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FULL PAPER

Strain differences of cerebral ventricles in mice: Can the MRL/MpJ mouse be a model for hydrocephalus?

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Abstract

Hydrocephalus is an intractable disease characterized by the excessive accumulation of cerebrospinal fluid (CSF) in the cerebral ventricles. There are many cases in both human and animals; however, the cause and mechanism of it's development is not clearly understood. In this study, differences of cerebral ventricles in 5 inbred mice strains (MRL/MpJ, C57BL/6, C3H/He, DBA/2 and BALB/c) were investigated by histological techniques to determine the possibility of a new animal model for hydrocephalus. Our analysis showed that significant differences in the volume and the surface area of lateral ventricles in the 5 inbred strains, with MRL/MpJ mice having the largest lateral, third, aqueduct and fourth ventricles. In addition, when MRL/MpJ mice were compared to BALB/c mice on 0 day after birth, the former already had larger lateral ventricles than the latter. Although there were no significant difference in the ratios of ependymal cell types in MRL/MpJ mice and BALB/c mice, the number and the diameter of lipid droplets in MRL/MpJ mice were, interestingly, smaller than those in BALB/c mice. It is well known that ependymal cells absorb nutritional substances in CSF by endocytosis, suggesting the possibility that their decrease may relate to the larger cerebral ventricles in MRL/MpJ. In conclusion, MRL/MpJ mice have greater volumes in cerebral ventricles than other strains and may be useful for a model showing high susceptibility to hydrocephalus.

Key words: cerebral ventricle, ependymal cells, hydrocephalus, mouse, MRL/MpJ

Introduction

Hydrocephalus is due to blockage of cerebrospinal fluid (CSF) circulation, resulting in excessive accumulation in the cerebral ventricles, which causes convulsions and mental retardation due to increased intracranial pressure. Previous pathological investigation showed that normal drainage of CSF is mainly blocked by obstruction of the cerebral aqueduct originating from tumors, inflammation and/or congenital malformation[23]. However, there are many cases in which the cause is unknown, which complicates the mechanism of hydrocephalus. Hydrocephalus commonly affects

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many mammalian species, including humans, with an estimated incidence of one in 500 births (http://domino.lancs.ac.uk/info/lunews.nsf/r/313a). In the veterinary field, it is a representative disease in canine species, especially the Chihuahua and Yorkshire terrier (http://www.pawfectchihuahuas.com/Hydrocephalus.html). Although hydrocephalus can be ameliorated by injection of drugs such as steroidogenic chemicals or by surgical placement of a catheter from the cerebral ventricles into the peritoneal cavity, the mechanism of it's development needs to be investigated since there are many complications such as side effects derived from drugs and unexpected diseases such as blockage of the shunt and infection.

It has been reported that several genes are implicated in the development of hydrocephalus[23]. In the lineage of human X-linked recessive inheritable hydrocephalic syndrome, abnormality of the *L1 gene* involving a nerve adhesion molecule was previously found[22]. However, this disease is thought to be caused by multiple mutations of several genes, including the *L1 gene*, although some lineages do not contain any abnormality of the *L1 gene*. In experimental animal models of hydrocephalus, there are mouse lines with deletion of the *L1 gene*[9], mice that develop hydrocephalus which were induced by administration of drugs such as kaolin[7], and mutant mice (*hyh* strain) and rats (H-Tx strain) in which they were caused by unknown factors[12,18].

Recently, it has been reported that the functional decrease of ependymal cells lining the walls of cerebral ventricles is partly associated with development of hydrocephalus[4,12,13]. The ependymal cells play some roles in the circulation and absorption of CSF, for example, their apical cilia activate CSF outflow and their microvilli assist CSF absorption via the subventricular region into intracerebral capillaries[10,20,21]. Additionally, recent investigation has reported that wounded ependymal cells could be replaced by differentiation and proliferation of neuroglia located just beneath the ependymal cell layer, and the possibility has been considered that their functional decrease gives rise to the development of hydrocephalus[17].

