Agent Replication Dynamics in a Long Incubation Period Model of Mouse Scrapie

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SUMMARY

The dynamics of scrapie agent replication in brain and spleen following intracerebral or intraperitoneal infection were investigated in a model with particularly long incubation periods [IM mice (Sincp7) infected with 87V scrapie]. In contrast to other mouse scrapie models previously investigated, there was no delay in onset of replication ('zero phase') in spleen using either route of infection. For both routes infectivity levels reached a plateau in spleen, which was maintained for at least 250 days in intraperitoneally infected mice. An infectivity plateau was also apparent in brain following intracerebral infection, suggesting that there may be constraints on agent replication in brain during the later part of the incubation period. In addition, the efficiency of the intraperitoneal route to produce disease was found to be particularly low for this model.

INTRODUCTION

There have been a number of previous reports on the replication dynamics of scrapie agent in mice following infection by various routes (e.g. Eklund et al., 1967; Dickinson & Fraser, 1969; Dickinson et al., 1969; Kimberlin & Walker, 1979a). Most of these have involved either ME7 or 139A (Chandler) strains of scrapie in mouse strains that give relatively short incubation periods. In general, these studies have shown that, following peripheral infection, scrapie agent undergoes an extraneural replication phase before infectivity can be detected in the central nervous system (CNS) (Dickinson & Outram, 1979; Kimberlin, 1979). During this phase the agent replicates and accumulates in lymphoreticular organs such as spleen, in which it reaches a plateau level. Subsequently, infective agent enters the spinal cord, probably via sympathetic nerves, and ascends to the brain (Kimberlin & Walker, 1980, 1982). With an intracerebral (i.c.) route, agent from the inoculum directly establishes infection in the brain, although there is also extraneural replication initiated by the spillover of inoculum into the bloodstream. It has been suggested that, by all routes, clinical disease occurs when infectivity reaches a threshold level in certain 'clinical target areas' in the brain (Fraser & Dickinson, 1973; Kimberlin & Walker, 1983).

Many strains of scrapie have been identified, each with characteristic incubation period properties in inbred mice of particular genotypes (Dickinson & Fraser, 1977). The present paper describes a preliminary study of scrapie pathogenesis in a long incubation period combination, the 87V strain of scrapie in IM mice, following i.c. and intraperitoneal (i.p.) infection. Long incubation period models such as this are particularly suitable for studies on the constraints on agent replication at various stages in pathogenesis.

The 87V/IM model is of interest for two other reasons. Firstly, it has been used extensively in studies on the development of cerebral amyloid plaques, which provide a model for the amyloid-containing senile plaques of Alzheimer's disease (Fraser & Bruce, 1983). Secondly, serum IgG levels have been shown to become elevated during the incubation period of both i.c. and i.p. infected mice (Collis, 1983). The present investigation provides background information on replication dynamics, needed for the interpretation of these studies. A similar study of
pathogenesis in this model, but differing in detail, has also been completed recently by colleagues working in another laboratory (Collis & Kimberlin, 1985).

METHODS

Inbred mice of the IM/Dk, VM/Dk and MB/Dk strains were used. These all carry the p7 allele of the Sinc gene, which controls scrapie incubation period in mice (Dickinson & Meikle, 1971) and therefore have approximately equal incubation periods with 87V (Bruce & Dickinson, 1985). The 87V strain of scrapie was derived from a natural case in a Cheviot x Border Leicester sheep, by serial i.c. passage at high dose in VM mice.

Efficiency of the i.p. route. In a retrospective study the results of 12 experiments in which 87V brain inoculum had been injected i.p. into IM, VM and MB mouse strains were combined. 87V was used at the sixth, seventh or eighth mouse passage. Each mouse received 0.02 ml of inoculum at either 10^-1 or 10^-2 dilution. All mice were monitored for clinical signs and were killed or died either at the clinical scrapie endpoint or for other unrelated reasons. Brains were taken from all animals for histopathological diagnosis as described below.

