Pathogenesis of Mouse Scrapie: Evidence for Spread of Infection from Central to Peripheral Nervous System

(Accepted 13 October 1982)

SUMMARY

The onset of replication has been studied in spinal cord, dorsal root ganglia and spinal nerves of CW mice infected intraperitoneally with the 139A strain of scrapie. The patterns obtained suggest a centrifugal spread of infection from central to peripheral nervous systems. Infectivity titres in the peripheral nervous system reached a plateau long before the end of the incubation period, and the maximum titres were much lower than in the central nervous system. This suggests that there is a restriction of replication in the peripheral nervous system similar to that already known in extraneural tissues.

In previous studies, we used a short incubation model of scrapie [strain 139A in Compton White (CW) mice: Sine^+] to investigate how the agent enters the central nervous system (CNS) after replication in spleen and other visceral lymphoid organs. In the absence of direct methods of study, we compared the times of onset of replication in different tissues, and in various parts of the CNS, on the assumption that the sequences obtained reflect the gradual spread of infectious agent. These studies used different routes of infection [e.g. intravenous, intraperitoneal (i.p.), subcutaneous] and relatively low doses of infectivity so that the detection of the onset of replication would not be obscured by any uptake and persistence of the original inoculum. Consistent patterns were obtained showing, successively, replication in spleen and thoracic spinal cord and then, simultaneously, in brain and lumbar cord (Kimberlin & Walker, 1979, 1980). In more detailed studies with i.p. infected mice, the patterns suggested entry of agent into spinal cord between thoracic vertebrae 4 and 9, followed by gradual spread to lower spinal cord and simultaneous forward spread to upper cord, medulla, various mid-brain regions and finally, anterior brain (Kimberlin & Walker, 1982). The initial onset of agent replication in mid-thoracic cord is strong circumstantial evidence for centripetal invasion of the CNS by scrapie via sympathetic nerves serving spleen and visceral lymph nodes.

The present paper is concerned with the possibility that invasion of the CNS by scrapie agent may be followed by centrifugal spread of infection to other parts of the peripheral nervous system (PNS). It seemed likely that this would occur since many neurotropic viruses spread both centripetally and centrifugally (see below). We therefore tested the prediction that spread of scrapie agent posteriorly to lumbar cord would lead to a later occurrence of replication in lumbar dorsal root ganglia (DRG). We also examined DRG and spinal nerves of mid-thoracic segments to test two possibilities: either a similar pattern of centrifugal spread to DRG in both lumbar and thoracic segments, or a pattern of centrifugal spread in lumbar segments contrasting with centripetal spread in thoracic segments, which might suggest that the initial invasion of the CNS occurred via autonomic afferent pathways in DRG and spinal nerves.

Two experiments were carried out using CW mice (Sine^+) infected i.p. with 0.1 ml of brain homogenates containing 100 to 1000 LD50 i.p. units of the 139A strain of scrapie agent. Groups of donor mice were infected each week to provide animals at different stages of incubation on a given day (Kimberlin & Walker, 1982). Three mice from each group were taken and tissues removed in the following sequence: brain, thoracic cord (between vertebrae T5 and T11), lumbar cord (between vertebrae L1 and L5) followed by the corresponding thoracic spinal nerves (1 experiment), thoracic DRG and lumbar DRG. With the last three tissues, pairs of spinal nerves or ganglia were taken from four segments to provide 3 to 11 mg samples from three mice. The samples of spinal nerve were distal to the DRG. The ganglia samples contained up to
Fig. 1. Agent (139A) replication in CNS (solid lines) and PNS (broken lines) of donor mice infected i.p.; brain (▲), thoracic cord (●), lumbar cord (■), thoracic ganglia (○), thoracic spinal nerves (△) and lumbar ganglia (□). The ordinate shows the incubation period in recipient groups of mice inoculated intracerebrally (i.c.) and indicates relative amounts of infectious agent in tissue homogenates. The curves are formed from recipient groups where all mice tested developed scrapie. Points on the abscissa denote recipient groups in which less than 100% of mice developed scrapie, indicating very low levels of agent in tissues. The abscissa shows sample times as a percentage of the incubation period of donor mice; this was 160 ± 1 (days ± s.e.) for experiment 1 (a to c) and 169 ± 1 for experiment 2 (d to f).

Half their weight of attached spinal nerve roots. Different sets of dissection instruments were used for different tissues to minimize cross-contamination of scrapie agent between samples (Kimberlin & Walker, 1979). The pooled tissues from three mice were homogenized in saline at a concentration of 0.5% wet weight (experiment 1) or 0.25% (experiment 2). The homogenates were injected i.c. into recipient groups of eight female CW mice, which were observed for the development of clinical scrapie for up to a maximum of 300 days after injection. The average incubation period was calculated for all groups with 100% clinical cases. Because of the inverse relationship between concentration of agent and length of incubation period (Kimberlin & Walker, 1978), replication in donor tissues can be readily detected by a rapid reduction in the average incubation period of consecutive groups of recipient mice. (It is difficult to prove that a progressive increase in infectivity within a tissue is due to replication and not to accumulation from elsewhere, but it is a reasonable assumption.)

