Scrapie strain-specific interactions with endogenous murine leukaemia virus

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The finding that a senescence-accelerated mouse (SAMP8) shows early brain ageing, with histopathological changes resembling those seen in scrapie, combined with the discovery of high levels of endogenous murine leukaemia virus (MuLV) in brains of SAMP8 mice prompted us to examine the effect of scrapie infection on MuLV titres in this strain and in one of its progenitors, the AKR strain. Three scrapie strains (ME7, 22L and 139A) that had a comparatively short incubation period in SAMP8 and AKR mice caused an increase in brain MuLV titres that was scrapie strain-specific: in each mouse strain, the greatest effect was with 139A, and the least with ME7. The 22A scrapie strain, which has a long incubation period in SAMP8 mice, did not affect MuLV titres in brains of this mouse strain. Previous analyses of scrapie incubation periods in AKR, SAMP8 and another strain derived from an AKR cross (SAMR1) showed an inverse relationship between brain MuLV titres and scrapie incubation periods. This finding, combined with the effect of scrapie on MuLV titres, suggests an interaction between the scrapie infectious process and MuLV replication.

Introduction

The transmissible spongiform encephalopathies (TSEs), also referred to as prion diseases, yield a variety of strain-specific changes in the host, but all strains have brain as their target organ. For TSEs, the brain is the major area of histopathological damage and is the organ that has the highest level of infectious titres (Eklund et al., 1965; Dickinson et al., 1969; Fraser, 1979; Dickinson & Fraser, 1979). Analyses of the interaction between TSE agents and host is accomplished most effectively using the archetype of these diseases, scrapie. One of the key advantages of the scrapie model system is the availability of numerous, well-characterized strains which produce distinctive effects (Dickinson & Meikle, 1971; Bruce et al., 1976; Dickinson & Fraser, 1979; Carp et al., 1984; Kim et al., 1987; Carp & Rubenstein, 1991). An additional advantage in the mouse-adapted scrapie strains is gained by the use of inbred mouse strains. A variety of markers (e.g. incubation period, profile of brain lesions, induction of obesity and diabetes) can be used to assess the different biological effects of various scrapie strains (Dickinson & Fraser, 1979; Fraser, 1979; Carp et al., 1984, 1989; Carp & Rubenstein, 1991). For each marker, the brain plays a major role in yielding the scrapie strain-specific biological effects.

The brain is also the key organ involved in a mouse model of accelerated ageing in which early deficits in learning and memory are a salient feature (Miyamoto et al., 1986; Takeda et al., 1994, 1997; Hosokawa, 1994; Flood et al., 1995; Flood & Morley, 1998). The SAMP8 mouse strain was one of a number of strains produced by an inadvertent cross between AKR mice and an unknown strain or strains, and subsequent inbreeding of the resultant litters (Takeda et al., 1997). Some of the strains that were developed were prone to accelerated senescence (e.g. SAMP8), whereas others were resistant to accelerated senescence (e.g. SAMR1). The histopathological changes that are seen at an early age in the brains of SAMP8 mice are similar to those seen in a variety of mouse strains infected with the archetype TSE, scrapie: these include vacuolation, astrocytosis, neuronal loss, alteration in hippocampal dendritic spines and changes in the blood–brain barrier (Fraser, 1979; Yagi et al., 1989; Akiguchi et al., 1994). SAMP8 mice have a short life-span compared to normal mice. SAMR1 mice have very few brain lesions, which develop late in life, do not show deficits in learning and memory, and have a normal life-span. In a previous study, it was shown that adult SAMP8 mice have high levels of endogenous ecotropic murine leukaemia virus (MuLV) in their brains (Meeker & Carp, 1997). As early as 1 week of age low levels of MuLV were present in brains of SAMP8 mice and the titres increased throughout life. In contrast, SAMR1 mice had little or no MuLV in their brains at
any age. The MuLV titres in spleen and blood of SAMP8 mice were also higher than the titres in these organs from SAMR1 mice.

In a recent study (Carp et al., 1998), infection of AKR, SAMP8 and SAMR1 mice with three scrapie strains revealed a number of key findings: the incubation periods of each of three scrapie strains (ME7, 22L and 139A) were shortest in AKR and longest in SAMR1. For two additional biological markers, the pattern of vacuolation in the brain (lesion profile) and the induction of obesity, the outcomes appeared to be a function of the infecting scrapie strain to a greater extent than a function of mouse strain.