Replication dynamics. The 87V strain used had been passaged i.c. at high dose six times in VM mice and once in IM mice. Brain material from a single terminally affected female IM mouse was homogenized in physiological saline at 10^-1 dilution and centrifuged at 500 g for 10 min. Female IM mice were injected with 0.02 ml of the supernatant, either i.c. into the right hemisphere or i.p. This represented a dose of approximately 10^4.5 i.c. LD50 units or less than 10 i.p. LD50 units of infectivity.

At the time of injection mice were randomly allocated to be killed at intervals through the incubation period. This allocation was strictly adhered to for the i.c. group. However, two mice were lost from the i.p. group, for reasons unrelated to scrapie, before their allocated killing dates (440 and 495 days) and substitutions were made from a group of 'spare' mice put aside for this purpose at the time of injection. Incubation periods were determined for each route of infection from additional groups of approximately ten animals, which were held until they developed clinical scrapie. These mice were scored for clinical signs and were killed at a standard endpoint, as previously described (Dickinson et al., 1968), when they were in extremis or had shown severe clinical signs of scrapie for 3 consecutive weeks. The diagnosis of scrapie was confirmed histologically and incubation periods were calculated as the interval between injection and endpoint.

Tissues for assay were collected from all serially killed mice and from the first animal injected by each route to be killed with terminal scrapie. No further tissues were taken for assay after this first mouse had reached the scrapie endpoint. Mice were killed by cervical fracture and brains and spleens were removed aseptically, using separate instruments for each tissue to avoid cross-contamination. Brains were sliced vertically, parallel to and slightly to the left of the midline. Spleens and left half-brains for assay were stored frozen at -25 °C, in separate bottles. Right half-brains were fixed in 10% formol saline, sliced at four standard coronal levels as previously described and embedded in paraflin wax (Fraser & Dickinson, 1968). Six-μm sections were stained with haematoxylin and eosin.

Infectivity levels were measured by incubation period assay, using the spleen and, where appropriate, the left half-brain from a single animal taken at each killing date. The tissues were homogenized in physiological saline at 10^-1 dilution; 0.02 ml of homogenate was injected i.c. into groups of 12 IM mice, including equal numbers of males and females, and incubation periods were determined as described above. As there is an inverse relationship between dose of infectivity and incubation period (Dickinson et al., 1969; Kimberlin & Walker, 1978), the mean incubation periods for the recipient groups were plotted inversely against the time after injection at which the tissues were collected. This gives 'replication curves' comparable to those described by Kimberlin & Walker (1979a).

To provide a reference standard for calculating infectivity titre from incubation period a conventional endpoint titration was carried out as follows. Terminal VM 87V brain was homogenized in physiological saline and centrifuged at 2000 g for 15 min. Serial tenfold dilutions in saline were prepared. For each dilution, 0.02 ml was injected i.c. into six IM mice (Table 1). It is assumed that the 87V dose–response curves in IM assay mice using IM and VM brain do not differ and also that spleen homogenates have the same dose–response characteristics as brain.

RESULTS

Efficiency of the i.p. route

The results of 12 experiments in which 87V terminal brain inoculum was injected i.p. into IM, VM and MB mice were combined (Fig. 1). As there were no consistent differences between mouse strains all three were considered together. Following injection of 10^-1 inocula, about two-thirds of the mice had positive scrapie pathology and some reached the clinical phase of the disease, with incubation periods ranging from 460 to 720 days. However, interspersed with the positive cases there were mice with no pathology, even up to 700 days post-injection. In contrast,
Fig. 1 Pooled results from experiments in which 87V brain was injected i.p. into IM, VM and MB mice at $10^{-1}$ or $10^{-2}$ dilution. Mice were killed either at the clinical scrapie endpoint or because of senility or other unrelated conditions. The brains of all mice were examined histologically. Individual mice were classified as follows on the basis of clinical and pathological diagnosis for scrapie: $\blacksquare$, clinically and pathologically positive; $\bullet$, clinically negative, pathologically positive; $\bigcirc$, clinically and pathologically negative. No infectivity was detected in the spleen of the mouse indicated by an arrow.