Since it is hypothesized that various factors are associated with the onset and development of hydrocephalus, the use of previous animal models is not sufficient to analyze the etiology and pathology of inherited hydrocephalus and for the development of effective new medicine. In the present study, we investigated strain differences of cerebral ventricles among several inbred mice strains in an attempt to find a new hydrocephalic model mouse, and herein we report the usefulness of the MRL/MpJ strain.

Materials and Methods

Mice: Adult MRL/MpJ, BALB/c, C57BL/6, C3H/He and DBA/2 mice aged 8 weeks (three or more males and females for each) and infant, MRL/MpJ and BALB/c mice aged 0 days, 1 week, and 3 weeks (three or more of both males and females) were used in the present study. All animals were purchased from Japan SLC (Hamamatsu, Japan) and maintained in our animal facility. In the experimental protocols for animal care and handling, the investigators adhered to the Guide for the Care and Use of Laboratory Animals, Hokkaido University, Graduate School of Veterinary Medicine.

Histological procedure: After weighing, adult ani mals were peritoneally anesthetized with pentobarbital (4.0 mg each, Kyoristu Shoji, Tokyo, Japan), and fixed by vascular perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 20 min, and then the brain was dissected and immersed in the same fixative overnight. After weighing the fixed brains were cut transversally based on the bregma position (crossing of the coronal suture and the sagittal suture), according to the mouse brain atlas (http://www.mbl.org/atlas165/atlas165_frame.html), with a brain slicer (Muromachi Kikai Co., Tokyo, Japan). This cutting plane at the bregma was estimated as position zero, and the rostral and caudal planes were shown

with plus and minus symbols, respectively. A 3-μm thick paraffin section was transversally cut in slices 100 μm in thickness from the rostral to the caudal position, and stained with hematoxylin-eosin.

To examine the strain differences of volume and surface area in the cerebral lateral ventricle, the areas $\{Are\ (i)\}$ and circular lengths $\{Cir\ (i)\}$ of lateral ventricles in all sections were measured by using image-analysis software (Image-J, NIH http://rsbweb.nih.gov/ij/). Finally, the volume and surface area of the lateral ventricle in each animal were estimated by using the following calculations and compared among mouse strains.

$$Volume\ of\ lateral\ ventricle \fallingdotseq \sum_{i=1}^{n}\{Are(i)\times 0.1\ (mm)\}$$

$$Surface\ area\ of\ lateral\ ventricle \fallingdotseq \sum_{i=1}^{n}\{Cir(i)\times 0.1\ (mm)\}$$

Instead of quantitative analysis of the third, aqueduct and fourth ventricles, the degree of dilation was qualitatively estimated by the comparison of adequate section levels in each strain.

MRL/MpJ and BALB/c mice at the day of birth and 3 week-old were sacrificed by CO_2 gas inhalation and then decapitated. The brains were fixed with 4% paraformaldehyde, transferred into graded sucrose and embedded in OCT compound (Tissue-tek, Sakura Finetek Co., CA, USA) for cryosectioning by a routine procedure. Five-micrometer-thick cryosections were made and stained with hematoxylin-eosin.

Ependymal cell types in lateral ventricle: The brains of MRL/MpJ and BALB/c mice aged 8 weeks were fixed by perfusion with 3% glutaraldehyde and then were immersed in 1% osmium tetraoxide. Samples embedded in epoxy resin were made by a routine procedure and double-stained ultrathin sections were examined by electron microscopy (JEM-1210, JEOL, Tokyo, Japan). Based on the observation of more than 50 ependymal cells, the ratios of 6 types of ependymal cells classified according to their morphology were compared between MRL/MpJ and BALB/c mice.

Lipid droplets in ependymal cells: To confirm the existence of lipid droplets in ependymal cells, cryosections obtained from MRL/MpJ and BALB/c mice aged 3 weeks were stained with oil red-O. Briefly, the sections were incubated for 30 min in a mixture of 6 volumes of isopropyl alcohol saturated with oil red-O and 4 volumes of distilled water. For histoplanimetry of lipid droplets, more than 50 ependymal cells obtained by electron microscopy in MRL/MpJ and BALB/c were selected and the maximum diameters of lipid droplets were measured using image-analysis software (Image-J).

