Slow Transmissible Diseases of the Nervous System: Volume 1

ON THE ORIGIN OF TRANSMISSIBLE MINK ENCEPHALOPATHY[1]

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ABSTRACT Studies on mink susceptibility to sources of scrapie from the United States, but not from the United Kingdom, indicate that transmissible mink encephalopathy (TME) most likely originates from mink being fed scrapie-infected sheep or goat tissues. Experiments further suggest that the shortest natural route of infection is via bite wounds inflicted by littermates rather than by the oral route per se. Other studies, on the biologic characterization of the TME agent from Sawyer County, Wisconsin, indicate that this particular source of TME is composed of a mixture of subpopulations which include a hamster pathogen and a mink-monkey pathogen.

INTRODUCTION

In 1965, Hartsough and Burger reported a new disease of commercially reared mink which appeared to have a long incubation period based on epizootiologic observations on affected farms (1). These investigators were then successful in demonstrating that the disease was caused by a virus-like agent which produced a spongiform encephalopathy 6-9 months after experimental inoculation, the length of incubation being dependent on the route of exposure (2). This information in hand, it was immediately suggested that the new disease of mink was related to scrapie disease of sheep and goats.

Subsequent studies over the next five years extended the scrapie analogy in areas of agent characterization (3),

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pathogenesis (4), and host immune response (5). The disease was named transmissible mink encephalopathy (TME) by Marsh et al. (4) and this term now seems to be widely accepted, although it is still occasionally referred to as mink encephalopathy or encephalopathy of mink.

With so many disease features in common, it would seem a simple matter to demonstrate that TME results from feeding scrapie-infected tissues to mink. But such has not been the case. Epizootiologic studies of the 14 worldwide occurrences of TME have revealed probable exposure to scrapie in only one instance, a 1965 incidence in Finland in which the affected farm was the only one in the area feeding sheep heads (Kangas, personal communication). Experimentally, mink have been found to be susceptible to some sources of scrapie and the disease produced was indistinguishable from TME (6). However, in these instances the incubation periods after intracerebral inoculation were one year or longer and do not seem to fulfill the criteria demanded by observation of natural occurrences of TME. Five of the 14 affected farms have experienced TME during or shortly after whelping season (May-June). In all of these cases the mortality rate has approached 100% of the adult animals, including first-year breeders not more than one year of age. Since TME is not congenitally transmitted, we must conclude that the incubation periods in these instances were one year or less after oral exposure, a route requiring a longer incubation period than intracerebral inoculation.

The purpose of these present studies was to attempt to explain differences between field and experimental observations, and to further characterize the biologic properties of the Sawyer County, Wisconsin, isolate of TME. Our results indicate that mink are more susceptible to sources of scrapie present in the United States than those found in the United Kingdom, and that bite wounds from littermates may represent a significant route of natural exposure. We further report that the TME source from Sawyer County can be separated into two components, a hamster pathogen and a mink-monkey pathogen.

MATERIALS AND METHODS

TME Agent. Originally recovered from a natural occurrence of TME on a Sawyer County, Wisconsin, mink farm in 1963 (2). The agent was passaged twice in mink before adaptation to hamsters (7).

Scrapie Agents. Various subpopulations of scrapie

agents, and their sheep or goat sources, were obtained from Dr. Alan Dickinson, A.R.C. Animal Breeding Research Organization, Edinburgh, Scotland. These agents were originally recovered from scrapie-affected animals in the United Kingdom, then separated into individual subpopulations by their biologic behavior in inbred strains of mice (8). We thank Dr. Dickinson for kindly supplying this material.

Additional sources of scrapie were obtained from the USDA Scrapie Field Trial in Mission, Texas. These included B-834, a naturally infected Nubian X Toggenburg goat; B-957, a Nubian X goat inoculated intracerebrally with brain from a scrapie-affected Suffolk sheep; and PR-81, a Suffolk sheep inoculated similarly to B-957. We thank Drs. Hourrigan, Klingsporn, and Clark for their cooperation and continuing interest in this work.

Inoculation. All inocula were 5% or 10% brain suspensions in saline. Intracerebral inoculations on mink and squirrel monkeys were performed using an electric drill to perforate the calvarium, then the inoculum (0.1 ml or 0.2 ml, respectively) was injected into the right cerebral hemisphere using a 22 g needle.

