribution of EP (A to C, purple) is different from those of leukocyte common antigen-labeled microglia (A, brown), SMI31-labeled neuronal axons (B, brown), and MAP-2B-labeled neuronal elements (C, brown). QD-9-positive structures (D, purple) do not overlap with glial fibrillary acidic peptide-positive astroglia (D, brown). All of these sections are from case M3. Bars, 50 μ m (A to D).

DAKO, Glostrup, Denmark), anti-glial fibrillary acidic peptide (rabbit polyclonal antibody, 1:20,000; DAKO), anti-neurofilaments (SMI-31, mouse monoclonal antibody, 1:2,000; Sternberger Monoclonals Inc., Lutherville, MD), and anti-MAP2 (mouse monoclonal antibody, 1:2,000; Sigma) were used as specific markers for microglia, astrocytes, neuronal axons, and neuronal dendrites, respectively. After incubation with primary antibody, the sections were washed and reacted at room temperature with a biotinylated antibody against the immunoglobulin G of the appropriate species (1:1,000, Vector Laboratories, Burlingame, CA) for 2 hours and then for 1 hour with the avidin-biotin-peroxidase complex (diluted 1:2000, Vector Laboratories). They were rinsed and incubated in a staining mixture containing 0.001% 3,3'-diaminobenzidine (Sigma), 0.6% nickel ammonium sulfate (Fisher Scientific, Pittsburgh, PA), 0.05% imidazole, and 0.0003%

H_2O_2 in 0.05 mol/L Tris-HCl buffer, pH 7.6, until a purple reaction product appeared. Where a brown reaction product was desired, the same mixture without nickel ammonium sulfate was used. The reaction was terminated by transfer of the sections to PBS-T. Sections were mounted on glass slides, dehydrated with graded alcohol, and coverslipped with Entellan (Merck, Darmstadt, Germany). Some sections were double immunostained before slide mounting. After the first staining procedure, they were pretreated with 1.0% H_2O_2 for 1 hour to destroy excess peroxidase from the first cycle and then processed with the second primary antibody and appropriate secondary antibody as described above. The final diaminobenzidine staining step was chosen to produce a color different from that of the first step, by addition or elimination of nickel ammonium sulfate. Control sections were stained without primary antibody or with a mouse

Figure 3. Various patterns of anti-EP/QD-9-positive oligodendroglial elements observed in MSA tissue. **A:** Long, thickened fibers (case M5). **B:** Densely stained cell bodies of irregular size and shape (arrow). Sometimes abnormally appearing fibers extend from them (case M3). **C:** Thickened or short, misshapen fibers extend from a positively staining oligodendrocyte (arrow) (case M5). Bars, 50 μm (A to C).

monoclonal antibody indifferent to brain tissue. All such control sections showed no staining.

Image Analysis

To compare quantitatively the intensities of Klüver-Barrera staining and density of EP/QD-9-positive elements, we used image analysis as follows. Severity of demyelination can be identified in Klüver-Barrera-stained tissue sections. To determine standard values, we measured the intensity of Klüver-Barrera staining in 80 frames from the middle cerebellar peduncle of four control cases (cases C1, C4, C5, and C6), in which Klüver-Barrera staining was microscopically normal. We used the sections of middle cerebellar peduncle from six MSA cases

