Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 349 (2006) 122–130
BBRC
www.elsevier.com/locate/ybbrc

Scrapie infection activates the replication of ecotropic, xenotropic, and polytropic murine leukemia virus (MuLV) in brains and spinal cords of senescence-accelerated mice: Implication of MuLV in progression of scrapie pathogenesis

Kyung-Hee Lee [a,1], Byung-Hoon Jeong [a,1], Jae-Kwang Jin [a], Harry C. Meeker [b], Jae-Il Kim [b], Richard I. Carp [b], Yong-Sun Kim [a,*]

[a] *Ilsong Institute of Life Science, Hallym University, 1605-4 Gwanyang-dong, Dongan-gu, Anyang, Kyounggi-do 431-060, South Korea*
[b] *New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, USA*

Received 26 July 2006
Available online 11 August 2006

Abstract

Senescence-accelerated mice (SAMP8) have a short life span, whereas SAMR1 mice are resistant to accelerated senescence. Previously it has been reported that the Akv strain of ecotropic murine leukemia virus (E-MuLV) was detected in brains of SAMP8 mice but not in brains of SAMR1 mice. In order to determine the change of MuLV levels following scrapie infection, we analyzed the E-MuLV titer and the RNA expression levels of E-MuLV, xenotropic MuLV, and polytropic MuLV in brains and spinal cords of scrapie-infected SAM mice. The expression levels of the 3 types of MuLV were increased in scrapie-infected mice compared to control mice; E-MuLV expression was detected in infected SAMR1 mice, but only in the terminal stage of scrapie disease. We also examined incubation periods and the levels of PrP^{Sc} in scrapie-infected SAMR1 (sR1) and SAMP8 (sP8) mice. We confirmed that the incubation period was shorter in sP8 (210 ± 5 days) compared to sR1 (235 ± 10 days) after intraperitoneal injection. The levels of PrP^{Sc} in sP8 were significantly greater than sR1 at 210 ± 5 days, but levels of PrP^{Sc} at the terminal stage of scrapie in both SAM strains were virtually identical. These results show the activation of MuLV expression by scrapie infection and suggest acceleration of the progression of scrapie pathogenesis by MuLV.

Keywords: Murine leukemia virus; Aging; Senescence-accelerated mouse; Scrapie; Prion

The transmissible spongiform encephalopathies (TSEs), also referred to as prion diseases, are fatal degenerative diseases of the central nervous system (CNS) characterized by loss of motor control, dementia, and paralysis. Infectious, inherited, and sporadic forms of these diseases are all due to conformational conversion of a normal cell-surface glycoprotein called PrP^{C}, expressed in neurons and glia, to a protease-resistant isoform termed PrP^{Sc} [1]. The process of conversion has been postulated to require the binding of PrP^{C} to a still unidentified "protein X" before its conversion to PrP^{Sc} can occur [2]. The conversion process is the key molecular event in the pathogenesis of prion diseases, but the mechanism of conversion is poorly understood.

The prion protein can interact with a number of molecules, including sulfated glycans [3], large nucleic acids [4–6], and possibly with retroviral RNA [7]. Recently, there were reports that nucleic acids can induce structural changes in the prion protein and act as a cofactor for its conversion to the polymeric structure [5]. Also, DNA converts cellular prion protein into the β-sheet conformation [8] and RNA molecules stimulate prion protein conversion [9]. Furthermore, prion protein induces dimerization of RNA and has DNA strand transfer properties similar to retroviral nucleocapsid protein [10,11]. PrP can be recov-

* Corresponding author. Fax: +82 31 388 3427.
E-mail address: yskim@hallym.ac.kr (Y.-S. Kim).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.08.016

ered in association with retroviral particles [7,12,13]. The possible involvement of retroviruses in prion disease has been suggested previously [7,14].

Scrapie infection of a senescence-accelerated mouse strain (SAMP8) caused an increase of MuLV titer [15]; the extent of the increase was a function of the scrapie strain used, suggesting that the informational molecule of different scrapie strains interacts differentially with replication of the retrovirus [16]. In another study [17], it was shown that strains with high titers of MuLV in brain, SAMP8, and AKR, had shorter incubation periods than a strain without brain MuLV, SAMR1.

These observations prompted us to investigate the relationship between scrapie disease and the activation of endogenous retrovirus replication. In the present study, we analyzed the expression levels of endogenous ecotropic, xenotropic, and polytropic MuLVs in scrapie-infected SAM mice, and examined the incubation period and the level of PrP^{Sc} in scrapie-infected SAMR1 and SAMP8 mice. Also, we analyzed the cellular localization of MuLV and $PrP^{C/Sc}$.

Materials and methods

Animals. SAMR1 and SAMP8 mice were originally obtained from Dr. T. Takeda (Kyoto University, Kyoto, Japan) and have been maintained as inbred strains in Korea Ginseng and Tobacco Research Institute (Daejun, South Korea).

Scrapie strains and injection procedures. Control inoculum was prepared from normal brains of SJL mouse (NMB). Mice were divided into control and infected groups. Mice were inoculated by the intraperitoneal route with 100 μl of 2% brain homogenate in 0.01 M phosphate-buffered saline (PBS). After inoculation with the 139A scrapie agent, mice developed clinical symptoms that included initial hyperactivity, followed by tremor, paresis, and ataxia. Incubation period was defined as the time when complete hindlimb paralysis was seen. Animals were sacrificed at the terminal stage of disease. Brains were removed and put on ice and then stored at −70 °C prior to use. NMB-injected control mice were age-matched to scrapie mice.

Tissue preparation. Mice were sacrificed by cervical dislocation and brains were removed rapidly and stored frozen at −70 °C for Western blots and RT-PCR. For immunostaining, the animals were sacrificed under 16.5% urethane at the terminal stage of disease. Age-matched control mice had been inoculated with NMB. The animals were perfused transcardially with cold PBS followed by 4% paraformaldehyde in PBS. The brains and spinal cords were immediately removed, postfixed in the same fixative overnight at 4 °C, rinsed with PBS, dehydrated with ethanol, and then embedded in paraffin.

