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Expression of Myelin-Associated Glycoprotein (MAG) in the Aged Rat Cerebrum

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노화된 흰쥐대뇌에서 Myelin-Associated Glycoprotein (MAG)의 발현

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ABSTRACT

Myelin-associated glycoprotein (MAG) has been known to have a crucial role to the formation of myelin sheath during initial stage of myelination. In the present study, we investigated the aging-related expressional changes of MAG in the rat cerebrum. MAG expression was markedly decreased in cerebral cortex by aging. In the adult rat cerebrum, MAG-positive cells were process-bearing cells with large nucleus, and extensively distributed. However, in the aged rat brain, MAG-positive cells showed small and round morphology with little cytoplasm and few processes. MAG was co-expressed with galatocerebroside, but not with Iba-1, or GFAP. These results suggest that the expressional change of MAG-positive cells is associated with degeneration of oligodendrocyte-myelin system by aging, and that MAG is likely to be a reliable marker for the mature oligodendrocytes in the aged rat brain.

Key words : Aging, Myelin-associated glycoprotein (MAG), Oligodendrocyte

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INTRODUCTION

Myelin-associated glycoprotein (MAG), a heavily glycosylated transmembrane protein, is a member of immunoglobulin (Ig) superfamily with five Ig-like domains (Arquint et al., 1987). MAG is expressed in the cytoplasm and process of oligodendrocytes prior to and during myelination when axons are being enwrapped (Bartsch et al., 1989; Trapp et al., 1989). After formation of myelin, MAG is restricted to the paranodal region of myelin sheaths, and is expressed in the periaxonal region of myelinated axons (Bartsch et al., 1989; Trapp et al., 1989). In the studies of MAG null mutant, the onset of myelination was delayed and morphological abnormalities were detected. Oligodendroglial cytoplasm at the inner aspect of most myelin sheaths was reduced and some axons were surrounded by two or more myelin sheaths (Montag et al., 1994). Based on these reports, it has been suggested that MAG plays a critical role for 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).

Age-related changes in the oligodendrocytes and myelin sheath of the brain may play an important role in the impairment of cognitive function (Peters, 2002). Based on the ultrastructural analysis of the aged monkey, myelin sheaths appear to undergo local splitting of the major dense line, ballooning, formation of redundant myelin, and increase of thickness (Peters, 2000, 2001). In addition, oligodendrocytes show swelling along their processes and gain dense inclusions (Peters, 2002). These morphological changes are associated with loss or degradation of myelin proteins and oligodendrocyte proteins (Kwiecien et al., 1998; Montague et al., 1999; Quarles, 2002). These reports suggest that oligodendrocytes have some morphological changes with aging and reconstruction of myelin.

No study has been devoted so far to the morphological evaluation of possible modifications occurring in aged animals in the expression and synthesis of the MAG, an important components of oligodendrocyte-axon system. Here, we report the results of morphological analyses of MAG in aged rat brain associated with oligodendrocytes and myelin sheath.

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) for adult and aged animals, respectively. For immunoblotting analysis, rats were anaesthetized with inhalation of diethyl-ether, and cerebral cortex was quickly dissected out. For immunohistochemistry, rats were perfused through the ascending aorta with 0.9% NaCl containing heparin (20 IU/mL). This solution was then replaced by cool 4% paraformaldehyde in 0.2 M phosphate buffer (PB, pH 7.4). Brain was removed, and was overnight fixed in the same fixative at 4°C. After soaking the brain in 0.02 M phosphate-buffered saline (PBS, pH 7.4), 20 μm-thick vibratome sections were obtained from the forebrain of the cerebrum through the coronal planes.

Immunoblotting

Cerebral cortex was homogenized in the ice-cold 25 mM tris-HCl buffer (pH 7.5) containing 1 mM EDTA. The homogenates were centrifuged at 25,000 rpm for 20 min, and the supernatant was assayed for protein content. Samples were loaded (20 μg protein/lane) onto 7.5% SDS-polyacrylamide gels and transferred to polyvinyllidne difluoride (PVDF) western blotting membrane (Roche diagnostic Co., Germany) for immunostaining with mouse anti-MAG antibody (1 : 1,000). After incubation with horseradish peroxidase-labeled anti-

mouse IgG antibody (Amersham Pharmacia Biotech, Sweden), the proteins were detected by chemoluminescence (ECL system; Amersham Pharmacia Biotech, Sweden) following the manufacturer's instruction.

