한국전자현미경학회지 제 36 권 제 2 호 2006
Korean J. Electron Microscopy 36(2), 119~130(2006)

Morphometric and Ultrastructural Change of Myelin-Associated Glycoprotein (MAG)-Immunoreactive Oligodendrocytes by Aging

Ik-Hyun Cho[1,6], Chang-Hyun Park[2], Jong-Hwan Lee[1],
Chun-Sik Bae[3], Sang-Kyu Ye[4], Beobyi Lee[5], Seung-Hwa Park[5],
Ki-Seok Koh[5], Jin-Suk Kim[1] and Byung-Joon Chang[1,*]
[1]College of Veterinary Medicine, Konkuk University
[2]Electron Microscope Facility, College of Medicine, Korea University
[3]College of Veterinary Medicine and Biotechnology Research Institutes, Chonnam National University
[4]Department of Pharmacology, Seoul National University College of Medicine
[5]Department of Anatomy, College of Medicine, Konkuk University
[6]Department of Physiology, College of Dentistry and Dental Research Institute,
Seoul National University
(Received May 12, 2006; Accepted June 18, 2006)

노화에 의한 Myelin-Associated Glycoprotein (MAG) 면역반응 희소돌기아교세포의 형태계측학적 및 미세구조적 변화

조익현[1,6], 박창현[2], 이종환[1], 배춘식[3], 예상규[4],
이법이[5], 박승화[5], 고기석[5], 김진석[1], 장병준[1,*]
[1]건국대학교 수의과대학, [2]고려대학교 의과대학 전자현미경실,
[3]전남대학교 수의과대학 및 생물공학연구소,
[4]서울대학교 의과대학 약리학교실, [5]건국대학교 의과대학 해부학교실,
[6]서울대학교 치학연구소 및 치과대학 생리학교실

ABSTRACT

To investigate the role of myelin-associated glycoprotein (MAG) in the normal aging process, aging-related morphometric and ultrastructural analyses of the MAG-positive (MAG-(+)) oligodendrocytes were carried out in the cerebral cortex of the Sprague-Dawley rats. In the aged rats, the density of MAG-(+) oligodendrocytes was significantly decreased in the cortical layer (IV-VI) compared with that of the adult rats. However, the percentage of medium and dark types of oligodendrocytes was significantly increased by aging. In the aged rats, the mean nuclear area of the MAG-(−) oligodendrocytes was interestingly reduced compared with that of MAG-(+) oligodendrocytes. In addition, MAG immunoreactive products were markedly decreased in the medium-dark type of oligodendroglial cytoplasm and processes, and were scarcely localized in the dark type of oligodendrocytes of the aged rats. These results suggest that degeneration of oligodendrocytes-myelin system

* Correspondence should be addressed to Byung-Joon Chang, Ph.D., Department of Anatomy, College of Veterinary Medicine, Konkuk University Seoul 143-701, Korea. Ph.: (02) 450-3711, FAX: (02) 450-3037, E-mail: bjchang@konkuk.ac.kr

by aging is associated with down regulation of MAG, and that may contribute to further understanding of the biology of MAG in the oligodendrocytes-myelin system.

Key words : Aging, Melin-associated glycoprotein (MAG), Oligodendrocytes

INTRODUCTION

Age-related changes in the oligodendrocytes and myelin sheath of the brain may play an important role in impairment of cognitive function (Peter, 2002). Ultrastructural analysis of the aged monkey showed that increase of thickness of the myelin sheaths, local splitting of the major dense line, formation of redundant myelin, dense inclusions and swelling along oligodendroglial processes (Peter, 2002). Age-related morphological changes are associated with loss or degradation of myelin proteins and oligodendrocyte proteins, such as myelin-associated oligodendrocytic basic protein (MOBP), myelin basic protein (MBP), myelin-oligodendrocyte glycoprotein (MOG), and myelin-associated glycoprotein (MAG) (Montague et al., 1999; Quarles, 2002). Surprisingly, no study has been devoted so far to the morphological evaluation of possible modifications occurring in aging-related expression and synthesis of the MAG, an important components of oligodendrocyte-axon system.

MAG, a heavily glycosylated transmembrane protein, is a member of immunoglobulin (Ig) superfamily with five Ig-like domains (Arquint et al., 1987). MAG was exclusively expressed in the cytoplasm and processes of oligodendrocytes during the early stage of myelination. However, MAG expression is restricted in the paranodal regions of myelin sheaths and the periaxonal region of myelinated axons after myelination (Trapp et al., 1989). The studies of MAG deficient mice reported the subtle morphological abnormalities in the oligodendroglial cytoplasm at the inner aspect of myelin sheaths (Montag et al., 1994), a delayed onset of myelination, a dilated periaxonal space for some myelinated CNS axons (Li et al., 1994; Montag et al., 1994), nerve conduction abnormalities, and axonal degeneration (Weiss et al., 2001). Based on these reports, it has been suggested that MAG plays a critical role to the formation of myelin sheaths during initial stage of myelination, and has a role in stabilizing oligodendrocyte-axon contacts in mature myelin sheath (Bartsch, 1996). In addition, it has been known that MAG enhances survival of oligodendrocytes and promotes myelin-like membranogenesis (Gard et al., 1996), and that MAG inhibits neurite outgrowth (1994; Mukhopadhyay et al., 1994).

As described above, although studies on the MAG related oligodendrogliogenesis and myelinogenesis are relatively abundant, the best of our knowledge, no work stating the normal aging-related changes on the MAG expression has ever been published so far. Here, we report that the morphometric and ultrastructural degeneration of oligodendrocytes-myelin system by ageing is associated with the down regulation of the MAG.

MATERIALS AND METHODS

Animals and Sample Preparation

Male Sprague-Dawley rats, 2 months (n=5; 269±27 g) and 32 months (n=5; 355±23 g) after birth, were obtained from Dae-Han Biolink Co. Ltd (Seoul, Korea). Rats were perfused according to Cho et al. (2006) with saline containing heparin (20 IU/mL) and cool 4% paraformaldehyde in 0.2 M phosphate buffer (PB, pH 7.4). Removed brain was overnight fixed in the same fixative at 4°C. After wash, 20 μm- and 60 μm-thick vibratome sections were obtained from the coronal planes of the forebrain.

