Analysis of linkage between scrapie incubation period and the prion protein gene in mice

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A single gene is known to have a predominant influence on scrapie incubation period in mice. In crosses between strains that give a short incubation period, such as NZW mice, and those which give a long incubation period, such as I/LnJ mice, long incubation period was dominant using a Chandler scrapie agent isolate. Recently a close linkage was found between the incubation period gene and the prion protein (PrP) structural gene in I/LnJ mice crossed to NZW mice. Because this linkage suggested an important role for PrP in the pathogenesis of scrapie we sought to verify the linkage between these genes and extended the analysis to three additional mouse strains. All four of the mouse strains that we evaluated, I/LnJ, P/J, MA/MyJ, and RIIIS/J, had incubation periods longer than those of the NZW mice to which they were crossed. In addition, all four strains shared an XbaI restriction enzyme polymorphism, which suggested that all four strains might also exhibit linkage between the incubation period and the PrP structural gene. Very strong linkage between PrP and incubation period was found in I/LnJ and P/J mice crossed to NZW mice, whereas less obvious linkage was demonstrated for MA/MyJ mice crossed to NZW mice. In MA/MyJ mice genes other than PrP also had an obvious influence on incubation period. In RIIIS/J mice no linkage was shown. Although linkage between PrP and incubation period was very significant in I/LnJ and P/J mice, a few animals were identified in both crosses that represented potential recombinants in which PrP and incubation period did not segregate together. Therefore, although these phenotypes are certainly linked in I/LnJ and P/J mice, it is possible that PrP and incubation period are controlled by separate genes.

Scrapie is a spongiform encephalopathy of sheep and goats caused by an agent that is resistant to ionizing radiation and to chemical treatments that inactivate most viruses (Alper et al., 1967; Bellinger-Kawahara et al., 1987; Dees et al., 1985; Latarjet, 1979). Preparations that contain large amounts of scrapie infectivity contain proteinase K-resistant fibrils (Merz et al., 1981; Diringer et al., 1983) or rods (Prusiner et al., 1983) containing an aggregated 27K to 30K protein, generally referred to as prion protein (PrP 27–30) (Bolton et al., 1982; Hope et al., 1986) or scrapie-associated fibril protein (Diringer et al., 1983; Hope et al., 1986). Considerable controversy surrounds the relationship between PrP and the infectious agent of scrapie. Some investigators have suggested that proteinase K-resistant, aggregated PrP 27–30 is the scrapie agent (Bolton et al., 1982; Prusiner, 1982). Recent reports have shown that a close linkage exists between the gene for PrP and a gene controlling scrapie incubation period in I/LnJ mice (Carlson et al., 1986) and in VM mice (Hunter et al., 1987). The incubation period gene has been designated Prn-i (Carlson et al., 1986) and is analogous to the Sinc gene described earlier (Dickinson et al., 1968). It has been suggested that this gene may determine both PrP genotype and incubation period (Carlson et al., 1986; Hunter et al., 1987). This linkage provides strong evidence for an important role for PrP in the pathogenesis of scrapie. However, in the original report many mice died before they could be evaluated for PrP genotype (Carlson et al., 1986). More importantly, a few mice were identified in the original and subsequent reports (Carlson et al., 1988) that represented mice with potential recombinants between the incubation period and PrP genes. Therefore, we repeated the original study in order to verify the linkage between these genes. In addition we extended the analysis to three other mouse strains originally thought to be similar to I/LnJ mice (Carlson et al., 1986) in order to determine whether linkage between these genes was common among various mouse strains.

I/LnJ mice were known to have very long scrapie incubation periods after inoculation of Chandler scrapie agent (Carlson et al., 1986) and I/LnJ, P/J, MA/MyJ and RIIIS/J mice all shared an XbaI restriction enzyme site polymorphism located approximately 0.9 kb upstream of...
the PrP structural gene (Westaway et al., 1987; Carlson et al., 1988). In the present experiments each of these strains was crossed with NZW mice, a strain known to have a short scrapie incubation time following inoculation of this isolate (Carlson et al., 1986). After inoculation of the Chandler scrapie agent, we observed long incubation periods in F1 crosses between short and long incubation period strains (data not shown). Therefore we analysed the possible genetic association between the PrP-linked XbaI site and long or short incubation period phenotype in backcross mouse populations between NZW and the various F1 mice where genes for these characteristics were segregating.