RT-PCR analysis. Total RNA from NMB and 139A scrapie-infected mouse brains and spinal cords was isolated by single-step guanidinium thiocyanate phenol–chloroform method [18]. RT-PCR was performed as described [19]. cDNA was synthesized from 2 μg of total RNA by reverse transcription using AMV reverse transcriptase (Promega, USA) and oligo(dT) primer. After 1-h incubation at 42 °C, the reaction was heat-inactivated and kept at 4 °C until use. An aliquot (5 μl) of cDNA of each sample was used for PCR with primers for either ecotropic, xenotropic, or polytropic MuLV or glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene. Total PCR mixtures were subjected to electrophoresis in 1.8% agarose gels including ethidium bromide. Separated PCR products were visualized under U.V. illuminator. The PCR primers used are shown in Table 1.

XC-1 UV plaque assay for ecotropic MuLV (E-MuLV). In order to quantitate the titers of MuLV present in brain and spinal cord from the SAM mice, tissues were homogenized at 10% (w/v) in DMEM by 20 strokes in ground glass tissue homogenizers. E-MuLV was quantified using the XC/UV plaque assay as previously described [16,20].

Western blot analysis. Whole brains or spinal cords were homogenized with a Teflon-glass homogenizer in 20 volumes of 1.15% KCl-10 mM phosphate buffer with 5 mM EDTA solution (pH 7.4). Protein was measured by the BCA protein assay kit (Pierce, USA). For each sample, 30 μg of total protein was separated on 13% SDS–PAGE and transferred to nitrocellulose membrane. The membranes were blocked with 5% skim milk in PBST (phosphate-buffered saline, 0.5% Tween 20) for 1 h and incubated with mouse monoclonal anti-PrP antibody 10E4 (1:5000), kindly provided by Dr. Richard Rubenstein (New York State Institute for Basic Research, Staten Island, New York, USA) [21], for 1 h at room temperature. The blots were incubated with secondary antibody conjugated with horseradish peroxidase for 1 h at room temperature. The visualization of blots was performed by using ECL detection system (Amersham, USA). At the end of each step, the membranes were washed three times for 10 min with PBST.

Immunohistochemical analysis. Immunohistochemical procedures were carried out using the ABC kit (Vector, USA) by a modification of the avidin–biotin–peroxidase method. Briefly, sections (6 μm) of the brain were deparaffinized with xylene, hydrated with ethanol, and then treated with 0.3% (v/v) hydrogen peroxide in methyl alcohol for 20 min to block endogenous peroxidase. After three washes in PBS buffer, the sections were exposed to normal horse/donkey serum for 1 h at room temperature, and then incubated with antibody raised against the MuLV P30 protein (1:100, goat antibody to Rausher virus p30 antigen, Quality Biotech, Inc., USA) or anti-PrP monoclonal antibody 10E4 (1:200) overnight at 4 °C. After washing, the sections were treated sequentially with biotinylated anti-goat or anti-mouse immunoglobulin and avidin–biotin–peroxidase complex. Diaminobenzidine–hydrogen peroxide solution (0.003% 3,3-diaminobenzidine and 0.03% hydrogen peroxide in 0.05 M Tris buffer) was used to visualize antibody boundary, and finally the sections were counterstained with hematoxylin. For PrP^{Sc} staining, the sections were treated with proteinase K (PK, 10 μg/ml) for 7 min at room temperature and the same procedures described above were followed.

Table 1
Oligodeoxyribonucleotide primer sequences used for RT-PCR

Primer names		Sequence 5′ → 3′	Size (mer)	Product size (bp)
Ecotropic	Sense	ATGGAGAGTACAACGCTCTCA	21	605
MuLV	Antisense	GAGGTTAGATTGTTGCTTACTG	22	
Xenotropic	Sense	GGACGATGACAGACACCTTC	20	250
MuLV	Antisense	TGATGATGGCTTCCAGTATG	20	
Polytropic	Sense	TGGGGACAATGACCGATG	18	664
MuLV	Antisense	CCTGTCTCCCGTCCCAGGTTG	21	
GAPDH	Sense	TGGTATCGTGGAAGGACTCATGAC	24	189
	Antisense	ATGCCAGTGAGCTTCCCGTTCAGC	24	

Results

Incubation period of scrapie was shorter in scrapie-infected SAMP8 mice compared to scrapie-infected SAMR1 mice

SAMP8 mice have high levels of endogenous E-MuLV in their brains, and the brains of SAMR1 mice have little or no virus. In order to investigate whether scrapie incubation periods in SAMR1 and SAMP8 mice differ, the two strains were inoculated with 139A scrapie strain by the intraperitoneal route. This experiment showed that while SAMP8 mice were clinically positive at 210 ± 5 days, SAMR1 mice were positive at 235 ± 10 days (Table 2). It appears that in this limited set, E-MuLV replication correlates with the progression of scrapie pathogenesis.

The expression levels of the three types of MuLV were increased in brains of scrapie-infected mice

Infectious E-MuLV was increased in brains of scrapie-infected SAMP8 mice compared to uninfected mice as analyzed by plaque assay (Fig. 1A). To assess the effect of scrapie on MuLV expression, we analyzed the expression levels of ecotropic, xenotropic, and polytropic MuLV RNAs in scrapie-infected SAM mice ($n = 5$) and control mice ($n = 5$) by RT-PCR. For the RT-PCR, we sacrificed all SAMP8 mice when scrapie-infected SAMP8 mice were at the terminal stage and all SAMR1 mice when scrapie-infected SAMR1 mice were at the terminal stage. Expression of the three types of MuLV was increased in the brains of scrapie-infected SAMR1 and SAMP8 mice compared with control mice (Fig. 1B). At the incubation time when scrapie-infected SAMP8 mice were at the terminal stage, scrapie-infected SAMR1 mice had not yet shown clinical symptoms. At that time, expression of E-MuLV in scrapie infected SAMR1 was not detected (data not shown). In contrast, we found that expression of E-MuLV was detected in scrapie-infected SAMR1 mice at the terminal stage (Fig. 1B). The fact that the level of E-MuLV mRNA is significantly higher in scrapie-infected SAMP8 than SAMR1 is related to the absence of the Akv provirus in SAMR1 mice [22]. These data show that the expression levels of the three types of MuLV were increased in scrapie-infected mice showing clinical disease.

