Scrapie in the central nervous system: neuroanatomical spread of infection and Sinc control of pathogenesis

J. R. Scott,* D. Davies and H. Fraser

Institute for Animal Health, AFRC and MRC Neuropathogenesis Unit, West Mains Road, Edinburgh EH9 3JF, U.K.

Following bilateral intraocular (i.o.) infection of Sinc s7 mice with ME7 scrapie, sequential tissue pools were taken from retina, optic nerve, superior colliculus (SC), dorsal lateral geniculate nucleus (dLGN), visual cortex and cerebellum. The infectivity levels in these pools were estimated by intracerebral (i.c.) assay in C57BL/FaBtDk mice. Infectivity was first detected in retina at 35 days post-injection (as an increase above residual injected inoculum), SC at 56 days, dLGN at 77 days and in optic nerve, visual cortex and cerebellum at 98 days. Pathological lesions were shown to develop in the same sequence later in the incubation period. Comparison of sequential retina and SC assays in congenic mice, which differ only in the vicinity of the Sinc locus, revealed a difference in the initial detection and progression of i.o. infection of between 60 and 100 days, indicating that Sinc acts by delaying the initiation of replication. Higher levels of infectivity were found in retina and SC of mice infected with 79A scrapie, which destroys the photoreceptor layer in the retina, than with ME7 scrapie, which does not. Retrograde transport of infection was indicated by the levels of infectivity in the retina after i.c. infection with ME7 or 79A scrapie. These results indicate that scrapie spread within the central nervous system is restricted to neuroanatomical pathways, and that Sinc controls the initiation, but not the rate of replication.

Introduction

Scrapie, a natural disease of sheep, is one of a group of unconventional 'slow virus' infections which have also been recognised in man, mink, cattle, several species of North American deer, elk and most recently in cats (Wyatt et al., 1991). All result in fatal neurodegenerative disease, characterized by vacuolar pathology in the central nervous system (CNS). Much of the present knowledge of unconventional viral infections comes from experimental scrapie models in inbred mice, in which over 15 different strains of scrapie have been isolated on the basis of their incubation periods and vacuolar lesion distribution (Bruce & Fraser, 1991).

The incubation period, which can range from 120 days up to mouse life-span, is strictly determined by the strain of scrapie, the genotype of the mouse and the route and dose of infection. Mouse genotype controls the incubation period mainly through the Sinc gene, although its mode of action has not been elucidated; inbred mice carry either the s7 or p7 allele of this gene, and mouse lines congenic for Sinc have been produced in this laboratory (Bruce et al., 1991).

The route of infection determines the pathogenesis of the disease; a parenteral route produces infection of the lymphoreticular system, which then spreads to the nervous system, although factors controlling access to the CNS are not clear (Kimberlin & Walker, 1988). Direct infection of the CNS produces the shortest incubation periods, and intracerebral (i.c.) infection is used for tissue assay as there is no other means of detecting infectivity. Scrapie has been shown to spread slowly within the CNS, notably in the spinal cord following intraperitoneal infection (Kimberlin & Walker, 1982), and through the optic nerve to the brain following intraocular (i.o.) infection (Buyukmihci et al., 1983; Fraser & Dickinson, 1985; Kimberlin & Walker, 1986). Stereotaxic infection of different brain areas also produces significant changes in incubation period (Kim et al., 1987). We have estimated the rate of transport of infectivity in the optic nerve at just over 1 mm per day, by serial enucleation following i.o. infection (Scott & Fraser, 1989). This concords with previous assessments of the transport rate in sciatic nerve (Kimberlin et al., 1983) and spinal cord (Kimberlin et al., 1987).

