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GROWTH OF ADENO-ASSOCIATED SATELLITE VIRUS IN DOGS IN THE PRESENCE OF INFECTIOUS CANINE HEPATITIS VIRUS

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Pups were inoculated by various routes with adeno-associated satellite virus (ASV) of canine origin, either alone or accompanied by infectious canine hepatitis virus (ICHV). ASV was not recovered from the pups inoculated with ASV alone; however, it was recovered from one of the 5 pups inoculated simultaneously with ASV and ICHV that died on the 4th day after inoculation and showed a fever and necropsy findings typical to infectious canine hepatitis. Recovery of ASV in this pup was positive from the blood, liver, gall bladder, parotid, and bronchial lymph node; from these ICHV recovery was also positive. Although the establishment of ASV infection in dogs was not so easy, the present results suggest that ASV requires the helper virus to grow even in the natural host.

INTRODUCTION

ASV has been known to replicate only in the presence of a helper virus, such as adenovirus, in tissue culture (ATCHISON et al., 1965; HOGGAN, 1970). It is not clear whether ASV requires a helper virus also in the natural host. This was investigated in the present study by inoculating ASV, which was originally associated with a strain of ICHV (DOMOTO & YANAGAWA, 1969), into dogs. Dogs have been considered to be one of the natural hosts of ASV from the data of a serological survey (ONUMA & YANAGAWA, 1972).

MATERIALS AND METHODS

Viruses: ASV strain M, which was associated with ICHV strain Matsuda, (DOMOTO & YANAGAWA, 1969) and identified with ASV type 3 (ONUMA & YANAGAWA, 1972), and ICHV strain Woc 4, which was free of ASV and virulent to dogs, were used for the inoculation. Another ICHV strain FD, which was also ASV free, was used as a helper for recovery of ASV in vitro. Non-association of ASV with ICHV strains Woc 4 and FD was already shown (DOMOTO & YANAGAWA, 1969). These viruses were grown in primary dog kidney monolayer cell cultures (DKC), as previously reported (DOMOTO & YANAGAWA, 1969).

Pups: Fifteen 2 to 7-day-old mongrel pups from 7 different litters were used for inoculation after having been raised for about 3 weeks. At the time of inoculation, the antibodies against ASV and ICHV were shown to be negative in all the pups by a complement fixation test (CFT). Each pup was kept in a cage in a room with a temperature of about 20°C and the cage was further warmed with an electric lamp, except for a summer experiment when each was kept in an isolated, unwarmed kennel. Commercial milk for pups was given 3 times a day. Care was taken to prevent natural infection of ASV or ICHV during the entire of the experiments. Pups from each litter were divided into those for inoculation and those for control for each experiment.

Inoculation and recovery of ASV and ICHV: The inoculum of ASV was the heated culture of ICHV strain Matsuda, which contained ASV strain M. A heat of 60°C was applied for 10 minutes to achieve selective inactivation of ICHV. Electronmicroscopic count of ASV was done in the manner described elsewhere (ONUMA & YANAGAWA, 1972). ASV and ICHV were inoculated alone or simultaneously. The inoculation of ASV was done subcutaneously, in the stomach through a catheter, intravenously, or intramuscularly. The inoculation in the stomach through catheter was applied because detection of ASV from anal specimens of children has been reported (HOGGAN, 1970). The inoculation of ICHV was done subcutaneously.

To recover the viruses, the following materials were collected from the pups at the time of natural or induced death: the blood, liver, spleen, kidney, lung, heart, pancreas, thymus, gall bladder, urinary bladder, tonsil, parotid, brain, spinal cord, testicle, ovary, uterus, stomach, duodenum, jejunum, ileum, caecum colon, rectum (the intestine was collected with its contents), mandibular, inguinal, axillar, bronchial and mesenteric lymph nodes. Feces and urine were collected from each pup every other day, and sera weekly. Recovery of the ASV and ICHV was done from a 10% suspension of the organs and feces as well as from the blood and urine. The suspensions were made in phosphate buffered saline (pH 7.2) containing 200~1,000 units of penicillin, 200~1,000 μ g of streptomycin, and 2.5~12.5 μ g of amphotericin (fungizon) per ml. Each 0.5 ml of the specimens was inoculated into 2 tubes of DKC; one was preinfected with the helper ICHV at a multiplicity of one TCID₅₀ for the recovery of ASV, and another was not preinfected for recovery of ICHV. The former tube was harvested when CPE due to helper ICHV reached its maximum, and the culture fluid was passed through DKC at least twice. Finally, coverslips were tested by the indirect immunofluorescent technique (BLACKLOW et al.³, 1967) for identification of ASV, using antisera of guinea pig against ASV strain M and fluorescein isothiocyanate-labeled anti-guinea pig γ globulin rabbit γ globulin. When CPE did not occur until the 7th day in the former tube, then

