Studies on Feline Panleukopenia

II. Antigenicities of the Virus

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Abstract. Infectivity, hemagglutination (HA) activity and complement-fixation (CF) activity of feline parvovirus (FPV) were investigated with 11 strains of the virus. The peaks of infectivity, HA activity and CF activity were observed similarly at a buoyant density of 1.32–1.35 g/ml in CsCl gradient. The eleven strains of FPV had the same CF antigenicity and were not distinguished from one another. Both infectivity and HA activity were affected with potassium periodate (KIO_4) and formaldehyde. Since a receptor for viral hemagglutinin on the porcine erythrocyte was inactivated almost completely by trypsin, KIO_4 and α -amylase, and to some extent by formaldehyde, it was supposed to have the same character as a glycoprotein. The agglutinability of erythrocytes by the virus was reduced when treated with neuraminidase as well.

There have been a few reports on the hemagglutination (HA) of feline parvovirus (FPV; feline panleukopenia virus) [6, 10–12, 15]. It has been reported that HA activity on porcine erythrocytes was considered to be a common property of the virus strains [15], that HA activity was parallel with infectivity [6], and that HA occurred with the fractions at different viral density levels of 1.44, 1.41, 1.36 and 1.31 g/ml in CsCl gradient [12]. In regard to the receptor for the virus on porcine erythrocytes and complement-fixation (CF) antigenicity of the virus, no information has been found as yet.

The present report describes the relationships among infectivity, HA activity and CF activity, with special reference to HA activity of the virus.

Materials and Methods

Virus production

One hundred ml of secondary feline kidney (FK-2) or a lined feline lung (FL) cell suspension at a concentration of $2 \times 10^{5.0}$ cells/ml in a 1,500-ml culture bottle was inoculated with 10 ml of stock virus fluid containing about $10^{3.5} \text{TCID}_{50}/\text{ml}$, and incubated at 37°C. On the 4th day after inoculation, multiplication of the virus was checked with the level of intranuclear inclusion body formation on stained coverslips. By that time, about 50% of the cells had been detached from the glass surface and almost all the remaining cells had inclusion bodies. After the infected cell culture in the bottle was frozen and thawed 3 times, it was centrifuged at 1,600 g for 15 minutes. The resulting supernatant was passed through a membrane filter (Millipore Corp.) 450 nm in pore size. The filtrate obtained was used as virus fluid.

Complement-fixation test

The virus was adsorbed on erythrocytes at 4°C and eluted from them at 37°C. Purification of the virus was performed effectively on the basis of this phenomenon. The CF test was done by the micro-

titer system (Cooke Laboratory Products) and, rabbit immune serum [15], or guinea-pig immune serum prepared with the purified virus was used after heat-inactivation at 63°C for 20 minutes or 56°C for 30 minutes, respectively. The CF antigen and antibody titers were expressed as the reciprocal of the highest dilution of the sample that had reduced hemolysis to 25% or less when 4 units of immune serum and virus fluid, respectively, had been used.

Virus concentration and viral fractionation in CsCl density gradient

About 600–700 ml of virus fluid was always concentrated and subjected to density gradient centrifugation in CsCl. The virus fluid was centrifuged at 163,500 g (R_{max}) for 3 hours in a Hitachi RP42/152 rotor of the Hitachi 65 ultracentrifuge. The pellet was resuspended in 1/300 of the original volume of phosphate-buffered saline solution (PBSS; 0.01 M). Samples were usually layered on preformed CsCl (Merck Co.) gradients ranging from 20 to 50 w/v % in 0.01 M Tris-HCl buffered solution and, if needed, mixed with saturated CsCl solution to a density of 1.35 g/ml. These gradients were centrifuged at 121,900 g (R_{max}) in a Hitachi RPS65T/224 rotor or at 156,000 g (R_{max}) in a Hitachi RPS40T rotor for 18–24 hours at 4°C. Fractions were collected by the density gradient fractionator (Hitachi Ltd.), assayed for density, infectivity, HA activity and CF activity, and observed by electron microscopy.