Statistical analysis: Results were expressed as mean ± S.E. Student's t-test was used for comparing sex differences ($p < 0.05$). Two-way ANOVA was used for comparing strain differences, and multiple comparisons were performed using Tukey's method when a significant difference was observed ($p < 0.05$).

Results

Strain differences of cerebral ventricles among adult mice

For both sexes, MRL/MpJ mice had the heaviest body and cerebral weights, with significant differences compared with other strains ($p < 0.05$) (Table 1). Sex differences of the weights were detected in some strains, with higher levels in males than in females ($p < 0.05$). The cerebro-body weight ratio in MRL was significantly lower than in other strains ($p < 0.05$). The histology of the lateral ventricles in all strains examined is shown in Fig. 1. Marked dilation of lateral ventricles was observed in MRL/MpJ and followed by C57BL/6 and C3H/He, whereas no dilation was detected in DBA/2 and BALB/c. In all strains, no thinning of the cerebral cortex was caused by ventricular dilation. Both sexes of MRL/MpJ mice had the highest volumes and the surface areas of lateral ventricles, with significant differences in

Table 1. Body weight, cerebral weight, its ratio, volume and surface area in lateral ventricle in all strains examined

		Body weight (g)	Cerebral weight (mg)	Cerebro-body weight ratio (%)	Volume of lateral ventricle (mm^3)	Surface area of lateral ventricle (mm^2)
MRL/MpJ	male	38.1 ± 0.2^{BCDA}	561 ± 5^{BCDA}	1.48 ± 0.02^{BCDA}	0.81 ± 0.16^{BDA}	7.8 ± 0.5^{BDA}
	female	$31.4 \pm 0.8^{*BCDA}$	$520 \pm 6^{*BCD}$	1.66 ± 0.06^{BCDA}	0.77 ± 0.24^{DA}	6.9 ± 1.0^{DA}
C57BL/6	male	21.7 ± 0.3^{MCA}	451 ± 2^{MD}	2.08 ± 0.03^{MCD}	0.23 ± 0.07^{M}	4.0 ± 0.1^{MC}
	female	19.4 ± 0.7^{M}	473 ± 7^{MD}	$2.44 \pm 0.07^{*MD}$	0.39 ± 0.11	4.5 ± 0.5
C3H/He	male	25.4 ± 0.9^{MCD}	437 ± 10^{MA}	1.72 ± 0.06^{MBA}	0.55 ± 0.08^{D}	6.2 ± 0.3^{BDA}
	female	$19.6 \pm 0.2^{*M}$	442 ± 7^{MDA}	$2.25 \pm 0.02^{*M}$	0.37 ± 0.10	5.4 ± 0.5
DBA/2	male	22.1 ± 0.2^{MC}	408 ± 9^{MBA}	1.84 ± 0.04^{MB}	0.12 ± 0.02^{MC}	3.1 ± 0.4^{MC}
	female	$18.6 \pm 0.2^{*MA}$	382 ± 9^{MBCA}	2.05 ± 0.04^{MBA}	0.11 ± 0.03^{M}	3.1 ± 0.2^{M}
BALB/c	male	24.5 ± 0.8^{MB}	479 ± 9^{MCD}	1.95 ± 0.05^{MC}	0.19 ± 0.05^{M}	4.1 ± 0.4^{MC}
	female	$21.2 \pm 0.4^{*MD}$	500 ± 12^{CD}	$2.36 \pm 0.06^{*MD}$	0.07 ± 0.01^{M}	3.4 ± 0.2^{M}

Significant strain differences were confirmed by two-way ANOVA in both sex groups ($p < 0.05$).
*Significantly different from the male of the same strain (Student's t-test. $p < 0.05$).
^{M,B,C,D,A}Significantly different from MRL/MpJ, C57BL/6, C3H/He, DBA/2 and BALB/c, respectively, by Tukey's multiple comparison test ($p < 0.05$).

comparison with DBA/2 and BALB/c ($p < 0.05$). The histology of the third, aqueduct and fourth ventricles is shown in Fig. 2, in all cases the same position was cut transversally, revealing that these cerebral ventricles in MRL/MpJ strain were also larger than those in other strains. In the MRL/MpJ strain, outflow of CSF was not impaired histologically at the interventricular foramen and the aperture of the fourth ventricle.