Table 1. Relationship between dose of infectivity and incubation period for IM mice injected i.c. with 0.02 ml terminal 87V brain inoculum

<table>
<thead>
<tr>
<th>Dilution of inoculum</th>
<th>Dose of infectivity (log$<em>{10}$ LD$</em>{50}$ units)*</th>
<th>Incubation period (days ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-2}$</td>
<td>3.5</td>
<td>299 ± 6</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>2.5</td>
<td>323 ± 10</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>1.5</td>
<td>347 ± 5</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>0.5</td>
<td>429 ± 33</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>–</td>
<td>All negative</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>–</td>
<td>All negative</td>
</tr>
</tbody>
</table>

* The LD$_{50}$ of the undiluted homogenate, calculated by Kärber's method (Parker, 1961), was $10^{-5}$. No clinical signs of scrapie were observed in any of the mice injected with $10^{-2}$ inocula; animals were killed or died due to unrelated conditions or senility. Only one mouse, which died at 410 days post-injection, showed positive scrapie pathology, consisting of very mild vacuolar lesions.

This was a retrospective study and tissues for passage had not been routinely taken. Therefore, it was not possible to determine whether the negative mice were true survivors or whether they were incubating scrapie in peripheral organs and heading for incubation periods longer than their lifespan. However, spleen had been collected from one of these negative mice in the $10^{-1}$ group, killed 790 days after injection. VM mice, injected i.c. with a $10^{-1}$ homogenate of this spleen, remained healthy and were killed at 607 days post-injection, at which stage they were pathologically negative. Assuming the majority of the other negative animals to be true survivors, the LD$_{50}$ using terminal 87V brain tissue would be between $10^{-1}$ and $10^{-2}$, differing from the i.c. LD$_{50}$ by a factor of about $10^{4}$ (Table 1; Bruce & Dickinson, 1985). It should also be noted that the titre of 87V in terminal brain measured by i.c. titration is itself rather low when compared to other scrapie models; this has previously been shown not to depend on mouse strain differences between injected tissue and recipients (Bruce & Dickinson, 1985).

Replication

The i.c. injected mice had a mean incubation period of $289 \pm 2$ days with individual values ranging from 286 to 300 days. Replication curves for brain and spleen are shown in Fig. 2(a). The level of infectivity in both organs dropped initially, probably due to the clearance of much of
the infectivity from the original inoculum, but at no stage was infectivity undetectable in either brain or spleen. In brain there was a steady increase in infectivity starting soon after injection, indicating that agent was replicating. However, it must be pointed out that plotting the results in this way exaggerates these early events because of the non-linearity of the dose–response curve. In the later part of the incubation period infectivity levels appeared to reach a plateau which was maintained until the endpoint. Vacuolar degeneration was first seen in the brain after this plateau had been reached, at 215 days after injection. In spleen, infectivity increased at about the same rate as in brain up to about half way through the incubation period and then remained fairly constant until the endpoint. The plateau level in spleen was lower than that in brain, corresponding to about a tenfold difference in infectivity titres.

Incubation periods in the i.p. injected mice covered a wide range, from 505 to at least 587 days. Three mice died between 587 and 622 days having shown no clinical signs, their brains being too autolysed to make any histopathological judgement. It is therefore not possible to set an upper limit on the incubation period range for this group, or to determine whether there were any true survivors, although the results of the serial study described below suggest that all mice were incubating scrapie in their spleens. Infectivity was assayed in spleen at intervals
Scrapie agent replication

throughout the incubation period, and in brain at 415, 440, 470, 495 and 505 days (Fig. 2b). In spleen, after an initial drop, infectivity increased until a plateau was reached about half way through the incubation period and this was maintained until the endpoint. Results for brain were more erratic; brains taken at 415, 440 and 470 days were positive for infectivity but the brain taken at 495 days was negative. Positive pathology was seen only in the mice killed at 440 and 505 days. The latter had clinical signs of scrapie and severe neuropathological lesions. This erratic pattern is consistent with the wide range of incubation periods seen in this i.p. injected group.