Fraser & Dickinson (1985) showed that the i.o. route of infection produces discrete vacuolar lesions in the retinal projections of the CNS. Following i.o. infection of mice with ME7 scrapie, for example, vacuolation is first seen in the superior colliculus (SC) contralateral to the infected eye (the major retinal projection) at about halfway through the incubation period of 240 days.
Subsequent lesions are seen in the dorsal lateral geniculate nucleus (dLGN) (which receives a collateral of the projection to the SC) and in the visual cortex, before the lesions become more generalized. It has also been shown that photoreceptor cell loss occurs in the retina as a result of infection with certain strains of scrapie in mice (Foster et al., 1986a, b) and in hamsters (Buyukmihci et al., 1982, 1985), where this lesion is associated with high levels of infectivity (Buyukmihci et al., 1980).

The way in which infection disseminates within the CNS, and its relationship with later vacuolar lesions remain to be clarified. The experiments described here were designed to test the hypothesis that the initial spread of infectivity following i.o. infection is restricted to the neuroanatomical pathways of the visual system, and to examine the spread of infection and thus relate scrapie replication to subsequent pathological changes. The i.o. model has several advantages; firstly, much is known of the neuroanatomy of the visual system in rodents; secondly, the contralateral projection in the mouse is almost total which effectively reduces the complexity of the route of infection to a relatively simple anterograde pathway; lastly, secondary relays such as the efferent projection from the dLGN to the visual cortex can be examined.

Methods

**Mouse strains.** Four strains of inbred mice were used: C57BL/1aTdk (C57BL), VM/Dk[Vm(p7)], VM/Dk-Sinc s7 [VM(s7)] and RIII/FaDk (RIII). C57BL, VM(s7) and RIII mice have the s7 allele of the Sinc gene; VM(p7) has the p7 allele. VM(p7) and VM(s7) are congenic strains which differ only at the Sinc locus (Bruce et al., 1991).

**Scrapie strains.** Mice were infected with two strains of scrapie: ME7 or 79A*. Both have been purified by biological cloning, i.e. at least three consecutive passages through mice infected with inoculum of high dilution (Kimberlin & Walker, 1978; Dickinson & Outram, 1983).

**Inoculation of donor mice.** Inoculum was prepared as a 10% suspension in saline of brain from a terminally affected mouse. The suspension was centrifuged at 500 g for 10 min, and the supernatant used as inoculum. Under pentobarbitone anaesthesia, mice were injected with 1 μl of inoculum at the scleral margin of each eye using a 27-gauge needle. Care was taken to avoid damage to the retina. For each experiment, a group of mice were infected intracerebrally with the same dose of inoculum to give an estimate of the dose of infection from an appropriate dose-response curve (see below).

**Infectivity assays.** At intervals after infection (specified in Fig. 1 to Fig. 4 captions), tissues for assay were removed immediately after cervical dislocation. Before removal of the eyes, the roof of the cranium was removed and the forebrain eased up until the optic chiasma was visible. The optic nerve was cut close to the chiasma; when the globes were gently removed with curved forceps, the optic nerve remained attached. The nerves were cut just behind the hilus, and gently pulled from the surrounding sheath of muscle and connective tissue. The retina was removed with watchmakers’ forceps after the globes had been bisected at the scleral margin. The SC, dLGN and visual cortex were removed using fresh blades for each. The left sagittal half of the cerebellum was taken. These tissues were stored at -30 °C as a pool from three to six mice. A standard weight for each tissue was derived by histological examination. Surviving assay mice were killed at intervals during the incubation period for a group of mice is given as the mean + S.E.M. The number of infectious units in each tissue at each time was estimated by comparing the mean incubation periods from titrations of brain tissue from terminal mice infected with ME7 or 79A*.

