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ADDITIONAL CHARACTERIZATION OF THE ADENOSATELLITE VIRUS ASSOCIATED WITH THE INFECTIOUS CANINE HEPATITIS VIRUS

Misao ONUMA and Ryo YANAGAWA

Department of Hygiene and Microbiology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo

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The adenosatellite virus (ASV) strain M, associated with the infectious canine hepatitis virus (ICHV) strain Matsuda, was propagated in a dog kidney cell culture and purified. The purified ASV, at a density of 1.37~1.38 g/cm^3 in CsCl, did not agglutinate human 0 red cells and was identified by the complement fixation test as ASV type 3.

INTRODUCTION

The small virus which was found to be associated with the ICHV strain Matsuda[12] was recently proved to be a member of ASV[5]. This paper is to report additional properties of the ASV, including serological type.

MATERIALS AND METHODS

Viruses ASV strain M, found in the stock culture of ICHV strain Matsuda, was used. Four strains of ASV (types 1, 2, 3 and 4) were provided by M. ITO, National Institute of Health, Tokyo. ASV types 1, 2, 3 and 4 were grown in the culture of dog kidney cells (DKC), co-infected with ASV-free ICHV strain FD as a helper.

Purification of the ASV strain M The culture fluid of DKC infected with the ASV strain M, associated with the helper ICHV strain Matsuda, was harvested when the cytopathic effect due to ICHV reached maximum. The fluid, frozen and thawed three times, was centrifuged differentially, at 11,000 g for 20 min and at 77,500 g for 90 min. The differential centrifugation was done several times. The resultant viral material of strain M was purified by banding three times in isopycnic CsCl density gradient ultracentrifugation at 120,000 g for 30 to 40 hours. Purification of ASV types 1, 2, 3 and 4 was carried out in the same way.

The counting of the virus particles A drop of ASV samples, 0.01 ml in amount, was put on a carbon-coated collodion membrane grid. After the sample was dried, approximately 0.004 ml of 3% sodium silicotungstate (pH 7.2) was placed on the same grid. After the staining solution was dried, the specimen was examined with a JEM-7 electron microscope (Japan Electrical Opticus Laboratory Co.) at an instrumental magnification of 50,000 ×. Virus particles were counted in 90 different fields (1 field = 1 mμ^2) and scored as the particles per mμ^2.

The immunization of a guinea pig against strain M One ml of the purified strain M, which contained 350 particles per $m\mu^2$ under the electron microscope at 50,000 × magnification, was mixed with an equal volume of complete Freund adjuvant. This mixture was injected into the foot pad and muscle of a guinea pig. Three weeks later, the same volume of the virus material without adjuvant was given as a booster. The serum was collected a week after the booster inoculation.

Preparation of ASV antigens for the complement fixation (CF) test Four units of the strain M antigen, determined by the CF test using anti-strain M guinea pig serum, contained 70~80 particles per $m\mu^2$ under the electron microscope at 50,000 × magnification. Antigens of ASV types 1, 2, 3 and 4 were adjusted so as to contain an almost equal number of virus particles.

The CF test The CF test was carried out according to the microtiter technique described by KRAFT & MELNICK.

The hemagglutination (HA) test The HA test was done according to the microtiter technique described by ITO & MAYOR.

RESULTS

The number of ASV particles associated with ICHV strain Matsuda was usually 10^5~10^6 particles per ml, 10 to 100 times smaller than that of ASV associated with human and simian adenoviruses. This was mainly due to the relatively low yield of the helper ICHV. This fact made it difficult to prepare the antigen of strain M for the CF test and for preparing hyperimmune serum. Therefore, for the purpose of increasing the yield of strain M, infectivity titer of the helper ICHV (strain Matsuda) was elevated by 3 limiting dilution passages.

As shown in table 1, inoculation with the original culture of strain Matsuda on to DKC yielded 71 ASV particles and only 0.3 ICHV particles per 10 electron microscopic fields, whereas inoculation with a 10^{-7} dilution of the same culture yielded a decreased number of ASV particles (11.7) and increased the number of ICHV (2.7). At the 3rd limiting dilution experiment, DKC inoculated with a 10^{-9} dilution of strain Matsuda yielded no ASV particles and 20 ICHV particles per 10 fields. The infectivity titer of ICHV was also increased.

The culture of strain Matsuda thus elevated in ICHV titer was mixed with an equal volume of the original culture of the same strain, containing many ASV and fewer ICHV, and inoculated on to DKC in order to obtain increased yield of ASV. The resultant viral material contained several times as many ASV particles as the original culture of strain Matsuda (fig. 1).

The purified ASV strain M is shown in figure 2.

The density in CsCl of strain M was 1.37~1.38 g/cm^3, whereas that of ICHV was 1.34~1.35 g/cm^3.

The guinea pig serum immunized against strain M reacted with the homologous antigen and ASV type 3, but not with ASV types 1, 2 and 4 (tab. 2). Strain M did not agglutinate human 0 red cells. These results indicate that strain M belongs to ASV type 3.

TABLE 1 *Result of 3 applications of the limiting dilution in strain Matsuda*

DILUTION (−LOG 10)	1ST LIMIT. DILUT.			3RD LIMIT. DILUT.		
	ASV	ICHV		ASV	ICHV	
	No.*1	No.*1	Titer*2	No.	No.	Titer
0	71	0.3	4.5	0.7	16.3	8.25
1	58	0.7	5.25	0	14.3	8.25
3	34	1.3	5.57	0	14.3	9.0
5	27	3.3	6.0	0	18.7	9.25
7	11.7	2.7	5.5	0	22	9.5
9				0	20	9.5

The highest dilution where CPE appeared in the first application was used for the second application. The third application was carried out using the highest dilution where CPE appeared in the second application.