Table 2
Incubation period in days to terminal stage in SAMR1, SAMP8, scrapie-infected SAMR1 (sR1), and scrapie-infected SAMP8 (sP8) mice after intraperitoneal injection of the 139A scrapie strain

Strains	Terminal stages (days)
R1 ($n = 10$)	Over 300
P8 ($n = 10$)	Over 300
sR1 ($n = 10$)	235 ± 10
sP8 ($n = 10$)	210 ± 5

Expression levels of MuLV increased in spinal cords of scrapie-infected mice

In clinically positive scrapie-infected SAMP8 and SAMR1 mice, we observed hindlimb paralysis to a greater extent than in other infected mouse strains. This finding led us to analyze E-MuLV infectivity and the levels of MuLV expression in spinal cord. Infectious E-MuLV has increased in spinal cords of scrapie-infected SAMP8 mice (Fig. 1C). Regarding expression levels, ecotropic, xenotropic, and polytropic MuLVs were increased in spinal

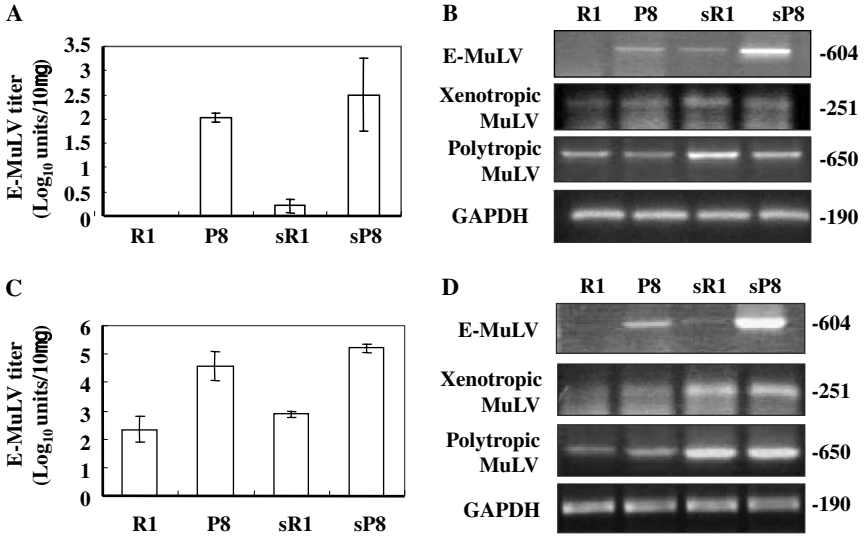

Fig. 1. E-MuLV titers in brains (A) and spinal cords (C) of SAMR1 (R1), SAMP8 (P8), scrapie-infected SAMR1 (sR1), and scrapie-infected SAMP8 (sP8). All mice were sacrificed at the terminal stage of scrapie clinical disease. RT-PCR analysis was used to determine the levels of MuLV mRNA in the brains (B) and spinal cords (D) of control and scrapie-infected mice. Both scrapie-infected SAMR1 and SAMP8 mice were sacrificed at terminal stage of scrapie. Age-matched uninfected SAM mice were used for comparison. As control, RT-PCR products were normalized by comparison to expression of the constitutive GAPDH gene.

cords of both scrapie-infected SAM strains compared to levels in uninfected SAM strains (Fig. 1D), similar to the increases shown for brain in Fig. 1A. A surprising finding was the much higher titers of infectious E-MuLV in spinal cord than in brain samples; the higher titers were seen in all groups.

E-MuLV immunostaining was detected predominantly in neurons of brain stem and spinal cords from scrapie-infected SAM mice

To characterize the expression and cellular localization of E-MuLV, we did immunohistochemical analysis using sections of brain and spinal cord from SAMRl, SAMP8, scrapie-infected SAMR1, and scrapie-infected SAMP8 mice. As shown in Fig. 2, the distribution of E-MuLV staining was correlated with the brain lesions in SAMP8 mice but there was no positive staining in uninfected SAMR1. As shown in Fig. 2, E-MuLV immunoactivity was detected in the brain stem and spinal cords of scrapie-infected SAMR1, as well as uninfected and scrapie-infected SAMP8 mice. The staining was seen primarily in neurons, however, a few positive reactive astrocytes were also present in infected SAMP8 and SAMR1 and in uninfected SAMP8 (Figs. 2B–D and I). These results show that E-MuLV is present predominantly not only in neurons of scrapie-infected SAMR1 and SAMP8 mice, but can also be found in reactive astrocytes in these groups.

Comparison of the levels of PrP^{Sc} in scrapie-infected SAMP8 mice and scrapie-infected SAMR1 mice

We detected PrP^{Sc} in the brains of scrapie-infected SAMR1 and SAMP8 mice. When scrapie-infected SAMP8 mice were at the terminal stage (210 days), the levels of PrP^{Sc} were significantly greater than in scrapie-infected SAMR1 mice (Fig. 3) at the same day after infection. However, at the terminal stage in SAMR1 mice (235 ± 10 days), PrP^{Sc} was present at comparable levels in the brains of

