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Hemagglutination Inhibition with Arboviruses: Relationship Between Titers and Source of Erythrocytes

NORMA E. METTLER, DELPHINE H. CLARKE, AND JORDI CASALS

Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510

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Antigens for Grand Arbaud, Hazara, and California arboviruses were able to agglutinate goose and either dog, hamster and guinea pig, or hamster red blood cells (RBC) to the same titer at the same *p*H; in hemagglutination-inhibition (HI) tests, titers for homologous and related sera were the same with these different types of RBC or occasionally one dilution higher with the mammalian cells. Antigens for St. Louis encephalitis and Eastern equine encephalitis viruses required use of lower antigen dilutions with human, guinea pig, and hamster RBC than with goose RBC. The results of comparative HI testing with these latter antigens and types of RBC indicate that HI titer is not directly related to the antigen dilution used with different types of RBC.

In a consideration of factors involved in the sensitivity of the hemagglutination-inhibition (HI) test, the effect of source of erythrocytes on the phenomenon of HI was investigated. Of the arbovirus antigens selected for the study, three (Grand Arbaud, Hazara, and California) were able to agglutinate red blood cells (RBC) of goose and certain other animal species to the same titer and at the same *p*H; the remaining antigens [St. Louis encephalitis (SLE) and Eastern equine encephalitis (EEE)], although likewise able to agglutinate RBC of animal species other than goose, gave significantly lower titers with these than with goose cells. On this basis, it was possible to carry out comparative HI tests in which the only variables were (i) type of RBC or (ii) type of RBC and antigen dilution.

MATERIALS AND METHODS

Virus antigens. These antigens were prepared by the sucrose-acetone method from brains of infected suckling mice and were held at 4 C in the lyophilized state. The following strains of the five viruses were used: Grand Arbaud, strain Argas 27, 6th mouse passage; Hazara, strain JC 280, 8th mouse passage; California, strain BFS 283, 15th mouse passage; SLE, Bellis strain, 5th mouse passage; and EEE, strains M and L, each initially isolated in chick embryo in Argentina (3) and then passaged three times in mice.

Erythrocytes. Goose cells were obtained from the flock maintained by Yale University and were prepared and standardized as described elsewhere (2). Hamster, guinea pig, and human RBC were obtained in our laboratories and were processed in the same manner as goose RBC, except that they were standardized volumetrically as 10% stock suspensions; for use in hemagglutination (HA)-HI tests, they were diluted to 0.5% in appropriate adjusting diluents. Dog RBC were obtained commercially in Alsever's solution and were washed and standardized volumetrically as above.

Immune fluids. The immune sera used were available in these laboratories. Immune ascitic fluids were produced in mice by use of the sarcoma 180/TG strain (5). Both sera and ascitic fluids were extracted with acetone and adsorbed with the type of RBC to be tested.

HA-HI tests. The procedure followed that described by Clarke and Casals (2), except that the tests were carried out in Lucite microplates, with diluent, sera, and antigens measured in drops (delivered by a calibrated dropper) and dilutions made with loops (6).

The optimal *p*H for each type of RBC was that at which the lowest concentration of antigen produced complete HA. This was determined by making titrations at *p*H values of 5.8 to 7.2 at 0.2 intervals and then testing in the indicated *p*H range at 0.1 intervals.

For HI tests, the plates with antibody-antigen mixtures were held overnight at 4 C. The following day RBC were added in the indicated adjusting diluents and allowed to settle at room temperature (37 C with Grand Arbaud antigen). Results were recorded 1 hr after the cells were added.

RESULTS

Antigens for Grand Arbaud, Hazara, and California viruses shared the property of agglutinating RBC of goose and either dog, hamster

TABLE 1. *HI tests with Grand Arbaud antigen, comparing goose and dog RBC*

Mouse serum	Antigen 1:8			
	*p*H 5.8 (4 units)		*p*H 6.0 (2 units)	
	Goose RBC	Dog RBC	Goose RBC	Dog RBC
Grand Arbaud	80[a]	160	160	320
Uukuniemi (S 23)	20	40	40	40
Uukuniemi (PO 63)	40	40	80	80
Pak Argas 461	20	20	40	40
Control, Silverwater	0	0	0	0

[a] Reciprocal of serum titer; 0 = <1:20, lowest dilution used.