The fraction with the topmost activity that needed further density gradient examination was pooled, vacuum-dialysed against PBSS with a collo-dion bag (Brinkmann Instrument Inc.), and stored

at –20°C until used.

Treatment of the virus and porcine erythrocytes with various agents

The TUI strain of the virus was used. The concentrated virus suspension mentioned above was diluted with PBSS. This diluted virus fluid had an infectivity of $5 \times 10^{3.0}$ TCID₅₀/ml and an HA titer of 1:512 (512 HAU/ml). It was treated with various agents.

The following materials and methods used were essentially the same as described previously [15, 18, 19]: 1) preparation of FK-2 and FL cells and media, 2) virus strains, 3) coverslip staining method, 4) virus assay of HA activity and infectivity, 5) hemagglutination-inhibition (HI) and serum-neutralization (SN) tests, 6) electron microscopy, 7) selection of porcine erythrocytes and 8) all the biochemical reagents and enzymes, and the procedures and conditions of treatment.

Results

Relationships among infectivity, HA activity, CF activity and buoyant density

As shown in Fig. 1, the highest infective and HA titers of the TUI strain were observed in fractions at a density of 1.34 g/ml (1.32–1.37 g/ml) and 1.32–1.35 g/ml, respectively. Those of the TU4 strain were observed in fractions at the same density of 1.33 g/ml, as shown in Fig. 2. The infective and HA peak titers of the TUI strain were in a somewhat wider range of density

Fig. 1. Distribution of infectivity and hemagglutinating activity of the TU 1 strain in the primary CsCl gradient

Fig. 2. Distribution of infectivity and hemagglutinating activity of the TU 4 strain in the primary CsCl gradient

Fig. 3. Distribution of infectivity and hemagglutinating activity of the TU 1 strain in the secondary CsCl gradient

Remarks.

Fractions, Nos. 15 to 35, in Fig. 1 were pooled and subjected to this CsCl gradient.

Fig. 4. Distribution of hemagglutinating and complement-fixation activities of the TU 1 strain in CsCl density gradient

Remarks.

*: Each fraction was diluted 1:100 in gelatin-veronal-buffered saline solution and tested.

Table 1. Effects of various agents on infectivity and hemagglutinating (HA) activity of virus*

Agent	Condition of treatment	Final concentration	% Reduction after treatment**	
			Infectivity	HA activity
Trypsin	37°C, 60 min, pH 7.4	0.2%	0	0
Dithiothreitol	30°C, 60 min, pH 7.8	0.002M	0	0
KIO ₄	37°C, 60 min, pH 7.4	0.25%	99.4	62.5
Sodium deoxycholate	37°C, 60 min, pH 7.4	0.5%	0	0
Formaldehyde	30°C, 60 min, pH 7.2	0.2%	68.0	25.0
2-Mercaptoethanol	37°C, 60 min, pH 7.4	5×10 ⁻³ M	0	0
Ethylenediaminetetraacetic acid	37°C, 60 min, pH 7.4	5×10 ⁻² M	0	0
Ether	4°C, 18 hr, pH 7.2	50%	0	0
Chloroform	4°C, 30 min, pH 7.2	50%	0	0
α-Amylase	37°C, 60 min, pH 7.4	0.5%	0	0
β-Glucosidase	37°C, 60 min, pH 7.4	2.0%	0	0
Heating	56°C, 30 min, pH 7.4	—	0	0

Remarks.

*: The TU 1 strain of feline parvovirus.

** : Mean value of 2 experiments.

than those of the TU4 strain. The secondary CsCl density gradient examinations for each peak fraction of both strains were performed. The results obtained from the TU1 strain are illustrated in Fig. 3. They resembled those obtained from the primary CsCl density gradients. Another low peak of infectivity at a high density of 1.40–

1.45 g/ml, however, was newly observed in both strains.

Fig. 4 shows the distribution of HA and CF activities of the TU1 strain in CsCl density gradient. The highest CF titer was observed in a fraction at a density of 1.32 g/ml in accordance with the HA activity of the virus.