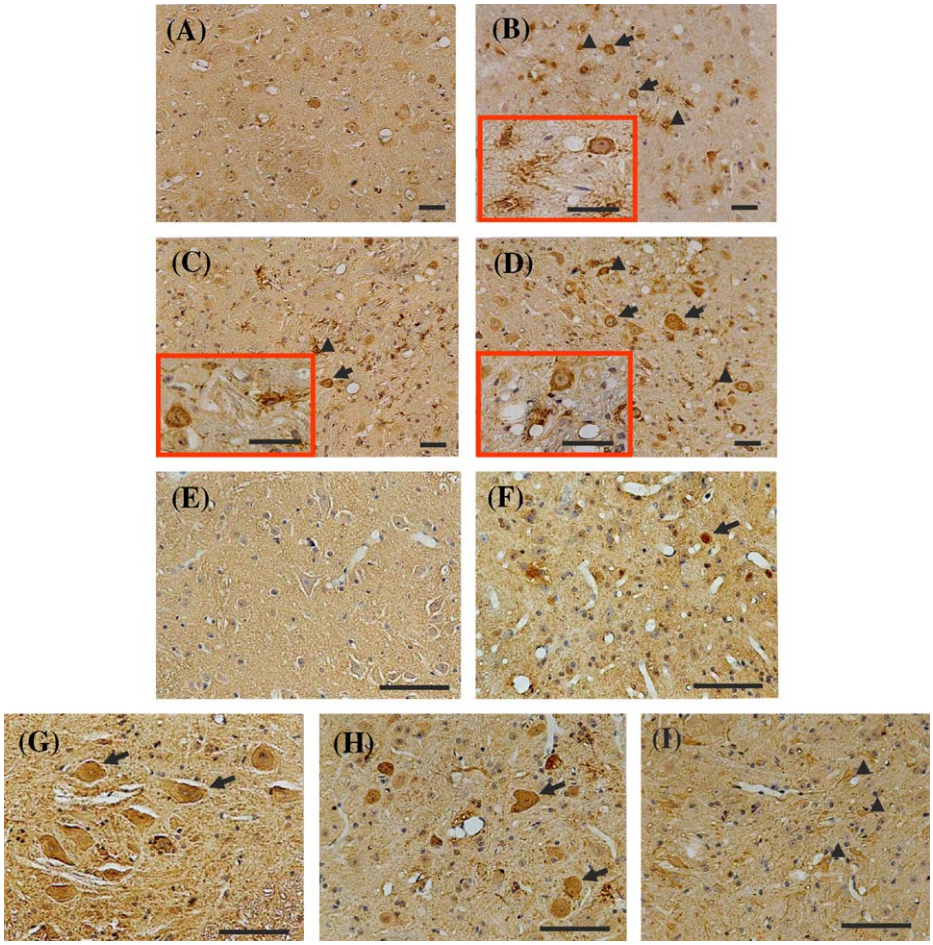

Fig. 2. Immunohistochemical staining of E-MuLV P30 antigen in thalamus region (A–D) and spinal cords (E–I) of control and scrapie brains. SAMR1 mice (A,E), SAMP8 mice (B,F), scrapie-infected SAMR1 mice (C,G), and scrapie-infected SAMP8 mice (D, H, and I). Note that MuLV immunoreactivity is present in neurons (arrows) and activated astrocytes (arrowheads) of uninfected SAMP8, scrapie-infected SAMR1 and scrapie-infected SAMP8. Neurons are immunostained more intensely than astrocytes. Both scrapie-infected SAMR1 and SAMP8 mice were sacrificed at terminal stage of disease. Age-matched uninfected SAMR1 and SAMP8 mice were used for comparison. Each insert in B, C, and D is a higher magnification of MuLV-positive cells. Scale bar = 10 μm.

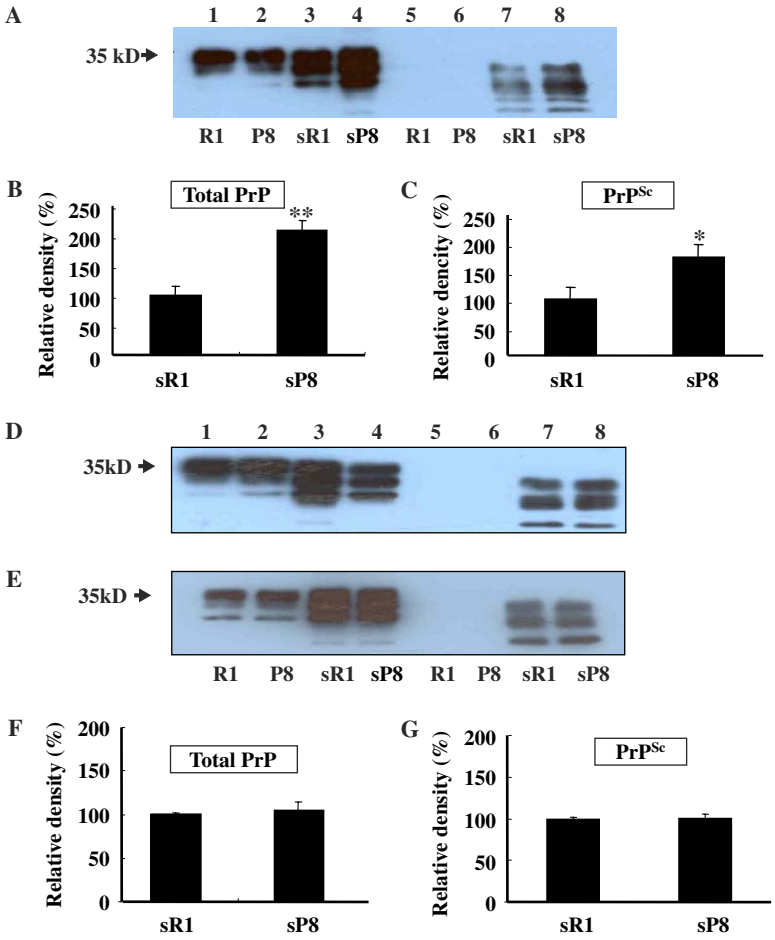

Fig. 3. The Western blot analysis of total PrP and PrP^{Sc} in the brains and spinal cords of scrapie-infected SAM mice. (A) Brain homogenates without PK digestion (lanes 1–4) and the same samples treated with PK (lanes 5–8) were obtained from mice sacrificed when scrapie-infected SAMP8 mice were at the terminal stage of disease, 210 days. (B) The total PrP (B) and PrP^{Sc} (C) were quantitated by scanning densitometry in scrapie-infected SAMR1 mice ($n = 4$) and SAMP8 mice ($n = 3$). Western blot of brain homogenates (D) and spinal cord homogenates (E) without PK digestion (lanes 1–4) and the same samples treated with PK (lanes 5–8). Both scrapie-infected SAMR1 mice and SAMP8 mice were sacrificed at terminal stage of disease, day 235 and day 210, respectively. Age-matched uninfected mice were used for comparison. In (F,G), results of (D) quantitated by scanning densitometry. Relative densities of immunoblots from scrapie-infected SAMR1 mice and SAMP8 mice were compared by Student's t test: $^{*}P < 0.05$: $^{**}P < 0.01$.

scrapie-infected SAMR1 and terminal stage scrapie-infected SAMP8 (210 ± 5 days) mice (Fig. 3).

