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Hemagglutination with transmissible gastroenteritis virus

Brief Report

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Summary

Transmissible gastroenteritis virus grown in primary swine kidney cell cultures agglutinated erythrocytes from chicken, guinea pig and cattle but not erythrocytes from mouse and goose. The optimal incubation temperature was at 4° C. The hemagglutination (HA) reaction was inhibited by specific antiserum. Some factors involved in the HA and HA-inhibition (HI) were investigated and standard HA and HI tests were established. HI antibody titers of individual pig sera showed a significant positive correlation with their neutralizing antibody titers.

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Some viruses of the familiy Coronaviridae, such as human coronavirus (8, 9) bovine coronavirus (12, 13) hemagglutinating encephalomyelitis virus (6, 7, 11) and avian infectious bronchitis virus (1, 3), are already known to have hemagglutinating (HA) activity. These observations encouraged us to investigate the possibility of HA by transmissible gastroenteritis (TGE) virus, a member of the family Coronaviridae, as this has not been previously reported. This paper describes our observations on the HA with the virus and HA-inhibition (HI) by specific antiserum.

The TGE virus strains used were the TO and SH strain (5) supplied by Dr. S. Furuuchi, National Institute of Animal Health, Tsukuba, Japan, and the Ukiha strain (4) supplied by Dr. M. Eto, Chemo-Sero-Therapeutic Research Institute, Kumamoto, Kumamoto, Japan. The h-5 and U strains, which were recovered from the attenuated TGE vaccines purchased from

the Nippon Institute for Biological Science, Oome, Tokyo, and the Chemo-Sero-Therapeutic Research Institute, Kumamoto, Kumamoto, Japan, respectively, were also used.

The passage levels in primary swine kidney (SK) cell cultures of the TO, SH, Ukiha, h-5 and U strains used were 165, 166, 128 and 72, respectively. The TO strain was mainly used.

Primary SK cells and CPK cells, a continuous cell line derived from pig kidney, were prepared as described previously (10) and used for preparation of HA antigens and titration of viruses, respectively. The growth medium was Earle's solution containing 0.5 per cent lactalbumin hydrolysate, 10 per cent bovine serum, 1 per cent of 7 per cent $NaHCO_3$, 100 units/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml kanamycin and 2 μg/ml fungizone for SK cells and Eagle's minimum essential medium (MEM) containing 10 per cent bovine serum, 10 per cent tryptose phosphate broth (TPB), 1 per cent of 7 per cent $NaHCO_3$ and antibiotics as above for CPK cells. The maintenance medium for both cells was MEM containing 10 per cent of TPB, 2 per cent of 7 per cent $NaHCO_3$ and antibiotics as above.

Confluent cultures of SK cells prepared in 500 ml bottles were inoculated with TGE virus, and incubated at 37° C with 30 ml of maintenance medium for 3 days or longer until the cytopathic effect was complete. In some preliminary experiments, culture fluid harvested from infected cells was used as HA antigen after centrifugation to remove cellular debris. In subsequent experiments, however, HA antigens concentrated from infected culture fluid were used.

The microtiter method was used for the HA and HI tests. The diluent was PBS (phosphate buffered saline, pH 7.2), unless otherwise stated. Blood was obtained in Alsever's solution and stored at 4° C. Chicken erythrocytes were used in 0.5 per cent suspension, unless otherwise stated. In HA tests, serial 2-fold dilutions of HA antigen were prepared in 0.025-ml amounts, and mixed with 0.025 ml of erythrocyte suspension after adding 0.025 ml of the diluent. The mixtures were incubated at 4° C for 1 hour before the results were read, unless otherwise stated. The HA titer was expressed as the reciprocal of the highest antigen dilution showing complete HA. In HI tests, the serum was treated with receptor-destroying enzyme (RDE) (Takeda Chemical Industries, Ltd., Osaka, Japan), unless otherwise stated. One tenth ml of the serum and 0.3 ml of RDE were mixed and incubated at 37° C for 18 hours. The mixture was then heated at 56° C for 30 minutes, mixed with 0.05 ml of packed erythrocytes and incubated at 37° C for 1 hour. At the end of the incubation, the mixture was centrifuged to sediment the cells and the supernatant fluid was used for the test as a 4-fold dilution of the serum. Four units of HA antigen in 0.025 ml and 0.025 ml of serial 2-fold dilutions of the treated serum were mixed, incubated at 4° C overnight, and then mixed with 0.025 ml of erythrocyte suspension. These mixtures were then incu-

bated at 4° C for 1 hour and the results were read. The HI titer was expressed as the reciprocal of the highest serum dilution showing complete HI.

Neutralization (NT) tests were carried out in tube cultures of CPK cells as described previously (2).

Antisera against the TO strain of TGE virus were prepared in rabbits and guinea pigs by the method described previously (2). Immune pig sera and serum samples from pigs experimentally infected with TGE virus, which were prepared by one of the authors (2), were also used.