The similarity of brain histopathological changes seen in scrapie mice, and in older uninfected SAMP8 mice, combined with the fact that scrapie brains have high titres of scrapie agent and SAMP8 brains have high titres of MuLV, prompted an investigation of the possible effect of scrapie infection on SAMP8 MuLV titres. Also included in the analyses were the only known progenitor of the SAM lines, AKR, plus SAMR1, a strain with low titres of MuLV in brain. The possibility that there is an interaction between the scrapie infectious process and MuLV replication is discussed.

Methods

**Animals.** SAMP8 and SAMR1 mice were originally obtained from T. Takeda (Kyoto University, Kyoto, Japan) and have been maintained as inbred strains in the Institute for Basic Research animal colony. Pathogen-free SAMP8 and SAMR1 animals were obtained subsequently from J. F. Flood and J. E. Morley and have been housed in Thoren cages (Flood & Morley, 1992; Flood et al., 1995). AKR/J mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). All mice were on a 12 h light–dark cycle and were fed and watered ad libitum.

**Scrapie strains and injection procedures.** The ME7, 22A and 22L scrapie strains were obtained from Alan Dickinson (Neuropathogenesis Unit, Edinburgh, UK) and have been passaged by intracerebral (i.c.) injection of C57BL/6j mice. The 139A strain was obtained from Richard Kimberlin (Neuropathogenesis Unit, Edinburgh, UK) and has also been passaged by i.c. injection of C57BL/6 mice. Mice 2–3 months old were injected i.c. with 25 μl of 1% brain homogenate prepared from mice infected with one of the scrapie strains or with homogenate from mice injected with normal mouse brain (NMB).

**Scrapie assessment and brain removal.** Mice were examined weekly by monitoring motor coordination on a grid apparatus containing parallel bars 3 mm in diameter, placed 7 mm apart from each other (Carp et al., 1984). An animal was scored positive when it failed to walk on the grid without foot slippage between bars. In positive mice, this initial sign was followed by increasing locomotor difficulties, with eventual weakening and wasting. The incubation period was defined as ending on the third consecutive weekly positive score, and is expressed as either mean (in days) ± SEM or mean. Scrapie-infected mice were sacrificed at the end of the scrapie incubation period by intraperitoneal injection of 5 mg sodium pentobarbital. Brains were removed and put on ice until storage at −70 °C. NMB-injected and non-injected control mice, age matched to scrapie mice, were handled in the same fashion.

**Culture of cell lines.** SC-1 cells (ATCC CRL 1404) were grown in Dulbecco’s modified Eagle’s medium (DMEM) + 10% foetal bovine serum (FBS) + 100 u/ml penicillin + 100 μg/ml streptomycin (DMEM10). The XC cell line (ATCC CCL 165) was grown in DMEM + 10% FBS without antibiotics (DMEM10). SC- and XC cells were harvested for use in plaque assays by trypsinization, washed once with DMEM, and resuspended in the appropriate medium for assay. All cell-growth and plaque assays were done at 37 °C in a humidified 5% CO₂ incubator.

**SC-1 UV plaque assay.** Ectropic MuLV was quantified using the SC-1/UV plaque assay (Rowe, 1975–1976). SC-1 cells were plated onto 60 mm plastic Petri dishes at 10⁶ cells per dish in 4 ml DMEM10A. The next day, approximately 1 h before addition of brain homogenates, medium in the dishes was discarded and replaced with 3 ml DMEM + 5% FBS + penicillin and streptomycin + 25 μg/ml DEAE-dextran (DMEM5A–DEAE). One ml volume of brain homogenate diluted in the same medium was then added to plates. One to two days after addition of brain homogenate, medium was removed and replaced with 4 ml per dish DMEM5A. Five days after addition of brain homogenate, medium was removed and cultures were exposed to 30 s of UV irradiation. Immediately after UV irradiation, 4 ml of a suspension containing 2.5 × 10⁸ XC cells/ml in DMEM10 was pipetted into each dish. After 24 h incubation, medium was discarded. Cultures were washed once with PBS, fixed with 100% methanol for 5 min and stained with haematoxylin for 5 min. Haematoxylin was discarded, the cultures washed twice with tap water, and the plaques counted under a dissecting microscope. Concentrations of brain homogenates higher than 1 ml of 1% (w/v) in 4 ml culture medium were found to be toxic to SC-1 cells. Therefore, brain homogenates were diluted in serial tenfold dilutions with 1% the highest concentration used. MuLV titres were expressed as p.f.u./10 mg brain (NMB, ME7, 22L, 139A) or p.f.u./1 mg brain in Table 1.

