Pathogenesis of Mouse Scrapie: Evidence for Neural Spread of Infection to the CNS

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SUMMARY

The replication of infectious agent has been studied in brain, thoracic cord, and lumbar cord of Compton white mice (Sinc7) infected with the 139A strain of scrapie. Nine experiments were carried out using four different peripheral routes of injection. A highly consistent pattern of results was obtained in which replication in the CNS started in the thoracic cord after about 35% of the total incubation period had elapsed (range 25 to 42%). This was followed by the simultaneous onset of replication in brain and lumbar cord which occurred 2 to 4 weeks later. It is difficult to explain these results on the basis of haematogenous spread of infection from peripheral sites of replication (e.g. in spleen) to the CNS. However, the data are consistent with spread of infection along peripheral nerves and, in particular, along nerves of the sympathetic nervous system. It is suggested, therefore, that this may be the major route by which scrapie agent invades the CNS.

The pathogenesis of scrapie has been studied in a variety of experimental models of the disease in mice (for reviews, see Dickinson & Fraser, 1977; Dickinson & Outram, 1979; Kimberlin, 1976, 1979; Outram, 1976). There appears to be no specific immune response to scrapie infection and pathogenesis is dominated by agent replication which is under the control of the mouse gene Sinc. In those models of scrapie with a relatively short incubation period, infection by a peripheral route leads to an early phase of replication in certain lymphoid organs of which the spleen seems to be particularly important as shown by splenectomy studies. Several weeks later, there is a prolonged period of replication in the brain culminating in the sharp onset of clinical disease. The entry of agent into the central nervous system (CNS) is an important stage in pathogenesis but the way in which this occurs is not known.

Our recent studies have established some features of one of the short incubation models of scrapie, namely, 139A strain of agent injected by peripheral routes into young adult Compton white mice (Sinc7). Replication proceeds rapidly in the spleen to reach a plateau concentration several weeks before agent can be detected in the CNS (Kimberlin & Walker 1979). Replication of agent in the brain occurs at a highly predictable time after infection and it is always preceded by replication in the spinal cord (Kimberlin & Walker, 1979). Except immediately after infection, agent is not detectable in blood (see Millson et al. 1979). These findings are consistent with the spread of agent along neural pathways from spleen (and other peripheral sites of replication) to spinal cord and then to the brain (Kimberlin & Walker, 1979). In the present study further evidence has been sought for this hypothesis by investigating the dynamics of agent replication in brain and in thoracic and lumbar cord.

Large groups of donor mice (Compton white, young adult females) were infected with diluted brain homogenates containing the 139A (Chandler) strain of mouse-passaged (intracerebrally) scrapie agent. Four routes of infection were used: intraperitoneal (i.p.), subcutaneous in the dorsal area behind the head (s.c.) or under the hind footpad (f.p.) and intravenous (i.v.) in the tail vein. Relatively low doses of agent were used (10 to 500 LD50 i.p., s.c., f.p. or i.v. units) so that replication of agent in tissues would not be masked by...
uptake or persistence of the original inoculum. Previous findings (Kimberlin & Walker, 1978, 1979) enabled us to adjust the dose to give similar incubation periods irrespective of the route of infection and also to limit observations to the early part of the incubation period. At weekly intervals after infection, three animals were taken from each group and various parts of the CNS removed. Great care was taken to avoid cross-contamination between samples with scrapie agent (Kimberlin & Walker, 1979). Thoracic cord was taken between the 4th or 5th and the 11th thoracic vertebrae. Lumbar cord was taken between the 1st and the 5th lumbar vertebrae. Pools of each tissue from three mice were homogenized in isotonic saline at a tissue concentration of 5% wet weight and homogenates were injected intracerebrally (i.c.) into recipient groups of eight mice, which were observed up to a maximum of 300 days after injection.

The incubation period of scrapie was measured as previously described (Kimberlin & Millson, 1976). Because of the inverse relationship between dose of agent and incubation period (Kimberlin & Walker, 1978), agent replication in donor tissues can be readily detected by a rapid reduction in the mean incubation period of consecutive groups of recipient mice as shown in Fig. 1. Further details of this method for assaying relative amounts of scrapie are given in Kimberlin & Walker (1979).

A total of nine experiments was carried out over a period of 3 years. The results are summarized in Table 1, and four experiments are illustrated in Fig. 1. There were two major findings. Firstly, agent replication in brain started at the same time as in lumbar cord, to within a week. Secondly, agent replication in the thoracic cord started 2 to 3 weeks earlier. This pattern was the same for all routes of infection, supporting the conclusion from previous studies that although the efficiency of infection by different extraneural routes may vary by up to 1000-fold, the course of pathogenesis is similar thereafter (Kimberlin & Walker, 1978, 1979).

The replication curves shown in Fig. 1 only indicate relative differences in the concentration of agent in different parts of the CNS. In one experiment, agent concentration was estimated by titration of samples that had been stored as 5% homogenates for 11 months at −20 °C. The samples, taken at 8, 9, 10 and 11 weeks after i.p. infection, were re-homogenized and titrated by injecting serial 10-fold dilutions into mice i.c. as described by Kimberlin & Walker (1978). The infectivity titres in thoracic cord were ≤ 2.5, 2.9, 3.6 and 3.7 log10 i.c. LD50 units/0.03 g of tissue at 8, 9, 10 and 11 weeks, respectively. These values are 10 to 100 times greater than the corresponding titres in lumbar cord (≤ 1.5, ≤ 1.5 ≤ 1.6, ≤ 2.0 log10 units) and in brain (≤ 1.5, ≤ 1.6, ≤ 1.9, 2.5 log10 units).

