Genetic Control of Scrapie: Incubation Period and Plaque Formation in I Mice

By Richard I. Carp,* Roger C. Moretz, Michael Natelli and Alan G. Dickinson

New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York 10314, U.S.A. and AFRC & MRC Neuropathogenesis Unit, West Mains Road, Edinburgh EH9 3JF, U.K.

(Accepted 6 October 1986)

SUMMARY

The host component of control of scrapie incubation period in the mouse is manifested largely through the action of the Sinc gene. Only one mouse strain (VM) has been found that is p7p7 (prolonged incubation for ME7 agent) and two other strains have been derived from VM. All other strains, designated s7s7, have a short incubation for ME7. In the present study, the I strain was shown to fulfill the criteria that are characteristic of mouse strains with the p7 allele of Sinc: (i) a comparatively long incubation period for ME7 and a short incubation period for 139A, (ii) the incubation period for F1 hybrid mice (s7s7 × p7p7) either fell between the incubation periods for the parental strains (with ME7) or were longer than either parent (with 139A and 22A), (iii) amyloid plaques occurred following injection of ME7 and 87V but not after 22A or 139A, (iv) lesion profiles for four scrapie strains were similar in I mice and p7p7 mouse strains, and (v) injection of 87V led to disease in less than 300 days. Finally, allelism tests using F1 hybrid mice (I × a p7p7 mouse strain) and progeny of backcrosses between these F1 mice and I mice failed to reveal the segregation of additional major genes affecting scrapie incubation period.

INTRODUCTION

The mouse gene Sinc, an acronym for scrapie incubation, controls the length of the incubation period of all known strains of scrapie agent (Dickinson & Meikle, 1971). Almost all inbred mouse strains carry the s7 allele of Sinc which leads to a comparatively short incubation period for the ME7 scrapie strain. For inbred mice carrying the p7 allele the incubation period is prolonged for ME7. Many scrapie strains have a similar pattern in terms of these two alleles, but other strains (e.g. 22A and 87V) have an opposite pattern with a longer incubation period in s7 than in p7 inbred mouse strains (Dickinson & Meikle, 1969, 1971; Dickinson & Fraser, 1977; Dickinson et al., 1984). Other genes are known to influence incubation period, but their effects are trivial compared to Sinc; also they have never been found to alter the ranking of incubation periods in different Sinc genotypes (Outram, 1976; Kingsbury et al., 1983; Bruce & Dickinson, 1985). Scrapie strain–mouse strain combinations also differ in various aspects of the brain lesions, including the presence and frequency of amyloid plaque formation.

The first documented p7 mouse strain (VM) was derived by selective inbreeding from a colony of mice at the Moredun Institute (Edinburgh, U.K.) in which the s7 and p7 alleles were segregating. Two additional inbred p7 strains (IM and MB) have been derived from the VM strain by crossing and selective inbreeding. All other inbred mouse strains thus far tested are homozygous for s7 (Carp et al., 1985). In the current work, a commercially available inbred mouse strain, I, has been shown to carry an allele of Sinc similar to or the same as p7.
METHODS

Mice. Both male and female I mice and female C57BL mice were obtained from Jackson Laboratories (Bar Harbor, Me., U.S.A.); I mice are sometimes listed as I/Ln. IM mice were maintained as an inbred strain in the Institute for Basic Research in Developmental Disabilities animal colony by brother–sister matings and for these experiments female mice were used. F₁ hybrid mice were produced by crossing C57BL female and I male mice. In a second series of experiments male and female I × IM F₁ hybrid mice were used as well as mice produced from the backcross of these F₁ with I mice. All mice were injected within 1 to 3 weeks of weaning. Mice were maintained in temperature (21 to 24 °C)- and humidity (40 to 50 %)-controlled rooms, with a 12 h on, 12 h off cycle of artificial light. Food and water were given ad libitum.