RESULTS

UK Scrapie. The response of 16-month old mink to intracerebral inoculation with various sources of scrapie from the United Kingdom is shown in Table 1. Only one animal developed a progressive neurologic disease during an observation period of three years. This female sapphire mink had been injected with brain tissue from the scrapie-affected Suffolk sheep from which the subpopulation 138A was isolated. Histopathologic examination revealed an absence of spongiform degeneration in the brain of this mink, but widespread astrocytic hypertrophy in the frontal cortex, striatum, thalamus, and hippocampus. These lesions were considered to be indicative of a TME-like disease process, especially since mink of this genotype (homozygous Aleutian) show very little endstage microvacuolation when affected with TME after two years of age (9). To further examine this finding, mink of varying ages and genotypes were inoculated intracerebrally with a 10% brain suspension from this animal. Incubation periods on second passage reduced to 9-10 months, and mink heterozygous for the Aleutian gene had severe spongiform degeneration in the cerebral hemispheres.

Table 1. Susceptibility of mink to 5% or 10% brain suspensions from various subpopulations of scrapie or their sheep or goat sources in the United Kingdom.

INOCULUM	RESPONSE*
SSBP/1	0/3
22A	0/4
22C	0/4
Sheep Source	0/4
87A	0/2
87V	0/3
Sheep Source (RLE)	0/3
Sheep Source	0/2
51C	0/3
Sheep Source	1/2 (22 months)
138A	0/4
Sheep Source	0/3
104A	0/2
ME7 (Q)	0/2
Drowsy Goat	0/4
79A	0/3
79V	0/3
58A	0/3
Chandler	0/3
ME7 (J)	0/4
125A	0/4

*Number of mink developing TME-like disease within three years after intracerebral inoculation/number inoculated (incubation period in parentheses).

American Scrapie. The susceptibility of 12-month old mink to three sources of American scrapie administered by various routes of inoculation is shown in Table 2. B-834 was the most pathogenic source of scrapie for mink injected intracerebrally, producing incubation periods of 11-12 months. This Nubian X Toggenburg buck was naturally infected

Table 2. Mink susceptibility to 10% brain suspensions from three sources of American scrapie.

INOCULUM	ROUTE*	RESPONSE**
B-834	IC	6/6 (11-12 months)
	IM	5/5 (17-22 months)
	per os	0/5
B-957	IC	5/5 (18-24 months)
	IM	0/5
	per os	0/5
PR-81	IC	5/5 (16-24 months)
	IM	0/5
	per os	0/5

*Mink injected with either 0.1 ml intracerebrally (IC), 0.5 ml intramuscularly (IM), or 3.0 ml per os using an oral dosing needle.

**Number of mink developing a TME-like disease within two years/number inoculated (range of incubation periods in parentheses).

via exposure to scrapie-contaminated pasture at Mission, Texas; the pasture being previously occupied by a flock of scrapie-affected Suffolk sheep. At 6 months of age, animal B-834 was removed from exposure and placed in a pen where he subsequently developed signs of scrapie at 40 months of age.

Mink inoculated with B-834 brain exhibited a typical clinical course of TME from behavioral changes, wasting and roughened fur, to progressive incoordination, somnolence and total debilitation. Neuropathologic lesions were indistinguishable from those seen in TME and included extensive microvacuolation in the cerebral hemispheres accompanied by reactive astrocytic hypertrophy. Lesions were bilaterally symmetrical and limited entirely to gray matter.

Both B-957 and PR-81 produced TME-like disease in mink after incubation periods of up to 2 years. However, there was a marked difference in the distribution of lesions as compared to TME or B-834. The microvacuolation and astrocytic hypertrophy were not limited to the gray matter, but could be found with equal intensity in both white and gray matter in the cerebral hemispheres as well as in brain stem, cerebellum and spinal cord.

Ten per cent brain suspensions from B-834, and from two mink developing TME-like disease after inoculation with B-834, were injected intracerebrally into weanling,

random-bred white mice. Mice inoculated with B-834 brain developed scrapie after 12 months. Mice inoculated with either of the mink brain suspensions remained unaffected for their lifespan of approximately 24 months.