![Table 1. Mean incubation periods (+ S.E.M.) from titrations of brain tissue from terminal mice infected with ME7 or 79A*](image)

<table>
<thead>
<tr>
<th>Dilution</th>
<th>ME7 in C57BL</th>
<th>ME7 in SV</th>
<th>79A in C57BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻¹</td>
<td>-</td>
<td>164 ± 3 (7)</td>
<td>-</td>
</tr>
<tr>
<td>10⁻²</td>
<td>162 ± 2 (10)</td>
<td>180 ± 3 (8)</td>
<td>142 ± 6 (4)</td>
</tr>
<tr>
<td>10⁻³</td>
<td>167 ± 2 (10)</td>
<td>198 ± 4 (8)</td>
<td>147 ± 3 (4)</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>186 ± 3 (12)</td>
<td>208 ± 4 (8)</td>
<td>-</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>207 ± 3 (13)</td>
<td>238 ± 7 (12)</td>
<td>162 ± 3 (4)</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>257 ± 18 (15)</td>
<td>284 ± 11 (9)‡</td>
<td>187 ± 3 (6)</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>229 ± 15 (5)‡</td>
<td>309 ± 60 (21)‡</td>
<td>0‡</td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>258 (1)‡</td>
<td>0‡</td>
<td>0‡</td>
</tr>
</tbody>
</table>

* The dose-response curves derived from these data were used to estimate the amount of infectivity in the assayed tissues. The dose was 20 μl injected intracerebrally.

† Days ± S.E.M. The numbers of mice in each group are given in parentheses.

‡ There were survivors in these groups.

**Inoculum was injected intracerebrally into 12 assay mice; the injection volume was 20 μl. Experiments 1, 3 and 4 were assayed in C57BL mice, and experiment 2 was assayed in VM(p7) mice.**

**Inoculation period and histological assessment.** Incubation period was measured from the day of infection to the clinical endpoint of the disease (Dickinson et al., 1968). All clinical assessments were substantiated by histological examination. Surviving assay mice were killed about 700 days post-injection (p.i.), or at least 200 days after the previous positive case in the group. Mice killed at this time had no pathological signs of disease were considered to be survivors. The incubation period for a group of mice is given as the mean ± S.E.M. Pathological examination was carried out on selected groups of C57BL mice infected intraocularly with ME7, and killed at intervals during the incubation period. Semi-serial coronal sections were cut from the level of the inferior colliculus to the paraterminal body, stained with haematoxylin and eosin, and examined for vacuolar lesions.

**Dose-response curves and sequential infectivity graphs.** Three titrations of terminal brain were made to provide appropriate dose-response curves from which infectivity levels were estimated. ME7 scrapie in C57BL mice, ME7 scrapie in VM(p7) mice, and 79A scrapie in RIII mice. The mean incubation periods for each dilution, and the Kärber (1931) estimates of titre are shown in Table 1. The number of infectious units in each tissue at each time was estimated by comparing the mean incubation period of the recipient assay mice with the appropriate dose-response curve, and expressed as units of infectivity per 20 μl of inoculum injected. Full titrations were carried out on selected tissues in order to compare the Kärber estimate of titre with the incubation period assay.
The dose–response curves and sequential infectivity curves were plotted using Graphwriter II software on an Elonex PC.

Results

Four related experiments were carried out. In the first key experiment, the levels of infectivity in five visual system areas were estimated by sequential assay throughout the incubation period of ME7 scrapie in a standard Sinc s7 mouse strain. The second experiment compared the replication dynamics in retina and SC in two strains of mice which are congenic for the Sinc gene; this gene has a dramatic effect on incubation period (Bruce et al., 1991) and the timing of lesion development, which is known to be in proportion to i.o. incubation period length (Fraser & Dickinson, 1985; Scott, 1990). Experiment 3 compared infectivity titres in the retina and SC after infection with ME7 or 79A scrapie, since 79A produces a degeneration of the photoreceptor layer, and ME7 does not (Foster et al., 1986a, b). The development of infectivity in the retina following i.c. infection with ME7 and 79A scrapie was explored in experiment 4. The dose of infection for each experiment, estimated in ID50 units from the appropriate dose–response curve, is shown in Table 2.