Inocula

Scrapie strains. The ME7 and 139A scrapie strains had been passaged serially by intracerebral (i.c.) injection of C57BL female mice. At the time of clinical disease, brains were removed under sterile conditions and 10 % homogenates prepared in phosphate-buffered saline containing Ca²⁺ and Mg²⁺ salts (PBS). The same procedure was followed for the 22A and 87V strains which had been serially passaged in IM mice. All homogenates were stored at −70 °C prior to use.

New isolates from scrapie sheep. Scrapie was transmitted to mice from two natural cases in Suffolk sheep. One sheep brain homogenate (10 %) was passaged in IM mice (isolate C603), the other in C57BL mice (isolate C608). Subsequent mouse-to-mouse passages were performed by i.c. injection of 5 % brain homogenates into the same strain of mouse.

Homogenates (1 %) prepared from the second mouse passage were analysed in terms of their relative incubation periods in different strains of mice following i.c. injection.

Injection. For the established scrapie strains, homogenates of mouse brain were prepared in PBS at 10 % concentration (w/v) and then stored at −70 °C. Dilutions were prepared in PBS and mice were injected with 0.03 ml in the right cerebrum.

Incubation period. Mice were monitored weekly for clinical disease starting at 10 weeks post-infection by observing their activity levels and competence on a set of parallel bars as previously described (Carp et al., 1984). The scrapie incubation period was designated as the day after infection on which the mouse had shown clinical symptoms for the 3rd consecutive week. At this stage they were within 1 to 4 weeks of death.

Histopathological evaluation. The technique for evaluation of vacuolation by lesion profiles has been described previously (Fraser & Dickinson, 1973). Brains were removed and placed in 10 % neutral formalin for at least 2 days. Dehydration of brains, paraffin embedding and staining with haematoxylin and eosin were done by standard procedures. Vacuolation was evaluated in nine positions of the grey matter as described (Fraser & Dickinson, 1973). Amyloid plaque incidence at four standard levels was assayed in sections stained with Masson’s trichrome.

RESULTS

Incubation periods for four scrapie strains in I mice and in representative s7 and p7 mouse strains, C57BL and IM respectively, are shown in Table 1. Incubation periods for each of the four scrapie strains in I mice were close to those seen in IM and quite different from those in C57BL mice. The crucial comparison for assessing the action of Sinc is the relative incubation period in s7s7 and pTp7 mice. In contrast, the absolute incubation period in any one genotype is relatively uninformative on its own, because it is very dose-dependent and influenced to a minor extent by genes other than Sinc (Outram, 1976; Kingsbury et al., 1983; Bruce & Dickinson, 1985). In the key comparisons, ME7 had a shorter incubation period and 22A a longer incubation period in C57BL than in either IM or I mice. The 40 to 50 day difference between I and IM mice with ME7 is of minor importance for the reasons just given. It is, for example, of the same magnitude as the difference between RIII and BALB/c mice, both of which carry the s7 gene (Outram, 1976). Incubation periods for the 139A strain were similar in IM and I, and significantly longer than in the s7 mouse strains. For 87V, incubation periods in both I and IM mice were much shorter than for C57BL (Table 1).

Incubation periods in IM and C57BL mice were determined for 1 % homogenates prepared from the second passage of isolates from two sheep, C603 and C608. For C603, the primary isolation was in IM mice and the second passage also in IM mice, whereas for C608 primary isolation and second passage were made in C57BL mice. The results (Table 2, experiment 1) show that the C608 isolate had incubation period values similar to those for the ME7 scrapie strain, whereas incubation periods for the C603 isolate were quite different, being shorter in the p7 strain (IM) than in the s7 strain (C57BL), results which are characteristic of the 22A group of
Table 1. Incubation periods of various scrapie strains in C57BL, IM and I mouse strains

<table>
<thead>
<tr>
<th>Scapie strain</th>
<th>Incubation period (days ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C57BL (s7s7)</td>
</tr>
<tr>
<td>ME7</td>
<td>145 ± 1</td>
</tr>
<tr>
<td>139A</td>
<td>125 ± 1</td>
</tr>
<tr>
<td>22A</td>
<td>356 ± 4</td>
</tr>
<tr>
<td>87V</td>
<td>&gt;600*</td>
</tr>
</tbody>
</table>

* Following i.c. injection of 10% homogenates some C57BL mice became positive for clinical disease between 600 days and the end of their natural life span.