DISCUSSION

The replication dynamics for 87V injected i.e. or i.p. were broadly similar to those previously reported for other scrapie strains. After i.c. infection, replication proceeded in the brain and spleen from early in the incubation period. With both routes, the level of infectivity in the spleen rose to a plateau which was maintained until endpoint. However, there are some striking differences between these and previously reported replication curves. Following i.c. injection, infectivity titres in brain and spleen rose simultaneously at the same rate, in contrast to previous results for mice infected with scrapie or with mouse-passaged Creutzfeldt-Jakob disease agent (an analogue of scrapie), in which spleen titres increased rapidly to a plateau, followed later by a slower replication in brain (Dickinson & Fraser, 1969; Dickinson et al., 1969; Kimberlin & Walker, 1979a; Kuroda et al., 1983).

The present study and the results of Collis & Kimberlin (1985) also suggest that there is a plateau of infectivity in brain as well as in spleen. A definite brain plateau has so far only been observed using one other model, 22A injected i.c. into C57BL mice (D. M. Taylor & A. G. Dickinson, unpublished results). Apart from this, most controls on scrapie incubation period are thought to operate at an early stage, for example by delaying the onset of agent replication. The existence of a brain plateau under some circumstances is evidence that there can also be constraints on agent replication at a late stage in the incubation period.

With both i.c. and i.p. routes, 87V infectivity could be demonstrated in spleen at all times after injection, that is, there was no obvious 'zero phase' as described by Kimberlin & Walker (1979b). The zero phase is the delay in onset of replication, seen in some models, during which time infectivity is undetectable by available assay systems. Previous work has suggested that the zero phase is more prominent in long incubation period models. For example, a zero phase in spleen of at least 100 days has been reported in C57BL mice injected i.p. with a high dose of 22A scrapie, a model with an incubation period of about 560 days; this zero phase could be greatly extended by reducing the dose of 22A (Dickinson et al., 1975). Similarly, a zero phase of several weeks was observed in VM mice infected i.p. with a high dose of ME7, with an incubation period of about 540 days (Dickinson & Fraser, 1969). It is therefore surprising that no zero phase is apparent using 87V, with i.p. incubation periods almost as long as these two models, particularly as the effective i.p. dose appears to be very low. Another notable feature of the i.p. data, as presented here and as found by Collis & Kimberlin (1985), is the extended period of time that infectivity is present at high titre in spleen before being detected in the brain. The previous study of the early stages of peripheral pathogenesis in VM mice with ME7 suggested, but did not directly demonstrate, a spleen plateau of comparable length (Dickinson & Fraser, 1969).

In general, in experimental scrapie the i.p. route of infection is less efficient than the i.c. route, usually by a factor of $10^2$ or $10^3$ (Kimberlin & Walker, 1978, 1983). The results reported here show that for 87V the i.p. route is particularly inefficient in terms of causing disease, differing in efficiency from the i.c. route by a factor of about $10^4$. This is partly due to a low neuroinvasiveness once infection has been established peripherally. However, the results also suggest an extremely low initial efficiency of infection; the absence of infectivity in the spleen of one of the apparent survivors, killed when senile, supports this possibility. If a low efficiency of infection is confirmed by further work, this model may provide insights into the initial processing and inactivation of infectivity from the inoculum.

By selecting short incubation period strains, a number of 'quick' scrapie models have been developed, which have proved to be valuable in outlining the sequence of events during
pathogenesis. However, it is likely that ‘quick’ models are quick because some of the constraints on agent replication are bypassed. Therefore, long incubation period models, such as the one described here, are more appropriate in investigations into the control of pathogenesis at various stages in the sequence. These models are also more likely to be relevant to the natural disease, which invariably follows a slow course.

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REFERENCES


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