(cases M1, M2, M4, M5, M6, and M7) to compare Klüver-Barrera staining with EP/QD-9-positive staining. The intensity of Klüver-Barrera staining was measured, as was the cross-sectional tissue area taken by cell processes and cell bodies of EP or QD-9-labeled oligodendroglia in adjacent sections. Monochromatic photo images were taken using an Olympus BH-2 microscope with a $\times 10$ objective and a $\times 3.3$ eyepiece connected to a photo camera (Olympus, C-35AD). After development of the negative films, these images were examined with a Nikon 35-mm film scanner (LS-1000) set at 1350 dpi and 256 gray scale. The image files obtained were converted into PICT files and analyzed using the NIH image analyzer program on an Apple personal computer Power PC7500. Each image measured $1060.6 \times 757.6 \mu\text{m}$ and consisted of 1893×1197 pixels. Five or six images from each Klüver-Barrera-stained section and adjacent EP/QD-9 sections were analyzed in a rectangular frame (512×512 pixels; $82,737 \mu\text{m}^2$). In the Klüver-Barrera-stained sections, the average score in each rectangular frame was taken as a measure of the severity of demyelination. The Klüver-Barrera scores were first determined on sections of the middle cerebellar peduncle of four control cases. The mean $\pm$ standard deviation (SD) of these controls was used to classify the degree of demyelination in each MSA case into five groups, ranging from 1 (very severe, less than -6 SD from control) to 5 (not demyelinated, $\geq$ mean). In the EP/QD-9 sections, the cross-sectional area of each positive structure was measured according to the definition of individual cell profiles based on manual thresholding. Whether there were significant differences in the EP/QD-9 data depending on the degree of demyelination was assessed by the Kruskal-Wallis test for the five groups and by the Mann-Whitney *U* test between all 10 pairs (ie, group 1 versus 2, group 1 versus 3, etc.). *P* values < 0.05 were considered statistically significant.

Western Blotting

For Western blotting, frozen tissues from the white matter of MSA cases were homogenized in five volumes of ice-cold 10 mmol/L Tris-HCl (pH 7.4) plus 150 mmol/L NaCl (Tris-buffered saline (TBS)), containing 1 mmol/L EDTA, 1 mmol/L ethylene glycol tetraacetic acid, phenylmethylsulfonyl fluoride (100 $\mu\text{g}/\text{ml}$), leupeptin (1 $\mu\text{g}/\text{ml}$), and pepstatin (1 $\mu\text{g}/\text{ml}$). The homogenates were centrifuged at 13,000 rpm for 20 minutes at 4°C . The pellets were suspended in TBS containing 1% sodium dodecyl sulfate. Aliquots containing approximately 100 μg of protein were electrophoresed on 15% sodium dodecyl sulfate-polyacrylamide gels and then transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA). The membranes were blocked by incubating with TBS containing 0.5% Tween-20 and 5% skim milk for 30 minutes at room temperature before being incubated overnight at room temperature with anti-EP (1:10,000) or QD-9 (1:50,000). The rabbit polyclonal and mouse monoclonal antibodies were reacted with alkaline phosphatase-la-

Figure 4. Anti-EP/QD-9-positive oligodendroglial elements observed in various regions in the MSA brains. **A:** Cerebellar cortex (case M6). Long fibers extend from the Purkinje cell layer to the granular layer. Bar, 100 μ m. **B:** Positive fibers in the middle cerebellar peduncle (case M4). **C:** Thick fibers around neurons of the dentate nucleus are stained (case M4). Bar, 25 μ m. **D:** Both longitudinal and transverse bundles at the base of the pons contain positive structures (case M3). **E:** Myelinated fiber bundles are intensely stained in the posterior lateral part of the putamen (case M4). **F:** The intensity of staining in the caudate is less than that in the putamen (case M4). **G:** Globus pallidus, pars externa (case M4). **H:** Internal capsule (case M6). Bar in H, 50 μ m (applies to B and D to H).

Figure 5. Comparison between anti-EP staining (A) and the Klüver-Barrera method (B) in nearby sections from the temporal cortex of an MSA patient (case M4). Stars in A and B identify the same vessel. Notice that the anti-EP antibody stains abnormal oligodendroglial structures, whereas the Klüver-Barrera method shows little or no demyelination. Bar, 100 μ m.

beled antibody against the immunoglobulin G of the appropriate species (1:1,000, Vector Laboratories) for 2 hours at room temperature. Alkaline phosphatase labeling was visualized by incubating with nitroblue tetrazolium (0.33 mg/ml; Life Technologies, Inc., Gaithersburg, MD) and 5-bromo-4-chloro-3-indolyl phosphate (0.165 mg/ml, Life Technologies) in 100 mmol/L Tris-HCl (pH. 9.5) containing 100 mmol/L NaCl and 50 mmol/L $MgCl_2$.