These findings could be explained in two ways. First, by haematogenous spread of agent directly to the CNS or, alternatively, to the dorsal root and autonomic ganglia which are more readily penetrated by several compounds injected i.v. (Jacobs et al. 1976; Jacobs, 1977). However, to account for the earlier replication of scrapie agent in thoracic cord it would be necessary to postulate structural differences in blood vessels at different levels of the CNS or in anatomically related ganglia. We have been unable to find any evidence of this in the literature. The concept of haematogenous spread of scrapie agent to the CNS is also weakened by the fact that infectivity has not been consistently detected in blood (except immediately after infection; Millson et al. 1979) and by the difficulty of explaining the highly predictable onset of agent replication in spinal cord (Table 1; Kimberlin & Walker, 1979).

The second explanation is neural spread. Although haematogenous spread of infection to the CNS cannot be excluded, we suggest that neural spread may be a more important route. Furthermore, it is possible that the earlier onset of agent replication in the thoracic cord is due to a preferential spread of scrapie agent from spleen and other visceral sites of replication along sympathetic nerves which connect with the CNS in this region, via the splanchnic nerves.
Fig. 1. Agent (139A) replication in thoracic cord (○), lumbar cord (●) and brain (▲) of (donor) mice infected by different routes (i.p., i.v., f.p. and s.c.) and at different dilutions (10⁻³, 10⁻⁴ and 10⁻⁵) of scrapie brain homogenate. Mice were infected (a) i.p. at 10⁻³ dilution, incubated for 165 ± 3 days; (b) i.v. at 10⁻⁵ for 183 ± 1 day; (c) f.p. at 10⁻⁴ for 184 ± 1 day; (d) s.c. at 10⁻² for 179 ± 2 days. The ordinate shows the incubation period in recipient groups of mice and indicates the relative amounts of infectious agent in tissue homogenates. The curves are formed from recipient groups where all mice tested developed scrapie. Points shown along the abscissa denote recipient groups in which less than 100% of mice developed scrapie, indicating very low levels of agent in tissues.

Table 1. Proportion of the incubation period that elapses before the onset of agent replication in thoracic and lumbar cord and in brain

| Route of injection | Dilution of scrapie inoculum | Incubation period (days ± s.e.) | % of incubation period before onset of agent replication in:*
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<td></td>
<td></td>
<td></td>
<td>Thoracic cord</td>
</tr>
<tr>
<td>i.p.</td>
<td>10⁻³</td>
<td>165 ± 3</td>
<td>25</td>
</tr>
<tr>
<td>i.p.</td>
<td>10⁻²</td>
<td>169 ± 1</td>
<td>30</td>
</tr>
<tr>
<td>s.c.</td>
<td>10⁻³</td>
<td>179 ± 2</td>
<td>34</td>
</tr>
<tr>
<td>f.p.</td>
<td>10⁻₅</td>
<td>175 ± 2</td>
<td>34</td>
</tr>
<tr>
<td>f.p.</td>
<td>10⁻⁴</td>
<td>184 ± 1</td>
<td>36</td>
</tr>
<tr>
<td>i.v.</td>
<td>10⁻⁻</td>
<td>183 ± 1</td>
<td>42</td>
</tr>
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* Data taken from Fig. 1 and from other experiments. The onset of agent replication is taken from the time when all the mice in the recipient groups developed scrapie, e.g. in Fig. 1 (b), 13 weeks for brain and 11 weeks for thoracic cord.
† Separate assays were carried out on tissues from four individual mice instead of on pools from three mice.
This preference could be due to some intrinsic property of sympathetic fibres. However, it is not known whether scrapie agent is transported within neurones, or in other, non-neural cells. More probably, it could be due to the fact that spleen appears to be the first site of replication of peripherally injected agent in this particular model of the disease (Clarke & Haig, 1971; Kimberlin & Walker, 1979; R. H. Kimberlin & C. A. Walker, unpublished data) and it should be noted that the murine spleen is innervated mainly, if not entirely, by adrenergic nerves (Reilly et al. 1976, 1979). We also suggest that agent replication in spleen and in other visceral sites served by sympathetic fibres in the splanchnic nerves may be quantitatively more important than agent replication elsewhere (e.g. sub-maxillary salivary glands, cervical lymph nodes and thymus). Possible evidence for this is the failure of thymectomy to alter incubation period (McFarlin et al. 1971; Fraser & Dickinson, 1979), whereas asplenia and splenectomy lengthen it (Fraser & Dickinson, 1970; Clarke & Haig, 1971; Dickinson & Fraser, 1972).

The hypothesis of neural spread of scrapie agent along sympathetic nerves is based on studies of only one model of scrapie and it may be premature to generalize too widely. However, a single experiment using the quite different 263K strain of agent injected i.p. into hamsters (R. H. Kimberlin & C. A. Walker, unpublished data) has given results similar to those reported here. Furthermore, histological studies have been made of vacuolation in the spinal cords of sheep, experimentally or naturally infected with scrapie (Wight, 1960). The author concluded that ‘the most commonly affected cells were the intercalated neurones and the central neurones of the sympathetic system’ and that ‘the intermediolateral nucleus, which contains the preganglionic neurones of the sympathetic nervous system was frequently and sometimes severely affected’. These findings indicate that neural spread of infection in the sympathetic nervous system may be a feature of the natural disease.

This hypothesis has a bearing on the unanswered question of ‘targeting’ of agent to specific cells and areas of the brain, damage to which leads to clinical disease. Because of the asymmetry of some components of the sympathetic nervous system, the hypothesis may also be relevant to the occurrence of asymmetric lesions in brain in certain models of mouse scrapie (Fraser, 1976). A study of the sequential development of histological lesions in brain and spinal cord, using different models of scrapie and peripheral routes of infection, might, therefore, be particularly rewarding.

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REFERENCES


Short communications


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