Transmissible Mink Encephalopathy: Infectivity of Corneal Epithelium

Abstract. *Corneal epithelium from hamsters dying of transmissible mink encephalopathy contained a virus titer of $10^{4.8}$ times the 50 percent lethal dose ($10^{4.8}$ LD_{50}) per 0.05 milliliter when assayed as a cell suspension derived directly from the infected animal. After one passage in tissue culture, an equivalent concentration of cells contained only $10^{0.8}$ LD_{50} per 0.05 milliliter. It is concluded that corneal tissues are infectious; the infectivity may be mainly associated with free nerve endings. However, the most important immediate inference is that corneas from human beings affected with Creutzfeldt-Jakob disease are likely to be lethal if transplanted to healthy recipients.*

Transmissible mink encephalopathy (TME) is a natural slow virus disease of commercially reared mink which has been experimentally transmitted to seven other species. Scrapie, kuru, Creutzfeldt-Jacob disease described the form a single nosologic entity known as the subacute spongiform encephalopathies (*1*). A report (*2*) on the possible person-to-person transmission of Creutzfeldt-Jakob disease described the development of a fatal spongiform encephalopathy in an individual 18 months after receiving a corneal transplant from a donor later diagnosed as having died of Creutzfeldt-Jacob disease. Since the highest estimate for the incidence of Creutzfeldt-Jakob disease in the United States is only 200 cases per year (*3*), it is unlikely that both donor and recipient developed the same disease by chance. To investigate the infectivity of corneal tissue, we examined an animal model, hamster TME, a disease with etiologic and clinicopathologic features similar to Creutzfeldt-Jacob disease.

Five outbred Syrian hamsters (Lakeview Hamster Colony, Newfield, New Jersey) showing advanced clinical signs of TME were killed, and the aqueous humor was collected by introducing a 26-gauge needle into the anterior chamber at the corneoscleral conjunction. The corneas were then removed and placed in saline. The epithelial layer was separated from the cornea propria with the aid of a dissecting microscope. The epithelium dissected easily, most remaining in large tissue sheets. These epithelial fragments were centrifuged at 500*g* and the pellet was washed twice in medium 199 containing 10 percent calf serum and antibiotics (100 units of penicillin and 100 μg of streptomycin per milliliter). The epithelium was then gently aspirated through a 22-gauge needle until the large tissue fragments were reduced to free cells or small aggregates. This suspension was adjusted to a concentration of 2×10^6 cells per milliliter and divided into two portions. One was subjected to four freeze-thaw cycles, then frozen at -70°C for later animal inoculation. The second portion was incubated in plastic tissue-culture petri dishes at 37°C in room air containing 5 percent CO_2.

After 38 hours, small foci of cells were seen to adhere to the plastic. At this time, the original tissue culture fluid was carefully decanted and fresh medium 199 was added. The cells from the individual foci grew rapidly, becoming almost confluent by 15 days. Most cells retained an epithelioid appearance and were of varying sizes, some approaching 30 μm in diameter. No cytopathic effect was observed. The cells were scraped from the petri dishes after 3 weeks in culture, adjusted to a concentration of 2×10^6 cells per milliliter, subjected to four freeze-thaw cycles, and frozen at -70°C.

Cell suspensions from both intact and cultured epithelium, aqueous humor, and brain tissue from the same animals were simultaneously assayed by intracerebral inoculation of weanling hamsters. The results (Table 1) show a moderate amount of infectivity in uncultured epithelium which was not sustained after tissue culture. Brain contained a high concentration of the TME agent, as observed earlier (*4*). No infectivity was detected in aqueous humor.

Table 1. Infectivity of corneal epithelium and brain tissue from hamsters with transmissible mink encephalopathy. The data are presented as number of hamsters developing disease within 6 months of inoculation per number of animals inoculated. The 50 percent lethal dose (LD_{50}) was calculated by the Spearman-Kärber method (*11*); NT, not tested.

Dilution	Epithelium (2×10^6 cells per milliliter)		Brain
	Uncultured	Cultured	
10^{-1}	4/4	1/4	NT
10^{-2}	4/4	0/4	NT
10^{-3}	4/4	0/4	NT
10^{-4}	3/4	0/4	NT
10^{-5}	2/4	0/4	4/4
10^{-6}	0/4	0/4	4/4
10^{-7}	NT	NT	4/4
10^{-8}	NT	NT	3/4
10^{-9}	NT	NT	2/4
10^{-10}	NT	NT	0/4
LD_{50} per 0.05 ml	$10^{4.8}$	$10^{0.8}$	$10^{8.8}$

The discrepancy between the infectivity of uncultured and cultured epithelium may be explained by the failure of infected epithelial cells to replicate in vitro. Corneal epithelial cells of animals have a high rate of mitosis in vivo, with a life-span of only 7 to 10 days (*5*). Since the replication of the scrapie agent in vitro coincides with cell division (*6*), it is possible that corneal epithelial cells may be capable of supporting a moderate level of infection. A second, and perhaps more probable, explanation is that infectivity is associated with the numerous nerve fibers in corneal tissue (*7*). Corneal epithelium is richly innervated with free nerve endings present as naked axis cylinders with no myelin, no sheaths of Schwann (*8*). It is logical to suspect that these unorthodox neuropathic agents are capable of replicating in nerve fibers, as has been speculated in previous studies on TME (*9*) and scrapie (*10*).

This report illustrates the value of studying animal models of human disease. In this instance, the results are not only applicable to explaining a clinical observation, but they may also be of significance in further understanding the pathogenesis of these diseases.

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References and Notes

1. C. J. Gibbs, Jr., and D. C. Gajdusek, *Science* **165**, 1023 (1969).
2. P. Duffy, J. Wolf, G. Collins, A. G. DeVoe, B. Streeten, D. Cowen, *N. Engl. J. Med.* **290**, 692 (1974).
3. D. C. Gajdusek, *Am. J. Clin. Pathol.* **56**, 352 (1971).
4. R. F. Marsh and R. H. Kimberlin, *J. Infect. Dis.*, in press.
5. G. K. Smelser and V. Ozanics, in *Symposium on the Cornea* (Mosby, St. Louis, 1972), p. 3.
6. M. C. Clarke and D. A. Haig, *Res. Vet. Sci.* **11**, 500 (1970).
7. F. C. Rodger, *Br. J. Ophthalmol.* **34**, 107 (1950).
8. C. I. Thomas, *The Cornea* (Thomas, Springfield, Ill., 1955), p. 39; M. Whitear, *J. Anat.* **94**, 387 (1960).
9. R. F. Marsh, J. M. Miller, R. P. Hanson, *Infect. Immun.* **7**, 352 (1973).
10. G. W. Outram, A. G. Dickinson, H. Fraser, *Nature (Lond.)* **249**, 855 (1974).
11. R. M. Dougherty, in *Techniques in Experimental Virology*, R. J. C. Harris, Ed. (Academic Press, New York, 1964), p. 183.

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