Infectivity spread in the visual system

Sequential infectivity levels were estimated in retina, optic nerve, SC, dLGN and visual cortex. Cerebellum was included as a control area as it has no direct connection with the retina. Fig. 1 shows a clear progression in the initial detection of infectivity, starting with retina at 14 to 21 days p.i. (as an increase above background level of residual inoculum), SC at 56 days p.i., dLGN at 77 days p.i. and optic nerves, visual cortex and cerebellum at 98 days p.i. In the retina (Fig. 1a), infectivity reached a plateau at about 104.3 infectious units from 140 days p.i. until the end of the incubation period, 100 days later. The titre in SC also rose rapidly to 103.4 units at 98 days p.i., then increased gradually by about 10-fold between 119 and 224 days p.i. Infectivity in optic nerves also increased rapidly between 98 and 140 days p.i., although there was evidence of a low level of infectivity in optic nerve from 21 days p.i. Between one and five of the 12 assay mice killed at 21, 35, 56 and 77 days p.i. died with scrapie. These groups therefore received less than one ID50 infectious unit of inoculum. Optic nerve titres were corrected for dilution, but the plateau from 140 days remained about 10-fold less than that of retina and SC.

The infectivity curve for dLGN (Fig. 1b) was very similar to that of SC, except that infectivity was first detected in dLGN at 77 days p.i., one sampling time later than in SC. One mouse in the 56 day group was killed with scrapie at 230 days. The titre rose steadily from 98 to 224 days p.i., and did not show the plateaux apparent with retina and optic nerve. This may reflect the difficulties of dissecting this nucleus, as the technique used involved removing a quantity of tissue surrounding the dLGN.

Infectivity in visual cortex was detected at 98 days p.i., and rose to levels comparable to those in dLGN from 120 days p.i., with no clear evidence of a plateau. The infectivity curves for visual cortex and cerebellum are very similar, suggesting that the visual cortex data are only reflecting the levels of infectivity in the brain as a whole. The relatively crude dissection of the dLGN and visual cortex means that although a maximum level of infectivity may have been reached in these areas by 100 days, background levels in the surrounding tissues in the sample would still be rising, and plateau titres would not be achieved.

Sequential pathology in the visual system

Examination of semi-serial sections from Sinc s7 mice killed at intervals throughout the ME7 incubation period revealed a progression in lesion development similar to that previously described by Fraser & Dickinson (1985).

Table 2. Incubation periods (days ± S.E.M.) and estimated dose of infection of groups of mice given the same infection as those from which tissues were removed for assay

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Mouse strain</th>
<th>Scrapie strain</th>
<th>Incubation period* (i.o.)</th>
<th>Incubation period* (i.c.)</th>
<th>Estimated dose of infection (i.e. ID50 units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57BL</td>
<td>ME7</td>
<td>234 ± 3 (29)</td>
<td>166 ± 1 (10)</td>
<td>10⁴⁴</td>
</tr>
<tr>
<td>2</td>
<td>SV</td>
<td>ME7</td>
<td>254 ± 5 (18)</td>
<td>175 ± 1 (8)</td>
<td>10⁴¹</td>
</tr>
<tr>
<td></td>
<td>VM</td>
<td>ME7</td>
<td>474 ± 8 (6)</td>
<td>326 ± 3 (7)</td>
<td>10⁴⁶</td>
</tr>
<tr>
<td>3, 4</td>
<td>C57BL</td>
<td>ME7</td>
<td>256 ± 3 (3)</td>
<td>167 ± 2 (9)</td>
<td>10⁴³</td>
</tr>
<tr>
<td></td>
<td>C57BL</td>
<td>79A</td>
<td>205 ± 6 (3)</td>
<td>155 ± 8 (3)</td>
<td>10⁴⁴</td>
</tr>
</tbody>
</table>