Table 2. Effects of various agents on agglutinability of porcine erythrocytes by feline parvovirus* at pH 6.6

Agent	Condition of treatment	Final concentration	% Reduction after treatment**
Trypsin	37°C, 60 min	0.2%	>99.4
Dithiothreitol	37°C, 60 min	0.004M	0
KIO ₄	37°C, 30 min	5×10 ⁻⁶ M	>93.6
Sodium deoxycholate	20°C, 60 min	0.025%	0
Formaldehyde	37°C, 60 min	0.2%	33.3
2-Mercaptoethanol	37°C, 60 min	5×10 ⁻³ M	0
Ethylenediaminetetra-acetic acid	37°C, 60 min	5×10 ⁻³ M	0
Ether	37°C, 60 min	2.5%	0
α-Amylase	37°C, 60 min	0.5%	>99.4
β-Glucosidase	37°C, 60 min	2.0%	0
Neuraminidase***	37°C, 60 min	25%	91.7

Remarks.

*: The TU 1 strain of feline parvovirus.

**: Mean value of 3 experiments.

***: Commercial cholera filtrate (lyophilized vibrio culture fluid) which causes 97% reduction of agglutinability of chicken erythrocytes by equine influenza virus (A/Equi-2/Tokyo/71 strain) hemagglutinin at fourfold dilution.

Fig. 5. Complement-fixation (CF)* antibody response of rabbits against the TU 2 strain

Remarks.

*: Four units of erythrocyte-purified virus antigen were used in the test. CF antibody titer was expressed as the reciprocal of the highest dilution of serum that reduced hemolysis to 25% or less.

**: Serum-neutralization (SN) titer was expressed as the reciprocal of the highest dilution at which 50% of the cultures lacked in viral inclusions.

***: Hemagglutination-inhibition (HI) titer was expressed as the reciprocal of the highest dilution giving a complete HI pattern.

Results of SN and HI tests were cited from reference [15].

Electron microscopic observation

All the peak fractions of infectivity, HA activity and CF activity (Figs. 1–4) were checked by the negative staining method. The same results were observed from both strains. Particles in every sample were around 22 nm in average diameter. A few small doughnut-shaped particles 10 nm in diameter were also observed. Particles in fraction No. 9 (1.34 g/ml) of Fig. 3 are again shown in Fig. 6 to indicate that almost all of them are “full” particles. There was neither stated rule nor interpretation for the smaller particles.

Effects of various agents on infectivity and HA activity of the virus

As shown in Table 1, the virus was reduced in both infectivity and HA activity by the treatment with KIO_4 and formaldehyde.

Effects of various agents on agglutinability of porcine erythrocytes

As shown in Table 2, the agglutinability was affected strongly with trypsin, KIO_4 and α -amylase, and moderately with formaldehyde. It was also reduced after treatment with neuraminidase.

Complement-fixation test

The eleven strains of FPV had essentially the same antigenicity in CF and could not be distinguished from one another.

Fig. 5 presents the CF antibody response pattern in rabbits and comparison between SN and HI antibodies against the virus. When erythrocyte-purified virus antigen was used in the test, CF antibody titer was low, but was closely correlated with SN and HI antibodies.

Discussion

The present investigation clarified the following items. (1) Hemagglutinin of FPV was considered to be a close attendant on virus particles of density less than

1.46 g/ml, the principal one of which was an infective particle at a density of 1.32–1.35 g/ml in CsCl gradient. (2) The receptor on the erythrocyte for viral hemagglutinin had the same character as a glycoprotein. (3) The major part of CF antigen was derived from the intact virion at the same density.

Up to date, a few reports have been published on the relationship between buoyant density and antigenicity of FPV, including mink enteritis (ME) virus [12, 13, 29]. ME virus was purified from infected mink tissues by adsorption and elution with calcium phosphatate and DEAE cellulose column chromatography. Its density was found to be 1.30–1.33 g/ml in CsCl gradient by CF antigenicity. Besides, low CF antigenicity was also detected in this virus at a density of 1.38 g/ml [13]. In FPV grown in cell culture the maximum buoyant density in CsCl gradient was 1.40 g/ml, although infectivity was associated with low density (1.33 g/ml) as well [29].