To clarify from when MRL/MpJ mice possessed large cerebral ventricles, infant animals aged 0 days, 1 week and 3 weeks were examined in comparison with BALB/c mice. As shown in Fig. 3, MRL/MpJ mice had already large lateral ventricles at the day of birth (Fig. 3). These results suggested that dilation of cerebral ventricles in MRL/MpJ mice are controlled by some inherited factors.

Classification of ependymal cells based on ultrastructural characteristics

The ependymal cells that made up the lateral wall of the lateral ventricles in MRL/MpJ and BALB/c mice were examined by electron microscopy. Based on the ultrastructural characteristics, the ependymal cells were classified into 6 types (Fig. 4). Type I cells contained numerous microvilli and cilia including basal bodies in their apical membrane, and showed highly electron-dense cytoplasm because of numerous free ribosomes. Type II cells showed highly electron-dense cytoplasm like Type I, while there were some microvilli, but neither cilia nor basal bodies at the apical cell membrane. Type III cells possessed microvilli and cilia, though the electron density of their cytoplasm was lower than in Type I and II cells. Type IV cells contained microvilli but no cilia at the apical cell membrane, with less electron-dense cytoplasm like Type III cells. Type V cells did not contain microvilli or cilia, and the electron density of their cytoplasm was lower, as in Type III and IV cells. Type VI cells showed highly electron-dense cytoplasm with numerous microvilli but no cilia, and they were identified as so-called tanycytes because of their cytoplasmic processes toward the basal region.

As a result of measuring and classifying cell types in both MRL/MpJ and BALB/c mice, their ratios were found to be similar in the two strains, with the highest value for Type I cells (MRL

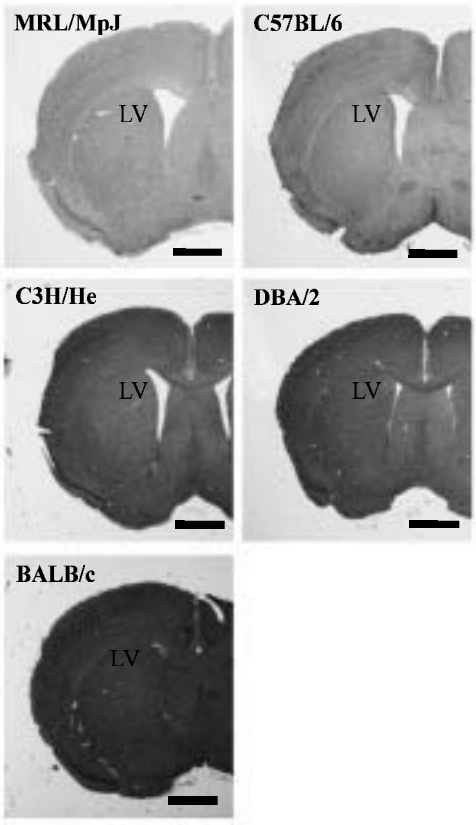

Fig. 1. Transverse sections (plus plane 100 μm from the bregma level) of lateral ventricles (LV) in all strains examined. Marked dilation is observed in MRL/MpJ and followed by C57BL/6 and C3H/He, whereas no dilation is detected in DBA/2 and BALB/c. Hematoxylin-eosin. Scale bars = 1 mm.

