Hemagglutination with Equine Arteritis Virus

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ABSTRACT. Equine arteritis virus (EAV) grown on RK₁₃ cell cultures was tested for hemagglutination (HA) with erythrocytes from a variety of species at 4°C, room temperature and 37°C. HA was observed at all temperatures with erythrocytes from mouse and chicken but not with those of cattle, horse, rabbit, guinea pig, mongolian gerbil, goose or chick embryo. Chickens showed an individual variation in agglutinability of their erythrocytes, requiring selection of birds to obtain erythrocytes for HA. The HA activity was enhanced by treatment of virus materials with Tween 80 followed by treatment with ether. The HA reaction was inhibited by specific antiserum. Higher HA-inhibiting (HI) antibody titers were obtained by the incubation of serum-HA antigen mixture at 4°C for 24 hr. HI antibody titers of individual horse sera showed a significant positive correlation with their neutralizing antibody titers. — **KEY WORDS:** equine arteritis virus, hemagglutination, hemagglutination-inhibition.

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We recently reported that porcine reproductive and respiratory syndrome virus (PRRSV), a member of the genus *Arterivirus*, has hemagglutinating activity [5]. This observation encouraged us to investigate the presence of hemagglutinating activity associated with equine arteritis virus (EAV), a representative member of the genus *Arterivirus* [6]. This paper describes the first observations on hemagglutination (HA) with EAV and HA inhibition (HI) by anti-EAV sera.

The Bucyrus strain of EAV [2], supplied by Dr. W. H. McCollum, University of Kentucky, Lexington, KY, U.S.A., was used.

The continuous cell line, RK₁₃ (ATCC CCL37), derived from rabbit kidney cells, was used. The cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% tryptose phosphate broth, 5% heat-inactivated calf serum, 0.12% NaHCO₃, and 100 units of penicillin, 100 µg of Kanamycin, and 2 µg of fungizone/ml. The maintenance medium (MM) was MEM supplemented with 0.5% lactalbumin hydrolysate, 0.2% bovine serum albumin (BSA), 0.12% NaHCO₃, and antibiotics as described previously [1].

Four kinds of HA antigen, Tween 80-ether (TE) treated cell (TE-cell) HA, non-TE treated cell (non-TE-cell) HA, TE-treated fluid (TE-fluid) HA, and non-TE treated fluid (non-TE-fluid) HA antigens were prepared from the RK₁₃ cell cultures infected with EAV by the method of Jusa *et al.* [5]. Similarly, control HA antigens were also prepared from the mock-infected RK₁₃ cell cultures.

Blood was obtained in Alsever's solution and stored at 4°C. When used, erythrocytes were washed 3 times with PBS (0.15 M NaCl, 0.02 M phosphate buffer; pH 7.2) and suspended in a diluent consisting of PBS, 0.2% BSA and

0.01% gelatin to make a 0.3% suspension. The ddY mouse erythrocytes were used, unless otherwise stated.

The HA and HI tests were carried out by a microtiter method using 96-round-well microtiter trays (Sanko Junyaku Co., Tokyo, Japan) by the method described in our previous paper [5].

Serum neutralization test (NT) was conducted by the 50% plaque reduction method using RK₁₃ cells and virus diluent containing 10% guinea pig serum (Denka Seiken, Tokyo) described in the previous paper [4].

In preliminary experiments with the concentrated antigens prepared from RK₁₃ cell culture infected with the Bucyrus strain of EAV, erythrocytes from mouse were found to be agglutinated, although the HA titer obtained was low. To confirm this finding, 4 kinds of HA antigen were prepared and titrated for HA activity with mouse erythrocytes. As shown in Table 1, HA was observed with all HA antigens tested at room temperature. However, the results with the TE-cell HA and TE-fluid HA antigens were comparatively better than those with the non-TE-cell HA and non-TE-fluid HA antigens. The highest HA titer of 64 was obtained with the TE-cell HA and TE-fluid HA antigens, and both the non-TE-cell HA and non-TE fluid HA antigens showed a considerably low HA titer of 16. All control HA antigens prepared from the mock-infected RK₁₃ cells treated with or without TE were HA negative (Table 1). In the following experiments, the TE-cell HA antigen was used, unless otherwise stated.

TE-cell HA antigen was tested for HA with erythrocytes from a variety of species at 4°C, room temperature and 37°C. Mouse, ddY and BALB/c strains, and chicken erythrocytes were agglutinated at all three temperatures, whereas erythrocytes from horse, cattle, guinea pig, mongolian gerbil, goose and 10-day old chick embryo failed to be agglutinated under the test conditions (Table 2). In addition we found that erythrocyte samples from individual chickens showed a wide variation in their agglutinability, ranging from negative to positive, with the HA antigen.