Expression of $PrP^{C/Sc}$ and PrP^{Sc} was significantly increased in reactive astrocytes of spinal cords from scrapie-infected SAM mice

To characterize the expression and cellular localization of $PrP^{C/Sc}$ in scrapie-infected SAMR1 and SAMP8 mice, we carried out immunohistochemical analysis using sections of thalamus (Figs. 4A and B) and spinal cord (Figs. 4C–H) from normal SAMR1 (Fig. 4C), normal SAMP8 (Fig. 4D), scrapie-infected SAMR1 (Figs. 4E and G), and scrapie-infected SAMP8 (Figs. 4F and H). In general, neurons and astrocytes of both the thalamus region and the spinal cord were immuno-positive for $PrP^{C/Sc}$ in scrapie-infected SAMR1 (Figs. 4A, E, and G) and SAMP8 mice (Figs. 4B, F, and H). $PrP^{C/Sc}$ and PrP^{Sc} immunoreactivities were detected predominantly in astrocytes of spinal cords of scrapie-infected SAMP8 mice (Figs. 4F and H), whereas astrocytic immunoreactivity was minimal in brain of the same group of mice (Figs. 4A and B). The staining in both regions was more extensive in scrapie-infected SAMP8 than in SAMR1.

Discussion

The present results show that the expression levels of E-MuLV, xenotropic MuLV, and polytropic MuLV are increased in scrapie-infected SAMP8 and SAMR1 mice.

Mice contain chromosomally integrated copies of MuLVs. These MuLVs, which are termed endogenous, are prototypical replication-competent retroviruses and RNA tumor viruses. Three host range groups of endogenous MuLV are found in mice; ecotropic viruses which are produced by mouse cells and can only infect other mouse cells [23,24], xenotropic viruses which are produced by mice but can only infect cells of other species

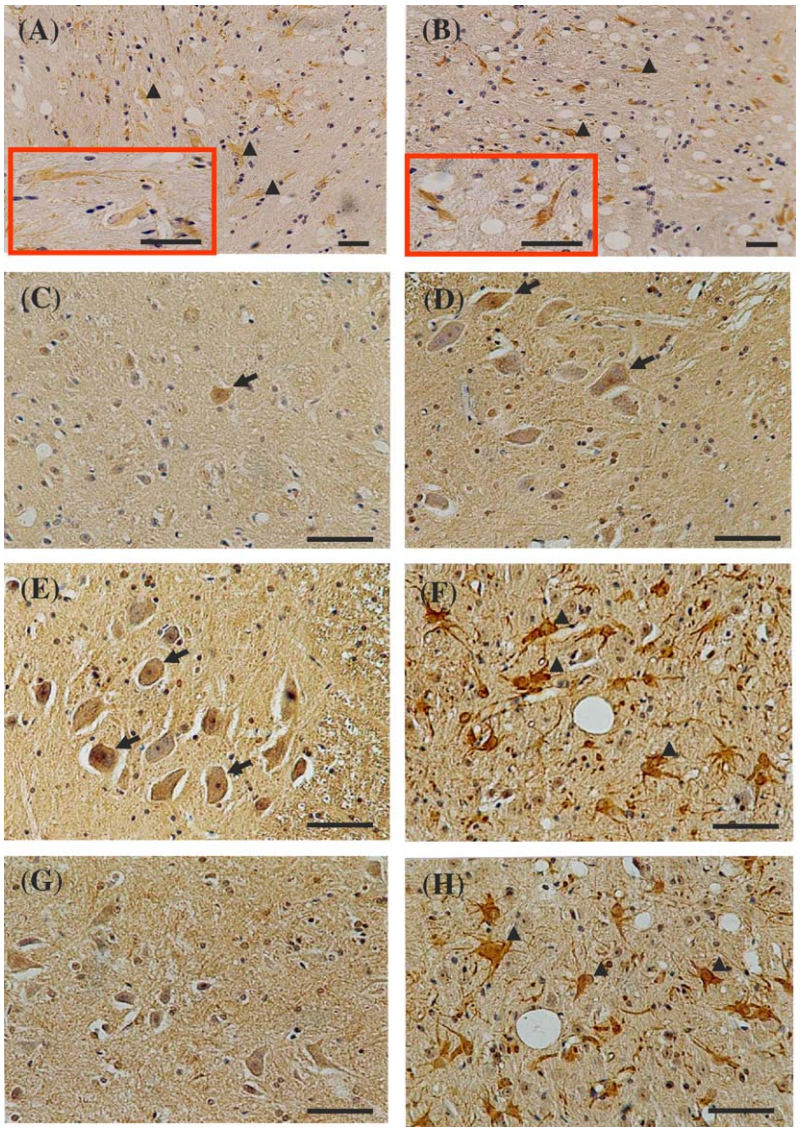

Fig. 4. Immunohistochemical staining of PrP^{Sc} and/or $PrP^{C/Sc}$ in thalamus region (A,B) and in spinal cords (C–H) from scrapie-infected SAM mice. PrP^{Sc} immunoreactivity was present in activated astrocytes (arrowheads) of thalamus region from scrapie-infected SAMR1 (A) and SAMP8 mice (B). $PrP^{C/Sc}$ (C–F) immunoreactivity was present in neurons (arrows) and reactive astrocytes (arrowheads) of spinal cords from scrapie-infected SAM mice. $PrP^{C/Sc}$ and PrP^{Sc} (G,H) immunoreactivity was more intense in reactive astrocytes of spinal cords from scrapie-infected SAMP8 than from scrapie-infected SAMR1 mice. Sections (A, B, G, and H) were treated with PK for 7 min at room temperature prior to immunostaining. SAMR1 mice (C), SAMP8 mice (D), scrapie-infected SAMR1 mice (E,G), and scrapie-infected SAMP8 mice (F,H). Both scrapie-infected SAMR1 and SAMP8 mice were sacrificed at the terminal stage of disease. Scale bar = 10 μm.

[25], and polytropic viruses that infect mouse cells as well as the cells of other species [26]. Distinct cell surface receptors are used by ecotropic and xenotropic/polytropic viruses. SAMP8 mice have high levels of endogenous E-MuLV in their brains [20]. Infectious E-MuLV was present in the brains of SAMP8 mice at the earliest time tested, 1 week, and the titers increased throughout life. However, little or no infectious E-MuLV was detected in the brain of SAMR1 throughout their life span. In our previous study, the E-MuLV provirus, termed Emv 11, from SAMP8 was shown to be that of the endogenous E-MuLV of the AKR mouse strain, Akv [27,28]. In our previous study [28], we showed that neuropathological changes seen in SAMP8 mice were strongly correlated with the expression of E-MuLV. These results indicate that E-MuLV may play an important role in neuronal cell death and be involved in the early changes that are seen in learning and memory that characterize the SAMP8. Xenotropic and polytropic genomes were expressed at equal levels in SAMR1 and SAMP8. In this study, we observed that the levels of expression of ecotropic, xenotropic, and polytropic MuLV were increased by scrapie infection in SAM mice (Fig. 1). These findings confirm and extend the concept that there may be involvement of TSE in retrovirus replication, as suggested previously [7,22,29].