General Immunohistochemistry

Free-floating sections were processed according to Cho et al. (2006). Briefly, sections were treated for 5 minutes with 3% H_2O_2, and then for 2 hrs with 10% normal horse at room temperature (RT). Sections were overnight incubated at 4°C with monoclonal anti-MAG antiserum (1 : 500; Chemicon, U.S.A.). Control sections were incubated without primary antiserum. After rinsing, the sections were incubated biotinylated anti-mouse IgG for 2 hrs at RT. The sections were washed and incubated with avidin-biotin-peroxidase complex (Vector Lab., U.S.A.) for 2 hrs at RT. The immune reaction was visualized with chromogen solution consisted of 0.05% 3, 3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma, U.S.A.) and 0.005% H_2O_2 mixed in 0.05 M Tris-buffer (pH 7.6).

Morphometric Analysis

Cell Count

The numerical MAG-immunoreactive (IR) oligodendrocytes was estimated by modified previous report (Peinado et al., 1998). Briefly, immunostained 20 μm-thick vibratome sections were mounted on the gelatin-coated slides, and coverslipped, and observed with light microscope (× 40 objective and × 10 eyepiece) fitted with a micrometer grid of 500 × 500 μm (250,000 μm^2); the grid was used as the count unit (CU). The upper edge of the grid was placed at the pial surface and lowered successively along 8 CU, which included all cortical layers from the motor area of the frontoparietal cortex (Paxinos & Watson, 1986). Only cells intersected by the right vertical and top grid bars were included in the counts; those intersected by the left vertical and bottom bar were not. This procedure was repeated along the 500 μm-wide cortical strips from the pial surface to the white matter in the vibratome sections from each animal group. Each time, all MAG-IR oligodendrocytes in the 800 CU from 100 strips per animal groups were counted.

Morphometry of Nuclear Area

Immunostained 60 μm-thick vibratome sections were examined with the light microscope. From these sections, 2 mm-wide tiny pieces were carefully obtained, which included all cortical layers from the motor area. These tiny pieces were fixed with 2.5% glutaraldehyde in PBS, and these pieces were postfixed with 1% OsO_4 dissolved in PBS for 30 minutes, then dehydrated with graded ethanol series, and immersed in propylene oxide, and flat-embedded in Epon resin between Teflon coated slides and polymerized at 60°C for 72 hrs (E100, TAAB embedding oven, U.S.A.). Serial semithin sections were cut with ultramicrotome (LKB-2088, Reichert-Jung Leica, Germany) through the surface zone of the embedded sections, and were mounted on the gelatin-coated slides. The obtained semithin sections were lightly counterstained with 1% toluidine blue and observed with a × 100 objective and a × 10 eyepiece lens. Randomly chosen 333 and 292 areas in the cortical sections of the adult and aged rat were photographed with an Olympus photomicroscope on 35 mm Kodak gold films, which were developed and printed at a final magnification of × 1337 at the same D. P. & E. room every time. The nuclear profile areas of all MAG-positive (MAG-(+)) and MAG-negative (MAG-(−)) oligodendrocytes on photographs were measured by hand drawing nuclear profiles with pen mouse under image analyzer system interfaced with appropriate software (KS400, Carl Zeiss Co. Ltd., Germany).

Ultrastructural Analysis

The remainder of Epon resin blocks of immunostained 60 μm-thick vibratome sections was used for taking ultrathin sections. Tangential serial ultrathin sections were cut through the 5 μm-thick surface zone of the embedded sections, and picked up on copper grids. They were contrasted with uranyl acetate and lead citrate. These sections were examined with transmission electron microscope Jeol 1200 EXII (Jeol Co., Japan)

under 60 Kv.

Statistical Analysis

The statistical significance of differences was determined by Student's t-test. All data were presented as the means±SEM, and differences were considered to be significant when p value was less than 0.05.

RESULTS

Light Microscopic Immunohistochemistry and Density of the MAG-positive Cells

Process-bearing MAG-positive (MAG-(+)) cells were diffusely distributed throughout cerebral cortex of adult rats. These cells had abundant cytoplasm and many processes, and the nuclei were relatively large and often irregular in shape (Fig. 1b, d). However, non-process-bearing MAG-(+) cells were abundant in the cerebral cortex of aged rats. The cytoplasm was little and has no processes, and the nucleus was small and generally round or oval (Fig. 1c, e). To investigate whether the MAG expression was affected by aging, the number of MAG-(+) cells was counted in the cerebral cortex of the adult and aged rats, and summarized in Table 1 and Fig. 2. In the aged rats, the number of MAG-(+) oligodendrocytes was significantly decreased in CU levels 4, 5, 6, and 7, located in layer IV-VI, compared with that of the adult rat. Whereas, the oligodendroglial numbers with aging were increased at CU levels 2 that corresponds to layer III (Table 1, Fig. 2).

Differential Count and Nuclear Area of the Oligodendrocytes

Differentiation

Based on the light microscopic features of the oligodendroglial nuclear chromatin and the texture and darkness of the cytoplasm (Mori and Leblond, 1970;