* The number of mice in each group is given in parentheses.
This is illustrated diagrammatically in Fig. 2, which shows lesion maps for individual mice. Vacuolation was first detected in the SC from around 140 days p.i., and subsequently in dLGN and visual cortex from 160 days p.i. onwards. Lesions in the visual cortex were never seen in the absence of lesions in the dLGN, but some individual lesions occurred in only the dLGN. All mice examined were infected in the right eye only, and the initial lesions were confined to the contralateral projections. Ipsilateral lesions could be seen in SC from 160 days p.i., and in dLGN and visual cortex from around 190 days p.i. At 190 days p.i., vacuolation also affected the ventral posteromedial thalamic nuclei. At later times, as vacuolation spread through the CNS, precise targeting became difficult to discern, and the lesion pattern in the terminal mouse was similar to that seen in an intracerebrally infected mouse, except for a residual asymmetry of lesions in the SC in some individuals. No pathological changes were seen in the retina following infection with ME7 scrapie, even in terminal mice (Foster et al., 1986a).

The effect of the Sinc gene

Comparison of the infectivity curves for the two Sinc genotypes (Fig. 3) indicates that Sinc delays the initiation of replication in the VM(p7) mice in retina and SC following i.o. infection with ME7 scrapie (the difference in incubation periods is shown in Table 2). At 48 days p.i., the first assay time, there was a low level of infectivity present in the retina of VM(s7) mice; this rose to a plateau level of around $10^{6.9}$ units from 104 days p.i. (Fig. 3a). In the retina of VM(p7) mice, a comparably low level of infectivity was found at 48 and 76 days p.i., but no infectivity was detected at 104 and 132 days p.i. Between 132 and 160 days p.i., the retinal titre reached...
10^5, and it increased by nearly one further order of magnitude before the final assay time at 328 days p.i. Initiation of replication in the retina of VM(p7) mice was therefore delayed by between 60 to 100 days, and reached a plateau around 60 days later than in the VM(s7) mice. This effect was also apparent in SC (Fig. 3b); infectivity was first detected at 76 days p.i. in the SC pool from VM(s7) mice, but not until 132 days p.i. in the VM(p7) SC. Infectivity levels in both VM(s7) and VM(p7) mice rose steadily to a maximum of 10^{6.7} at 216 days in VM(s7) mice, and 10^{6.2} in VM(p7) mice. There was a delay in replication of infection in SC pools between VM(s7) and VM(p7) mice of around 50 to 60 days. In both tissues, the rate of replication, estimated by the steepness of the curve, is very similar; the difference between the two genotypes lies in the timing of the initiation of replication.

**Intraocular infection with 79A scrapie**

Replication of 79A scrapie in C57BL mice was first detected in the retina between 20 and 60 days p.i., and at 60 days p.i. in the SC (Fig. 4a). The infectivity levels in both tissues stabilized from 100 days p.i. onwards at about 10-fold higher than with ME7 scrapie (Fig. 1a). Although nearly 14-fold fewer i.c. ID_{50} units were injected (Table 2), the initial 79A levels were similar to those of ME7. The titres in SC reached 10^{4.9} at 140 days p.i. and 10^{5.8} in terminal groups; these were confirmed by titrations of these tissues which gave Kärber estimates of 10^{4.7} and 10^{5.5} respectively. Photoreceptor degeneration was first detected at 100 days p.i., as previously reported by Foster et al. (1986a).

**Intracerebral infection with ME7 or 79A scrapie**

Following a 1 μl i.c. infection with either ME7 or 79A scrapie (see Table 2), infectivity was not detected in retina pools at 20 days p.i., but both strains of scrapie could be detected at 60 days p.i. (Fig. 4b). As with i.o. infection, the levels rose more rapidly in the 79A-infected mice, reaching a titre of 10^{6.1} at 155 days p.i. in retinas removed from terminal mice. In mice infected with ME7, the levels were consistently lower, reaching 10^{3.4} at 140 days (retinas from terminal mice were not assayed.)

**Discussion**

The central enigma of scrapie pathogenesis is the control of the timing of the clinical disease, which can be precisely predicted even over several hundred days. Incubation period length is known to vary with the strain of scrapie, the Sinc genotype of the host, and the dose and route of infection. Differences in pathogenesis resulting from a change in the site of infection have produced mounting evidence for neural spread of infectivity, both in the peripheral nervous system and CNS; this is supported by the results of this study, which shows that infectivity spreads through the visual system projections in a sequence which is consistent with synaptic or transneuronal transfer, and also with the progression of the subsequent vacuolar pathology.