We found that E-MuLV expression was detected in scrapie-infected SAMR1 mice at the terminal stage of scrapie disease (Fig. 1). We did not detect E-MuLV expression in scrapie-infected SAMR1 mice before they showed clinical symptoms. The finding that the induction of E-MuLV RNA expression in scrapie-infected SAMP8 is far greater than in SAMR1 (Fig. 1) is probably a function of the presence of the provirus for Akv in the SAMP8 genome and its absence in the SAMR1 genome [22]. The increased expression of E-MuLV in infected vs. control SAMR1 cannot be Akv, since the Emv 11 provirus for this virus is not present in SAMR1 mice [20]. Other infectious E-MuLV viruses are present in uninfected SAMR1, but at low infectivity titers [20]. Therefore, the increased E-MuLV expression found in scrapie-infected SAMR1 mice must be caused by the induction of these non-Akv E-MuLV proviruses that are ordinarily either poorly expressed or not expressed at all in uninfected SAMR1. It is known that some proviruses are not actively transcribed when hosts are young but are expressed during aging [30]. Gene expression of MuLVs shows a qualitative change during aging [31] and MuLV levels can be increased by radiation [32]. Once these proviruses become transcriptionally activated, the resulting infectious virions may play a crucial role in pathogenesis of a number of clinical syndromes [33], including lymphoma–leukemia in AKR mice [34]. In relation to the current study, the increased expression of non-Akv E-MuLV in scrapie-infected SAMR1 mice may be a function of the interaction of PrP^{Sc} with the replication mechanism of E-MuLV or the effect could relate to the cellular and cytokine response to scrapie infection.

The scrapie incubation period gene, *sinc* [35–37], also referred to as *Prn-i* [38], is the mouse gene that has a major influence on the length of the scrapie incubation period. Mice with the s7s7 allele have a comparatively short incubation period for scrapie strains such as 139A, ME7, and 22L, whereas mice with the p7p7 allele have long incubation periods for these scrapie strains. SAMP8, SAMR1, and AKR mice, one of the SAM progenitor strains, are all s7s7. Also, scrapie incubation period differences within the s7s7 group of mice have been shown to be a function, in part, of H-2D locus [39,40]. It is known that for SAMP8 and AKR strains the H-2D locus is the same, but the H-2D locus of SAMR1 is unknown [41]. In the current experiment, the incubation period was shorter in scrapie-infected SAMP8 mice, which have high levels of E-MuLV expression, compared to SAMR1 mice (Table 2). AKR mice yielded even shorter incubation periods than SAMP8 mice [17]. The E-MuLV titers in the brains of AKR mice were higher than those in brains of SAMP8 mice [15]. Thus, in these 3 strains, there is an inverse relationship between the level of E-MuLV expression and the length of scrapie incubation period. The fact that all mammals contain various types of retro-elements suggests that there could be interaction with TSE agents affecting agent replication, spread or induction of disease [15]. The membrane-bound prion protein mainly localizes on the cell surface, however, PrP is also found in several cell compartments including cytoplasm, golgi, and nucleus, and could be affected by retrovirus replication in one of these compartments.

It has been suggested that prion protein is involved in nucleic acid metabolism [11,13], and that it can also be a factor in nucleic acid internalization into the cytoplasm [42]. Recent work from several groups has demonstrated that nucleic acid binding alters the biochemical properties of PrP [8,9,43]. Moreover, the binding of PrP to nucleic acid results in the formation of condensed nucleoprotein structures similar to those obtained with retroviral nucleocapsid proteins and these nucleoproteins share many of the specific functional characteristics of retroviral nucleocapsid protein, NCp7, in the context of retrovirus maturation [10]. The NCp7 is a retroviral gag polyprotein and is membrane-associated during virion formation and budding at the cell surface [44–46]. The fact that both gag protein and PrP^{C} interact with viral RNA [10] suggests that these two proteins could influence retrovirus replication in either an antagonistic or additive way. The fact that, in general, there is an increase in ecotropic retrovirus replication and xenotropic and polytropic expression in scrapie-infected mice argues that the structure or concentration of the PrP molecule can influence MuLV production. It should be noted that there is an exception to increased expression of MuLV following scrapie infection, in that 22A-infected mice do not show an increase in E-MuLV titer [15].

In experiments examining the cellular localization of MuLV, we observed that expression occurred predominantly in neurons (Fig. 2). However, immunoreactivity of MuLV was increased in astrocytes of scrapie-infected SAMP8 mice (Fig. 2). Interestingly, PrP^{Sc} immunoreactivity was also seen in astrocytes and neurons of scrapie-infected mice (Fig. 4). The fact that increases in both MuLV expression and PrP^{Sc} staining occur in the same cell types indicates that either a synergistic interaction may have occurred or that the potential for intracellular interaction is augmented or both. The concept that there is potential for interaction between PrP and components of MuLV is supported by a number of findings: when homogenates with high levels of scrapie infectivity are treated *in vitro* with PK for a limited time, several fragments of prion protein (PrP) remain, as well as full-length retroviral gag proteins [4]. Protected long nucleic acids, including retroviral RNAs, can also be recovered in CJD homogenates [4] treated with PK and retroviral sequences also co-sediment with infectivity in scrapie [12]. Small, highly structured RNAs have the capacity to participate in the conversion of human recombinant PrP^{Sen} to PrP^{Res} [43] and RNA molecules stimulate prion protein conversion [9].

Furthermore, there is some evidence that E-MuLV expression directly correlates with prion infection. Previous studies showed that the *in vivo* infection with three scrapie strains caused an increase of E-MuLV titers in brains [15]. Recently, Stengel et al. [47] reported that prion infection influenced murine endogenous retrovirus expression *in vitro*. Leblanc et al. (2006) showed that moloney MuLV

(MoMuLV), which belongs to E-MuLV, strongly enhances the release of scrapie infectivity as well as the accumulation of PrP^{Sc} *in vitro*, and MoMuLV virions were specifically immunoprecipitated by anti-PrP antibodies [48,49]. Thus, these data combined with our finding suggest that E-MuLV appears to be closely associated with prion diseases and could be a cofactor involved in pathological prion agent.