Hamster-adapted TME. In an attempt to study the effects of host modification on species susceptibility, hamster-adapted TME brain was backpassaged into mink after both the third (10^{10} LD_{50}/gram of brain tissue) and sixth ($10^{9.3}$ LD_{50}/gram of brain tissue) hamster passage. Results are summarized in Table 3.

Table 3. Effect of hamster-adaptation on mink susceptibility to TME.

PASSAGE	INCUBATION*	TITER**
3rd	26 weeks	6.8
6th	58 weeks	2.0

*Length of incubation period after intracerebral inoculation with 10% brain suspensions.
**$Log_{10}LD_{50}$/gram of brain tissue as calculated by the Spearman-Kärber method after titration in mink.

Brain suspensions from mink affected with TME after injection with third passage hamster-adapted TME were inoculated back into seven hamsters intracerebrally. These animals appeared normal for a period of 18 months at which time three developed a TME-like disease while four remained unaffected for their remaining lifespan.

A similar experiment to the above was performed in squirrel monkeys in which two animals were each inoculated intracerebrally with third passage hamster-adapted TME. These animals developed TME-like disease in 55 and 60 weeks. Hamster backpassage of brain material from one of these monkeys resulted in an incubation period of 44 weeks in hamsters inoculated with a 10% brain suspension and an endpoint titer of 10^3LD_{50}/gram of brain tissue.

DISCUSSION AND ADDITIONAL RESULTS

Sources of scrapie from the United Kingdom had little mink pathogenicity whether tested as individual subpopulations or from their sheep or goat origins. Conversely, American scrapie, both in this study and in others (6), is

consistently pathogenic for mink, although relative susceptibility varies depending on the source of inoculum. Since we do not as yet fully understand all factors influencing species susceptibility, one can only speculate as to the reasons for this difference. Almost all of the scrapie in the United States occurs in the Suffolk breed of sheep. An obvious explanation would be that this breed potentiates the disease for carnivores, either by host modification or selection of subpopulations.

These are the first studies to compare sheep and goat scrapie in mink. Although goat B-834 did produce incubation periods of only 11-12 months, it is apparent that this increased pathogenicity is not due to a goat effect alone since both B-957 and PR-81 produced similar responses in mink. Furthermore, the different distribution of lesions produced by these later two inocula show that scrapie can have different pathologic expressions in mink than typically recognized as TME. Therefore, it should be expected that the pathology of natural TME will vary depending on the source of scrapie to which mink are exposed. Johannsen and Hartung have reported an incidence of TME occurring in East Germany in 1967 in which affected mink had diffuse cerebral "edema" and widespread lesions in the spinal cord (10).

Even though B-834 produced short incubation periods when inoculated intracerebrally, exposure by the oral route was ineffective during an observation period of two years. Thus, we once again seem to have a conflict between field and experimental data. However, Gajdusek has suggested that the main route of entry for these transmissible agents is not the oral route per se, but rather via breaks or abrasions of skin and mucosal surfaces (11). To examine this possibility, we tested third mink passage TME brain for neuro-invasiveness after intra-

Table 4. Comparison of infectivity of a 10% TME mink brain suspension tested by intracerebral (IC), intramuscular (IM), or intradermal (ID) inoculation.

INOCULATION	RESPONSE**
IC (0.1 ml, right cerebral hemisphere)	5/5 (20-23 weeks)
IM (0.5 ml, right hind leg)	5/5 (24-30 weeks)
ID (0.05 ml, right foreleg)	17/17 (24-40 weeks)

*Number of mink developing TME/number inoculated, excluding intercurrent deaths (range of incubation periods in parentheses).

dermal inoculation. The results are presented in Table 4.

The intradermal route appears to be an efficient means of exposure and one with a high potential for occurrence, considering mink husbandry practices. Mink kits are born in May and kept together as litters until August. During this period there is considerable fighting among littermates, especially at feeding time. It is easy to imagine how the scrapie agent could be introduced through bite wounds from feeding animals; each tooth a tiny inoculation needle. If this scenario is correct, the devastating occurrences of TME seen in the spring have not happened by chance, but are the result of mink being exposed to scrapie the previous summer, before separation into individual cages.