Results

Characterization of the Anti-EP and QD-9 Antibodies

The anti-EP antibody and QD-9 detected the same bands on Western blots of human cerebellar white matter. These were at 18.5 and 17.2 kd, which corresponded to normal hMBP isoforms (Figure 1A).^{14,15} In immunohistochemistry, each antibody stained identical structures in the putamen of MSA brains. These were abnormal-appearing oligodendrocytic elements (Figure 1B (anti-EP) and 1C (QD-9)). In control brains, no structures were stained by either anti-EP or QD-9 (Figure 1D).

Double staining with specific markers and EP or QD-9 was performed to determine which type of cells contained structures positive for EP or QD-9. The distribution of leukocyte common antigen (Figure 2A), SMI31 (Figure 2C), or MAP2 (Figure 2D) was clearly different from that of

EP. Glial fibrillary acidic peptide-positive astroglia never overlapped with QD-9-positive structures (Figure 2B).

Most anti-EP/QD-9 positive structures were thickened, misshapen fibers extending from positively staining oligodendrocytes (Figure 3A). In some areas, densely staining cell bodies of irregular size were prominent (Figure 3B). Sometimes these cell bodies had thin, apparently degenerating fibers extending from them (Figure 3C).

Distribution of EP/QD-9-Positive Structures in MSA Brains

In the olivopontine cerebellar system of MSA brains, anti-EP/QD-9-positive structures were observed in the cerebellar cortex, middle cerebellar peduncle, pons, and lower olivary complex. In the cortices of the cerebellar hemisphere, positive fibers were located in the granular layer (Figure 4A). They seemed to be degenerating myelin sheaths of axons of Purkinje cells. The middle cerebellar peduncle contained various densities of positive fibers (Figure 4B). The cerebellar nuclei had anti-EP/QD-9-positive fibers around the neurons (Figure 4C). In the ventral portion of the pons, portions of both transverse and longitudinal fiber bundles were stained by anti-EP/QD-9 (Figure 4D). In the olive complex, there were some anti-EP/QD-9 positive fibers.

In the striatonigral system, the putamen contained intense anti-EP/QD-9-positive fibers, especially in its lateral ventral portion (Figure 4E). The caudate nucleus also had positive structures, but the density was sparser than in the putamen (Figure 4F). The globus pallidus was rich in anti-EP/QD-9-positive structures, particularly in the external segment (Figure 4G). The internal capsule showed a moderate density of anti-EP/QD-9 staining (Figure 4H). Anti-EP/QD-9 staining was sparse in the pars reticulata of the substantia nigra. Anti-EP/QD-9-positive fibers could be observed in the locus ceruleus, where there was clear neuronal loss.

The neocortices have been considered to be relatively spared in MSA. However, the anti-EP/QD-9 antibodies could detect abnormal-appearing oligodendroglial elements in the temporal, prefrontal, and occipital cortices, which had a normal appearance with the Klüver-Barrera method (Figure 5A and B).

Comparison between EP/QD-9 Immunohistochemistry and Klüver-Barrera Staining

The density of anti-EP/QD-9 positive structures seemed to depend on the severity of demyelination. Many anti-EP/QD-9-positive fibers were observed in areas where myelin was moderately affected (Figure 6, C and D). In some MSA cases, the anti-EP/QD-9 antibodies stained some fibers even in areas that appeared normal by the Klüver-Barrera method (Figure 6, E and F). On the other hand, positive structures were sparsely located in very severely demyelinated sites (Figure 6, A and B).

Figure 6. Comparison between the Klüver-Barrera method (A, C, and E) and the anti-EP method (B, D, and F) in the cerebellar medulla of an MSA patient (case M4). A and B, C and D, and E and F are nearby sections. Stars and asterisks identify the same vessels. Anti-EP-positive structures are more intense in the moderately demyelinated area (C and D) than in the severely affected (A and B) or slightly affected (E and F) regions. Bar in F, 100 μ m (applies to A to F).

