Vacuolization, Incubation Period and Survival Time Analyses in Three Mouse Genotypes Injected Stereotactically in Three Brain Regions with the 22L Scrapie Strain

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Abstract. In previous studies we showed that C57BL mice injected stereotactically in the cerebellum with the 22L scrapie strain had a significantly shorter incubation period than those injected with the same agent in other brain regions. In mice injected in the cerebellum, vacuolization was limited to the cerebellum, medulla and mesencephalon, whereas injection into forebrain regions resulted in vacuolization in all brain regions. The studies suggested that the cerebellum had a selective vulnerability for 22L. In this study we examined the interaction between host genotype and selective vulnerability of specific brain regions. The mouse gene that has the most profound effect on pathogenesis, particularly incubation period, is termed Sinc (scrapie incubation). Groups of mice with three genotypes of Sinc (s7s7, p7p7 and their F1 cross, s7p7) were injected with 22L into the cerebral cortex, thalamus or cerebellum. Analysis of incubation periods showed that, regardless of the host genotype, the cerebellum injection group had a significantly shorter incubation period than groups injected in other regions. After cerebellum injection vacuolization was limited to the cerebellum, medulla and mesencephalon in all three host genotypes. The location of vacuoles within the cerebellum differed depending upon the host strain. Vacuolization developed almost exclusively in grey matter in s7s7 mice, mainly in white matter in p7p7 mice, and in both grey and white matter in F1 mice. These results demonstrate that the selective vulnerability of the cerebellum to induction of clinical disease by 22L does not depend on host genotype, but host genotype does affect lesion distribution within the cerebellum.

Key Words: Genotypes, mouse; Incubation period; Scrapie; Stereotactic injection; Vacuolization.

INTRODUCTION

Scrapie agent causes a slow degenerative neurological disease in sheep and goats (1). The agent has been passaged in a number of laboratory animal species, including mice and hamsters (2, 3). During the past 20 years, a variety of pathogenetic aspects of scrapie disease have been analyzed using inbred mouse strains injected with well-defined scrapie strains by different routes (4, 5).

We showed recently that C57BL mice injected stereotactically with 22L in the cerebellum had a significantly shorter incubation period than those injected with the same agent in other brain regions (6). In mice injected in the cerebellum, vacuolization was limited to the cerebellum, medulla and mesencephalon and these mice also developed a distinctive ataxic gait prior to the appearance of the typical motor signs of scrapie disease. Injection into forebrain regions resulted in vacuolization in...
all brain regions and mice did not develop the early ataxia. These clinical and pathological findings suggested that the cerebellum, or a closely connected region is most susceptible to the induction of clinical disease by 22L scrapie agent.

In the mouse several aspects of scrapie pathogenesis are controlled by a gene termed Sinc (7, 8). In particular, this gene affects the scrapie incubation period. For example, in mice with the s7s7 genotype (e.g. C57BL), the incubation periods of scrapie strains such as ME7 and 22L are shorter than in mice with the p7p7 genotype (e.g. IM). For these scrapie strains, the incubation period for F1 mice is between those for the homozygotic parents. In the present study we examined the interaction between host Sinc genotype and selective vulnerability of specific brain regions to 22L scrapie agent. Three different strains of mice (s7s7, p7p7 and s7p7) were injected stereotactically with 22L into the cerebral cortex, thalamus or cerebellum and we ascertained the incubation period, survival time and distribution of brain vacuolization in the different combinations.

MATERIALS AND METHODS

1. Mouse Strains and Their Maintenance: Female weanling C57BL mice were obtained from Jackson Laboratories, Bar Harbor, ME. For the IM strain, female mice were obtained from our breeding stock; the original IM breeding pairs were kindly provided by Dr. Alan Dickinson, MRC and AFRF Neuropathogenesis Unit, Edinburgh, Scotland. The IM strain has been maintained by random brother-sister mating in our breeding colony. The F1 hybrid mice were produced by crossing C57BL female and IM male mice. Experimental mice were housed in two rooms in our animal colony; breeding mice were in a separate room. Temperature, humidity and light cycle (12 hours (h) on, 12 h off) were controlled in all rooms. Mice were given rodent chow and water ad libitum.

2. Scrapie Strain: The 22L scrapie strain was kindly provided by Dr. Alan Dickinson, MRC and AFRF Neuropathogenesis Unit, Edinburgh, Scotland. This strain was passaged in our laboratory using C57BL mice. Passages were done by routine intracerebral injection of 30 μl of brain homogenate prepared in cold (4°C) phosphate-buffered saline (PBS). Homogenization was done using 20 strokes of a hand-operated Ten-Broek homogenizer. Mice injected with normal mouse brain served as a source for normal brain homogenate and these homogenates were prepared as described for scrapie preparations.