Finally, to analyze the effect of E-MuLV on the accumulation of PrP^{Sc} in brains, a useful approach would be to determine if anti-E-MuLV viral drugs can reduce the accumulation of PrP^{Sc}. The recent findings on the enhancement of PrP^{Sc} infectivity by MoMuLV infection *in vitro* provide some supporting evidence for this approach [48,49]. In conclusion, we suggest that E-MuLV replication could play an important role in the progression of scrapie pathogenesis.

Acknowledgment

This work was supported by the MRC program of MOST/KOSEF (R-13-2005-022-01002).

References

[1] D.A. Harris, Cell biological studies of the prion protein, cell biological studies of the prion protein, Curr. Issues Mol. Biol. 1 (1999) 65–75.
[2] S.B. Prusiner, Prions, Proc. Natl. Acad. Sci. USA 95 (1998) 13363–13383.
[3] B. Caughey, K. Brown, G.J. Raymond, G.E. Katzenstein, W. Thresher, Binding of the protease-sensitive form of PrP (prion protein) to sulfated glycosaminoglycan and congo red, J. Virol. 68 (1994) 2135–2141.
[4] A. Akowitz, T. Sklaviadis, L. Manuelidis, Endogenous viral complexes with long RNA cosediment with the agent of Creutzfeldt–Jakob disease, Nucleic Acids Res. 22 (1994) 1101–1107.
[5] P.K. Nandi, E. Leclerc, Polymerization of murine recombinant prion protein in nucleic acid solution, Arch. Virol. 144 (1999) 1751–1763.
[6] T. Sklaviadis, A. Akowitz, E.E. Manuelidis, L. Manuelidis, Nucleic acid binding proteins in highly purified Creutzfeldt–Jakob disease preparations, Proc. Natl. Acad. Sci. USA 90 (1993) 5713–5717.
[7] G. Mudoch, T. Sklaviadis, E. Manuelidis, L. Manuelidis, Potential retroviral RNAs in Creutzfeldt–Jakob disease, J. Virol. 64 (1990) 1477–1486.
[8] Y. Cordeiro, F. Machado, L. Juliano, M.A. Juliano, R.R. Brenani, D. Foguel, J. Silva, DNA converts cellular prion protein into the β-sheet conformation and inhibits prion peptide aggregation, J. Biol. Chem. 52 (2001) 49400–49409.
[9] N.R. Deleault, R.W. Lucassen, S. Supattapone, RNA molecules stimulate prion protein conversion, Nature 425 (2003) 717–720.
[10] C. Gabus, S. Auxilien, C. Pechoux, D. Dormont, W. Swietnicki, M. Morillas, W. Surewicz, P. Nandi, J.L. Darlix, The prion protein has DNA strand transfer properties similar to retroviral nucleocapsid protein, J. Mol. Biol. 307 (2001) 1011–1021.
[11] C. Gabus, E. Derrington, P. Leblanc, J. Chnaiderman, D. Dormont, W. Swietnicki, M. Morillas, W.K. Surewicz, D. Marc, P. Nandi, J.L. Darlix, The prion protein has RNA binding and chaperoning properties characteristic of nucleocapsid protein NCP7 of HIV-1, J. Biol. Chem. 276 (2001) 19301–19309.
[12] L. Manuelidis, T. Sklaviadis, A. Akowitz, W. Fritch, Viral particles are required for infection in neurodegenerative Creutzfeldt–Jakob disease, Proc. Natl. Acad. Sci. USA 235 (1995) 5124–5128.
[13] M. Moscardini, M. Pistello, M. Bendinelli, D. Ficheux, J.T. Miller, C. Gabus, S.F. Le Grice, W.K. Surewicz, J.D. Darlix, Functional interactions of nucleocapsid protein of feline immunodeficiency virus and cellular prion protein with the viral RNA, J. Mol. Biol. 318 (2002) 49–159.
[14] M.L. Labat, Possible retroviral origin of prion disease: Could prion disease be reconsidered as a preleukemia syndrome? Biomed. Pharmacother. 53 (1999) 47–53.
[15] R.I. Carp, H. Meeker, V. Caruso, E. Sersen, Scrapie strain-specific interactions with endogenous murine leukaemia virus, J. Gen. Virol. 80 (1999) 5–10.
[16] R.I. Carp, H.C. Meeker, P. Kozlowski, E.A. Seren, An endogenous retrovireuses and exogenous scrapie in a mouse model of aging, Trends Microbiol. 8 (2000) 39–42.
[17] R.I. Carp, H. Meeker, E. Sersen, P. Kozlowski, Analysis of the incubation periods, induction of obesity and histopathological changes in senescence-prone and senescence-resistant mice infected with various scrapie strains, J. Gen. Virol. 78 (1998) 2863–2869.
[18] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acidguanidium thiocyanate–phenol chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[19] J.M. Irving, L. Chang, F.J. Castilli, A reverse transcriptase-polymerase chain reaction assay for the detection and quantitation of murine retroviruses, Biotechnology 11 (1993) 1042–1046.
[20] H.C. Meeker, R.I. Carp, Titers of murine leukemia virus are higher in brain of SAMP8 than SAMR1 mice, Neurobiol. Aging 18 (1997) 543–547.
[21] J.I. Kim, C. Wang, S. Kuizon, J. Xu, D. Barengolts, P.C. Gray, R. Rubenstein, Simple and specific detection of abnormal prion protein by a magnetic bead-based immunoassay coupled with laser-induced fluorescence spectroifluorometry, J. Neuroimmunol. 158 (2005) 112–119.
[22] E.E. Manuelidis, W.W. Fritch, J.H. Kim, L. Manuelidis, Immortality of cell cultures derived from brains of mice and hamsters infected with Creutzfeldt–Jakob disease agent, Proc. Natl. Acad. Sci. USA 84 (1987) 871–875.
[23] J.M. Horowitz, R. Risser, Molecular and biological characterization of the endogenous ecotropic provirus of BALB/c mice, J. Virol. 56 (1985) 798–806.
[24] C.A. Kozak, Retroviruses as chromosomal genes in the mouse, Adv. Cancer Res. 44 (1995) 295–336.
[25] J.A. Levy, Xenotropic virus: murine leukemia viruses associated with NIH Swiss, NZB and other mouse strains, Science 182 (1973) 1151–1153.
[26] J.W. Hartley, N.K. Wolford, L.J. Old, W.P. Rowe, A new class of murine leukemia virus associated with the development of spontaneous lymphomas, Proc. Natl. Acad. Sci. USA 74 (1977) 789–792.
[27] R.I. Carp, H.C. Meeker, R. Chung, C.A. Kozak, M. Hosokawa, H. Fujisawa, Murine leukemia virus in organs of senescence-prone and -resistant mouse strains, Mech. Ageing Dev. 123 (2002) 575–584.
[28] B.H. Jeong, J.K. Jin, E.K. Choi, E.Y. Lee, H.C. Meeker, C.A. Kozak, R.I. Carp, Y.S. Kim, Analysis of the expression of endogenous murine leukemia viruses in the brains of senescence-accelerated mice (SAMP8) and the relationship between expression and brain histopathology, J. Neuropathol. Exp. Neurol. 61 (2002) 1001–1012.
[29] E.L. Oleszak, L. Manuelidis, E.E. Manuelidis, In vitro transformation elicited by Creutzfeldt–Jakob-infected brain material, J. Neuropathol. Exp. Neurol. 45 (1986) 489–502.
[30] D.R. Lowy, Transformation and oncogenesis: retroviruses, Raven Press, New York, 1985, 235–263.
[31] Y. Wada, M. Tsukada, S. Kamiyama, A. Koizumi, Retroviral gene expression as a possible biomarker of aging, Int. Arch. Occup. Environ. Health 63 (1993) S235–S240.
[32] R.S. Narin, B.W. Mclntyre, E.R. Richie, J.P. Allison, Characterization of env gene recombination in X-ray induced thymomas of C57BL/6J mice, Mol. Carcinog. 2 (1989) 126–130.
[33] J.P. Stoye, J.M. Coffin, The four classes of endogenous murine leukemia virus: structural relationships and potential for recombination, J. Virol. 61 (1987) 2659–2669.

