Temporary and Permanent Modifications to a Single Strain of Mouse Scrapie on Transmission to Rats and Hamsters

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SUMMARY

The interspecies transmission of scrapie is frequently associated with exceptionally long incubation periods at first passage in the new host compared to later passages (the species barrier effect). The basis of this was investigated using the 139A strain of scrapie which had been cloned by three serial passages in mice at limiting infectious doses. Cloned scrapie was passaged through hamsters (twice) or rats (thrice) and then reisolated in mice. Large species barrier effects were encountered on mouse-to-hamster and hamster-to-mouse passage resulting in the isolation of a mutant strain, 139-H/M, with properties very different from 139A. In contrast, the strain reisolated from rats was indistinguishable from 139A. However, a large species barrier was encountered at the mouse-to-rat passage but not at the rat-to-mouse passage. It is suggested that the transmission of scrapie between species may be associated with (i) no change in properties or (ii) a permanent change in the scrapie genome due to the selection of mutants. A third possibility, the donor species effect, is a temporary change occurring only at first passage in the new host species which is largely or entirely caused by the introduction of material from the previous host. We speculate that the donor species effect could be explained if some host protein forms a functional part of the infectious agent.

INTRODUCTION

There have been many successful transmissions of sheep scrapie to rodents and about 20 different strains of scrapie agent have been isolated, mostly in mice (Dickinson et al., 1984). However, some transmissions fail (Fraser, 1983) and, even when successful, they are usually accompanied by extremely long incubation periods at the first passage in the new host compared to subsequent passages (the species barrier effect; Dickinson, 1976; Kimberlin, 1979). Attempts to increase the efficiency of primary isolations are hampered by an inadequate understanding of the biological basis of this barrier (Kimberlin, 1979) and of the reasons why it does not always occur (see Discussion).

One of the earliest studies was by Pattison & Jones (1968) who studied the ‘Chandler’ isolate of scrapie in mice (the main passage line of which is now designated 139A) which had been serially passaged five times in Wistar and white rats and then reisolated in mice. On reisolation they observed a permanent alteration of properties, which included loss of vacuolation in the cerebellar white matter. The authors concluded that the alteration was due to ‘induced differences in the transmissible agent’. However, the nature of these ‘induced differences’ was not known at the time.

Subsequently, considerable evidence has accrued for the mutation of scrapie strains (Bruce & Dickinson, 1987) and it is clear that mutation is not a rare event (Kimberlin et al., 1986; Bruce & Dickinson, 1987). Other studies have shown that a process of strain selection occurs on passaging mixtures of scrapie strains in hamsters (Kimberlin & Walker, 1978). These developments suggest that the ‘induced differences in the transmissible agent’ observed by...
Pattison & Jones (1968) were not 'induced' events but permissive ones which can be explained if passage in rats caused a selection of one or more strains, either from a pre-existing mixture in mice, or from mutants that arose in rats.

In the present study we have investigated these possibilities by repeating the Pattison & Jones experiment with the important difference that 139A scrapie was first cloned by serial passage at limiting dilution in mice. We have also studied the effects of passage in hamsters in order to create a potentially different species barrier. The results clarify the significance of previous observations (Pattison & Jones, 1968; Kimberlin et al., 1975, 1986; Kimberlin & Walker, 1978, 1979) based on uncloned strains of scrapie and suggest at least two different processes underlying the species barrier effect: the selection of mutants over several passages, and a donor species effect acting on a single scrapie strain but only at first passage in the new species.

METHODS

Golden hamsters, albino rats and Compton White (CW) mice (Sine\textsuperscript{+}) were outbred. Females only were inoculated intracerebrally (i.c.) as weanlings (30 \textmu l of inoculum into mice; 50 \textmu l into hamsters and rats). The 139A strain of scrapie was serially cloned in mice at the 28th, 29th and 30th i.c. passages using, respectively, $10^{-8}$, $10^{-7}$ and $10^{-6}$ dilutions in saline of whole brain homogenates from single clinical cases (Kimberlin & Walker, 1978); before dilution, the homogenates were centrifuged at 1800 g for 10 min. After cloning, all i.c. passages were made with uncentrifuged, 1\% homogenates of whole brains (stored at $-20 \, ^\circ \text{C}$) from at least two animals except at the second passage in rats which was made from single brains. To avoid cross-contamination between brains (and inocula) from different passage lines, all instruments and equipment were either disposable or given one pre-cleaning plus two post-cleaning cycles of autoclaving at 126 \textdegree C for 30 min before re-use. Only coded information was displayed on the cages of injected animals to reduce observer bias when assessing clinical signs of scrapie. Incubation periods were calculated to the time of the first appearance of definite and consistent clinical signs of scrapie. All animals at all passages developed the clinical disease. The clinical signs in mice, hamsters and rats were not altered by the previous passage history.