When backcross mice were 18 to 25 days old a 2-5 cm piece of tail was removed from each mouse and frozen at −70 °C until DNA could be prepared. Mice were then inoculated intracerebrally with 10^6 LD_{50} of the Chandler strain of scrapie (Eklund et al., 1967). DNA from the tail sections was isolated, cut with XbaI, Southern-blotted and hybridized against a mouse-derived cDNA PrP probe (Chesebro et al., 1985). Mice were clinically evaluated weekly and the onset of scrapie was documented for each mouse. Mice were allowed to live until near death. They were then killed and a portion of liver was removed, stored at −70 °C, and used to prepare DNA in order to confirm results obtained from tail DNA, as liver DNA usually gave a more distinct XbaI restriction pattern than did tail DNA. The interval from inoculation to death was determined for each mouse and then compared to the XbaI restriction pattern for each respective mouse.

We first evaluated 44 progeny from an NZW × (I/LnJ × NZW) F1 backcross population. Twenty-four mice had long intervals to death (211 ± 12 days, the mean ± s.d.) and by Southern blot all of these gave two PrP bands of 3-8 kb and 5-5 kb (F1 pattern). Twenty mice had short intervals to death (134 ± 9 days) and 19 of these had a single 3-8 kb band (NZW pattern). One mouse gave two bands on Southern blot (Fig. 1, lane 4) and a short interval to death (146 days) and represented potential recombination between the PrP gene and incubation period gene (Fig. 2). The ratio of mice with a short interval to death to those with a long interval to death was close to 1:1, suggesting that a single gene controlled the interval to death and, in these experiments, there was strong statistically significant evidence for linkage between interval to death and the PrP-associated XbaI site polymorphism. However, the occurrence of one possible recombinant mouse suggested that these genes might be separate.

We found a similar situation when P/J mice were studied. Among 34 NZW × (P/J × NZW) F1 backcross mice that were evaluated, 13 mice gave short intervals to death (140 ± 6 days) and one 3-8 kb band on Southern blots, whereas 19 mice gave long intervals to death (208 ± 11 days) and two bands of 3-8 kb and 5-5 kb on Southern blots (Fig. 3). Two potential recombinant mice were identified that had short intervals from inoculation...
Fig. 2. Segregation of interval to death and PrP restriction fragment polymorphism in NZW and (I/LnJ × NZW) F1 parental mice and NZW × (I/LnJ × NZW) F1 female backcross mice. The parental mice are shown on the top part of the figure and the backcross progeny on the bottom. XbaI-digested DNA was analysed by Southern blot hybridization using a mouse PrP cDNA probe to determine the PrP-associated restriction pattern (Chesebro et al., 1985). Open bars represent F1 restriction pattern (bands at 3-8 and 5.5 kb); shaded bars represent NZW parents and backcross mice with NZW restriction pattern (single band at 3.8 kb). Open circles represent mice for which no XbaI pattern was obtained. The interval from inoculation to death of scrapie-inoculated mice is shown on the horizontal axis, and the cumulative number of mice dying is shown on the vertical axis. All mice were inoculated intracerebrally with 10^7 LD_{50} of 7th mouse passage Chandler scrapie agent (Eklund et al., 1967). Scrapie incubation periods for I/LnJ, P/J, MA/MyJ and RIIIS/J mice inoculated with this agent were 255, 295, 170 and 136 days respectively (Carlson et al., 1988). Linkage between the PrP-associated restriction pattern and interval to death was statistically significant (P < 0.001), based on a χ^2 test for a 2 × 2 table. Arrows indicate mice in which potential recombination between PrP genotype and interval to death has occurred.