Table 2. Incubation periods of two sheep scrapie isolates in C57BL, IM and I mice

<table>
<thead>
<tr>
<th>Sheep isolate</th>
<th>C608</th>
<th>C603</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>151 ± 2</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>300 ± 8</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>145 ± 0</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>255 ± 4</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3. Incubation periods of ME7, 139A and 22A in C57BL, I and C57BL × I F1 hybrid mice

<table>
<thead>
<tr>
<th>Scapie strain</th>
<th>C57BL</th>
<th>I</th>
<th>C57BL × I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mice</td>
<td>Days ± S.E.M.</td>
<td>No. of mice</td>
</tr>
<tr>
<td>ME7</td>
<td>32</td>
<td>145 ± 2</td>
<td>10</td>
</tr>
<tr>
<td>139A</td>
<td>20</td>
<td>125 ± 1</td>
<td>7</td>
</tr>
<tr>
<td>22A</td>
<td>12</td>
<td>356 ± 4</td>
<td>9</td>
</tr>
</tbody>
</table>

scrapie strains (Outram, 1976). The comparative incubation period values for these sheep isolates in C57BL and I mice were essentially similar to those seen in the C57BL and IM strains (Table 2, experiment 2). The C603 strain had a shorter incubation period in I than in C57BL mice whereas the C608 strain had a shorter incubation period in C57BL than in I mice. Thus, the results for I mice were similar to those for the representative p7 strain, IM.

In previous studies of incubation periods in s7p7 F1 hybrid mice, some scrapie strains had values that were between those for the two parental mouse strains, whereas the F1 incubation periods for other scrapie strains were longer than the values for either parental mouse strain (Dickinson & Meikle, 1971). Incubation periods for ME7, 139A and 22A were analysed in C57BL × I F1 mice and the results (Table 3) were compared to the incubation periods for the same scrapie strains in the parental mouse strains. The incubation period for ME7, 200 days, fell midway between the values obtained for the two parental strains, whereas the values for the 139A and 22A strains were higher in F1 mice than in either of the parental strains. Similar relationships have been reported for the incubation periods of these three scrapie strains in C57BL, IM (and VM) and their F1 cross (Dickinson, 1975; Scott & Dickinson, 1985).

It next needed to be established whether the similarity of control of scrapie incubation period is due to the same gene in the I stock as in the IM and VM stocks of mice. Substantial evidence in favour of this stems from the comparison of incubation periods in I × IM F1 mice with those in the two parental strains (Table 4): for 22A the F1 incubation was 187 ± 3 days, for ME7 it was...
Fig. 1. Fluorescence micrographs of thioflavin S-stained sections from I mice infected with ME7 (a, c, e) and 87V (b, d, f) scrapie agents: (a, b) thalamus, (c, d) corpus callosum, (e, f) cortex. Bar markers represent 100 μm.
Table 4. Incubation periods of ME7, 22A and 87V scrapie strains in I × IM F1 mice and F1 × I backcross mice