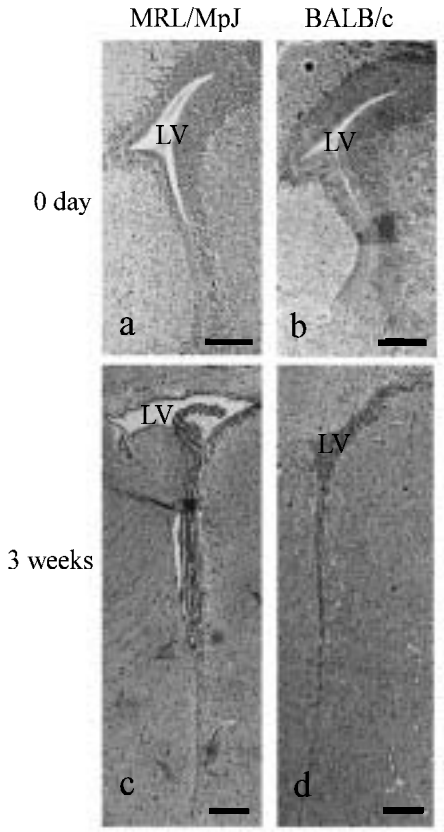

Fig. 3. Transverse sections (plus plane 100 μm from the bregma level) of lateral ventricles (LV) in infant MRL/MpJ (a: 0 day, c: 3 weeks) and BALB/c (b: 0 day, d: 3 weeks). Both aged MRL/MpJ mice show dilation of lateral ventricles in comparison with BALB/c mice. Hematoxylin-eosin. Scale bars = 500 μm.

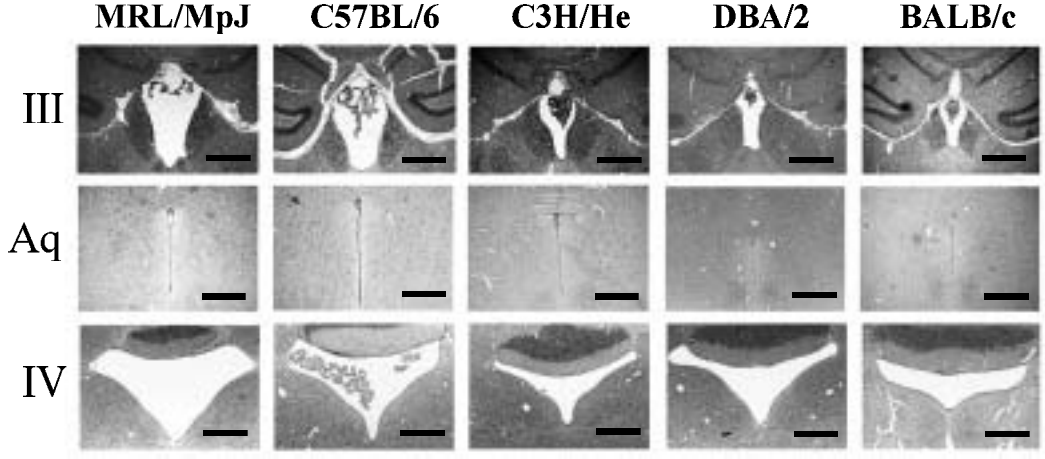

Fig. 2. Transverse sections of the third, aqueduct and fourth ventricles in all strains examined. Sections correspond to minus planes 400, 1200 and 2500 μm from the bregma level, respectively. In all positions, the MRL/MpJ strain has the largest cerebral ventricles. III: Third ventricle, Aq: Aqueduct, IV: Fourth ventricle. Hematoxylin-eosin. Scale bars = 500 μm.