Fig. 1 (a, d) confirms the results of previous experiments with i.p. infected mice (Kimberlin & Walker, 1980, 1982) that replication in thoracic cord precedes that in brain and lumbar cord.
Table 1. Infectivity titres in various tissues during the incubation period of scrapie

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Percent of incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>57*</td>
</tr>
<tr>
<td>Brain</td>
<td>3.8†</td>
</tr>
<tr>
<td>Thoracic cord</td>
<td>5.6</td>
</tr>
<tr>
<td>ganglia</td>
<td>≤2.9</td>
</tr>
<tr>
<td>nerves</td>
<td>ND†</td>
</tr>
<tr>
<td>Lumbar cord</td>
<td>≤3.2</td>
</tr>
<tr>
<td>ganglia</td>
<td>≤2.6</td>
</tr>
</tbody>
</table>

* Data taken from experiment 1; the other times from experiment 2.
† Infectivity titres expressed as $-\log_{10}$ LD$_{50}$ i.e. units per 0.03 g of tissue.
‡ ND, Not done.

There were three new findings. First, the onset of replication in both lumbar and thoracic DRG was 4 to 13% of the incubation period (1 to 3 weeks) later than the onset of replication in the corresponding segments of cord (Fig. 1b, c, e, f). This suggests that there is a centrifugal spread of infection from CNS to PNS, and the observation in experiment 2 that replication in thoracic spinal nerves occurred later than in thoracic ganglia (Fig. 1e) supports this view. Hence the initial centripetal invasion of the CNS in mid-thoracic cord (Kimberlin & Walker, 1980, 1982) probably occurs via pathways not associated with DRG and spinal nerves; the most likely pathway from spleen is via postganglionic fibres to the coeliac ganglion and then along preganglionic fibres to the sympathetic trunk and ventral spinal roots.

The second finding was that the curves for brain, thoracic cord and lumbar cord tended towards plateaus of similar height, representing similar concentrations of infectivity in these tissues at the end of incubation (Fig. 1d). Thirdly, the curves for thoracic spinal nerves, thoracic ganglia and lumbar ganglia reached definite plateaus quite early in the incubation period but at levels much lower than in the CNS.

The third finding was unexpected and led us to carry out complete titrations of infectivity in selected samples that had been stored as 0.5 or 0.25% homogenates at $-20^\circ$C for 7 to 10 months. The results showed that after 87 and 95% of the incubation period, the infectivity titres in brain, thoracic cord and lumbar cord were similar (5.8 to 6.3 $\log_{10}$ units/0.03 g; Table 1). The titres of agent in ganglia and spinal nerves were also similar to one another (4.3 to 4.8 $\log_{10}$ units/0.03 g) but were on average 1.5 $\log_{10}$ units lower than in the CNS.

The present and previous studies suggest that both centrifugal and centripetal spread of infection occurs between CNS and PNS, but along different pathways. In this respect, scrapie may resemble neurotropic viruses such as rabies (Murphy, 1977), herpes simplex (Wildy et al., 1982) and pseudorabies (Field & Hill, 1974). There is one study (with a different combination of scrapie strain and mouse genotype) showing intraneuronal transport of agent from eye to contralateral superior colliculus (Fraser, 1982), but otherwise nothing is known of which cells transport scrapie in nerves or in the CNS. However, the apparent rate of spread of scrapie seems generally to be much slower than with the neurotropic viruses. For example, retrograde axonal transport of herpes simplex, rabies, pseudorabies and polio occurs at rates of 50 to 250 mm/day (Kristensson, 1978; Wildy et al., 1982) whereas the estimated rate of spread of scrapie agent within the CNS is only 0.5 to 1.0 mm/day (Kimberlin & Walker, 1982). A similar slow rate probably applies to the centrifugal spread of scrapie agent, otherwise the patterns shown in Fig. 1 would not have been detectable using 1 week intervals between samples. Assuming that there is intraneuronal spread, the estimates for scrapie are approximately equal to the slow axoplasmic transport of cytoskeletal proteins (Black & Lasek, 1980).

The infectivity titres in ganglia and spinal nerves were different from those in the CNS in two respects; they reached a plateau long before the end of the incubation period and the plateau titres were about 30-fold less than the maximum CNS titres (Fig. 1, Table 1). Other studies, of the same scrapie model, have shown that these two features also occur in extraneural tissues where the agent replicates, namely spleen (Clarke & Haig, 1971; Kimberlin & Walker, 1979),...
cervical lymph nodes (R. H. Kimberlin & C. A. Walker, unpublished observations) and submaxillary salivary gland (Millson et al., 1979). The differences in infectivity titres between spleen and brain have been seen in other models of scrapie and interpreted as evidence for a more limited number of replication sites in extraneural tissues compared to the CNS (Dickinson & Outram, 1979). It is therefore of great interest that a similar restriction seems to apply to replication in the PNS. What this means, however, in terms of different populations of cells in CNS, PNS and extraneural tissues is completely unknown.

Previous studies indicated that scrapie agent eventually spreads to most, if not all, parts of the CNS (Kimberlin & Walker, 1982). Spread of infection from brain to optic nerve and retina has been suggested recently for a hamster model of scrapie (Buyukmihci et al., 1980; Hogan et al., 1981). The present studies indicate that scrapie infection may spread to much of the PNS as well. Hence, the pruritus which frequently occurs in natural sheep scrapie could be a consequence of agent in the PNS, or even in skin, rather than in the CNS, as is often assumed. If our observations with scrapie apply to the related diseases, then spread of Creutzfeldt–Jakob agent to the PNS could account for the recent findings of autonomic dysfunction in two cases of Creutzfeldt–Jakob disease in man (Khurana & Garcia, 1981).

REFERENCES


(Received 12 August 1982)