**Statistical analyses.** The results were analysed using the statistical package Statistica 5.0 (Statsoft, Tulsa, OK, USA). Titres from the paired assays of NMB-injected and scrapie-injected mice were compared as a within subject factor in a multivariate analysis of variance with mouse strain and scrapie strain as between subject factors. Other group comparisons were performed using the Mann–Whitney U-test.

Results

The titres of MuLV in the brains of SAMP8 and AKR mice injected with homogenates of NMB or with one of three scrapie strains (ME7, 139A and 22L) were analysed. For each mouse strain, assays for an NMB-injected mouse and for a mouse infected with a particular scrapie strain were paired; there were seven pairs for ME7 and for 22L in each mouse strain and five comparisons for 139A in each mouse strain. The mean differences in titres between each group of pairs are presented in Fig. 1. In a multivariate analysis, the titre differences between mouse strains and scrapie strains were analysed with mouse strain (AKR vs SAMP8) and scrapie strain (ME7, 139A and 22L) as between subject factors and inoculum (NMB vs scrapie-infected brain) as within subject factors. Other group comparisons were performed using the Mann–Whitney U-test.
Fig. 1. Bars represent the difference in brain MuLV titres expressed in log₁₀ units between mice injected with the designated scrapie strain and mice injected with normal mouse brain (NMB). For each instance the assays were paired, MuLV titres in a scrapie-infected mouse minus the titre in an NMB-injected mouse. For both SAMP8 and AKR mice, there were seven pairs for ME7 and 22L, whereas for 139A there were five pairs.

**Values:** * < 0.05; *** < 0.001.

Fig. 2. Bars represent brain MuLV titres expressed in log₁₀ units (mean ± SEM) of all SAMP8 mice injected with either NMB or scrapie (left bar) compared to all AKR mice injected with either NMB or scrapie (right bar). **Value:** *** < 0.001.

### Table 1. Effect of infection with the 22A scrapie strain on MuLV titres in brains of SAMP8 mice

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>No.</th>
<th>MuLV titre (mean ± SEM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7</td>
<td>2.99 ± 0.13</td>
</tr>
<tr>
<td>22A</td>
<td>6</td>
<td>2.63 ± 0.11†</td>
</tr>
</tbody>
</table>

* Values are log₁₀ p.f.u./1 mg brain homogenate.
† Not significantly different from the uninoculated group (Mann–Whitney U-test, P = 0.23).

The brains of SAMR1 mice were also analysed for MuLV after injection with either NMB or one of the scrapie strains. None of the five mice injected with NMB had MuLV plaques at a 10⁻² dilution of brain homogenate. Sixteen of 18 mice injected with scrapie (seven with 22L, five with ME7 and four with 139A) were negative at a 10⁻² dilution also; one animal injected with ME7 had four plaques at a 10⁻² dilution and one SAMR1 mouse injected with 139A had more than 100 plaques at 10⁻³.

In contrast to the ME7, 139A and 22L scrapie strains, which have a comparatively short incubation period in these mouse strains, the 22A scrapie strain has a long incubation period. For example, the incubation periods for ME7, 139A and 22L ranged from 128 to 193 days (Carp *et al*., 1998), whereas for 22A, the incubation periods were 422 ± 17 in SAMP8, and 505 ± 13 in SAMR1. Scrapie-injected AKR mice died of leukaemia–lymphoma well before the conclusion of the scrapie incubation period. The results of MuLV analysis, in Table 1, show that 22A infection of SAMP8 mice did not cause an increase in MuLV titre, nor was there a consistent effect on MuLV levels in 22A-infected SAMR1 mice (data not shown). The 22A-infected mice were assessed for MuLV at the end of the scrapie incubation period.

### Discussion

Results of the present study demonstrate an interaction between MuLV replication and the scrapie infectious process. The effect on MuLV titre is a function of scrapie strain which is demonstrated by two points. First, in those scrapie strains that have a comparatively short incubation period in both AKR and SAMP8 mice, the MuLV titre differences between scrapie-infected and NMB-injected values within each mouse strain. This is the first quantitative assessment of the expression of endogenous MuLV in brains of AKR mice.