The severity of vacuolation was scored by light microscopy in anatomically defined grey and white matter areas of brain according to published methods (Fraser & Dickinson, 1968, 1973) and as recently described (Cole & Kimberlin, 1985). Note that the scale of white matter vacuolation is different from the grey matter scale. Successive integers on the grey matter scale represent approximately 10-fold differences in the number of vacuoles/unit area. The grey matter areas examined are: 1, dorsal half of the medulla; 2, cerebellar cortex adjacent to the fourth ventricle; 3, cortex of the superior colliculus; 4, hypothalamus; 5, central nuclei of the thalamus; 6, hippocampus; 7, septal nuclei of the paraterminal body; 8, posterior and 9, anterior cingulate cortex. The white matter areas are: 1, cerebellar peduncles; 2, tegmentum of mesencephalon; 3, pyramidal tracts at the level of the thalamus. The lesion profiles shown in Fig. 2 and 3 are based on a minimum of 10 and an average of 14 brains each. Comparisons between passage lines were made on the average lesion score for each area using Student's t-test.

RESULTS

139A was cloned in mice by three serial i.c. passages at limiting infectious doses (Methods). It was passaged once more in mice (31st passage) under the standard conditions employed for all subsequent passages, i.e. 1\% brain inocula. The 32nd passage was set up in hamsters, mice and rats (Fig. 1) and, for convenience, these passages are referred to H1, M1 and R1, respectively.

Effect of passage in rats

At R1, all the injected rats developed scrapie but a large species barrier was encountered (Fig. 1); incubation periods were over a year longer than at subsequent passages in rats, and they were also highly variable (468, 554, 610, 649 and 666 days). The incubation periods shortened to an average of 203 days when brain from the 649-day-incubation case was injected at R2 (Fig. 1). Average incubation periods of 203 to 244 days were also found when each of the other four brains at R1 were individually passaged in rats (data not shown) thus demonstrating the reproducibility of the difference between R1 and R2. At R2 and R3, incubation periods in rats were stable at about 200 days (Fig. 1).

On transmission back to mice, the incubation period was shorter than it had been in rats (Fig. 1). However there was still a small species barrier effect because the incubation period at R3M1 was 50 days longer than at R3M2 or R3M3 (Fig. 1). Also there was a highly abnormal lesion profile at R3M1 compared to the later passages in mice (Fig. 2a).
In the main comparison, the combined lesion profiles of R3M(2 + 3) (Fig. 2a) and their respective incubation periods (Fig. 1) were indistinguishable from those at the equivalent levels of continuous 139A passage in mice (M5 and M6). On this evidence, the rat-passaged reisolate in mice (designated 139-R/M) was similar to if not identical with 139A (see Discussion).

**Effect of passage in hamsters**

A large species barrier effect was found at the mouse-to-hamster passage (H1) with an average incubation period of 378 days (Fig. 1). This dropped to 178 days at H2 and thereafter stabilized at about 130 days (Fig. 1). Reisolation into mice was made after the second hamster passage. Surprisingly the average incubation period at H2M1 was even longer (449 days) than it had been...
Fig. 2. Effect of three serial passages in rats on the vacuolation profiles in mice and hamsters. Profiles from two successive passages were combined when the corresponding incubation periods were similar (see Fig. 1). (a) Profiles in mice caused by R3M1 (solid circles, dotted lines), R3M(2 + 3) (solid circles, solid lines) and M(5 + 6) (open circles, solid lines). Statistical differences between R3M1 and R3M(2 + 3) are indicated by 3 ($P < 0.001$) and 2 ($P < 0.01$). There were no significant differences between R3M(2 + 3) and M(5 + 6). (b) Profiles in hamsters caused by H(5 + 6) (open squares) and R3H(2 + 3) (solid squares); there were no statistically significant differences.