Immunohistochemistry

Free-floating sections were treated for 5 minutes with 3% H_2O_2 to suppress endogenous peroxidase activity, and then for 2 hrs with 10% normal horse serum to inhibit non-specific immunoreactions at room temperature (RT). Sections were overnight incubated at 4°C with monoclonal anti-MAG antiserum (1 : 500; Chemicon, U.S.A.). After rinsing with PBS, the sections were incubated with biotinylated mouse IgG for 2 hrs at RT. Sections were washed, and incubated with avidin-biotin complex (Vector Lab., U.S.A.) for 2 hrs at RT. 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) for 10～15 minutes. The immunostained sections were mounted on the gelatin-coated slides, and coverslipped.

For double immunofluorescent staining, floating sections were incubated overnight at 4°C with a mixture of mouse anti-MAG antibody and rabbit anti-galatocerebroside antibody (a cell type-specific marker for oligodendrocyte) (1 : 500; Novus Biologicals, U.S.A) or rabbit anti-Iba-1 (ionized calcium-binding adapter molecule 1) antibody (specific marker for microglia) (1 : 1,000; Wako, Japan) or rabbit anti-GFAP antibody (specific marker for astrocytes) (1 : 10,000: DACO, Denmark). The sections were then incubated for 1 hr at RT with mixture of FITC- and Cy3-conjugated mouse/rabbit IgG antibody (1 : 200; Jackson ImmunoResearch, U.S.A.). The sections were mounted with VectaShield (Vector Laboratories, U.S.A.). The double-stained images were analyzed with confocal laser scanning microscopy (LSM 5 PASCAL; Carl Zeiss, Germany).

Statistics

The Student's t-test was used to verify the change of expression of MAG by aging. Results are expressed as mean±S.E.M. Differences were considered to be significant when P value was less than 0.05.

RESULTS

Decrease of the MAG expression by aging

The monoclonal anti-MAG antibody recognizing the common parts of MAG was used to determine the changes of total MAG expression in the cerebral cortex of normal adult and aged rats. The expression of MAG was shown in Figure 1a. In the cerebral cortex, MAG was clearly detected at high levels, and decreased in the aged rat compared with adult (Fig. 1b).

Distribution of the MAG positive cells in the aged rat cerebrum

To examine the distribution of MAG, vibratome sections from normal adult and aged rat brain were stained with monoclonal anti-MAG antibody. The prominent MAG-labeled cells were found in the cerebrum of adult and aged rat brain, and these were identified as two types. Characteristically process-bearing MAG-positive cells were larger than non-process bearing MAG-positive cells and showed rather irregular morphology compared with the round shaped non-process bearing cells (Fig. 1c-j; Fig. 2).

Process-bearing MAG-positive cells were diffusely distributed throughout cerebral cortex and white mater tract (external capsule, anterior commissure, and axonal tract of striatum) of the adult rats (Fig. 1c, e-g). However, these cells were very rare in the aged rat brain (Fig. 1d, h-j). These cells in the adult rat brain had abundant cytoplasm and many processes, and the nuclei were relatively large and often irregular in shape. Nuclei