3. Stereotactic Injection: Microinjections were carried out under general anesthesia (sodium pentobarbital 50 mg/kg, intraperitoneal injection) using a stereotactic instrument (Stoelting Co.). Since no cerebral dominance for scrapie infection has been shown, injections were made in the right hemisphere. The stereotactic coordinates used for the three brain regions were as follows: the cerebral cortex, A+1.0, L2.0, H+1.5; thalamus, A-1.4, L0.8, H+3.2; cerebellum, A-6.5, L1.0, H+2.0 (9). Using a 30-gauge stainless steel needle, 5 μl of a 1% scrapie brain homogenate was injected into the appropriate brain region. In order to minimize pressure-induced spread of inoculum, one to two minutes were taken for each stereotactic injection. Injection of normal mouse brain homogenate was done using the same procedures as those used with scrapie material.

4. Clinical Evaluation: Starting from ten weeks post-injection, we examined all mice weekly for clinical symptoms. The clinical test consisted of monitoring motor coordination on a grid apparatus containing a series of parallel bars of 3 mm in diameter, placed 7 mm apart from each other (10). An animal was scored positive when it failed to walk on the grid without foot slippage between bars. The incubation period was defined as ending on the third consecutive weekly positive score. Mice were killed within one week of the end of the incubation period except in those experiments in which survival times were monitored.

5. Histopathological Evaluation: Brains were removed and fixed by immersion in 10% neutral formalin. After fixation, brains were cut coronally at four standard levels (11). All tissues were paraffin-embedded and 7 μm sections were stained with hematoxylin and eosin (H&E). Nine different regions of the grey matter were examined (without knowledge of the
TABLE 1
Incubation Periods and Survival Times Following Stereotactic Injection of 22L Scrapie Agent in C57BL, C57BL × IM, and IM Mice in Different Brain Regions

<table>
<thead>
<tr>
<th>Injection site</th>
<th>C57BL Incubation</th>
<th>Survival</th>
<th>C57BL × IM Incubation</th>
<th>Survival</th>
<th>IM Incubation</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>138 ± 2*</td>
<td>152 ± 3</td>
<td>160 ± 2</td>
<td>201 ± 3</td>
<td>201 ± 2</td>
<td>260 ± 4</td>
</tr>
<tr>
<td>Thalamus</td>
<td>135 ± 2</td>
<td>148 ± 1</td>
<td>162 ± 2</td>
<td>203 ± 3</td>
<td>191 ± 2†</td>
<td>250 ± 3</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>118 ± 1‡</td>
<td>137 ± 3‡</td>
<td>145 ± 1‡</td>
<td>188 ± 2‡</td>
<td>178 ± 2‡</td>
<td>235 ± 3‡</td>
</tr>
</tbody>
</table>

* Incubation period in days (mean ± standard error). For each injection site-mouse strain combination 12–15 animals were used.

For each mouse strain, comparisons were between groups injected in each region and those injected stereotactically in the cerebral cortex. † p < 0.01; ‡ p < 0.001.

...sample origin) under the light microscope: 1) dorsal medulla; 2) cerebral cortex; 3) mesencephalon; 4) hypothalamus; 5) thalamus, 6) hippocampus; 7) parietal body (sub callosal gyrus); 8) posterior cerebral cortex; and 9) anterior cerebral cortex. Each of the nine areas was given a score for the amount of vacuolization with zero for none and five for the maximum level. Three white matter areas were examined: (1) cerebellar white matter; (2) tegmentum of mesencephalon; (3) pyramidal tract at the level of the thalamus. Vacuolization was measured on a different scale of zero to three; consequently, scores in white and grey matter were not directly comparable to one another (11, 12).

6. Statistical Analysis: The data for incubation periods and survival times were evaluated using Student’s t-test. p values <0.05 were regarded as statistically significant.

RESULTS

Incubation Periods and Survival Times in the Three Mouse Genotypes Injected with 22L: Regardless of the host genotype, cerebellar injection groups had significantly shorter incubation periods and survival times than groups injected in the other regions (Table 1). In F1 mice, incubation periods and survival times were between those for the two parental strains regardless of the brain area injected.

The groups injected stereotactically in the cerebellum developed an ataxic gait approximately 15–20 days before the appearance of the typical motor signs of clinical scrapie disease (6). For the C57BL combination, the observation confirmed a previous study (6). The current results established that the other Sinc genotypes also have early ataxic signs after cerebellar injection.

Comparison of the differences between incubation periods and survival times revealed similar values for the three injection sites (cortex, thalamus and cerebellum) in mice of a specific genotype; however, there were marked differences between genotypes. For s7s7 mice the values ranged from 13 to 19 days for the three injections sites. In marked contrast, values for p7p7 mice ranged from 57 to 59 days. For s7p7 mice, the value for the incubation period to survival time differences (41–43 days) were between those seen for the homozygotes.