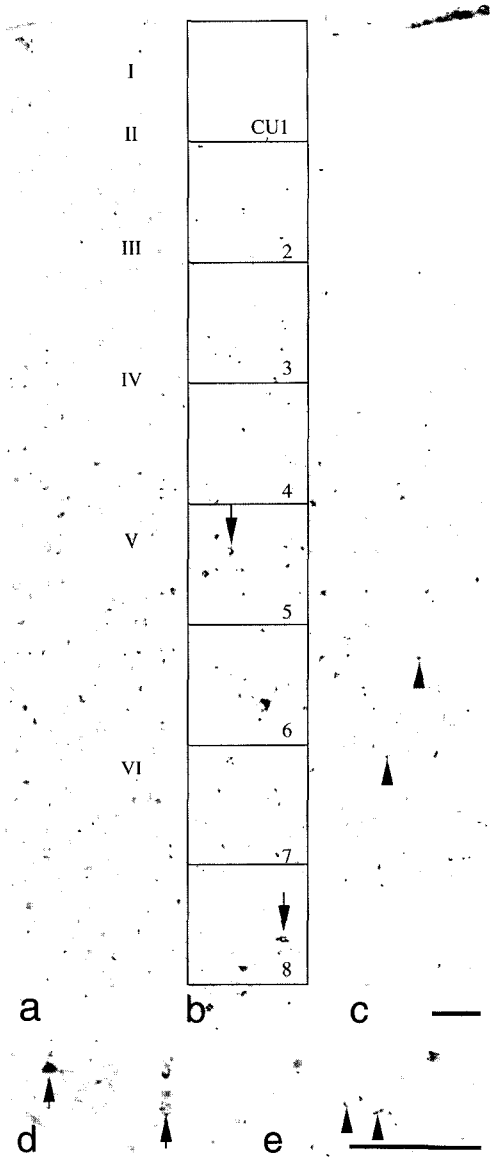

Fig. 1. Coronal sections of the adult and aged rat cerebral cortex stained with MAG antiserum (b-e) and Nissl stained (a). Sections show various density of MAG-(+) cells depending on the cortical layers. Process-bearing MAG-(+) cells (arrow) were abundant in the adult (Fig. b, d), whereas non-process-bearing MAG-(+) cells (arrowhead) were abundant in the aged rat cerebrum (Fig. c and e). Fig. d and e are the high magnification micrographs of the Fig. d and c. I, II, III, IV, V, and VI signify cortical layers. The lattices show counter unit (CU1-8) used in cell count. Scale bar=100 μm.

Table 1. Number of MAG-(+) oligodendrocytes per 250,000 μm² in the cerebral cortex of adult and aged rats. Values are presented as means±SEM. *, $p<0.001$; **, $p<0.01$ (Student's *t*-test)

Group	Total strips	CU1	CU2	CU3	CU4	CU5	CU6	CU7	CU8	Total
Adult	100	2.45 ±0.15	6.44 ±0.26	14.97 ±0.72	20.00 ±0.69	20.72 ±0.61	19.69 ±0.67	17.74 ±0.65	15.12 ±0.68	117.13 ±2.70
Aged	118	2.44 ±0.18	8.58 ±0.37*	15.18 ±0.58	16.08 ±0.57*	15.88 ±0.59*	14.70 ±0.56*	15.13 ±0.57**	13.90 ±0.56	101.89 ±2.49*

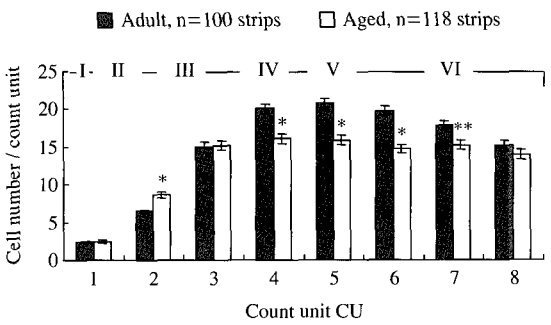

Fig. 2. Distribution of MAG-(+) oligodendrocytes (mean± SEM) obtained in each of the 8 count units (CU) in the cerebral cortex. I II-IV, V, and VI signify cerebral cortical layers. *, $p<0.001$; **, $p<0.01$ (Student's *t*-test).

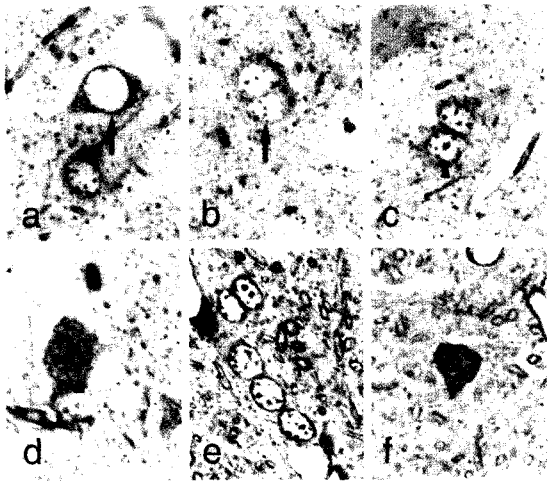

Fig. 3. Light microscopic photographs of the three types of oligodendrocytes in semithin sections stained with MAG antiserum and toluidine blue. Arrow, light of MAG-(+) oligodendrocytes; Arrow head, medium type of MAG-(+) oligodendrocytes; Thick arrow, dark type of MAG-(+) oligodendrocytes; White arrow, light type of MAG-(−) oligodendrocytes; White arrow head, medium type of MAG-(−) oligodendrocytes; White thick arrow, dark type of MAG-(−) oligodendrocytes. Scale bar=10 μm.

Ling and Leblond 1973), light, medium and dark types of oligodendrocytes were clearly identified in semithin sections of rat cerebral cortex, immunostained with anti-MAG antiserum and counterstained with toluidine blue. To avoid possible mistakes in identifying subtype of oligodendrocytes, light-medium cells were quantified as light type cells, and medium-dark cells as medium type cells.

The **light** type of oligodendrocytes were large and had a very pale oval or round nucleus, which showed a little or no distinct chromatin masses. The cytoplasm was extensive, and dark colored small dense structures were observed around the nucleus (Fig. 3a, b). The **medium** type of oligodendrocytes were smaller than light cells. The nucleus was smooth oval or elongated shape, and was smaller than that of light cells, and contained discrete chromatin masses, mainly at the periphery (Fig. 3c). The **dark** type of oligodendrocytes were recognized as the smallest and densest elements. The nucleus was round to oval or irregular and quite dark, although chromatin masses may still be distinguished. The cytoplasm was darker than that of medium cells, and may be concentrated on one side of the nucleus; it was very rarely showed the cytoplasmic extensions (Fig. 3d, f). Also, the light, medium and dark oligodendrocytes not stained for MAG antiserum were identified (Fig. 3e, f).