Johnson et al. [12] found in their studies on both FPV and ME virus that there were four main virus populations at a density level of 1.44, 1.41, 1.36 and 1.31 g/ml, respectively, in CsCl, as classified by radioactivity and HA. All the populations showed coincident “peaks” of both radioactivity and HA, except one at the lowest density level which was detected only by HA and which was apparently devoid of DNA.

The 3 reports mentioned above cannot be compared with one another because of the methodological differences among them. The relationship between antigenicity and buoyant density in the 2 strains of FPV used in the present investigation was clearly different from that indicated in the 2 reports, except for the ME virus study [13]. That is, in the present investigation the

major virus population was at a density of 1.32–1.35 g/ml. Moreover, there was a slight difference between the TU1 and TU4 strains. In neither strain there was a major particle at such a density of around 1.41 g/ml as that in the other reports [12, 29].

In the other parvoviruses, the relationship between antigenicity and buoyant density has been known [1, 5, 16, 24, 26, 28]. That is, parvoviruses make two big bands of particles when centrifuged to equilibrium in CsCl. One consists of “complete” particles containing DNA at a density of around 1.41 g/ml and has also HA activity. The other has empty capsids with no detectable DNA at a density of 1.30–1.32 g/ml. In it particles have high HA activity, but rarely infectivity. Additionally, it has been known that there are two other bands at a density of 1.44 and 1.38 g/ml, respectively. In the latter, there have been two observations; one is shown as a population of “complete” particles with DNA [7, 8, 17, 21] and the other as a mixture of “complete” and “incomplete” particles [24]. Furthermore, particles of higher density (1.47 g/ml) with DNA have been reported [3, 16].

As a whole, the parvovirus population is composed of two kinds of particles, which are infective particles at a density of 1.37–1.47 g/ml and non-infective particles at a density of 1.30–1.32 g/ml, and both of which have HA activity.

Whiteman and Hetrick [30] described Kilham rat virus. According to them, the virus band occurs at a density of 1.31 g/ml in potassium tartrate and consists of numerous homogeneous particles approximately 22 nm in diameter. Both HA and infectivity titers were encountered within a density range of 1.27–1.33 g/ml.

In the electron microscopic study of

parvovirus by negative staining, small doughnut-shaped particles 7 to 12 nm in diameter were sometimes observed [1, 2, 29]. Breese et al. [2] reported that these small particles found at a density of 1.24–1.29 g/ml in potassium tartrate had high HA activity and no infectivity. They assumed that such small particles might be either subunits of the large particle (20 nm, 1.32 g/ml) or abnormal protein formed in the process of multiplication. These small particles were not always observed in the present study.

All the parvoviruses, including FPV, examined so far have one major and two minor structural proteins in virions [3, 9, 12, 14, 25, 27, 28]. So that, in the present study, viral hemagglutinin was presumed to have a character of the protein.

A few papers [4, 6, 20] have been found on the receptor for parvoviruses on erythrocytes. The receptor for FPV on porcine erythrocytes was reduced in activity by treatment with trypsin, KIO_4 , α -amylase, formaldehyde or neuraminidase. Therefore, it was different from the receptor for rat parvoviruses [4] and considered to be a glycoprotein.

The CF test using such erythrocyte-purified virus antigen as prepared in the present study was also available like the SN and the HI tests. In the CF test, however, feline serum has occasionally induced some troubles [22, 23]. Therefore, the CF test is not supposed to be a practical serological tool for the actual diagnosis of feline panleukopenia.

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Explanation of Figures

Fig. 6. Virus particles observed in fraction No. 9
in Fig. 3 by negative staining. Arrow indicates

a small doughnut-shaped particle. Bar=50 nm.