The Sinc gene has a dramatic effect on incubation period length with most scrapie strains (Bruce et al., 1991), which is clearly illustrated by the incubation period differences between the two Sinc genotypes in experiment 2 (Table 2). The way in which this gene controls pathogenesis in the CNS is not known, but from the available evidence, Bruce et al. (1991) concluded that 'the Sinc gene exerts its effect either on the rate of cell-to-cell spread or on the rate of replication.' The results of experiment 2 indicate that replication occurs at a similar rate in retina and SC of both genotypes, implying that this gene acts by controlling the transneuronal transfer of infectivity. If replication is governed by restrictions on cell-to-cell transfer, then although the retinal ganglion cells transport scrapie, replication may take place post-synaptically in the bipolar cells of the inner nuclear layer in the retina, or in the neurons of the SC. The evidence that replication occurs in the photo-

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Fig. 4. Sequential titre estimates (a) from retina (●) and SC (■) pools taken from C57BL mice following i.o. infection with 79A scrapie. (b) Retina pools from C57BL mice following i.c. infection with ME7 (■) or 79A (●) scrapie. Tissues were taken at 24 h, 20, 60, 100 and 140 days, and from terminal infected groups. Arrows mark the groups in which the tissue was titrated to give an ID_{50}.
receptor cells following infection with 79A scrapie is discussed below.

The function of the Sinc gene is not known, but there is convincing evidence from genetic linkage (Hunter et al., 1987) and transgenic mouse studies (Scott et al., 1989) that PrP is the Sinc gene product. PrP is a host protein which becomes more resistant to proteases as a result of infection with scrapie or any of the spongiform encephalopathies. The normal metabolic turnover of PrP appears to be compromised by the disease process, and it aggregates to form deposits in the CNS in a scrapie strain-specific manner (Bruce et al., 1989). These can be seen in immunolabelled sections from as early as 35 days after i.c. infection with ME7 scrapie, several weeks before vacuolation can be detected (P. A. McBride, personal communication). The role of PrP in pathogenesis has not been resolved, and remains a subject of some controversy (see Bruce et al., 1991; Meyer et al., 1991; Weissman, 1991). However, as PrP is a neuronal membrane glycoprotein, it has been suggested that it could act as a binding site for infection and thus control the transfer and targeting of infectivity (Bruce et al., 1991; Hope & Baybutt, 1991). This hypothesis is consistent with the present results showing that Sinc acts by controlling the initiation of replication in retina and SC.

The limitations on pathogenesis of controlled neuron-to-neuron spread of infection through neuroanatomical routes is consistent with the protracted but predictable incubation periods. Kimberlin & Walker (1983) suggested that the duration of the replication phase in brain varies with the site of infection, indicating that this phase is determined by the complexity of the neuroanatomical pathway between entry to the CNS and infection of the postulated 'clinical target areas' which are essential for survival. Subsequent results involving several routes of infection have validated this hypothesis (Kimberlin et al., 1983, 1987; Kimberlin & Walker, 1986; Gorde et al., 1982), and the present study provides further evidence for neuron-to-neuron spread within the CNS. The prolongation of the i.o. incubation period compared with i.c. [30 to 60% longer in all mouse models examined (Scott, 1990)] has recently been shown to be due to intrinsic control of pathogenesis and not an effect of dose (Scott et al., 1991). It has been suggested previously that dissemination of infectivity within the CNS may be by other means such as haematogenous or glial spread; a viraemic phase has been detected in the hamster/263K scrapie model (Diringer, 1984), but there is no evidence that this leads directly to infection of the CNS (discussed by Kimberlin & Walker, 1988).