Differential Count

To confirm correlation with aging, the percentages of

each type of oligodendrocytes were calculated (Table 1, Fig. 2). The result of differential count showed decrease in the percentage of MAG-(+) oligodendrocytes with aging. In the adult rat, the percentages of MAG-(+) light (18.4%) and medium oligodendrocytes (40.4%) were higher, whereas in the aged rat, the percentages of MAG-(+) medium (26.5%) and dark (183.0%) oligodendrocytes were higher. In the aged rats, the percentage of MAG-(+) oligodendrocytes (46.3%) was lower than in the adult rats (71.5%), whereas the percentage of MAG-(−) oligodendrocytes (53.5%) was higher than in the adult rats (28.5%). In the adult rats, the percentage of MAG-(+) oligodendrocytes (71.5%) was higher than MAG-negative (MAG-(−)) oligodendrocytes (28.5%), whereas in the aged rats, the percentage of MAG-(−) oligodendrocytes (53.5%) was higher than MAG-(+) oligodendrocytes (46.3%).

Nuclear Area

To investigate whether the oligodendroglial nuclear area was affected by aging, the morphometric analysis of the nuclear area of different cell types was carried out (Table 2, Fig. 4). In the aged group, the mean nuclear area (MNA) of three types of MAG-(+) oligodendrocytes ($16.88 \pm 0.20\ \mu m^2$), three types of MAG-(−) oligodendrocytes ($15.97 \pm 0.20\ \mu m^2$), and total oligodendrocytes ($16.39 \pm 0.14\ \mu m^2$) was narrower than that of adult group ($19.84 \pm 0.23\ \mu m^2$, $19.05 \pm 5.46\ \mu m^2$, and $19.62 \pm 0.19\ \mu m^2$), respectively ($p < 0.001$) (Table 2, Fig. 4a). In the adult rats, the MNA of the light ($25.90 \pm 0.37\ \mu m^2$) and three ($19.05 \pm 0.33\ \mu m^2$) types of MAG-(−) oligodendrocytes was narrower than that of MAG-(+) oligodendrocytes ($27.09 \pm 0.40\ \mu m^2$, and $19.84 \pm 0.23\ \mu m^2$), respectively ($p < 0.05$). In the aged rat, the MNA of medium ($17.81 \pm 0.21\ \mu m^2$), dark ($12.62 \pm 0.20\ \mu m^2$) and three ($15.97 \pm 0.20\ \mu m^2$) types of MAG-(−) oligodendrocytes was narrower than that of MAG-(+) oligodendrocytes ($18.39 \pm 0.23\ \mu m^2$, $13.78 \pm 0.24\ \mu m^2$, and $16.88 \pm 0.20\ \mu m^2$), respectively (Table 2, Fig. 4b). There was no significant difference between medium (or dark) type of MAG-(+) oligodendrocytes and that of MAG-(−) oligodendrocytes within the adult group.

Pre-embedding Immunohistochemistry

Based on the ultrastructural features of the oligodendroglial nucleus (Mori and Leblond, 1970), MAG-(+) cells were identified as the characteristic features of either light, medium (medium-dark) and dark types of oligodendrocytes. The light and medium types of oligo-

Table 2. Differential counts and nuclear area of MAG-(+) and (−) cells of cerebral cortex in the adult and aged rats

Group		MAG(+) ODC				MAG(−) ODC				MAG(+)+(−) ODC
		Light ODC	Medium ODC	Dark ODC	Three types ODC	Light ODC	Medium ODC	Dark ODC	Three types ODC	Total ODC
Adult	Number (%)	184 (18.4)	404 (40.4)	127 (12.7)	715 (71.5)	66 (6.6)	173 (17.3)	46 (4.6)	285 (28.5)	1000 (100.0)
	Nuclear area (Mean±SEM) (μm^2)	27.09 ±0.40	18.40 ±0.17	13.93 ±0.29	19.84 ±0.23	25.90 ±0.37*	17.92 ±0.25	13.45 ±0.42	19.05 ±0.33*	19.62 ±0.19
Aged	Number (%)	18 (1.8)	265 (26.5)	180 (18.0)	463 (46.3)	32 (3.2)	273 (27.3)	230 (23.0)	535 (53.5)	998 (100)
	Nuclear area (Mean±SEM) (μm^2)	25.70 ±0.86	18.39 ±0.23	13.78 ±0.24	16.88 ±0.20###	24.39 ±0.70	17.81 ±0.21*	12.62 ±0.20**	15.97 ±0.20###,**	16.39 ±0.14###

###, $p < 0.001$ (compared to mean nuclear area of each type ODC (oligodendrocyte) of adult rats)

*, $p < 0.05$; **, $p < 0.01$ (compared to mean nuclear area of MAG-(+) ODC in adult and aged rats.

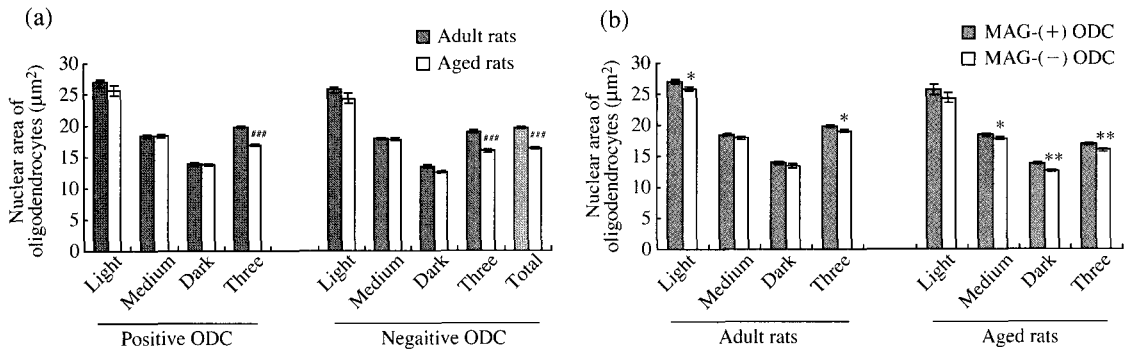

Fig. 4. Mean nuclear area (mean ± SEM) of oligodendrocytes of cerebral cortex in adult and aged rats. (**a**) The mean nuclear area (MNA) of three types of MAG-(+) ODC (oligodendrocytes), three types of MAG-(−) ODC, and total ODC was reduced in the aged rats. (**b**) The MNA of the MAG-(−) oligodendrocytes was interestingly reduced compared with that of MAG-(+) ODC. *, $p<0.05$; **, $p<0.01$ (Student's *t*-test).