Histopathological Changes in the Three Mouse Genotypes: As shown in Figure 1, the injection of cerebral cortex produced vacuolization in all brain regions regardless of host genotype. The vacuolization patterns following stereotactic injection of the thalamus were almost identical to those seen after cerebral cortex injection (data not shown) and these lesion profiles were similar to those seen after routine injection (12, 13, Kim et al, unpublished data). However, after cerebellar injection, vacuoli-
Fig. 1. Lesion profiles in the grey and white matter of C57BL (a), C57BL × IM (b) and IM (c) mice injected with 22L scrapie strain in the cerebral cortex (O—O) or cerebellum (●—●). Each point is the mean value from 5 to 7 mice and standard errors were <0.03.

Zonation was limited to the cerebellum, medulla and mesencephalon in all three host genotypes. Although all injections (cerebral cortex, thalamus and cerebellum) were on the right side of the brain, the patterns of vacuolization were similar on the right and left sides. The location of vacuolization within the cerebellum differed depending upon the host genotype. As shown in Figure 2, vacuolization developed almost exclusively in grey matter in s7s7 mice. In p7p7 mice there was a low level of vacuolization in the grey matter with more extensive vacuolization occurring in white matter areas. In F1 mice vacuolization occurred in both grey and white matter with some mice showing a predominance of grey matter vacuolization and other mice showing more in white matter.

DISCUSSION

The key findings in this study were 1) the incubation periods and survival times of mice injected stereotactically in the cerebellum were significantly shorter than for mice injected in the cerebral cortex or thalamus regardless of the Sinc genotype of the mouse; 2) the relationship of incubation periods among 22L-injected s7s7, p7p7 and s7p7 mice were the same regardless of the region (cerebral cortex, thalamus,
cerebellum) injected; and 3) there were differences in the distribution of vacuoles within the cerebellum depending on the Sinc genotype.

In a previous study (6), the results on incubation periods and survival times after stereotactic injection of five brain regions confirmed the concept of clinical target areas (3, 14). In this concept replication of agent in a given area depends upon agent reaching that area and upon the selective vulnerability of the cells in that area. The induction of clinical disease will then depend on replication of the agent, the ability of agent to damage the function of cells and the importance of those cells for normal function of the organism. A corollary of this concept is that replication in other areas may be irrelevant for the development of clinical disease. Following stereotactic injection of the cerebral cortex, substantia nigra, caudate nucleus, thalamus and cerebellum, the shortest incubation period for 22L injected C57BL mice was seen after cerebellar injection (6). A key finding was that the areas giving the shortest incubation period differed with different scrapie strains, e.g. for strain ME7 both thalamus and cerebellum injection yielded shorter incubation periods than the other three areas, whereas for the 139A strain the incubation periods for cerebellum, substantia nigra, caudate nucleus and thalamus were equivalent and shorter than for mice injected in the cortex. Results in the current study indicate that the greater vulnerability of the cerebellum with regard to the induction of disease by the 22L strain reported in s7s7 mice was also seen in p7p7 mice and in F1 mice produced by s7s7 × p7p7 cross. In previous studies it was shown that the incubation period for F1 mice injected by the routine intracerebral route with 22L were between those of s7s7 and p7p7 mice (15, Carp, unpublished data). Similar results were obtained with the groups injected stereotactically in the cerebral cortex, thalamus and cerebellum, i.e. in each case the incubation periods and survival times for F1 mice were between those for the homozygous parental strains.

In the three Sinc genotypes, vacuolization was limited to the posterior portion of the brain after intracerebellar injection but not after injection in the cerebral cortex (Fig. 1) or thalamus (data not shown). The reason for the absence of vacuolization in the forebrain regions after intracerebellar injection appears to be related to the time interval required for spread of agent from the injection site to the forebrain and to the fact that vacuolization occurs only after the titer of infectivity has reached high levels in a particular region. A delay in appearance of vacuolization compared to infectivity was observed after peripheral, i.e. non-central nervous system injection (16). In this study, brain infectivity was observed initially in the posterior portion of the brain and later in the forebrain (16). Similarly there was a sequential appearance of vacuolization with the first changes occurring in the posterior regions of the brain. In each region vacuolization occurred after the appearance of comparatively high levels of infectivity. The difference in time of appearance between infectivity and vacuolization was confirmed in a study using stereotactic injection of scrapie into cerebella that had been bisected into right and left hemispheres prior to injection (17).

Injection site had no effect on the different location of cerebellar vacuolization seen in different Sinc genotypes. For the three stereotactic injection sites there was virtually complete absence of white matter vacuolization in s7s7 mice, whereas in

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**Fig. 2.** Vacuolization distribution in cerebellum of C57BL (a), C57BL × IM (b) and IM (c) mice injected stereotactically in the cerebellum with 22L scrapie strain. H&E. ×100.
p7p7 mice there was more vacuolization in white matter than in gray matter. The differences in location of cerebellar vacuolization in the Sinc genotypes seen after stereotactic injection is similar to previous findings with routine intracerebral injection (12, 13, 17).

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