Fig. 3. Segregation of interval to death and PrP restriction fragment polymorphism in NZW and (P/J × NZW) F1 parental mice and NZW × (P/J × NZW) F1 backcross mice. The parental mice are shown on the top of the figure and the backcross progeny on the bottom. Linkage between the PrP restriction pattern and interval to death was statistically significant (P < 0.001). Technical details are the same as in Fig. 2.

Fig. 4. Segregation of interval to death and PrP restriction fragment polymorphism in NZW and (MA/MyJ × NZW) F1 backcross mice. The parental mice are shown on the top of the figure and the backcross progeny on the bottom. Technical details are the same as in Fig. 2.

Fig. 5. Segregation of incubation period and PrP restriction fragment polymorphism in NZW and (RIIIS/J × NZW) F1 parental mice and NZW × (RIIIS/J × NZW) F1 backcross mice. The parental mice are shown on the top of the figure and the backcross progeny on the bottom. No linkage between the PrP-associated restriction pattern and incubation period was shown. Technical details are the same as in Fig. 2.

to death of 142 and 145 days, but bands at both 3.8 kb and 5.5 kb on Southern blots (Fig. 1, lanes 5 and 6). Again, the ratio of short to long intervals to death was close to 1:1 and linkage between PrP type and the interval to death was very strong. However, the presence of two potential recombinant mice in this backcross supported the suggestion from the previous NZW × (I/LnJ × NZW) F1 backcross that the PrP and incubation period genes might be distinct.

In contrast to the previous two strains, backcross mice from the NZW × (MA/MyJ × NZW) F1 cross (Fig. 4) gave a range of intervals to death intermediate between those of the parental strains. Nevertheless, when the interval to death and XbaI polymorphism were compared, these two parameters appeared to be linked (Table 1). However, the statistical significance of this
linkage depended on exactly where one placed the distinction between long and short intervals to death. In any event, in this backcross non-PrP-associated genes appeared to play an important role in influencing the interval to death.

The fourth mouse strain studied was RIIIS/J, which was evaluated because it had an XbaI restriction pattern similar to the three strains discussed before. In contrast to the other strains studied, no linkage between the interval to death period and PrP was shown when progeny from the NZW × (RIIIS/J × NZW) F1 backcross mice were analysed. XbaI restriction patterns from 19 of these mice gave a ratio of 15:4 (two bands:one band) and no apparent correlation between restriction fragment size and interval to death (Fig. 5). Absence of linkage between the interval to death and the XbaI restriction fragment pattern in this combination could be explained if RIIIS/J and NZW mice are the same at all relevant genes in this region. Alternatively, it is possible that linkage was obscured by the nearly identical intervals to death of the parents of the backcross progeny.

Incubation period (interval to death) and PrP were closely linked in I/LnJ and P/J mice crossed to NZW mice. It is possible that this linkage could be explained by a BstEII restriction site difference and accompanying amino acid sequence difference at position 189 of the PrP protein of NZW mice, compared with I/LnJ and P/J mice (Westaway et al., 1987). However, in this study and previous work there have consistently been a few mice whose incubation period phenotype and PrP-linked restriction site appeared to be separable (Carlson et al., 1986, 1988). This could be explained in at least two ways. If scrapie incubation period and the PrP-linked restriction sites are separate genetic elements, these mice might represent recombinants at a recombination rate of about 2 to 5% between these genetic loci. Conversely, the aberrant scrapie incubation period might be caused by the statistically reproducible cosegregation of non-PrP-linked genes, which together might modify the effect of the PrP gene to produce the opposite incubation period phenotype. In this case there could still be a major gene influencing incubation period in the same genetic locus as the PrP-linked restriction site. Resolution of this issue requires that the true nature of potential recombinants be determined by progeny testing. Unfortunately, all mice have died of scrapie by the time the relevant ones have been identified. A prospective study to characterize these potential recombinants is theoretically possible, but the logistics of such a study are immense. Other studies using VM and VM(Sinc) congenic mice have already showed linkage of the PrP gene and the incubation period gene (Sinc) (Hunter et al., 1987). Unfortunately, using this approach the influence of genetic elements close to the PrP gene cannot necessarily be excluded from the PrP effect. Another possibility would be to transfer the cloned I/LnJ or P/J PrP gene to an NZW background by developing transgenic mice. This approach is quite precise in terms of genetic material transferred, but it may be difficult to achieve due to possible problems of regulation of tissue specificity and levels of expression in vivo.