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Table 1. HA titers of various HA antigens treated with or without Tween 80 and ether (TE)

HA antigen	HA titer
TE-cell ^{a)}	64
Non-TE-cell ^{b)}	16
TE-fluid ^{c)}	64
Non-TE-fluid ^{d)}	16
Control ^{e)}	<2

- a) The TE-cell HA antigen was prepared from the infected RK₁₃ cells by treatment with TE.
 b) The non-TE-cell HA antigen was prepared from the infected RK₁₃ cells by treatment without TE.
 c) The TE-fluid HA antigen was prepared from the infected RK₁₃ cell culture fluids by treatment with TE.
 d) The non-TE-fluid HA antigen was prepared from the infected RK₁₃ cell culture fluids by treatment without TE.
 e) Control HA antigens were prepared from the mock-infected RK₁₃ cells or culture fluids by treatment with or without TE.

Table 2. HA titers with erythrocytes from various species

Erythrocytes	HA titer		
	4°C	Room	37°C
Mouse			
ddY	64	64	64
BALB/c	64	64	64
Horse	—	—	—
Cattle	—	—	—
Guinea pig	—	—	—
Mongolian gerbil	—	—	—
Goose	—	—	—
Rabbit	—	—	—
Chicken	<1 ~ 64	<1 ~ 64	<1 ~ 64

Therefore, a selection of chicken was required to obtain erythrocytes for HA.

HI antibody titers of serum samples from a horse experimentally infected with the Bucyrus strain of EAV were determined by incubation of serum-HA antigen mixture at 37°C for 1 hr or at 4°C for 24 or 48 hr. As shown in Table 3, the results indicated the presence of slow-reacting HI antibody in the serum sample. The greatest sensitivity of the HI test was obtained by incubating serum-HA antigen mixture at 4°C for 24 hr.

In the following experiments, HI tests were carried out by incubating serum-HA antigen mixture at 4°C for 24 hr, unless otherwise stated.

HI and NT tests were carried out on serum samples from two horses experimentally infected with the Bucyrus strain of EAV. As shown in Table 4, these animals were negative (HI antibody titer: <5; NT antibody titer: <5) for antibody by either HI or NT tests before infection. The HI antibodies in both animals were first detected 2 weeks after infection, increased and reached a peak titer of 160 at 2 or 3 weeks after infection. Similar titer rises were observed for antibody by the NT test. The antibodies obtained by the NT test,

Table 3. Effect of incubation time on HI antibody titers of sera from a horse experimentally infected with EAV

Serum	HI antibody titer		
	37°C 1 hr ^{a)}	4°C 24 hr	4°C 48 hr
pre ^{b)}	<5	<5	<5
post ^{c)}	5	160	80

- a) HI antibody titers were determined by incubation of serum-HA antigen mixtures at 37°C for 1 hr or at 4°C for 24 hr or 48 hr.
 b) Before inoculation.
 c) Three weeks after inoculation.

Table 4. Antibody response of the experimentally infected horses measured by the HI and NT tests

Horse	Test	Weeks after inoculation				
		0	1	2	3	1M ^{a)}
1	HI	<5 ^{b)}	<5	160	160	160
	NT	<5	20	640	640	640
2	HI	<5	<5	80	160	160
	NT	<5	10	320	640	640

- a) One month after inoculation.
 b) Antibody titer.

however, were detected one week earlier and the titers were slightly higher than those obtained by the HI test at all stages tested after infection.

In the present study, we demonstrated the HA activity of EAV by treating viral samples with TE as well as that of PRRSV [5]. On the contrary, Doll *et al.* [3] reported that EAV did not agglutinate erythrocytes from horse, cow, chicken, sheep, guinea pig, hamster, rabbit and mouse when they used the saline extracts of fetus tissues infected with the Bucyrus strain of EAV as HA antigen. Their negative results might be attributable to use the crude viral samples as HA antigen.

All our findings, together with the fact that the virion component or structure of EAV is basically similar to that of PRRSV [6], suggest that the N polypeptide of EAV is responsible for the HA activity.

On the basis of the results presented here, HA and HI tests with EAV could be used for the application in the studies of the virus and its infection. Knowledge of the basic properties of EAV hemagglutinin is still far from complete and further detailed investigations are required.

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