The brains of SAMR1 mice were also analysed for MuLV after injection with either NMB or one of the scrapie strains. None of the five mice injected with NMB had MuLV plaques at a 10⁻² dilution of brain homogenate. Sixteen of 18 mice injected with scrapie (seven with 22L, five with ME7 and four with 139A) were negative at a 10⁻² dilution also; one animal injected with ME7 had four plaques at a 10⁻² dilution and one SAMR1 mouse injected with 139A had more than 100 plaques at 10⁻³.

The scrapie effect on MuLV titres is limited to those situations in which ample virus titres are found in the brain of normal (uninjected or NMB-injected) mice. For example, in
contrast to SAMP8 and AKR, the SAMR1 strain has virtually no MuLV in brain preparations (Meeker & Carp, 1997), and scrapie infection did not affect the endogenous MuLV levels in the brains of this mouse strain. Furthermore, although the SAM strains and AKR mice have a number of xenotropic proviruses (Kitado et al., 1994), scrapie infection did not affect xenotropic virus expression in any of the mouse strains (data not shown): xenotropic virus was not detected in any of the brain preparations from either scrapie-infected or NMB-injected mice.

Within the context of the three mouse strains used in this study, there was an inverse relationship between MuLV titres and the length of scrapie incubation period. Thus, the AKR mouse strain, which had the highest MuLV titres (Fig. 2), yielded the shortest scrapie incubation periods for the ME7, 139A and 22L strains: 146 ± 3, 131 ± 1 and 128 ± 3, respectively (Carp et al., 1998). The longest incubation periods were in SAMR1, which had virtually no MuLV titre in brain; scrapie incubation periods were 193 ± 6, 186 ± 8 and 180 ± 2 for ME7, 139A and 22L, respectively. SAMP8 mice showed intermediate incubation periods of 162 ± 2, 153 ± 2 and 149 ± 4 for ME7, 139A and 22L, respectively (Carp et al., 1998). The inverse relationship between MuLV titres and scrapie incubation periods is seen also with 22A, in which the scrapie incubation periods were 505 days. In addition, a recently developed strain (C603) (Carp & Callahan, 1991) had incubation periods of 215 ± 2 days in SAMP8 mice and 270 ± 11 days in SAMR1 mice.

The scrapie incubation period gene, Sinc (Dickinson & Meikle, 1971; Dickinson & Fraser, 1979; Carp & Callahan, 1991) [also referred to as Prn-1 (Carlson et al., 1986)], is the mouse gene that has a major influence on the length of the scrapie incubation period. Mice with the $s^5s^7$ allele have a comparatively short incubation period for scrapie strains such as ME7, 139A and 22L, whereas mice with the $p^p$ allele have a long incubation period for these scrapie strains. The 22A scrapie strain has the opposite pattern with a short incubation period in $p^p$ mice and a long incubation period in $s^5s^7$ mice (Dickinson & Meikle, 1971). Comparison of incubation periods for ME7, 139A and 22L versus that of 22A clearly establishes that the mouse strains used in these studies have the $s^5s^7$ genotype. It has been shown that different mouse strains within the same Sinc genotype, for example $s^5s^7$, can have different incubation periods for the same TSE strain (Kingsbury et al., 1983; Carp & Callahan, 1986; Dickinson & Outram, 1988; Mohri & Tateishi, 1989). Scrapie incubation period differences within the $s^5s^7$ group of mice have been shown to be a function, in part, of H-2D locus (Kingsbury et al., 1983; Carp & Callahan, 1986). The H-2D locus of SAMR1 mice is not known, but the locus is the same for AKR and SAMP8 (Takeda et al., 1994), which eliminates H-2D as a cause of the different scrapie incubation periods seen in these two mouse strains.