dendrocytes were confirmed mainly in the adult rat cerebrum, whereas dark type of oligodendrocytes was observed primarily in the aged rats. The light type of oligodendrocytes had a large and pale nucleus. In the nucleus, there was little or no mass of chromatin. The perinuclear cytoplasm contained many mitochondria, distinct Golgi apparatus, and cisternae of rough endoplasmic reticulum (Fig. 5a). The medium (or medium-dark) type of oligodendrocytes had abundant chromatin masses in the nucleus. The cells were quite small cells with moderately dense nucleus and cytoplasm containing well developed organelles (Fig. 5b-e). The dark type of oligodendrocytes was even smaller cells with very dense nucleus and cytoplasm containing prominent Golgi apparatus, structures referred to as lamellar bodies (Fig. 5f).

In the adult rat cerebrum, MAG-IR products were diffusely localized in light and medium (or medium-dark) oligodendroglial cytoplasm, and some intense labeling was observed in the Golgi complexes, and rough endoplasmic reticulum (rER), which occurred either in the vicinity of the nucleus or in the proximal processes. MAG-IR products were abundant in the oligodendroglial processes that extend to myelin internodes, in oligodendroglial cytoplasm of inner and outer tongue processes, and in periaxonal regions of myelinated fibers (Fig. 5a-c). However, in the aged rat brain, MAG-positive cells were mainly medium and dark oligodendrocytes, and MAG immunoreactions were remarkably decreased when it was compared with that of adult. The intense labeling of MAG was restricted to the perinuclear cytoplasm of medium oligodendrocytes, and diffuse staining was decreased in the cytoplasm and proximal processes, and staining was scarcely observed in the distal processes (Fig. 5d, e). In the dark oligodendrocytes, MAG-(+) labeling was observed in the cytoplasm directly underlying the nuclear membrane (Fig. 5f). The reactant was not observed almost in dark oligodendroglial cytoplasm and processes. The staining of rER and Golgi apparatus was more evident in the light and medium oligodendrocytes of the adult rat than that of the aged rat, probably due to the absence of diffuse cytoplasmic staining in aged rat. In the aged rats, intense immunostained membranes of the rER and Golgi apparatus were hardly detected. The neuclei and some inclusion bodies were devoid of reaction products (Fig. 5e). None of the labeled cells was identified as either astrocytes or neurons (data not shown).