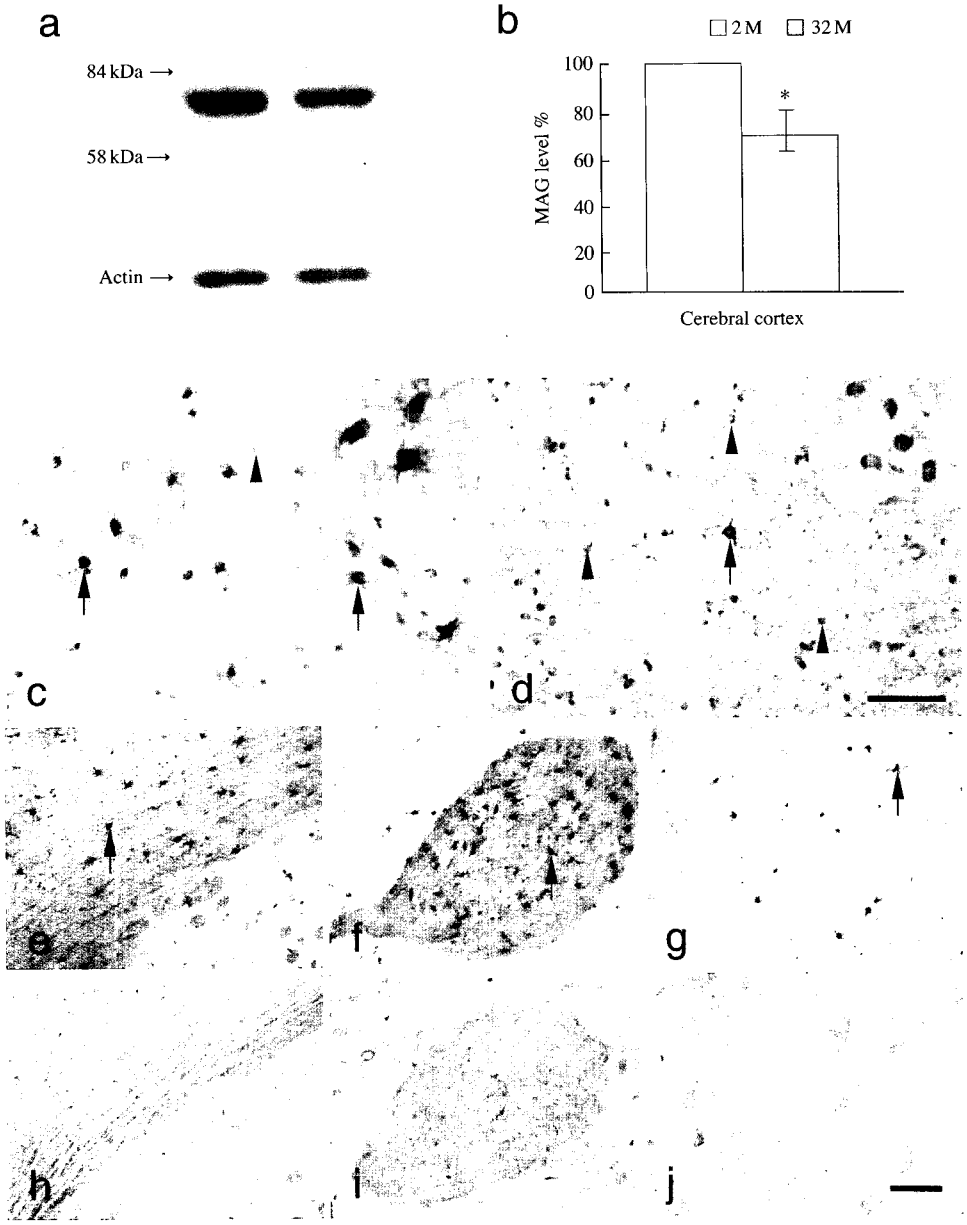

Fig. 1. (a-b) Immunoblotting analysis of adult and aged rat cerebral cortex with anti-MAG antibody. (a) MAG was detected at high levels in the cerebral cortex, but decreased in the aged rat compared with adult. Immunoblotting graph was quantified by densitometry. (1b) The values were expressed as the percentage of MAG in the adult rat cerebral cortex (n=5). Student's t-test, $P<0.01$. **(c)** Coronal section of the cerebral cortex of the adult rats stained with MAG antibody. Process-bearing cells (arrows) were abundant, and non-process-bearing cells (arrow heads) were also present. Inset shows process-bearing cells. **(d)** Coronal section of the cerebral cortex of the aged rat stained with MAG antibody. Process-bearing cells (arrows) are rarely found, however non-process-bearing cells (arrowheads) were abundant. Inset shows non-process-bearing cells. **(e-j)** Section of the white mater tract of the adult and aged rat stained with MAG antibody. MAG positive cells were abundantly appeared in the external capsule (e), anterior commissure (f), and striatum (g) of the adult rats, however were not appeared those of the aged rats (h-j). Scale bar=200 μm.