<table>
<thead>
<tr>
<th>Scrapie strain</th>
<th>IM</th>
<th>I</th>
<th>F1</th>
<th>Backcross</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Days ± S.E.M.</td>
<td>No. Days ± S.E.M.</td>
<td>No. Days ± S.E.M.</td>
<td>No. Days ± S.E.M.</td>
</tr>
<tr>
<td>ME7</td>
<td>9 300 ± 3</td>
<td>10 252 ± 3</td>
<td>11 293 ± 4</td>
<td>6 286 ± 3</td>
</tr>
<tr>
<td>22A</td>
<td>69 190 ± 1</td>
<td>9 184 ± 4</td>
<td>11 187 ± 3</td>
<td>9 173 ± 2</td>
</tr>
<tr>
<td>87V</td>
<td>105 274 ± 1</td>
<td>4 277 ± 2</td>
<td>10 300 ± 5</td>
<td>7 315 ± 10</td>
</tr>
</tbody>
</table>

293 ± 4 days, for 87V it was 300 ± 5 days, and for 139A (data not in table) it was 170 ± 3 days. These are essentially similar to the I and IM incubation periods for these scrapie strains in the non-contemporary experiment shown in Table 1. Backcrossing of the I × IM F1 mice to one of the parental strains (I) gave no evidence that gene segregation relating to incubation period had occurred and this is further evidence that the same gene is involved (Table 4).

The pattern of vacuolation intensity at nine positions in the brain is termed the lesion profile (Fraser & Dickinson, 1973; Fraser, 1976). The appearance of the profiles for ME7, 22A, 87V and 139A were similar in I and IM mice. The differences were no greater than those seen within other groupings of p7p7 or s7s7 mouse strains (Fraser, 1971).

An analysis of plaque formation in I mice yielded results that were similar to those seen in IM mice and other p7 inbred strains. The 22A and 139A strains failed to cause plaque formation in either I or IM mice. In contrast, ME7 and 87V produced extensive plaque formation in I mice. In Fig. 1, sections of thalamus, corpus callosum and cortex are shown for ME7 (Fig. 1 a, c, e) and 87V (Fig. 1 b, d, f). The extent of plaque formation in I mice injected with 87V and ME7 was similar to that seen in IM mice injected with these strains. The results for the 139A and 87V strains in I mice cannot be used to distinguish the Sinc genotypes since 139A does not form plaques in mouse strains carrying either the s7 or p7 allele and 87V produces plaques in both. In contrast, the complete absence of plaques in 22A-injected I mice and the presence of extensive plaque formation in ME7-injected I mice is different from results obtained in s7s7 strains in which 22A causes plaques and ME7 produces few if any.

DISCUSSION

The incubation period characteristics for all the scrapie strains tested clearly show that I mice carry an allele of Sinc either identical to p7 or a new allele very similar to it. In comparison to data obtained with s7 mouse strains such as C57BL, the relatively long incubation period for ME7 and the relatively short incubation period for 22A is the criterion that establishes the similarity of I mice to a p7 strain. The close similarity is confirmed by the patterns of dominance and overdominance displayed in F1 mice (I × C57BL and IM × C57BL) with scrapie strains ME7, 22A and 139A. The incubation period data obtained with I × IM F1 hybrid mice were similar with each scrapie strain to those observed in the parental stocks. This is strong evidence that the same gene, namely Sinc and not a p7-like mutant at a different locus, is operative. Further evidence on this point is provided by the fact that incubation periods for the progeny of a backcross between I and the I × IM F1 mice yielded incubation periods identical to those seen in both I and IM mice.

All other data support the above conclusion. First, 87V caused disease in I mice in less than 300 days, whereas in s7 strains 87V does not cause disease before at least 600 days, even after i.e. injection with 10% brain homogenate. Second, there was extensive amyloid plaque formation in ME7-injected I mice, a pathological lesion found in ME7-p7p7 but absent or at very low incidence in ME7-s7s7 combinations. Also, 22A did not produce plaques in I mice, whereas this strain does produce plaques in mice carrying s7. Third, the lesion profiles for I mice with ME7, 22A, 87V and 139A were broadly similar to those seen in p7 strains and quite distinct from s7 strains. Finally, the relationship of incubation periods of two new sheep isolates were similar in I compared to C57BL as in IM compared to C57BL mice.
In a previous study (Kingsbury et al., 1983) four I mice (referred to as ILN/J), injected more than 200 days previously with 'Chandler agent', died suddenly without any neurological symptoms characteristic of scrapie. No histopathological findings for these mice were given although they were said to have died from scrapie. In the present study, I mice at the clinical stage of scrapie showed typical scrapie symptoms, with locomotor and coordination signs the earliest seen (Carp et al., 1984). After their third consecutive positive score, the I mice tended to die more quickly than other strains, usually within 1 or 2 weeks.