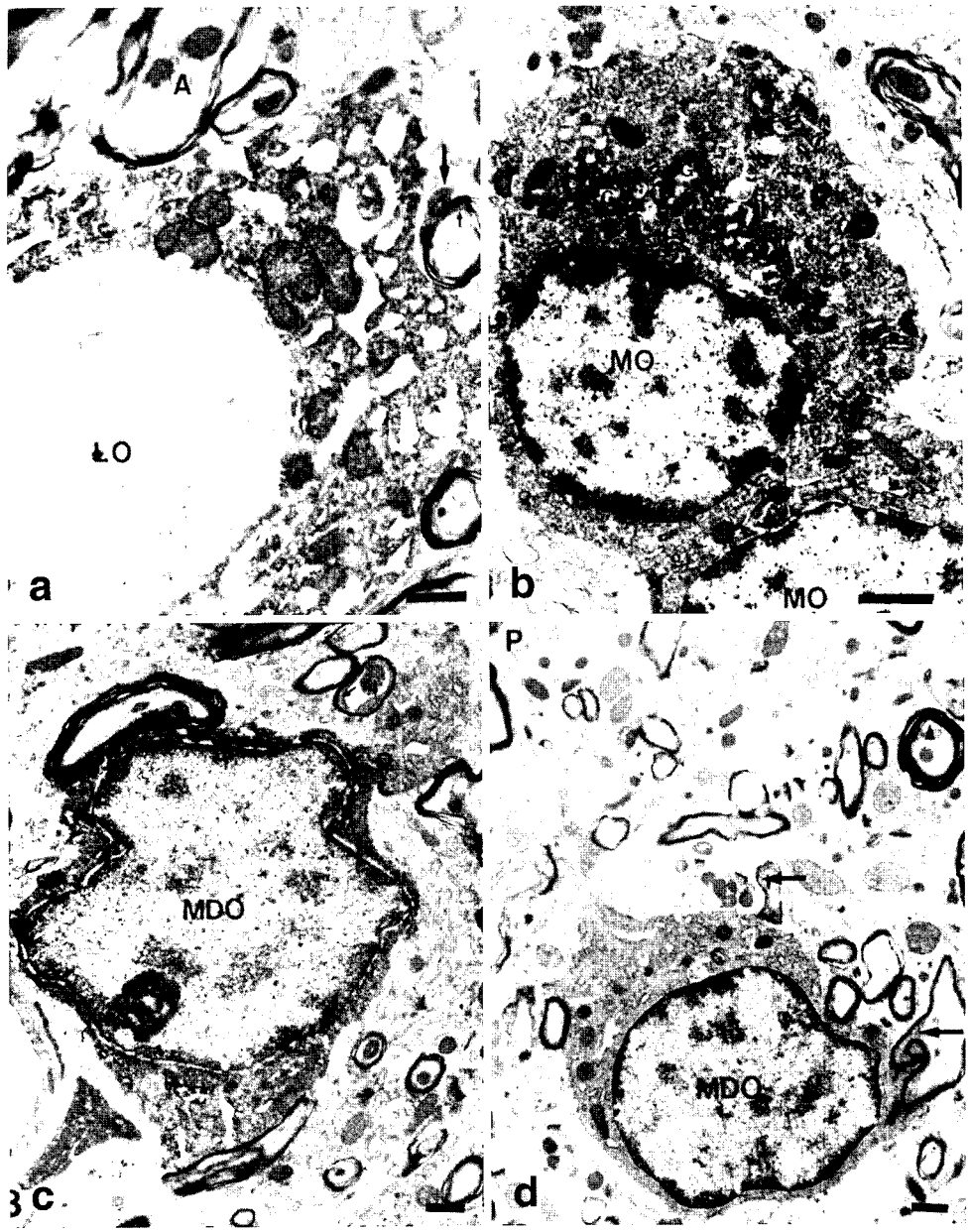

Fig. 5. Immunohistochemical localization of MAG in the adult and aged rat cerebral cortex. **(a-c)** Cell identified as light (LO), and medium (or medium-dark) (MO or MDO) types of oligodendrocytes in the adult. Intense immunostaining is observed throughout the cytoplasm, and is observed on the oligodendroglial membrane associated with myelin sheaths, periaxonal region (small arrows), inner tongue process (small arrows), and outer tongue processes (large arrow). Immunostaining was decreased in the processes of the medium oligodendrocyte (P). Axons (A) and nuclei are devoid of reaction products. **(d-f)** Cells identified as medium (or medium dark) (MO or MDO), and dark types (DO) of oligodendrocytes in the aged cerebral cortex. The diffuse and intense cytoplasmic staining is more obviously decreased. The intense immunostaining was restricted to the perinuclear cytoplasm (white small arrows), however not observed in the processes (P). Immunostaining was observed very lightly (small arrows), or was not observed (arrowheads) in the structures related with axon-myelin system. Nucleus, axons (A) and some inclusion bodies (asterisk) are not stained. Scale bar=500 nm.

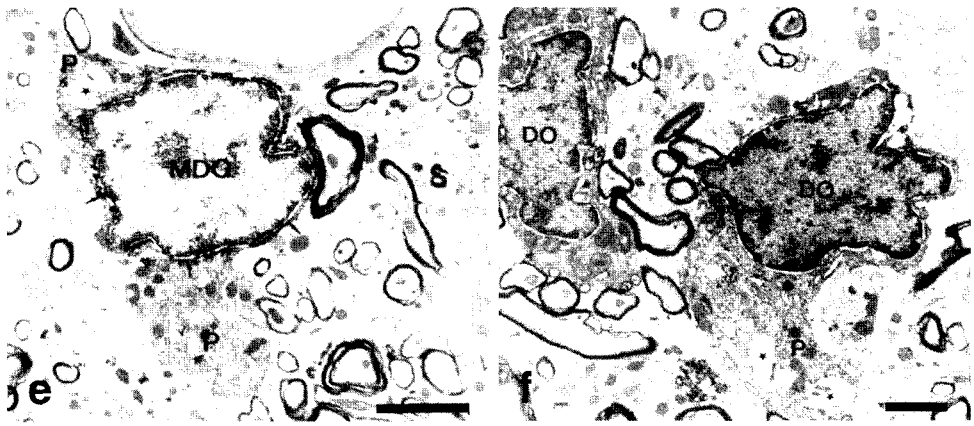

Fig. 5. To be continued.

DISCUSSION

The aging-related role of the MAG in the oligodendrocytes-myeline system has not yet been elucidated. In this study, the possible influence of aging on the morphometric and ultrastructural changes of MAG-(+) oligodendrocytes was analyzed in the cerebral cortex. The results showed that aging produced a decrease in the number of MAG-(+) oligodendrocytes, and that aging induced a significant decrease in the nuclear area of the medium and dark types of MAG-(−) oligodendrocytes, and that aging caused the decrease of the MAG-IR products in the medium and dark type of oligodendroglial cytoplasm and processes. These results suggest that MAG is associated with degeneration of oligodendrocyte-myelin system by aging, and that may contribute to the further understanding of the biology of this protein.

Previous studies of aging-related changes in the oligodendroglial cell population of the cerebral cortex have yielded conflicting results. Oligodendroglial populations were found no changes in the aging rat cerebral cortex (Diamond et al., 1977). The populations of the oligodendrocytes plus microglia showed stability with age in the rat parietal cortex (Peinado et al., 1998). And, studies in monkeys showed an increase with age in oligodendrocytes plus microglia (Peters et al., 1991, 1994). As described above, the studies on the population of oligodendrocytes were rare. In this study, in relation to the cell distribution throughout the thickness of the cerebral cortex, the number of MAG-(+) oligodendrocytes was significantly decreased in most of aged rat cortical layers, particularly, layers IV, V, and VI (Table 1, Fig. 2). However, the number of MAG-(+) oligodendrocytes was increased in layer II-III (CU2). These data suggest that MAG-(+) oligodendrocytes are unstable for effects of aging in dependent on the cortical layers.

It has been suggested that the light and possibly medium types of oligodendtrocytes are actively involved in the production and maintenance of myelin (Sturrock, 1974). However, the dark type of oligodendrocytes have been considered to be the mature non-dividing oligodendrocytes that are formed from the division (Mori & Leblond, 1970; Ling & Leblond, 1973) or the subsequent differentiation of the light and medium types of oligodendrocytes (Imamoto et al., 1978). It has also been previously reported that anti-myelin antiserum labels light and medium oligodendrocytes, but not the dark cells. These results indicate that the morphological classification of oligodendrocytes may be based on varying amounts of myelin antigen synthesis (Roussel &