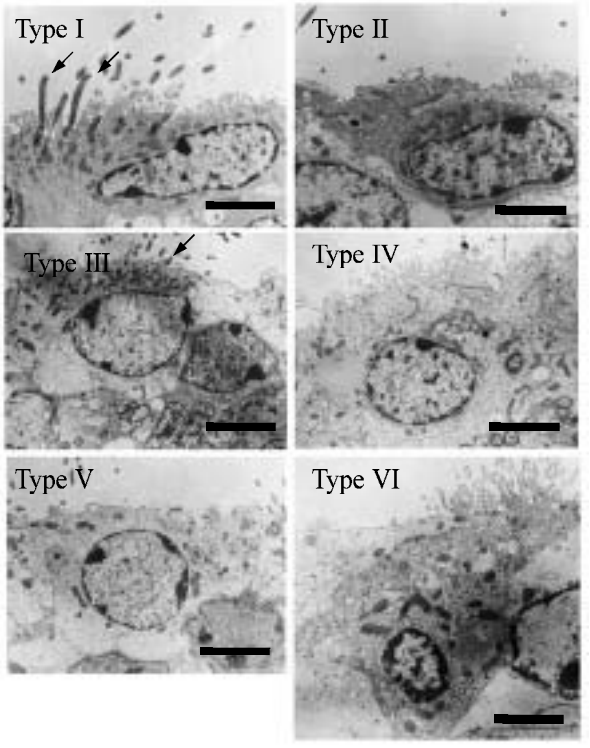

Fig. 4. Electron micrographs of the ependymal cells constructing the lateral wall of the lateral ventricle in MRL/MpJ. Based on the characteristics of electron density in cytoplasm and existence of cilia (arrows) in the apical membrane, the ependymal cells are classified into 6 types. The similarity of classification of cell types (Types I～VI) in MRL/MpJ and BALB/c is as follows: 75.8 ± 4.7%, 13.3 ± 2.2, 3.1 ± 2.6, 1.6 ± 0.4, 3.9 ± 2.4 and 2.3 ± 1.4 in MRL/MpJ, and 76.8 ± 3.0, 15.2 ± 0.1, 2.7 ± 0.9, 0.9 ± 0.8, 2.7 ± 0.8 and 1.8 ± 0.1 in BALB/c, respectively. Scale bars = 5 μm.

75.8 ± 4.7%, BALB 76.8 ± 3.0%) and the lowest for Type IV cells (MRL 1.6 ± 0.4%, BALB 0.9 ± 0.8%).

Comparison of lipid droplets in ependymal cells

By the oil red-O staining of cryosections, lipid droplets were detected in the ependymal cells (Fig. 5). Electron microscopically, they varied in size and showed homogeneous and electron-lucent contours. Histoplanimetrically, the ependymal cells in BALB/c mice contained significantly more lipid droplets than those in MRL/MpJ mice (MRL/MpJ 0.28 ± 0.08, BALB/c 0.12 ± 0.01. This value is the number of lipid droplets observed in each

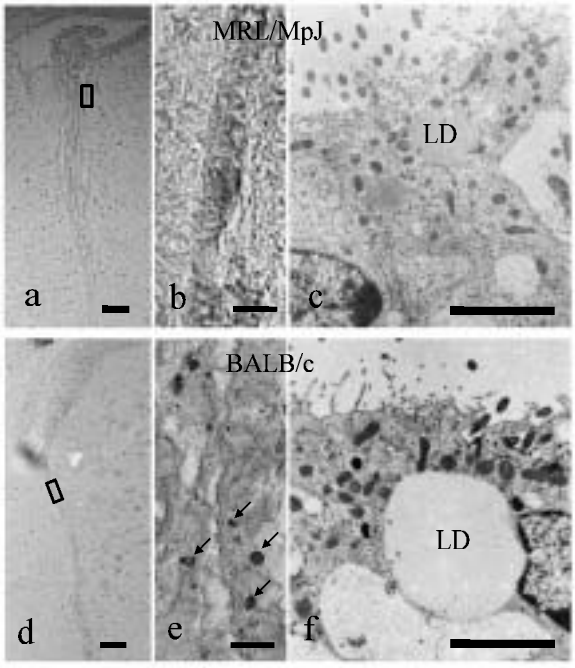

Fig. 5. Comparison of lipid droplets in the ependymal cells of MRL/MpJ (a, b, c) and BALB/c (d, e, f) (a and d: low magnification, b and e: enlargement of box in low magnification, c and f: electron micrograph). Light microscopically, the droplets stained with oil red-O are detected only in BALB/c (e, arrows). Electron microscopically, lipid droplets are smaller in MRL/MpJ. Scale bars: a and d = 200 μm, b and e = 10 μm, c and f = 5 μm.

ependymal cell). Additionally, the average lipid droplet diameter was larger in BALB/c than that in MRL/MpJ (MRL/MpJ 1.60 ± 0.20 μm, BALB/c 2.15 ± 0.03 μm). Lipid droplets more than 3 μm diameter were observed only in BALB/c mice. These results suggested that lipid droplets in ependymal cells of MRL/MpJ mice were fewer and smaller than those in BALB/c.