In preliminary experiments with supernatant fluid harvested from SK cell cultures infected with the TO strain, erythrocytes from chicken and some other animals were found to be agglutinated, although the HA titers obtained were low. To confirm this finding, we prepared concentrated HA antigens by ultracentrifugation. Infectious culture fluid, after low-speed centrifugation to remove cellular debris, was centrifuged at 40,000 × g for 1 hour, and the pellets were suspended in 1/100 the original volume of PBS.

The concentrated antigens thus prepared were tested for HA with erythrocytes from a variety of species at 4° C, 22° C and 37° C. All the five TGE virus strains tested gave positive HA. The HA titers, however, were affected significantly by the incubation temperature. The titer was considerably higher at 4° C than at 22° C and much lower or negative at 37° C (Table 1).

Table 1. HA titers with erythrocytes from various species incubated at different temperature

Virus strain	Erythrocyte species																	
	Chicken			Guinea pig			Cattle			Swine			Mouse			Goose		
	4°	22°	37°	4°	22°	37°	4°	22°	37°	4°	22°	37°	4°	22°	37°	4°	22°	37°
TO	2048	8	<2	1024	16	<2	1024	4	<2	1024	16	8	<2	<2	<2	<2	<2	<2
Ukiha	2048	16	<2	1024	16	<2	1024	8	<2	1024	32	8	<2	<2	<2	<2	<2	<2
SH	1024	2	<2	512	8	<2	256	4	<2	1024	–	–	<2	<2	<2	<2	<2	<2
h-5	2048	16	2	1024	16	4	512	4	<2	–	–	–	<2	–	–	<2	–	–
U	2048	16	2	1024	16	4	1024	4	<2	–	–	–	<2	–	–	<2	–	–

Incubation temperatures are in °C
– Not tested

Chicken erythrocytes gave the highest titer, followed by erythrocytes from guinea pig, cattle and swine. Erythrocytes from mouse and goose gave negative results. HA titers also varied between different samples from the same species.

No significant change of HA titer occurred, when PBS (pH 7.2), VBS (veronal buffered saline, pH 7.2) and 0.15 M NaCl solution (pH 7.2), respectively, were used as the diluent.

HA was observed when McIlvaine's buffered saline with various pH values ranging from 6.0 to 7.2 was used as the diluent and there was no significant difference in the HA titers.

On the basis of these results, chicken erythrocytes with PBS as the diluent, and incubation at 4° C became the standard method.

Culture fluid harvested from infected CPK cell cultures showing cytopathic changes has HA titers of between 2 and 8. Figure 1 illustrates a representative example of HA antigen production as well as that of viral infectivity in the fluid phase of tube cultures of CPK cells incubated at 37° C after infection at an input multiplicity of 0.1 $TCID_{50}$/cell. The HA was first demonstrated 3 days after inoculation, gradually increased and reached a plateau of 8 at 4 days, with a gradual decline thereafter. The curve of viral infectivity resembled that of HA production, although a rise was detected somewhat earlier.

Sera from pigs, rabbits and guinea pigs negative for NT antibodies to TGE virus were inactivated at 56° C for 30 minutes and showed high titer inhibitory activity to HA by TGE virus. Therefore serum treatments with RDE (see above), kaolin, acetone, trypsin, or potassium periodate were carried out by the method as described previously (14). These HI activities were completely removed by treatment with RDE. This treatment did not affect the HI activity of immune sera. On the other hand, the treatment of

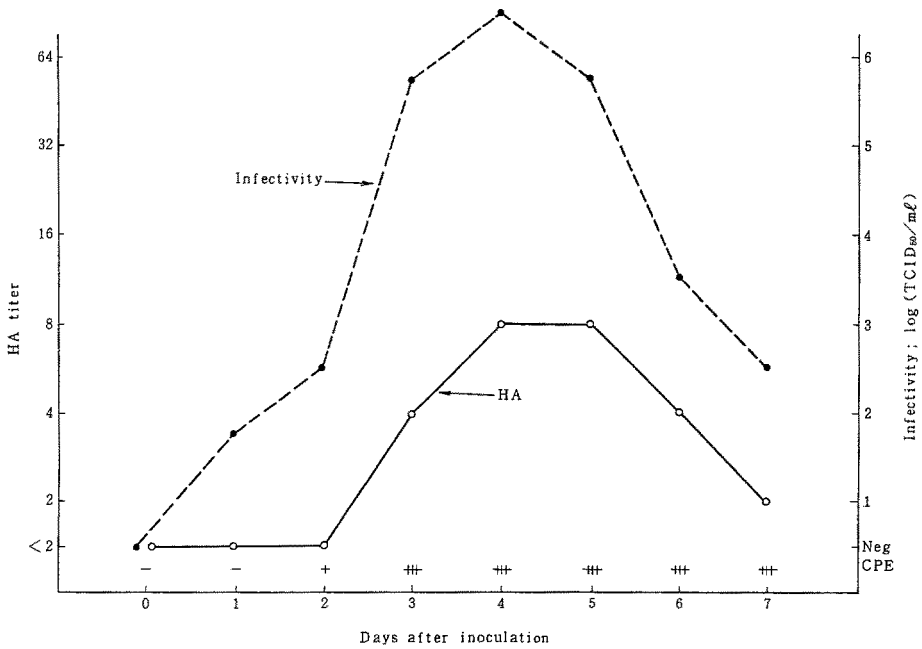

Fig. 1. Production of hemagglutinin and active virus in CPK cell cultures infected with the TO strain of TGE virus