Nussbaum, 1983). In the white matter of the adult mouse, Sturrock (1976) reported that percentages of light, medium, and dark types of oligodendrocytes were 2.4%, 10.9% and 86%, respectively. Mori and Leblond (1970) identified the percentage were 12.3%. 35.7% and 51.9%, respectively, in the corpus callosum of young rats. Monteiro et al. (1995) reported that type I (medium-dark type) was 93%, and type II (dark type) was 7% in 2 months rat cerebellum, and that type I was 26%, and type II was 74% in 24 months rats. In this study, the percentages of total light, medium and dark oligodendrocytes were 25.0%, 57.7% and 17.3%, respectively in the adult cerebral cortex, and 18.0%, 53.8% and 41.0%, respectively in aged. And, the percentages of the three types of the MAG-(−) oligodendrocytes were 6.6%, 17.3% and 4.6%, respectively in the adult cerebral cortex, and 3.2%, 27.3% and 23.0%, respectively in aged. That is, the percentage of the dark type of oligodendrocytes was significantly increased with aging (Table 2). Results and experimental conditions were not agreed with previous reports, but in aged group the distribution of dark oligodendrocytes was similarly increased to the results by Monteiro et al. (1995). To explain this, it was suggested that oligodendrocytes were capable of undergoing mitosis, and that they were prone to have morphological changes with aging, and that the physiology of oligodendrocytes may be eventually affected by aging. These results suggest that MAG expression may be associated with the morphological changes of the oligodendrocytes with aging.

Light microscopic studies in semithin sections have revealed that oligodendrocytes were classified into light, medium and dark types on the basis of fine morphological features, i.e. the density of nucleus, size of the cell and the development of cytoplasmic organelles in the rat (Ling et al., 1973). In this study, three subtypes of cells were readily identified among the oligodendrocytes of the rat cerebral cortex (Fig. 3). The most obvious difference among three subtypes was their nuclear chromatin patterns, although the cell size and number of fine processes were also useful criteria. The density of cytoplasm was less helpful, as immunoreaction products masked the protoplasm between organelles. However, some difficulty was experienced in deciding whether a cell should be classified as a medium or dark oligodendrocytes, as some transitional types were present. Thus, to avoid possible mistakes in identifying subtype of oligodendrocytes, medium-dark type cells were regarded as medium cells. Recognition of light oligodendrocytes was straight forward and presented no difficulties.

Ling et al. (1973) identified that the mean diameters of the light, medium and dark oligodendrocytes were 7.0 μm, 4.7 μm and 4.3 μm, respectively. And, Monteiro (1995) classified as type I (medium-dark) and type II (dark types) oligodendrocytes, and reported that the mean nuclear diameter of type I and of type II oligodendrocytes, were 7.8 μm and 6.9 μm, respectively at 2 months (Monteiro et al., 1995). In this study, the MNA of total oligodendrocytes ($16.39 \pm 0.14\ \mu m^2$) was significantly reduced in the aged rats compared with that of the adult rats ($19.62 \pm 0.19\ \mu m^2$). And, the MNA of the MAG-(−) oligodendrocytes were significantly decreased in the adult and aged rats compared with that of the MAG-(+) oligodendrocytes (Table 2, Fig. 4). Even though the parameter was different, the trends of the MNA of oligodendrocytes in the same group were similar to the results of previous study (Ling et al., 1973; Monteiro et al., 1995). The decreasing tendency of the nuclear area in the aged groups is thought as results of the mitosis by aging process, and as results of the down regulation of MAG.

Morphological changes in the oligodendrocytes and myelin sheath of the brain may play an important role in impairment of cognitive function (Peter, 2002), and are associated with loss or degradation of myelin proteins and oligodendrocyte proteins, such as MOBP, MBP, MOG, and MAG (Montague et al., 1999; Quarles, 2002). Studies on the brain of MAG-null mice revealed degeneration of the distal periaxonal oligodendroglial

processes in the 8-month-old (Lassmann et al., 1997; Schachner & Bartsch, 2000), and significant reduction in several oligodendroglial proteins like 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNPase), in the 14-month-old, consistent with a dying-back oligodendrogliopathy (Weiss et al., 2000). Down regulation of the expression of myelin genes (MBP, CNPase, MAG, PLP etc.) in oligodendrocytes is a typical feature of inflammatory demyelination diseases (Rodriguez et al., 1994). Interestingly, reduction in the expression of MAG protein has been observed in at least a subgroup of active multiple sclerosis lesions (Itoyama et al., 1980). Another important point which emerges from the present observations is that, at least in the case of MAG expression, the expression of MAG is dramatically decreased in the oligodendrocytes and myelin (Cho et al., unpublished data; Fig. 5) of the normal aged rat cerebrum. The results suggest that the MAG may be required for maintaining the structure and function of oligodendrocyte-myelin system, and that the decrease of MAG expression in the aged rats could be related to the degeneration of oligodendrocytes and myelin in association with demyelinating diseases, and that the changes in axonal morphology may occur.

REFERANCES

Arquint M, Roder J, Chia LS, Down J, Wilkinson D, Bayley H, Braun P, Dunn R: Molecular cloning and primary structure of myelin-associated glycoprotein. Proc Nat Acad Sci USA 84 : 600-604, 1987.

Bartsch U: Myelination and axonal regeneration in the central nervous system of mice deficient in the myelin-associated glycoprotein. J Neurocytol 25 : 303-313, 1996.

Cho IH, Park CH, Lee JH, Bae CS, Ye SK, Lee B, Park SH, Koh KS, Kim JS, Chang BJ: Expression of myelin-associated glycoprotein (MAG) in the aged rat cerebrum. Korean J Electron Microscopy 36(2) : 101-108, 2006.

Diamond MC, Johnson RE, Gold MW: Changes in neuron number and size and glia number in the young, adult, and aging rat medial occipital cortex. Behav Biol 20(4) : 409-418, 1977.

Gard AL, Maughon RH, Schachner M: *In vitro* oligodendrogliotrophic properties of cell adhesion molecules in the immunoglobulin superfamily: Myelin-associated glycoprotein and N-CAM. J Neurosci Res 46 : 415-426, 1996.

Imamoto K, Pterson JA, Leblond CP: Radioautographic investigation of gliogenesis in the corpus callosum of young rats: I. Sequential changes in oligodendrocytes. J Comp Neurol 180 : 115-137, 1978.

Itoyama Y, Sternberger NH, Webster H de F, Quarles RH, Cohen SR, Richardson EP Jr.: Immunocytochemical observation on the distribution of myelin-associated glycoprotein and myelin basic protein in multiple sclerosis lesions. Ann Neurol 7 : 167-177, 1980.