We have previously mentioned factors influencing species susceptibility to these transmissible agents. Terms such as "genetic predisposition", "host modification", and "selection of subpopulations" are freely used, but poorly understood. These experiments have shown once again that mouse pathogenicity of scrapie is markedly reduced after only a single passage in mink. This appears to be the best example yet for a possible host modification effect on scrapie agent. Such a dramatic change in host range could be explained by modification of a viral component after replication in a heterologous system. Perhaps, there is a critical host-contributed component to scrapie agent which modulates infectivity of susceptible cells.

However, while the effects of host modification remain questionable, there is a growing body of evidence to indicate that host selection of subpopulations of scrapie is an important mechanism by which these agents adapt to different populations of animals. Kimberlin and Walker, studying hamster-adapted scrapie, have been able to separate out two distinct populations, each with different host pathogenicities (12). A similar mechanism may explain our results with interspecies passage of hamster-adapted TME. The decrease in hamster pathogenicity after passage in mink or squirrel monkeys may indicate that the TME inoculum is composed of a mixture of agents and that passage in different hosts potentiates different subpopulations (Figure 1). This explanation becomes more attractive in light of evidence that hamster-adapted scrapie loses none of its pathogenicity for hamsters after passage in squirrel monkeys (R.F. Marsh, unpublished). This would argue against host modification since squirrel monkeys represent a common species which would be expected to alter both disease agents similarly.

To test this possibility we attempted to separate out the

hamster pathogen by two serial hamster passages of hamster-adapted TME at high dilution (10^{-7}). Hamster brain from the second serial passage was then inoculated as a 20% suspension into both mink and squirrel monkeys. Both of these species, which previously developed TME within 60 weeks after inoculation with unseparated hamster-adapted TME, remain unaffected after 108 weeks. These results, in combination with our previous studies showing that monkey-passaged TME retains a high mink LD_{50} endpoint, indicate that the Sawyer County source of TME is composed of a mixture of at least two subpopulations, a hamster pathogen and a mink-monkey pathogen. We would like to emphasize, however, that we do not consider these host restrictions to be absolute, nor that they infer a consistent relationship from one source of scrapie to another. In this particular instance, the mink and monkey pathogen appear to be one and the same, but other results (R.F. Marsh, unpublished) clearly show that different sources of scrapie vary in their mink and monkey pathogenicities and that, therefore, these host specificities segregate independent of one another.

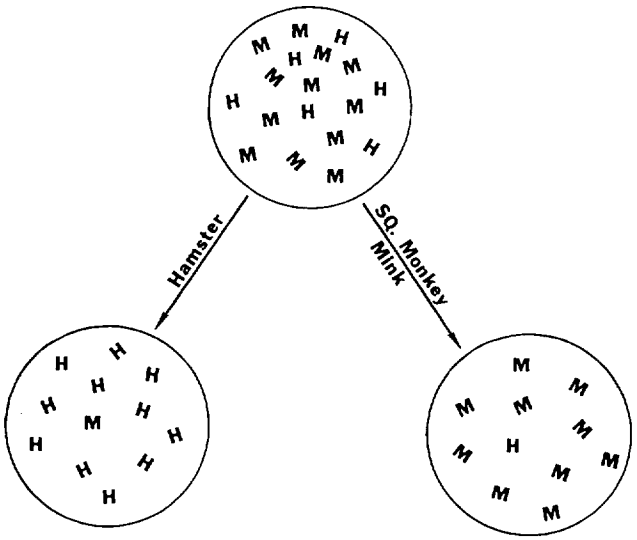

FIGURE 1. Diagrammatic illustration of mixture of a hamster pathogen (H) and mink-monkey (M) pathogen in the Sawyer County source of TME, and enrichment of subpopulations after animal passage.

In summary, our studies have shown a difference in mink pathogenicity of scrapie sources from the United Kingdom as compared to the United States. They also offer a reasonable explanation for differences observed between field incidences of TME and experimental studies on mink susceptibility to scrapie. Therefore, there appear to be no major obstacles remaining to the probable fact that TME originates from feeding mink scrapie-infected tissues, a conclusion Dr. Eklund reached many years ago.

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