*1 Virus particles per 10 $m\mu^2$ counted at magnification 50,000 ×

*2 Infectivity titer expressed as Log TCID/0.5 ml

TABLE 2 *Serological typing of strain M*

VIRUSES*1	ANTI-STRAIN M GUINEA PIG SERUM	HEMAGGLUTINATION
Strain M	512*2	20*3
ASV-1	< 32	ND*4
ASV-2	< 32	ND
ASV-3	256	ND
ASV-4	< 32	2560

*1 Purified viruses whose number of viral particles was almost equal as determined by electron microscopy.

*2 CF titer

*3 Hemagglutination titer using human 0 red cells

*4 Not done

DISCUSSION

It was necessary in this study to obtain a large amount of ASV strain M culture, because it usually occurred in ICHV strain Matsuda culture in an amount of less than 10^6 particles per ml. This fact was thought to be due to a relatively small yield of the helper virus strain Matsuda. It has been known that the yield of an adenovirus was decreased when associated with ASV[3,10]. The use of limiting dilution on strain Matsuda (containing ASV strain M) was accordingly first carried out and resulted in the increase of ICHV and decrease of ASV. The reason was determined as follows: There were cells infected only with

ICHV by inoculating the diluted culture, where ICHV grew without interference from ASV. After 3 applications of the limiting dilution, the strain Matsuda, containing many ICHV particles, was obtained. CASTO et al.[4] reported that adenovirus overcame the inhibition by ASV when the inoculated amount was larger than that of ASV. The strain Matsuda culture thus prepared was inoculated on to DKC after mixing it with an equal volume of the original culture containing much ASV. The resultant viral material contained several times as many ASV particles as the original culture of the strain Matsuda. BLACKLOW et al.[1] reported that the ASV yield was increased when ASV and adenovirus were coinfected with a sufficient multiplicity of infection, usually more than one.

Buoyant density in CsCl of strain M was 1.37~1.38 g/cm^3, which was similar to those reported by others[6,9,11].

It is interesting that strain M, the first ASV associated with ICHV, is ASV type 3, the most frequently found ASV in man[2].

Whether strain M derived from the dog is not known. The fact that antibodies against strain M was rather common in dogs, which will be reported elsewhere, suggests that the strain may be of canine origin.

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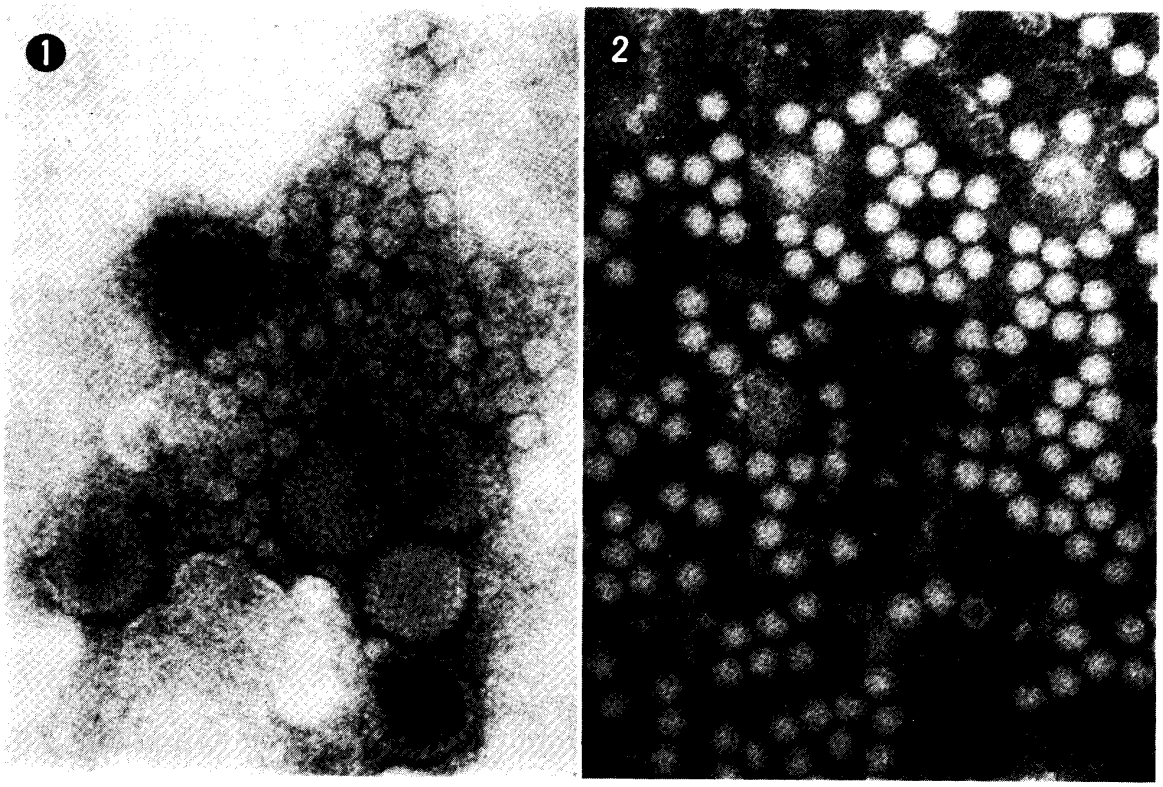
Explanation of plate

Fig. 1 An electron micrograph of the original culture of strain M
ASV (small) and ICHV (large) particles are seen. × 150,000

Fig. 2 An electron micrograph of the purified ASV strain M × 150,000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