TABLE 1 Summary of the data of virus inoculation and recovery in dogs

INFECTED WITH	PUP NO.	INOCULUM SIZE				ROUTE ^{e)}	TERMINATION ^{d)}	RECOVERY		
		ASV		ICHV				ASV	ICHV	
		Number ^{a)}	Vol. ml	TCID ₅₀ ^{b)}	Vol. ml					
ASV	31	15	1			sc	K13			
	17	35	10			is	K56	-	-	
	4	54	0.5			im	DF5			
	2	54	0.5			iv	DF11			
ASV and ICHV	13	35	0.5	5.5	0.5	sc ^{e)}	D4	+	+	
	21	53	2	6.0	1	is, sc ^{f)}	D6	-	+	
	26	1	0.5	6.5	0.5	sc ^{e)}	K44	-	+	
	27	1	0.5	6.5	0.5	sc ^{e)}	K43	-	-	
	16	35	10	1.5	1	is ^{g)}	K55			
				3.5	1	sc				
4.5				1						
ICHV	23, 24			6.5	1	sc	K7, K16	-	+	
Control: 4 puppies								-	-	

a) ASV particles per 10 electromicroscopic fields at the instrumental magnification of $\times 50,000$

b) Log TCID₅₀ per 0.5 ml

c) sc, subcutaneous; is, in the stomach through catheter; im, intramuscular; iv, intravenous

d) K, killed; D, died; DF, died from environmental failure
Number indicates day after inoculation.

e) Simultaneous inoculation of ASV-3 and ICHV

f) ASV was inoculated in the stomach; 2 days later ICHV was inoculated subcutaneously.

g) ASV was inoculated in the stomach, and 13, 22, and 40 days later, ICHV was inoculated subcutaneously.

Growth of ASV in dogs

the culture fluid was passed at least twice through DKC and similarly examined. Detection of ASV was also done by applying electronmicroscopy from the culture fluid which was passed 5 or more times through DKC. The latter tube without preinfection of helper was observed for 7 days. When CPE due to ICHV appeared, the fluid was tested for hemagglutination. When CPE did not appear, the fluid was passed through another DKC and again observed for 7 days. For identification of ICHV, hemagglutination and hemagglutination inhibition tests were used. Detection of serum antibodies of the pups against ASV and ICHV was done by CFT (ONUMA & YANAGAWA, 1972).

RESULTS

The results of the experiments are shown in table 1. Four pups inoculated with ASV alone showed no clinical effects. In 2 of them death was induced 13 and 56 days after inoculation, and the remaining 2 died from environmental failure (low temperature) at 5 and 11 days after inoculation. Recovery of ASV from all 4 pups was negative.

Five pups were inoculated with both ASV and ICHV. Recovery of ASV was positive in only one of these (No. 13), while that of ICHV was positive in 3 pups (Nos. 13, 21 & 26). Pup No. 13, which received a simultaneous inoculation, incurred a fever (40°C) on 2nd and 3rd days and died on the 4th day after infection. It showed upon necropsy a mottled appearance of the liver, edema of the gall bladder, increase of bloody ascites, and subcutaneous edema around the inoculation site. ASV was recovered from its blood, liver, lung, gall bladder, parotid, and bronchial lymph node. ICHV was recovered from almost all of the examined tissues and organs of this pup. No. 21 showed fever and rapid collapse before death. Recovery of ASV was negative. The gross lesion and recovery of ICHV was similar to No. 13, except that ICHV was not detected in the feces and urine. No. 26 showed only fever, lack of appetite, and subcutaneous edema. Recovery of ASV was negative but was positive for ICHV in feces at 2, 3, 13, and 30 days, and from urine at 12 and 22 days after inoculation, but not from tissues and organs collected at the time of autopsy. Nos. 27 and 16 showed no clinical effects, and recovery of ASV and ICHV was negative.

Two pups, Nos. 23 and 24, inoculated with ICHV alone, showed the typical clinical signs of infectious canine hepatitis, such as fever, loss of appetite, vomiting, and conjunctivitis. Recovery of ICHV was positive from almost all of the tissues and organs of No. 23 and from the rectum of No. 24.

Isolation of ASV and ICHV from 4 control pups, raised during the experiment and killed after 13 to 45 days, was negative.

The antibody against ASV was not detected throughout the period of the

experiment in any of the pups, including No. 13, which was positive in ASV recovery but died as early as 4 days after inoculation. The antibody against ICHV was detected in No. 26, whose titers were 1:32 or more from the 21st to the 44th day after inoculation, and in No. 16, which received repeated inoculations of ICHV.

The clinical findings and gross lesions did not differ between No. 13, which was inoculated with ASV and ICHV, and the pups inoculated with ICHV alone. This suggests that ASV may not produce its own clinical illness.

DISCUSSION

The fact that ASV was recovered from only one of the 5 pups inoculated with both ASV and ICHV may indicate that, contrary to ICHV, experimental infection of ASV in dogs is rather difficult. Perhaps the virulence of ASV, the age of the dog, the method of infection, and other factors should be considered further.

ASV has been known to replicate only in the presence of adenovirus in tissue cultures (ATCHISON et al., 1965; HOGGAN, 1970), and presumed to behave so in its natural hosts. Co-existence of antibodies against ASV and adenoviruses has been known in monkeys (RAPOZA & ATCHISON, 1967), man (BLACKLOW et al., 1968), and dogs (ONUMA & YANAGAWA, 1972). Children from whom ASV was isolated also experienced adenovirus infection (BLACKLOW et al., 1967⁴, 1968). As shown in the present experiment, ASV which was originally associated with ICHV did not replicate in pups when inoculated alone, but replicated in pup No. 13 when inoculated simultaneously with ICHV, and the organs and tissues that were ASV-positive were also ICHV-positive. Although the present experiment is a preliminary one, these findings may indicate that replication of ASV is dependent on ICHV not only in tissue culture, but also in its natural host.

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