Table 2. HI and NT titers of antisera from pig, guinea pig, and rabbit hyperimmunized with TO strain of TGE virus

Antiserum		Antibody titers against			
		TO strain		Ukiha strain	
		HI	NT	HI	NT
TO (swine)	preimmun	<4	<4	<4	<4
	postimmun	2048	4096	2048	2048
TO (guinea pig)	preimmun	<4	<4	<4	<4
	postimmun	512	512	512	1024
TO (rabbit)	preimmun	<4	<4	<4	<4
	postimmun	512	1028	512	1028

serum with kaolin, aceton, trypsin or potassium periodate did not remove nonspecific inhibitors completely. According, sera for the HI test were treated with RDE in the following experiments.

The HA reaction was shown to be virus specific, since it could be inhibited by specific antisera (Table 2). HI and NT tests were carried out on serum samples from pigs infected with the SH strain. All the animals developed antibodies for the virus. HI and NT antibodies exhibited a similar pattern of appearance and persistence, NT antibody reaching a plateau 20 days after inoculation. HI antibody became positive in 8 days and reached a maximum titer of 128 at 20 days, remaining constant thereafter (Table 3).

Individual serum samples from pigs vaccinated with an attenuated TGE vaccine available commercially were tested for HI and NT antibodies. As shown in Fig. 2, HI titers of individual sera showed a significant positive correlation with their NT titers, the correlation coefficient being 0.901 ($p < 0.01$).

Table 3. Production of serum HI and NT antibodies in pigs infected with SH strain of TGE virus

Pig no.	Antibody	Antibody titer against TO strain of TGE virus							
		Days after inoculation							
		0	4	8	12	16	20	24	28
1	HI	<4	<4	4	8	16	32	32	32
	NT	<2	<2	8	8	16	16	32	32
2	HI	<4	<4	8	8	16	32	64	
	NT	<2	<2	8	8	64	256	128	
3	HI	<4	<4	4	8	64	128	128	128
	NT	<2	<2	4	8	64	128	128	128
4	HI	<4	<4	4	4	8	16	16	16
	NT	<2	<2	16	16	128	128	64	64

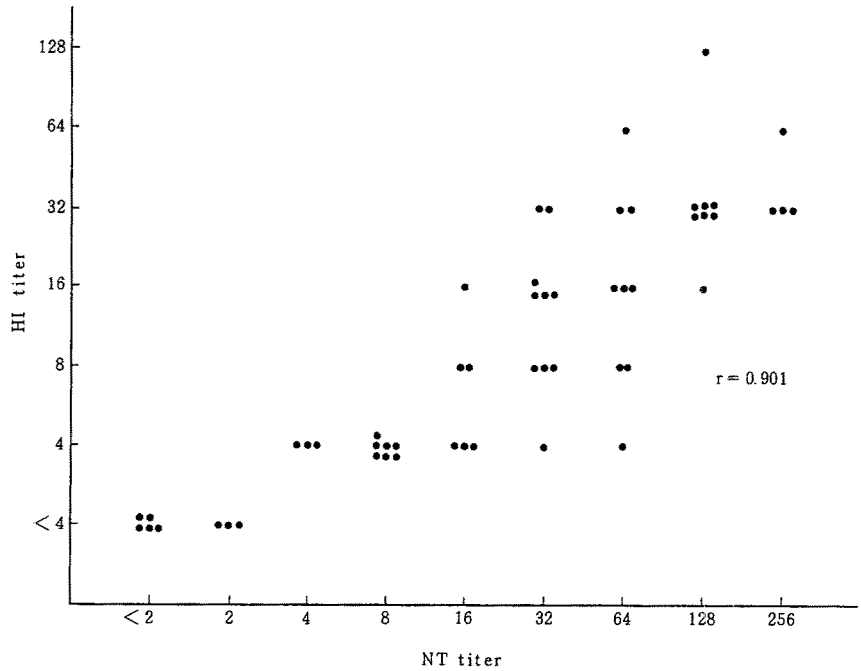

Fig. 2. Correlation between HI and NT titers in pigs vaccinated with a commercial TGE virus vaccine

The HA and HI tests developed in this study seem very useful and should find a wide application in studies on the virus and associated disease.

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