Which of the different characteristics within this group of mouse strains brings about the incubation period differences is unknown. The inherent concentration of an endogenous virus (MuLV) in brain, the target organ for scrapie, could certainly play a role in the replication, spread or induction of disease by these scrapie strains. These data, combined with the scrapie-strain effect on MuLV titres, suggest a two-way interaction between scrapie and MuLV in these scrapie-strain–mouse-strain combinations. The basis for the scrapie strain-specific increase in MuLV titres seen with the ME7, 139A and 22L scrapie strains is unknown. One hypothesis is that there is a direct interaction at the molecular level. This could occur at any phase of the MuLV–host interaction (e.g. passage through the blood–brain barrier, agent attachment to cells, entrance into cells, replication or maturation). The molecular-based effect would appear to function at the level of the scrapie informational molecule, because the effects seen are dependent on the scrapie strain injected. A second hypothesis is based on an indirect effect that would be a function, for example, of the numbers and/or activation levels of a specific cell type within the brain (e.g. astrocytes, microglia). Microglia would be a particularly likely target for this indirect effect. In the latter part of the scrapie incubation period there is evidence of marked microglial activation, demonstrated by increased levels of staining for F4/80, leukocyte-common antigen and complement receptor type 3 antigens (Williams et al., 1994). The increased microglial reactivity is associated with areas of vacuolation and neuronal loss; areas which also show accumulation of the modified host protein, PrPSc, which is associated with the occurrence of TSE (Williams et al., 1997). Microglia are one of the brain-cell types which support the replication of MuLV in a number of systems in which exogenous, ecotropic MuLV has been studied (Gravel et al., 1993; Lynch et al., 1996). It is difficult to assess the possible significance of scrapie-strain differences in microglial activation vs MuLV replication because the scrapie-strain–mouse-strain models are different from those that we studied and because for our MuLV analyses the entire brain was used, whereas microglia activation was measured in specific brain areas. For example, in Williams et al. (1994) microglia activation was examined in 22A-infected VM mice, and it was noted that activation was extensive in the thalamus. Other brain areas were not quantified in this system.

One suggested explanation for the fact that the 22A strain did not induce an increase in MuLV titre in brain of SAMP8 mice is that older, un.injected SAMP8 mice have higher MuLV titres than young mice (Meeker & Carp, 1997). Thus, if there is a maximum level of expression possible for the endogenous virus in SAMP8, aged mice may have reached that maximum and scrapie infection cannot increase the titre. This could be tested by infecting older SAMP8 mice with one of the scrapie strains that causes an increase in MuLV in younger animals, e.g. 139A.

The possible involvement of retroviruses in TSE pathogenesis has been suggested previously (Manuelidis et al., 1983,
1987; Oleszak et al., 1986; Murdoch et al., 1990). The concept put forward viewed the scrapie infectious agent as a retrovirus on the basis of tissue culture and molecular biology studies (Murdoch et al., 1990; Akowitz et al., 1994). For example, these authors characterized the marked astrocytosis, the robust growth of cells derived from TSE brain in culture and their own work on induction of transformation of mouse, hamster and guinea-pig cells in culture exposed to Creutzfeld–Jakob disease (CJD) material as biological evidence of a transforming moiety, probably an oncogenic retrovirus (Oleszak et al., 1986; Manuelidis et al., 1987). Furthermore, several cell lines derived from CJD brain cultured in vitro caused large tumours when injected into nude mice (Manuelidis et al., 1983). Previous workers had postulated that the histopathological and tissue culture evidence pointed to a transformation event and that the astrocytosis seen in brain is a form of neoplasms (Field & Peat, 1969). In the context of the concept advocated in this presentation, a possible mechanism for increased cell transformation after exposure to TSE agents would involve their stimulation of host-cell retro-elements. Interactions between MuLV and a number of infectious processes have been documented. For example, it was shown that persistent infection with lymphocytic choriomeningitis virus induces the appearance in blood of soluble antigen from Gross leukaemia virus (Oldstone et al., 1971). This occurred in mouse strains with low as well as high expression levels of the leukaemia virus. In other studies, the induction of paralysis by lactate dehydrogenase-elevating virus (LDV) required persistent infection of central nervous system tissue with MuLV; infection with either virus alone had no clinical effect (Pease & Murphy, 1980). Mouse strains that had high levels of N-tropic MuLV were susceptible to LDV-induced paralytic disease, whereas mouse strains that had the Fv-1b allele (susceptible only to B-tropic viruses) or had low levels of N-tropic virus expression were resistant (Pease et al., 1982).

The current presentation argues that the scrapie infectious agent contains a moiety, an informational molecule, which interacts with endogenous retro-elements. In the context of the mouse system, it is clear that scrapie replicates and causes disease in animals that do not contain endogenous ecotropic MuLV; for example, scrapie strains cause disease in NZB mice (Carp et al., 1985) which do not have endogenous ecotropic MuLV provirus (Risser & Horowitz, 1983). However, all mouse strains and, indeed, all mammals contain various types of retro-elements (Temin, 1992) that could interact with components of the scrapie agent to affect replication, spread or induction of disease.

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