Fig. 3. Effect of two serial passages in hamsters on the lesion profiles in mice. Profiles from two successive passages were combined when the corresponding incubation periods were similar (Fig. 1). (a) Profiles caused by H2M1 (solid circles, dotted line), H2M2 (dotted line only) and H2M(3 + 4) (solid circles, solid line). Statistical differences between H2M1 and H2M(3 + 4) are indicated by 3 ($P < 0.001$), 2 ($P < 0.01$) and 1 ($P < 0.02$). (b) Profiles caused by H2M(3 + 4) (solid circles) and M(5 + 6) (open circles). Statistical differences are indicated by 3 ($P < 0.001$), 2 ($P < 0.01$) and 1 ($P < 0.02$).

on first transmission from mice to hamsters (378 days; Fig. 1). The same observation was also made at H1M1 (416 ± 8 days) and at H3M1 (434 ± 1 days). Serial passage in mice reduced the average incubation period to 301 days at H2M2, and the incubation periods became stable at H2M3 and H2M4 (202 and 215 days, respectively; Fig. 1). The combined lesion profile at H2M(3 + 4) was very different from that obtained at H2M1 and at H2M2 (Fig. 3a) again demonstrating differences in profile associated with crossing the species barrier.

The major comparisons of this experiment are between H2M(3 + 4) and the equivalent levels of continuous passage in mice (M5 and M6). The effect of two passages in hamsters was to increase incubation periods in mice by about 75% (or 100 days; Fig. 1) and to alter the lesion profile; in particular there was a dramatic reduction of vacuolation in the white matter (Fig. 3b). We conclude that the scrapie strain isolated in mice after two passages in hamsters (line H2M) is different from 139A, and we designate it 139-H/M.

Comparisons in hamsters

In the course of this study, six serial passages of scrapie were achieved in hamsters (the H line) and stable incubation periods were observed in the last four, i.e. H3 to H6 (Fig. 1). Comparisons were made with the passage line established in hamsters after three serial passages in rats (R3H line). No differences were found between R3H(2 + 3) and H(5 + 6) in either incubation period (Fig. 1) or in lesion profile (Fig. 2b). This suggests that similar scrapie strains were isolated in the R3H line and in the H line. These isolates in hamsters are designated 139-R/H and 139-H.
Modifications to a single strain of scrapie

DISCUSSION

The first major finding of this study was that passage of cloned 139A from mice to rats and back to mice, did not cause a permanent detectable change in properties. We suggest that the ‘induced differences’ reported by Pattison & Jones (1968) were due to the selection, in rats, of a scrapie strain that arose by mutation or was a component of a pre-existing mixture in mice. The latter is consistent with the fact that the original passage line of Chandler (139A) scrapie in rats was established after only the third passage in mice when mixtures of strains would have been more likely than at later passages (Dickinson, 1976).

The second major finding was that passage of 139A through hamsters led to the isolation of a different strain in mice. This variant, 139-H/M, had twice the incubation period in mice of 139A and it is unlikely that it could have been present in the original cloned 139A because it would have been strongly selected against during the serial cloning procedure. Almost certainly, 139-H/M is a mutant strain that arose directly or indirectly from 139A after cloning. This could have occurred at the last (low dilution) passage in mice, on passage in hamsters or on reisolation in mice. At both the mouse-to-hamster and hamster-to-mouse species barriers, incubation periods did not stabilize until the third passage (Fig. 1; H2M line). A gradual reduction in incubation period over several passages in a new host has been associated with the selection, from a mixture, of the shortest incubation period variant until it becomes the sole or quantitatively dominant strain (Kimberlin & Walker, 1978; Bruce & Dickinson, 1987; Kimberlin et al., 1986). Therefore both of the species barriers encountered in the H2M line may have involved the selection of mutants and the differences in lesion profile observed between H2M1 and H2M(3 + 4) (Fig. 3a) probably reflect the gradual selection of 139-H/M. It is difficult to know when 139-H/M arose but if it arose from 139A in mice it would certainly not have been isolated without passage in hamsters. This illustrates how passage into other species increases the opportunities of isolating variants that might otherwise become lost. Another example of this is the ‘rescue’ of 431K in mice from a passage line in hamsters in which 263K eventually became the dominant strain (Kimberlin & Walker, 1978).

The likelihood of frequent mutations in scrapie (Dickinson et al., 1984; Kimberlin et al., 1986), especially with some strains of agent (Bruce & Dickinson, 1987), makes it difficult to assume that either 139-R/H or 139-H are in fact 139A in hamsters. However, by the criteria used, 139-R/H appears to be the same as 139-H and it is clear that both isolates are different from 263K, the very short incubation strain of scrapie in hamsters (Kimberlin & Walker, 1977, 1978). The possibility that 139-H/M might be the same as 79A is raised by the evidence that 79A can arise as a mutant of 139A (Dickinson et al., 1984). However 79A differs from 139-H/M in having a 100 day shorter incubation period in CW mice (1% inocula injected i.c.) and by the fact that 79A produces white matter vacuolation. Also the properties of 79A passaged in hamsters differed from those of 139-H in that the stable incubation period of 79A was about 50 days longer (R. H. Kimberlin & C. A. Walker, unpublished).