In the middle cerebellar peduncle of the four control cases, the mean ($\pm$ SD) of Klüver-Barrera intensity (KBI) measured was 148.58 ± 8.55 (in a 256 gray scale). Based on these data, the severity of demyelination in each region was grouped into five grades chosen to represent different degrees of SDs away from the control mean. They were: grade 1 (very severely demyelinated), $\text{KBI} < 97.28$; grade 2 (severely demyelinated), $97.28 \leq \text{KBI} < 114.38$; grade 3 (moderately demyelinated), $114.38 \leq \text{KBI} < 131.48$; grade 4 (slightly demyelinated), $131.48 \leq \text{KBI} < 148.58$; and grade 5 (not demyelinated), $148.58 \leq \text{KBI}$. Comparison between KBI and EP/QD-9 density was performed in the sections from the middle cerebellar peduncle of the six MSA cases (Table 2). The five grades of demyelination showed a significant difference in the percentage area stained by EP/QD-9 by the Kruskal-Wallis test ($P < 0.0001$). In paired comparisons between the five groups, 6 of 10 pairs showed a significant difference by the Mann-Whitney U test (Figure 7). The percentage of area covered by

EP/QD-9-positive structures in the moderately and slightly demyelinated regions (grades 3 and 4) showed significantly greater values than those in very severely or severely affected regions (grade 3 *versus* grade 1, $P < 0.001$; grade 4 *versus* grade 1, $P < 0.001$; grade 3 *versus* grade 2, $P < 0.05$; and grade 4 *versus* grade 2, $P < 0.001$). In addition, the percentage area positive for EP/QD-9 in the slightly demyelinated regions was significantly greater than in nondemyelinated regions ($P < 0.01$). Even nondemyelinated regions (grade 5) had some EP/QD-9-positive structures ($2.42\% \pm 1.74$).

Relationship between EP/QD-9-Positive Structure and GCIs

Double staining with QD-9 and anti-ubiquitin showed that GCIs did not contain the QD-9 epitope (Figure 8A). In regions that were relatively less affected, more anti-EP-positive fibers than GCIs could be seen (Figure 8B). In

Figure 7. Comparison between intensity of Klüver-Barrera staining and EP/QD-9 density in MSA middle cerebellar peduncle. Anti-EP/QD-9 density is expressed as a percentage of each cross-sectional area. Grading was performed according to the average KBI measured in adjacent sections. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. P values were calculated using the Mann-Whitney U test. See text for more details.

moderately affected areas, both anti-EP-positive structures and GCIs were observed (Figure 8A). In some severely affected areas, GCIs could be observed, whereas anti-EP-positive fibers were sparse (Figure 8C). Neither GCIs nor anti-EP/QD-9-positive structures were detected in some extremely damaged regions.

Discussion

The anti-EP antiserum used in this study was raised in a rabbit against guinea pig MBP residues 68 to 86. We previously reported that this antiserum stained damaged myelin selectively and that the staining was completely abolished by immunoabsorption with QDENPVV, corresponding to hMBP residues 82 to 88.¹⁰ This indicates that this sequence contains the key epitope exposed in areas of myelin damage. The QD-9 mouse monoclonal antibody was raised against hMBP 69 to 88, and the hybridoma cells were screened against QDENPVV. The results from the Western blots and immunohistochemistry

Figure 8. Double staining with QD-9 and anti-ubiquitin in MSA cases. The distribution of QD-9 (purple) is different from that of ubiquitinated GCIs (brown). **A:** Moderately affected cerebellar medulla (case M6). Ubiquitinated GCIs and QD-9-positive fibers are detected. Note that QD-9 does not stain the GCIs. **B:** QD-9-positive fibers located in the globus pallidus in a case of MSA (case M4). GCIs are rarely observed. **C:** Plenty of GCIs are observed in the putamen in a case of MSA (case M5), whereas QD-9-positive fibers are relatively sparse in this case. Bar in C, 100 μ m (applies to A to C).

Table 2. Comparison between Density of EP/QD-9-Positive Structures and Intensity of Klüver-Barrera Staining

Klüver-Barrera demyelination grade	Count	Anti-EP/QD-9 positive structures (percentage area*)
1	14	0.82 ± 0.49
2	15	1.96 ± 1.97
3	32	3.56 ± 2.77
4	24	3.96 ± 1.82
5	29	2.42 ± 1.74

*Mean ± SD.

showed that these two antibodies were identically specific for QDENPVV. We applied these unique antibodies to investigate demyelinating areas in MSA brains.