Lassmann H, Bartsch U, Montag K, Schachner M: Dying-Back oligodendrogliopathy: a late sequel of myelin-associated glycoprotein deficiency. Glia 19 : 104-110, 1997.

Li C, Tropak MB, Gerial R, Clapoff S, Abramow-Newerly W, Trapp B, Peterson A, Roder J: Myelination in the absence of myelin-associated glycoprotein. Nature 369 : 747-750, 1994.

Ling EA, Leblond CP: Investigation of glial cells in semithin sections. Variation with age in the numbers of the various glial cell types in rat cortex and corpus callosum. J Comp Neurol 149 : 73-82, 1973.

Montag D, Giese KP, Bartsch U, Martini R, Lang Y, Bluthmann H, Karthigasan J, Kirschner DA, Wintergerst ES, Nave K-A, Zielasek J, Toyka KV, Lipp HP, Schachner M: Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. Neuron 13 : 229-246, 1994.

Montague P, Kirkham D, McCallion AS, Davies RW, Kennedy PG, Klugmann M, Nave K, Griffiths IR: Reduced levels of a specific myelin-associated oligodendrocytic basic protein isoform in shiverer myelin. Dev Neurosci 21(1) : 36-42, 1999.

Monteiro RA, Conceicao LE, Rocha E. Marini-Abreu MM: Age changes in cerebellar oligodendrocytes: the appearance of nuclear filaments and increase in the volume density of the nucleus and in the number of dark cell forms. Arch Histol Cytol 58(4) : 417-425, 1995.

Mori S, Leblond CP: Electron microscopic identification of three classes of oligodendrocytes and a preliminary study of their proliferative activity in the corpus callosum of

young rats. J Comp Neurol 139 : 1-30, 1970.

Mukhopadhyay G, Doherty P, Walsh FS, Crocker PR, Filbin MT: A novel role for myelin-associated glycoprotein as an inhibitor of axonal regulation. Neuron 13 : 757-767, 1994.

Paxinos G, Watson C: The rat brain in stereotaxic coordinates. New York, Academic press, 1986.

Peinado MA, Quesada A, Pedrosa JA, Torres MI, Martinez M, Esteban FJ, Del Moral ML, Hernandez R, Rodrigo J, Peinado JM: Quantitative and ultrastructural changes in glia and pericytes in the parietal cortex of the aging rat. Microsc Res Tech 43(1) : 34-42, 1998.

Peters A: Structural changes that occur during normal aging of primate cerebral hemispheres. Neurosci Biobehav Rev 26(7) : 733-741, 2002.

Peters A, Josephson K, Vincent SL: Effects of aging on the neuroglial cells and pericytes within area 17 of the rhesus monkey cerebral cortex. Anat Rec 229(3) : 384-398, 1991.

Peters A, Leahu D, Moss MB: The effects of aging on area 46 of the frontal cortex of the rhesus monkey. Cereb Cortex 4(6) : 621-635, 1994.

Quarles RH: Myelin sheaths: glycoproteins involved in their formation, maintenance and degeneration. Cell Mol Life Sci 59(11) : 1851-1871, 2002.

Rodriguez M, Prayoonwiwat N, Howe C, Sanborn K: Proteolipid protein gene expression in demyelination and remyelination of the central nervous system: a model for multiple sclerosis. J Neuropathol Exp Neurol 53(2) : 136-143, 1994.

Roussel G, Nussbaum JL: Immunohistochemical study with an anti-myelin serum. A marker for all glial cells except 'dark' oligodendrocytes. J Neuroimmunol 5(3) : 209-226, 1983.

Schachner M, Bartsch U: Multiple functions of the myelin-associated glycoprotein MAG (siglec-4a) in formation and maintenance of myelin. Glia 29 : 154-165, 2000.

Sturrock RR: Histogenesis of the anterior limb of the anterior commissure of the mouse brain. III. An electron microscopic study of gliogenesis. J Anat 117 : 37-53, 1974.

Sturrock RR: Quantitative changes in neuroglia in the white matter of the mouse brain following hypoxic stress. J Anat 121 : 7-13, 1976.

Trapp BD, Andrews SB, Cootauco C, Quarles RH: The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes. J Cell Biol 109 : 2417-2426, 1989.

Weiss MD, Hammer J, Quarles RH: Oligodendrocytes in aging mice lacking myelin-associated glycoprotein are dystrophic but not apoptotic. J Neurosci Res 62(6) : 772-780, 2000.

Weiss MD, Luciano CA, Quarles RH: Nerve conduction abnormalities in aging mice deficient for myelin-associated glycoprotein. Muscle Nerve 24(10) : 1380-1387, 2001.

<국문 초록>

정상적인 노화과정에 있어서 마이엘린연합당단백질(MAG)의 기능을 알아보고자 Sprague-Dawley 계통 흰쥐의 대뇌피질에서 MAG 면역양성반응세포(희소돌기아교세포)에 대한 형태계측학적 및 미세구조적인 분석을 시행하였다. 노화된 흰쥐의 대뇌피질(IV-VI)에서 MAG 면역양성반응세포의 밀도는 정상 흰쥐에 비하여 유의하게 감소하였다. 그러나 medium과 dark 형의 희소돌기아교세포의 비율은 증가하였다. 노화된 흰쥐의 대뇌피질에서 MAG 면역음성반응세포의 핵의 평균면적은 MAG 면역양성반응세포의 핵의 평균면적보다 유의하게 감소하였다. MAG의 면역반응물은 노화된 흰쥐의 대뇌피질의 medium-dark 형의 희소돌기아교세포의 세포질과 돌기에서 뚜렷이 감소하였고 dark 형의 희소돌기아교세포에서는 거의 관찰되지 않았다. 이러한 결과는 노화에 의한 희소돌기아교세포와 마이엘린의 변성은 MAG의 감소와 관계가 있을 것으로 생각되며, 희소돌기아교세포와 마엘린 계통에서 MAG의 기능을 연구하는 기초자료로서 유용할 것으로 사료된다.