Rabies Virus in the Tonsils of a Carrier Dog

By

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With 4 Figures

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Summary

A female dog, inoculated with a rabies isolate from the saliva of an apparently healthy Ethiopian dog, developed rabies but later recovered without supportive treatment. Rabies virus was isolated from the saliva collected 42, 169 and 305 days after recovery. Sixteen months after it recovered, the dog suddenly died after giving birth to two stillborn puppies. At necropsy, viral antigen could be detected in the tonsils and the brain tissue, but viable virus was isolated from the Palatine tonsils only.

Introduction

The outcome of most rabies infections is death but, in rare instances, recoveries from clinical rabies have been noted in humans and animals, with or without sequelae (2, 10, 11, 16, 19, 25, 26). Intermittent excretion of rabies virus in the saliva of apparently healthy dogs in nature (7, 8, 12, 28) and by a dog which recovered from clinical rabies has also been documented (10).

We recently reported that two dogs which were inoculated with a rabies virus from the saliva of an apparently healthy Ethiopian dog, had developed disease symptoms but recovered without any supportive treatment (10). One of these surviving dogs was studied for 16 months; during this period the dog excreted rabies virus in the saliva on days 42, 169 and 305 after recovery (12). Fourteen months after recovery the dog was bred to study the possibility of maternal transmission of rabies to her puppies. The dog remained healthy during pregnancy, but she suddenly died after giving birth to two puppies. The efforts made to isolate rabies virus from various organs of this dog are the subject of this report.
Materials and Methods

Animals

The beagles used in this experiment were reared in a closed colony and pretested for serum rabies neutralizing antibodies prior to virus inoculation.

Virus Stock

Original virus was isolated from the saliva of an apparently healthy Ethiopian dog reported to excrete virus intermittently in the saliva (7, 8). The inoculum used in this study was a second mouse brain passage of saliva from an apparently healthy dog.

Experimental Inoculation

Dog No. 128 in this report was one of four beagles inoculated intramuscularly (im) into the right masseter muscle with 800,000 MICLD₅₀ as previously reported (10). All inoculated animals were observed daily.

Virus Isolation Attempts

Mouse Inoculation

Twenty percent tissue suspension of organs (Table 1) were prepared by grinding with mortars and pestles, using 0.75 percent bovine albumin in phosphate-buffered saline solution (PBS) as diluent, and then the suspension was inoculated intracerebrally (i.c.) into weanling mice. Inoculated mice were observed daily for 30 days for clinical signs of rabies (18).

Co-Cultivation

Tissues (Table 1) were minced into <1 cu mm pieces and homogenized in enriched Eagles medium (MEM) containing 10 percent fetal calf serum (FCS) and co-cultivated with mouse neuroblastoma cells (kindly supplied by Jean S. Smith, Viral and Rickettsial Zoonoses Branch, CDC, P.O. Box 363, Lawrenceville, GA 30046) (23). After incubation for 8 days at 37°C the culture was washed every 4 days with replacement of media. Cultures were also examined by immunofluorescence every four days.

Dissociation of Antigen-Antibody Complexes at High pH

Fifty percent suspension of brain, corneal and salivary gland tissues were prepared in PBS and then diluted in tenfold dilution in alkaline NaOH-glycine buffer pH 12.0 (15, 33) or in control diluent (MEM) incubated for 30 seconds at room temperature and then immediately further diluted in serial ten-fold dilution in MEM pH 7.6, and assayed for infectious virus by inoculation of mouse neuroblastoma cells and mice by the i.c. route.

Immunofluorescence

Acetone-fixed touch impressions of brain and spinal cord tissues were stained with fluorescein isothiocyanate (FITC)-conjugated equine antirabies globulin (14).

Fresh tissues from various organs were mounted in tissue mounting medium (Tissue-Tek II*, O.C.T. Compound, Lab-Tek Products, Division Miles Laboratories, Inc., Naperville, Illinois 60540), frozen on dry ice, and sectioned in a cryostat at a thickness of 4 μm. All tissue sections were air dried, fixed in acetone for 4 hours at −20°C, and stained with FITC. Stained sections were examined with a Zeiss microscope equipped with epi-illumination using a XBO 150 Xenon bulb and KP490 and LP510 filters. At least five sections from each tissue block were examined.

Histology

Tissues of organs examined (Table 1) were fixed in phosphate-buffered formalin, embedded in paraffin, sectioned at 4 to 6 μm, and stained with hematoxylin and eosin. The tonsils were removed and frozen for immunofluorescence and mouse inoculation studies only. Because rabies virus was not expected to be found in the tonsils, this

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