The double staining of specific markers and EP/QD-9 showed clearly that EP/QD-9-positive structures are not from astroglia, microglia, or neurons. Taking these results together with the specificity of these two antibodies against the partial sequence of MBP, We concluded that EP/QD-9-positive structures are oligodendroglial elements. The anti-EP/QD-9 antibodies stained the fibrils and cell bodies of damaged oligodendrocytes in the white matter of MSA brains, whereas no positive structures were observed in the control brains. Anti-MBP antibodies and the Klüver-Barrera method have been used to detect demyelinating lesions. They are, however, not always sensitive enough to visualize minor MBP changes that may occur in white matter. In fact, the anti-EP/QD-9 antibodies detected abnormal-appearing oligodendrocytic elements in areas where no apparent changes were observed by conventional methods. The anti-EP and QD-9 antibodies are, therefore, very sensitive tools for investigating myelin pathology in MSA as well as in multiple sclerosis.¹⁰

MSA is pathologically characterized by cell loss and gliosis occurring in selective regions such as the substantia nigra, caudate, putamen, globus pallidus, inferior olives, pons, and cerebellum.^{16,17} Structures positive for anti-EP and QD-9 antibodies were seen in these regions. In the middle cerebellar peduncle, where demyelination is always observed, anti-EP and QD-9 both detected intensely positive fibers and cell bodies intensely. All examined sections of the pons, caudate, putamen, and globus pallidus (especially the pars externa) from MSA brains contained richly positive structures. Because demyelination in these areas is thought to be secondary to neuronal loss, the anti-EP/QD-9-positive structures in these areas may result from secondary demyelination.

The intensity and density of anti-EP/QD-9-positive structures depended on the severity of demyelination. The image analysis gave quantitative support for this impression. Moderately or slightly affected regions had the most prominent staining with these antibodies. Severely affected regions showed little or no anti-EP/QD-9 staining. The epitope recognized by anti-EP/QD-9 may have been destroyed in these very severely affected regions. The same phenomena were observed in central regions of multiple-sclerotic plaques.¹⁰ Anti-EP/QD-9 positive structures were observed in neocortical white

matter that was examined, such as that of the temporal or frontal lobes, whereas the conventional Klüver-Barrera method failed to detect the myelin degeneration. Involvement of cerebral cortex in MSA has been studied by several groups. Motor cortex has been reported to be the most GCI-rich cerebral cortical area.^{2,7,18,19} Ubiquitinated neuronal inclusions have been found in the subiculum and CA1 of Ammon's horn.²⁰ A recent report indicated some neuronal damage in prefrontal cortex, dentate gyrus, and parahippocampal gyrus in MSA brains.²¹ These data showed that neuronal damage in the cerebral cortex in patients with MSA is more extensive than previously thought. Neuronal loss itself in the cerebral neocortices is, however, not as marked as the massively degenerated oligodendrocytes stained by anti-EP/QD-9. Because damage in these areas to projection fibers from brain stem, basal ganglia, or sick neurons of neocortices cannot be excluded, more studies are needed to prove that oligodendroglial involvement is one of the primary events in MSA. The present results, however, show that damage of oligodendroglia can be detected in the early stages of the degenerative process in MSA.

GCIs are one of the hallmarks of pathology in MSA brains.^{2,4,7,22} Immunohistochemical studies have shown that MBP is not a component of GCIs.^{23–25} The present study also showed that GCIs do not contain the anti-EP/QD-9 epitope, corresponding to hMBP 82 to 86. The appearance of GCIs is thought to precede the other changes of neuronal loss, gliosis, and myelin pallor.⁷ In this study, anti-EP/QD-9-positive structures in MSA brains were observed widely, including GCI-free regions. The presence of anti-EP/QD-9-positive structures and GCIs may be early signs of oligodendroglial degeneration in MSA. Further studies are obviously needed to reveal the precise relationship between the pathogenesis of MSA and early oligodendroglial degeneration.

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