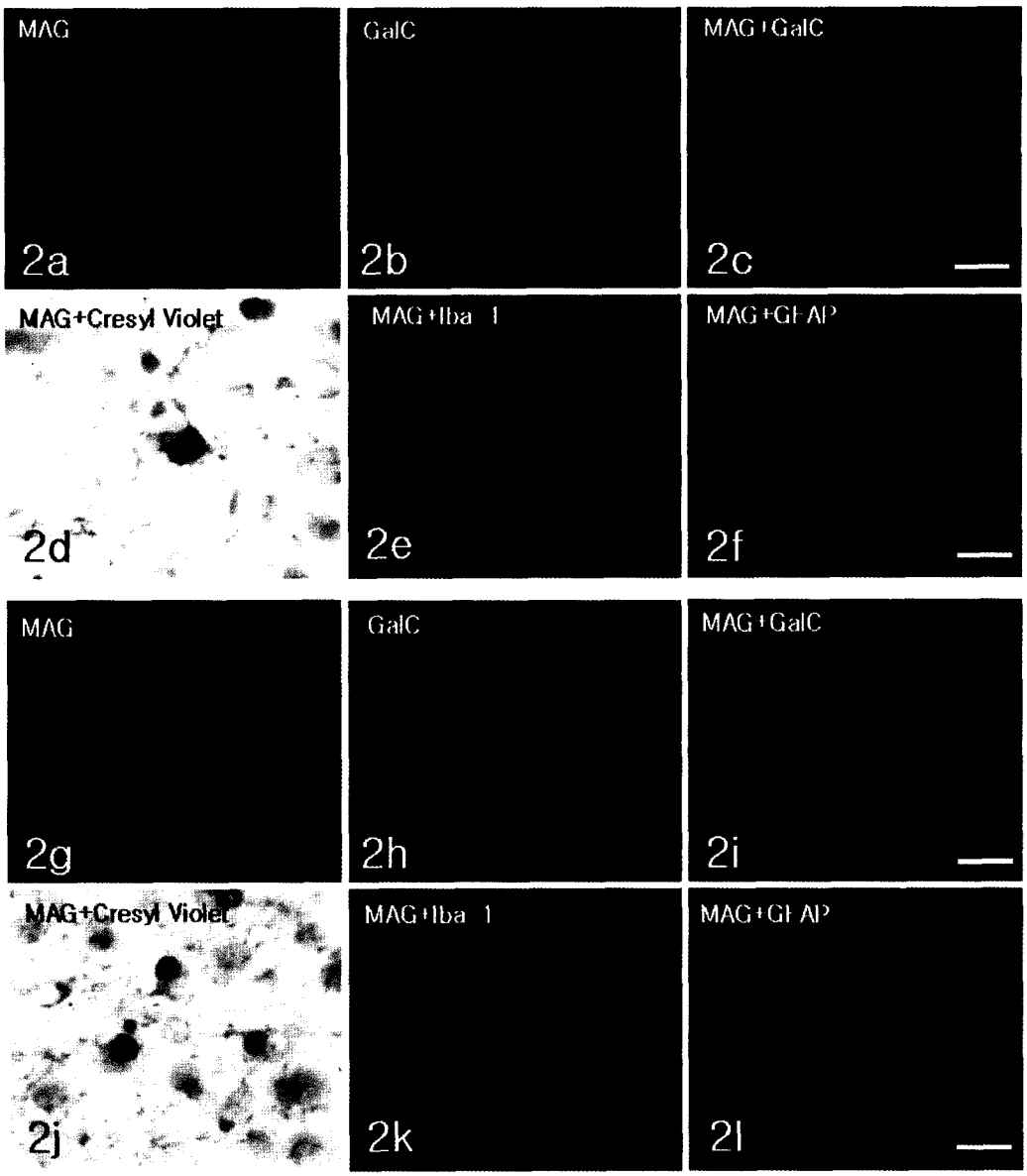

Fig. 2. MAG expression in the oligodendrocytes of the adult **(a-f)** and aged **(g-l)** rats. The MAG immunoreactivity (IR) was found exclusively in the galatocerebroside-IR oligodendrocytes (a-c, g-i), but not in neurons (d, j; counterstained with toluidine blue) or microglia (e, k; merged image; MAG-green, Iba-1-red) or astrocytes (f, L; merged image; MAG-green, GFAP-red). Scale bar=20 μm.

were not clearly stained and usually showed a clear space within the cytoplasm (Fig. 1c inset; Fig. 2a-f). Morphology of these cells resembled galactocerebroside-positive oligodendrocytes (Wolswijk, 2000).

Non-process-bearing MAG-positive cells were abundant in the cerebral cortex of aged rats and also present in the adult animals (Fig. 1c, d). The cytoplasm was little and has no processes, and the nucleus was small

and generally round or oval (Fig. 1d inset; Fig. 2g-l). These cells were supposed to belong to fully matured oligodendrocytes, and the previous study has emphasized it (Wolswijk, 2000). However, these cells were not observed in the white matter tract of the aged rat brain (Fig. 1h-j).

Process-bearing MAG-positive cells were generally occurred singly. However, the other type of MAG-positive cells was frequently seen in groups or distributed in rows in the cerebral cortex (Fig. 1d, white arrows). Compared with normal adult rats, the amount of MAG immunoreactivity was reduced in the aged rat cerebrum. MAG-positive cells were distributed throughout all the cortical laminae in the adult and aged rats, but observed in large quantities in the deeper cortical layers of brain (data not shown).