In previous studies comparing MA/MyJ and C57BL/6 mice no linkage between PrP and scrapie incubation period was observed (Carlson et al., 1988). NZW and C57BL/6 mice are believed to have many similarities in the PrP gene region (Carlson et al., 1988). Thus, it is unclear why our results comparing MA/MyJ and NZW mice showed a PrP-linked effect on the interval to death. The differences between these earlier results and ours might be due to the relatively small number of mice analysed in each instance and the added difficulty of observing PrP-linked effects on incubation periods in the face of the obvious influence of additional non-PrP genes in these backcrosses. Alternatively, there might be PrP-linked genetic differences between NZW and C57BL/6 mice, such that when compared to MA/MyJ only NZW mice would show the linkage observed. However, so far no differences between NZW and C57BL/6 have been detected in restriction mapping studies of PrP (Carlson et al., 1988). Furthermore, in contrast to I/LnJ and P/J mice, C57BL/6, NZW and MA/MyJ all have a BstEII site at the same position within the PrP open reading frame (Westaway et al., 1987). Thus, the linkage between PrP and incubation period in crosses of MA/MyJ and NZW mice cannot be explained on the basis of this particular genetic marker. However, linkage could be due to sequences within the PrP open reading frame other than the one defined by BstEII, or could be due to

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### Table 1. Linkage between interval to death period and PrP XbaI polymorphism in NZW × (MA/MyJ × NZW) F1 backcross mice

<table>
<thead>
<tr>
<th>XbaI pattern*</th>
<th>Incubation period†</th>
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<tr>
<td>3.8 kb band</td>
<td>&lt;150 days</td>
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<tr>
<td>3.8 kb + 5.5 kb band</td>
<td>12‡</td>
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<td></td>
<td>&gt;150 days</td>
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* Mice were grouped by their XbaI restriction enzyme pattern and interval to death after intracerebral inoculation of the Chandler isolate of scrapie agent (Carlson et al., 1986).
† Linkage was significant $P < 0.05$ ($X^2 = 5.34$) when groups of mice were separated on the basis of an interval to death of 150 days. If mice were distributed using a value of 155 days significance was questionable $P < 0.1$ ($X^2 = 3.7$) (Fig. 4).
‡ Numbers represent the number of mice.
some other genetic region linked to XbaI. More striking is the fact that unlike I/LnJ and P/J crosses to NZW all backcross mice from the NZW × (MA/MyJ × NZW) F1 had intervals to death intermediate between those of the F1 and NZW parents. This distribution in the backcross progeny strongly implies that other non-PrP linked genes are involved in determining the incubation period in this cross.

In all of the crosses that we evaluated it is unclear what role the inoculum might have played on the intervals to death. In the experiments that we described, as well as those done recently by others (Carlson et al., 1986, 1988), crude brain homogenates, possibly containing more than one scrapie strain, were used to transmit the scrapie agent (Bruce & Dickinson, 1979; Kimberlin & Walker, 1978). Thus it is conceivable that the interaction of various agent strains with different mouse host genotypes could influence the interval to death. Therefore, it might be worthwhile to repeat some of these studies using different scrapie stocks derived by passage at limiting dilution. Such studies might show whether the same host genes influence the incubation period of disease induced by scrapie agent strains with different biological properties (Bruce & Dickinson, 1987). It is also possible that immunological recognition of tissue alloantigens from brain tissue in the agent inoculum could influence the interval to death. To exclude this possibility it would be desirable to use an agent grown in NZW mice, so all NZW backcross mice would be tolerant of its dominant alloantigens from this mouse strain.

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References