[34] D.R. Lowy, S.K. Chattopadhyay, N.M. Teich, W.P. Rowe, A.S. Levine, AKR murine leukemia virus genome: frequency of sequences in DNA of high-, low-, and non-virus-yielding mouse strains, Proc. Natl. Acad. Sci. USA 71 (1974) 3555–3559.
[35] A.G. Dickinson, V.M. Meikle, Host-genotype and agent effects in scrapie incubation: change in allelic interaction with different strains of agent, Mol. Gen. Genet. 112 (1971) 73–79.
[36] A.G. Dickinson, H. Fraser, An assessment of the genetics of scrapie in sheep and miceSlow Transmissible Diseases of the Nervous System, 1, 1979, pp. 367–385.
[37] Y.S. Kim, R.I. Carp, S.M. Callahan, M. Natelli, H.M. Wisniewski, Vacuolization incubation period and survival time analyses in three mouse genotypes injected stereotactically in three brain regions with the 22L scrapie strain, J. Neuropathol. Exp. Neurol. 49 (1990) 106–113.
[38] G.A. Carson, D.T. Kingsbury, P.A. Goodman, S. Coleman, S.T. Marshall, S.J. DeArmond, D. Westaway, S.B. Prusiner, Prion protein and scrapie incubation time genes are linked, Cell 45 (1986) 503–511.
[39] R.I. Carp, S.M. Callahan, Scrapie incubation periods and end-point titers in mouse strains differing at the H-2D locus, Intervirology 26 (1986) 85–92.
[40] D.T. Kingsbury, K.C. Kasper, D.P. Stites, J.D. Watson, R.N. Hogan, S.B. Prusiner, Genetic control of scrapie and Creutzfeldt–Jakob disease in mice, J. Immunol. 131 (1983) 491–496.
[41] T. Takeda, M. Hosokawa, K. Higuchi, Senescence-accelerated mouse (SAM): a novel murine model of senescence. In the SAM Model of senescence, in: Proceedings of the First International Conference on Senescence, Elsevier, Amsterdam, 1994, pp. 15–22.
[42] E. Derrington, C. Gabus, P. Leblanc, J. Chnaidermann, L. Grave, D. Dormont, W. Swietnicki, M. Morillas, D. Marck, P. Nandi, J. Darlix, PrP^{C} has nucleic acid chaperoning properties similar to the nucleocapsid protein of HIV-1, C. R. Biol. 325 (2002) 17–23.
[43] V. Adler, B. Zeiler, V. Kryukov, R. Kascsak, R. Rubenstein, A. Grossman, Small, highly structured RNAs participate in the conversion of human recombinant PrP^{Sen} to PrP^{Res} in vitro, J. Mol. Biol. 332 (2003) 47–57.
[44] A. Aldovini, R.A. Young, Mutations of RNA and protein sequences involved in human immunodeficiency virus type 1 packaging result in production of noninfectious virus, J. Virol. 64 (1990) 1920–1926.
[45] R. Berkowitz, J. Fisher, S.P. Goff, RNA packaging, Curr. Top. Microbiol. Immunol. 214 (1996) 177–218.
[46] A. Cimarelli, S. Sandin, S. Hoglund, J. Luban, Basic residues in human immunodeficiency virus type 1 nucleocapsid promote virion assembly via interaction with RNA, J. Virol. 74 (2000) 3046–3057.
[47] A. Stengel, C. Bach, I. Vorberg, O. Frank, S. Gilch, G. Lutzny, W. Seifarth, V. Erfle, E. Maas, H. Schatzl, C. Leib-Mosch, A.D. Greenwood, Prion infection influences murine endogenous retrovirus expression in neuronal cells, Biochem. Biophys. Res. Commun. 343 (2006) 825–831.
[48] P. Leblanc, S. Alais, I. Porto-Carreiro, S. Lehmann, J. Grassi, G. Raposo, J.L. Darlix, Retrovirus infection strongly enhances scrapie infectivity release in cell culture, EMBO J. 25 (2006) 2674–2685.
[49] A. Ashok, R.S. Hegde, Prions and retroviruses: an endosomal rendezvous? EMBO Rep. 7 (2006) 685–687.