Extensive searches of available inbred lines of mice and a few randomly bred colonies (Outram, 1976; Carp et al., 1985; A. G. Dickinson, unpublished) had hitherto failed to find Sinc alleles other than s7, apart from the initial discovery of p7 in a randomly bred colony which had been used since the 1940s for work with scrapie (Dickinson & Meikle, 1971). The original p7 allele and that in the I mice must have arisen as independent mutations because the stocks involved have had completely separate histories. The I strain originated in 1926 in the U.S.A. (Festing, 1979); the VM strain, produced as a carrier of the p7 allele, was inbred in Edinburgh, U.K. from randomly bred animals stemming from the Institute of Animal Genetics in Edinburgh, which has never imported the I strain.

At present, the function of the Sinc gene in uninfected animals remains unknown, as does the chemical nature of any product for which it may code. Furthermore, the mechanism of action of the putative Sinc gene product, in determining the phenotypic expression seen following infection with various scrapie strains, is still a matter for speculation. It is known that the gene controls incubation period, regardless of the route of infection and that it acts throughout the incubation period, rather than only on the primary infective events (Dickinson & Fraser, 1977). It has been postulated that there are a finite number of replication sites for scrapie agent, coded by Sinc, and variation between the alleles presumably relates to the specificity of these sites for different strains of scrapie (Dickinson & Outram, 1979). The nature of the scrapie agent is still unknown, apart from substantial evidence that it has an independent genome which can mutate (Bruce & Dickinson, 1979; Dickinson et al., 1984; Scott & Dickinson, 1985). A small nucleic acid with only regulatory functions and protected by host-specified components is a plausible model for the agent (the virino hypothesis: Dickinson & Outram, 1983). The specificity of Sinc for different scrapie strains could relate to specific interactions with sequences of the agent genome. One possibility, though not particularly likely, is that agent replication is at the level of the host chromosome (Dickinson & Meikle, 1969). If this were so, the possibility has had to be entertained that a mutation from s7 to p7 in Edinburgh might have been induced directly by scrapie agent. One important outcome of the present work is that it excludes this explanation.

It has recently been reported (Carlson et al., 1986), using restriction fragment length polymorphism analysis of I mice, that the gene coding for the precursor to the protein (PrP) found in scrapie-associated fibrils (SAF) (Merz et al., 1981; Bolton et al., 1982; Oesch et al., 1985; Chesebro et al., 1985; Robakis et al., 1986) is on the same chromosome as Sinc. It will be important for understanding the control of scrapie agent replication to establish whether the SAF protein determinant and Sinc are part of a closely linked, organized genetic system or even a single gene with the precursor of the SAF protease-resistant protein as the Sinc product.

The existence of additional inbred strains carrying the p7 allele in a genetic background different from that in VM (and its derivatives, IM and MB) will assist in investigation of the mechanism of Sinc action in controlling the overall dynamics of agent replication. Some strains of scrapie (e.g. 87V) have a high amyloid plaque incidence in I mice, which will assist in cerebral amyloid investigations. Finally, the I strain can be used as a substitute for VM or IM mice in strain-typing new scrapie isolates.

The authors thank Mrs Adele Monaco for her excellent assistance in preparation of the manuscript and Ms Sharon Callahan for her technical expertise. This work was supported in part by grant NS21349-02.
REFERENCES


(Received 17 July 1986)