Discussion

Strain differences in cerebral ventricles

In the present study, it was noted that MRL/MpJ had the largest cerebral ventricles, including not only on the lateral ventricle but also the third, aqueduct and fourth ventricles among the strains examined. This is the first report indicating that

all cerebral ventricles in MRL/MpJ mice are larger than those in other major inbred strains, though a previous report showed the large lateral ventricle in MRL/MpJ compared with that in CD-1 mice[3]. Sex differences in lateral ventricles of the mouse strains were not clearly detected in the present study, as was the case in previous reports in humans[1,2,6]. In the present study, the fact that the individual differences in the volume and surface area of the lateral ventricle were markedly large may indicate the possibility of physiological differences such as in the blood glucose concentrations, which is reported to change the volume of cerebral ventricles in mice and humans[15,19].

Classification of cell types constructing cerebral ventricles

The ependymal cells lining the wall of lateral ventricle were classified into 6 types, Type I and III cells containing cilia and microvilli, and Type II, IV, V and VI cells possessing only microvilli. These classifications corresponded to those in previous reports[8,17]. The cilia in ependymal cells are strongly associated with the regulation of CSF circulation[10]. Additionally, these 6 types of cells were classified by electron density in the cytoplasm, electron-dense Type I, II and VI cells, and electron-lucent Type III, IV and V cells. It is reported that neuroglia located just beneath the ependymal cells play an important role for regeneration of ependymal cells[17]. When the ependymal cells are regenerated under normal conditions in mice, these neuroglia, which have electron-lucent cytoplasm like the Type III, IV and V cells observed in the present study, are actively transformed to ependymal-like cells after invading the ependymal cell lining.

The MRL/MpJ strain is known to have several unique characteristics in regenerative wound healing such as those observed in ear punch closure and in cardiomyocyte regeneration[5,16]. Although we started this research considering the possibility that the function of ependymal cells was related to this regenerative ability possessed in MRL/MpJ mice, there was no significant difference in cell proportions between MRL/MpJ and BALB/c mice. Therefore we attempted to examine and compare the cell organella in ependymal cells between MRL/MpJ and BALB/c, and finally found a difference of lipid droplets, the latter mice having more and larger ones than the former. In ependymal cells, it is known that transthyretin, a transporter for retinol, is taken from CSF up to vacuoles by endocytosis[14]. The present results led us to assume that the functional changes of ependymal cells would affect the volumes of cerebral ventricles in mice. It is thus necessary to investigate the direct relationship between the volumes of cerebral ventricles and the lipid catabolism in ependymal cells by using many mouse strains, including MRL/MpJ.

MRL/MpJ mouse as an animal model for hydrocephalus?

In the present study, although the MRL/MpJ mouse strain was found to have the largest cerebral ventricles among the strains examined, MRL/MpJ showed neither the rarefactions of cerebral cortex nor clinical symptoms due to large cerebral ventricles. In addition, the lateral ventricle in MRL/MpJ already had large lumens at the day of birth compared with other strains. In a previous report, it was found that the cerebral ventricles in C57BL/6 mice were larger than those in A/J mice, suggesting by linkage analysis that the causative genetic loci were major quantitative trait loci controlling variance in volume on chromosome 8 near the markers *D8Mit94* and *D8Mit189*[24]. Additionally, strong epistatic interaction affecting ventricular volume between loci on chromosome 4 (near *D4Mit237* and *D4Mit214*) and on chromosome 7 (*D7Mit178* and *D7Mit191*) was also detected. From these findings, we considered that the large cerebral ventricles of MRL/MpJ were one of physiological and innate phenotypes and had multiple causative factors relating to alter the cerebral ventricular volumes.

Interestingly, a previous study reported that C57BL/10, which is a closely related to C57BL/6, showed the dilations of lateral ventricles in methylmercury induced hydrocephalus, but DBA/2 failed

to develop this disease[11]. In the present study, not only MRL/MpJ but also C57BL/6 and C3H/He tend to have a large cerebral ventricles compared to DBA/2 and BALB/c strains. Therefore, strain differences in volumes of cerebral ventricles may affect the susceptibility and/or severity to hydrocephalus, especially, MRL/MpJ mice may have a key to elucidate these pathological mechanisms.

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