The relationship between replication in the CNS and subsequent vacuolar pathology is difficult to clarify in the absence of a marker for replication sites. Baringer et al. (1983) found high infectivity titres several weeks before vacuolation occurred in hamsters infected intracerebrally with 263K scrapie, and concluded that 'the presence of these agents in high concentrations is unrelated to the vacuolation of the nervous system.' The present results suggest that vacuolation occurs as a eventual consequence of replication, in areas where a high titre has been maintained for several weeks. This direct relationship would resolve the problem of clinical cases with little or no vacuolation which have been reported for scrapie and other spongiform encephalopathies (see Taylor, 1991). One inconsistency in this association between infectivity and degenerative pathology arises from studies on the retina. Ontogenetically, the retina arises as a local outgrowth from the lateral walls of the rostral part of the brain, and is considered to be a part of the CNS. Degenerative pathology does not occur in the ME7-infected C57BL retina (Foster et al., 1986a), despite the relatively high levels of infectivity detected in experiment 1. Following infection with 79A scrapie, which destroys the photoreceptor cell layer, replication in the retina starts at the same time as with ME7 scrapie, but rises faster and remains about 10-fold higher. A maximum retinal titre of $10^{4.4}$ is reached at 100 days p.i., which compares with the earliest detectable morphological changes at 120 days (Foster et al., 1986a) and electroneoptographic changes at 118 days (Curtis et al., 1989), both in this 79A scrapie model. Changes in the photoreceptors have been reported at 56 days p.i. (Hogan et al., 1981) but also as early as 8 days p.i. (Buyukmihci et al., 1982) in the same hamster/263K model which has an incubation period of about 50 to 60 days. Hogan et al. (1986) have shown that high titres precede retinal pathology in this model, and suggest that the retina is a primary site of replication.

Photoreceptor degeneration appears to result from the high titres produced by 79A infection, suggesting that neuronal loss occurs only in those cell groups which contain large amounts of infectivity. It is interesting that neither scrapie strain appears to affect the retinal ganglion cell bodies, although this remains to be assessed at an ultrastructural level.

One of the problems of infectivity bioassay is the inability to distinguish between replication of infectivity and its accumulation, except perhaps in the primary replication site. In the optic nerve, infectivity is not detected until high titres are reached in SC and dLGN, and it remains about one-tenth of that in other tissues even towards the end of the incubation period, suggesting that infectivity is not replicating in optic nerve but merely being transported in both directions. This sequence of detection was also found by Fraser & Dickinson (1985) in C3H mice; however, Kimberlin & Walker (1986) found infectivity in the optic nerve before...
SC in hamsters infected with 263K scrapie. They suggested that this difference may be due to more rapid accumulation of 263K infectivity in the optic nerve. Recent ultrastructural studies using this scrapie model confirm the neuronal localization of pathological changes (Jeffrey et al., 1991).

The optic nerve has been widely used to study the physiology of axoplasmic transport because of its accessibility and homogeneity. Essentially all axonal and dendritic constituents, and many exogenous materials such as lectins, horseradish peroxidase and viruses are conveyed by axonal transport (Weiss, 1982). We have used the anterograde pathway from retina to SC to assess the rate of spread of ME7 scrapie infection at slightly over 1 mm per day (Scott & Fraser, 1989). From the assays of retina and SC following i.c. infection, it can be seen that retrograde transport is equally important. The levels of infection in retina with ME7 and 79A are very similar to those of SC following i.o. infection. Retrograde axonal transport has not been analysed to the same extent as anterograde transport, but it appears to be an exaggerated manifestation of the endocytotic and degradative pathway operating in cells in general (Vallee et al., 1989). Recent evidence for the involvement of cellular degradation has come from the discovery of neuronal autophagic vacuoles associated with both scrapie (Boellaard et al., 1991) and Creutzfeldt-Jakob disease (Boellaard et al., 1989).

The i.o. route has enabled us to study the pathogenesis of scrapie at a cellular level, and offers the future possibility of defining scrapie strain-specific differences in targeting of infection and relating these to Sinc control of replication.

References


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