The most surprising finding of this study was the very large species barrier observed in the R3M line at the mouse-to-rat passage, even though passage through rats did not permanently affect the properties of 139A that were measured. This suggests that factors additional to mutation and strain selection may sometimes be important in the interspecies transmission of scrapie. We recognize the alternative possibility that the strain obtained in mice after rat passage may not have been 139A but a strain like it which was not distinguishable by the methods used. However, in contrast to what happened in the H and H2M lines, there was no indication of a gradual selection of a different strain in rats because the incubation periods at R2 and R3 were virtually identical (Fig. 1). It seems more likely that the greatly extended incubation period at R1 was a ‘donor species effect’ in which some host components in the inoculum impeded the transmission to rats.

The exceptionally large size of this species barrier (a difference of about 380 days in incubation period between first and subsequent passages) is almost certainly outside the normal dose–incubation range in rats, implying modifications to scrapie pathogenesis over and above any effective loss of injected titre. Two other examples have been reported of modified scrapie pathogenesis at first passage in the new host; the failure of scrapie to replicate in spleen and the
greatly delayed onset of agent replication in brain (Kimberlin & Walker, 1979). Another possible example was seen in the R3M line at the rat-to-mouse passage (Fig. 1). Here the species barrier effect was small (it was probably caused by a reduction in the effective scrapie titre that was injected) but there was also a highly atypical lesion profile at R3M1 compared to R3M2 and R3M3 (Fig. 2a).

There are at least two mechanisms for the donor species effect. One is that foreign host components in the crude inocula used in all these studies may sometimes interact with the infectious units of scrapie (e.g. by aggregation) in ways which reduce the effective titre in the new host or modify the early events of scrapie pathogenesis. So far, attempts to modify interspecies transmission by sensitizing the new host to tissue from the donor species have produced only slight effects (Kimberlin et al., 1975; Kimberlin, 1979) but further studies on this are needed. One approach would be to see whether the species barrier can be reduced by using scrapie of sufficient biochemical purity. However, any effects due to crude inocula would tend to be non-specific and this does not easily account for the very small species barrier observed at rat-to-mouse and rat-to-hamster passage (Fig. 1) and elsewhere (Kimberlin et al., 1986); in a study of hamster-to-mouse transmission the species barrier was minimal with the 431K isolate of scrapie and extremely large with the 263K strain (Kimberlin & Walker, 1978). We therefore speculate that a more specific way in which host components might give rise to the donor species effect is if some of them form a structural and functional part of the infectious agent.

In essence this is the 'virino' concept of the scrapie agent and the forms which the infective units may take. This concept postulates that a small, scrapie-specific genome (presumably nucleic acid) is associated with one or more host coded proteins that are needed for infectivity (Dickinson & Outram, 1983). In such an informationally hybrid agent, the host-coded protein could have one or more functions, involving infection of cells, replication, or the stability in vivo of the scrapie agent. Some of these functions could be affected when infectious agent with protein from one species is introduced into another. Depending on the nature of these proteins, their structural relationships with different scrapie genomes, and their ability to function in a different species, the species barrier could be large or small.

However it is important to emphasize the distinction between changes that affect the scrapie genome (such as mutation) and differences in the species of origin of host proteins that are not part of the scrapie genome but could be a functional part of the infectious agent. By definition changes in the genome will permanently affect the infectious agent whereas differences involving host proteins will only affect agent at the first passage in a new species of host. In the latter case, provided no other scrapie strains are present and inocula have similar infectivity titres, incubation periods at the second and subsequent passages in the new species will be constant for a given route of infection, exactly as was observed on transmitting 139A to rats (Fig. 1).

Full biochemical characterization of the infectious scrapie agent has not been achieved (Kimberlin, 1986) and it remains conjectural whether the donor species effect depends on host components acting extrinsically on the behaviour of scrapie agent or as an intrinsic part of its infectious structure. Further biological investigations should be carried out under two conditions. First, the passaging of cloned scrapie strains to different host species should be done at limiting dilutions of inoculum. This would maximize the removal of any prior mutants and allow a direct measurement of the effective scrapie titre that was injected. Secondly, more rigorous strain typing methods should be applied to improve the evidence for an apparent identity of a reisolate with the original strain. In this way the nature of the donor species effect could be better defined.

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REFERENCES

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