To determine the characteristic of the MAG positive cells in the aged rat brain, immuno-fluorescence stain was tested. MAG was co-expressed with galatocerebroside, marker for immature and mature oligodendrocytes (Wolswijk, 2000) in the cerebral cortex of the adult and aged rats (Fig. 2a-c, g-i), but not with neurons, or microglia, or astocytes (Fig. 2g-f, j-l), ependymal cells, and connective tissues (data not shown). MAG-positive cells were usually distributed near the neurons (Fig. 2d, j). In the adult rats, the vast majority of the MAG and galatocerebroside positive cells were a relatively large, round cells with pale nucleus, abundant cytoplasm and process. In the aged rats, double-labeled cells of the MAG and galatocerebroside were appeared small, round cells with little cytoplasm and few processes and thus appeared to belong to fully matured oligodendrocytes (Wolswijk, 2000).

DISCUSSION

Recently, studies of MAG associated with the oligodendrogliogenesis and myelinogenesis were reported (Schachner and Bartsch, 2000; Quarles, 2002), however researches of the expression of MAG in the normal aging step after maturation are rare (Sloane et al., 2003). We have studied the expression of MAG in the normal aged rat cerebrum by immunoblotting and immunohistochemical analysis.

The present data obviously show that MAG is expressed in the oligodendrocytes of both adult and aged rat cerebral cortex. MAG-positive cells were appeared as two distinct population of cells, those are, large process-bearing and small non-process-bearing oligodendrocytes. These results suggest that MAG is continuously produced in the oligodendrocytes and MAG is involved in the oligodendroglia-axon interaction.

In the aged rat of this study, most of the MAG-positive cells were non-process-bearing oligodendrocytes in the cerebral cortex (Fig. 2d). MAG immunoreactivity was restricted to the cytoplasm of oligodendrocytes and rarely expressed in the processes and myelinated axons. MAG positive cells were galatocerebroside positive oligodendrocyte (Fig. 2d). MAG was not expressed in the neurons, astrocytes, microglia, ependymal cells, and connective tissue elements (Fig. 2; Itoyama et al., 1980). These results are similar to the expression pattern of myelin/oligodendrocyte glycoprotein (MOG), which is a reliable marker for fully differentiated oligodendrocytes (Scolding et al., 1989; Coffey and McDermott 1997; Wolswijk, 2000). According to the present study, it is suggested that MAG is likely to be a reliable marker for the mature oligodendrocytes in the adult and aged rat brain. Actually, myelin basic protein (MBP) and proteolipid protein (PLP) are not necessarily a reliable indicator of oligodendroglial maturity (Scolding et al., 1989).

Biochemical studies on the brain of 14-month-old MAG null mice showed significant reduction in several oligodendroglial proteins (Weiss et al., 2000). Down regulation of the expression of myelin genes in the oligodendrocytes is a typical feature of inflammatory demyelination diseases (Jordan et al., 1989; Rodriguez et al., 1994). Interestingly, reduction of MAG protein

has also been observed in a subgroup of active multiple sclerosis lesions (Itoyama et al., 1980). In addition to these reports, our present result suggests that MAG might be required for maintaining the structure and function of the oligodendrocytes and myelin in the aging process. In this study, decreased expression of MAG in the cerebral cortex and white matter tracts of the aged rats might be related to the degeneration of oligodendrocytes and myelin in association with demyelinating diseases of the brain.

The present study showed that MAG was obviously expressed in the aged rat brain as well as the adult. The expression of MAG was decreased by aging and this down expression is supposed to be related to the degeneration of oligodendrocytes-myelin system.

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<국문 초록>

신경섬유의 수초화의 초기단계에 있어서 마이엘린의 형성에 중요한 역할을 한다고 알려져 있는 마이엘린연합 당단백질(MAG)이 정상적으로 노화된 흰쥐의 대뇌에서도 발현되는지를 알아보고자 하였다.

성숙흰쥐의 대뇌피질에서 MAG가 높은 농도로 발현되었으나 노화흰쥐의 대뇌피질에서는 유의하게 감소하였다. 대뇌에서 MAG 면역양성반응 세포는 두 성숙흰쥐의 대뇌피질에서 주로 돌기를 가진 큰 세포였으며, 노화흰쥐의 경우에는 주로 세포질과 돌기가 거의 없는 작고 둥근 세포였다. 성숙흰쥐의 백색질내 신경로에서 MAG 면역양성반응 세포는 많이 관찰되었으나 노화흰쥐에서는 거의 관찰되지 않았다. MAG 면역반응은 galatocerebroside의 면역반응과 일치하였다.

이상의 결과로부터 노화에 의한 MAG 발현의 변화는 노화시에 나타나는 희소돌기아교세포와 마이엘린 퇴행성 변화와 관계가 있을 뿐만이 아니라 MAG는 노화시에 희소돌기아교세포의 기능 연구를 위한 적절한 marker로서 사용될 수 있음을 의미하며 앞으로 이에 대한 자세한 연구가 필요할 것으로 사료된다.