JOURNAL OF CLINICAL MICROBIOLOGY, May 1978, p. 495-496
0095-1137/78/0007-0495$02.00/0

Vol. 7, No. 5
Printed in U.S.A.

Measurement of Neutralizing Antibody to Equid Herpesvirus 1 by Single Radial Hemolysis

B. KLINGEBORN[1]* AND Z. DINTER[2]

Department of Virology, College of Veterinary Medicine,[2] and National Veterinary Institute,[1] Biomedical Center, S-751 23 Uppsala, Sweden

Received for publication 9 January 1978

Antibody to equid herpesvirus 1, which mediates single radial hemolysis, is that responsible for neutralization. Hemagglutination inhibition antibody is not necessarily involved in neutralization or hemolysis.

Equid herpesvirus 1 (EHV-1) is the agent of equine rhinopneumonitis, abortion in mares, and equine paresis. Various serological tests are used for diagnosis of infection with EHV-1. The most common test is serum neutralization (SN), but this is laborious and time consuming. The SN test could not be replaced by hemagglutination inhibition (HI), since the HI test was found to be insensitive.

EHV-1 agglutinates horse erythrocytes (4). The hemagglutinin, probably a glycoprotein of the viral envelope, is poorly represented, since about 0.65 μg of the viral protein is needed to give 1 hemagglutinating unit (3). The poor immunogenicity of EHV-1 with respect to hemagglutinin is shown by the low HI titers in sera from immunized horses or rabbits (3, 4).

We then tried to replace the SN test by single radial hemolysis. Since EHV-1 is a hemagglutinating virus, two different techniques of attachment of the virus to erythrocytes, by hemagglutinin and by chromic cation, were compared. The results in Table 1 show that, independent of the technique applied, the antibody mediating single radial hemolysis is that responsible for neutralization. The experimental data (Table 1) are as follows.

The EHV-1 strain was the high-passage RAC-H strain (5) propagated in monolayers of a continuous line of pig kidney cells, PK-15. The virus was concentrated as described elsewhere (3). Serum B.R. was from a horse with paresis due to EHV-1. In this serum, antibodies to EHV-1 were detected by SN and HI but not by complement fixation. These tests were performed as described elsewhere (1, 3). To eliminate the HI antibody, serum B.R. was absorbed with EHV-1 in the following way. Equal volumes of serum and a virus concentrate (512 hemagglutinating units/0.05 ml) were mixed, and the mixture was incubated for 10 min at 37°C and then immediately chilled on ice. Virus and virus-antibody complexes were removed by centrifugation for 30 min at 100,000 × *g*. The supernatant fluid was the "absorbed serum" in Table 1; its HI antibody was eliminated, and the SN titer was reduced 16-fold. A horse serum without measurable antibodies to EHV-1 was used as a control.

The single radial hemolysis tests were carried out according to Hierholzer and Tannock (2) for non-hemagglutinating viruses or Russell et al. (6) for influenza, but with some minor modifications. Horse erythrocytes were used instead of sheep erythrocytes. Guinea pig serum (complement) was not incorporated into the gel but was poured, after incubation of the plates at 4°C, undiluted onto the gel. The volume of guinea pig serum was 1.0 ml/plate (Falcon, 10‡2 Integrid petri dish). The plates were then incubated further at 37°C. When the test was performed according to Russell et al. (6), the blood-KIO_4 mixture was washed twice to eliminate KIO_4, which in the concentration used inactivates the EHV-1 hemagglutinin (3).

Several immune horse sera, devoid of complement-fixing antibody, have been tested in HI, SN, and single radial hemolysis tests in parallel. The titers obtained in the two latter tests were equal and much higher than the HI titers. Also, the results in Table 1 show that the HI antibody is not necessarily involved in neutralization or

TABLE 1. *Comparison of HI, SN, and single radial hemolysis (SRH) as tests for detection of antibodies to EHV-1 in horse sera*

Horse serum	Test and antibody titer[a]			
	HI	SN	SRH	
			I[b]	II[c]
B.R., unabsorbed	64	2,048	2,048	2,048
B.R., absorbed with EHV-1	<2	256	256	256
Control	<2	<2	<1	<1

[a] Reciprocal of serum dilution.
[b] Technique of Hierholzer and Tannock (2).
[c] Technique of Russell et al. (6).

hemolysis; its function in immunity to EHV-1 is unknown.

A grant from the Albert Hjarre Fond is gratefully acknowledged.

LITERATURE CITED

1. **Dinter, Z., and B. Klingeborn.** 1976. Serological study of an outbreak of paresis due to equid herpesvirus 1 (EHV-1). Vet. Rec. **99**:10-12.
2. **Hierholzer, J. C., and G. A. Tannock.** 1977. Quantitation of antibody to non-hemagglutinating viruses by single radial hemolysis: serological test for human coronaviruses. J. Clin. Microbiol. **5**:613-620.
3. **Klingeborn, B., and Z. Dinter.** 1973. Equine abortion (herpes) virus: properties of the hemagglutinin in virus suspensions. Virology **56**:164-171.
4. **McCollum, W. H., E. R. Doll, and J. T. Bryans.** 1956. Agglutination of horse erythrocytes by tissue extracts from hamsters infected with equine abortion virus. Am. J. Vet. Res. **17**:267-270.
5. **Mayr, A., J. Pette, K. Petzoldt, and K. Wagener.** 1968. Untersuchungen zur Entwicklung eines Lebendimpfstoffes gegen die Rhinopneumonie (Stutenabort) der Pferde. Zentralbl. Veterinaermed. Reihe B **15**: 406-418.
6. **Russell, S. M., D. McCahon, and A. S. Beare.** 1975. A single radial hemolysis technique for the measurement of influenza antibody. J. Gen. Virol. **27**:1-10.