TABLE 2. *HI tests with Hazara antigen, comparing goose, hamster, and guinea pig RBC*

Mouse serum or ascitic fluid[a]	Antigen 1:4, *p*H 6.5 (4 units)		
	Goose RBC	Hamster RBC	Guinea pig RBC
Hazara, serum	160[b]	320	320
Congo, serum	40	40	40
CHF, serum	20	40	40
Controls			
Group A, ascitic fluid	0	0	0
Group B, ascitic fluid	0	0	0
EHD-New Jersey, serum	0	0	0

[a] CHF, Crimean hemorrhagic fever; EHD, epizootic hemorrhagic disease of deer.

[b] Reciprocal of immune fluid titer; 0 = <1:20, lowest dilution used.

TABLE 3. *HI tests with California antigen, comparing goose and hamster RBC*

Guinea pig serum or mouse ascitic fluid	Antigen 1:4, *p*H 6.3 (4 units)	
	Goose RBC	Hamster RBC
California, guinea pig serum	20[a]	20
Keystone, guinea pig serum	80	80
Jamestown Canyon, mouse ascitic fluid	20	20
San Angelo, mouse ascitic fluid	20	20
Snowshoe Hare, mouse ascitic fluid	20	40
Tahyna, mouse ascitic fluid	40	80
Controls		
Group A, mouse ascitic fluid	0	0
Group B, mouse ascitic fluid	0	0

[a] Reciprocal of immune fluid titer; 0 = <1:20, lowest dilution used.

TABLE 4. *HI tests with SLE (Bellis) antigen, comparing goose, human, and guinea pig RBC*

Serum	Antigen, *p*H 6.4 (8 units)			Antigen, *p*H 6.6 (8 units)		
	1:8,000	1:250	1:1,000	1:8,000	1:250	1:1,000
	Goose RBC	Human RBC	Guinea pig RBC	Goose RBC	Human RBC	Guinea pig RBC
SLE						
Mouse 1	640[a]	160	320	640	160	320
Mouse 2	80	40	40	80	40	80
Mouse 3	1,280	640	640	1,280	320	640
SLE						
Rabbit 1	1,280	160	640	2,560	320	640
Rabbit 2	80	40	80	80	20	80
Rabbit 3	80	80	80	160	80	80
SLE						
Guinea pig 1	160	80	160	160	80	160
Guinea pig 2	160	80	160	160	80	160
Guinea pig 3	80	40	80	160	40	80
SLE						
Human 1	320	160	160	640	160	320
Human 2	160	40	160	320	80	320
Human 3	80	40	80	80	40	80
Human 4	80	40	80	160	40	160
Human 5	80	20	80	160	20	80
Control						
Tahyna, mouse	0	0	0	0	0	0

[a] Reciprocal of serum titer; 0 = <1:20, lowest dilution used.

TABLE 5. *HI tests with EEE (strains M and L) antigens, comparing goose and hamster RBC*

Serum	Strain M antigen, *p*H 6.3 (8 units)		Strain L antigen, *p*H 6.3 (8 units)	
	1:1,280	1:320	1:640	1:160
	Goose RBC	Hamster RBC	Goose RBC	Hamster RBC
EEE (vaccine)				
Human 1	40[a]	20	20	20
Human 2	40	20	20	20
EEE (prototype)				
Rabbit 1	80	40	40	40
Rabbit 2	80	80	80	40
EEE (strain F), mouse	160	80	40	40
EEE (strain M), mouse	160	80	40	40
Group A, mouse	80	40	40	40
Control				
Tacaribe, mouse	0	0	0	0

[a] Reciprocal of serum titer; 0 = < 1:20, lowest dilution used.

and guinea pig, or hamster to the same HA titer at the same *p*H. The results of HI tests with these antigens are given in Tables 1 to 3. Each antigen was used in the same dilution with the different types of RBC tested, and the specificity of the reactions is shown with homologous, related, and unrelated (control) immune fluids.

SLE antigen, in contrast, gave consistently higher titers with goose RBC than with human and guinea pig RBC and, accordingly, for HI tests (Table 4) had to be used in more concentrated form with the mammalian cells. As between goose and human RBC, the HI titers for the immune sera showed a 2- to 8-fold difference, whereas a 32-fold difference existed in the antigen dilutions required for these two types of RBC. As between goose and guinea pig RBC, the HI titers mostly agreed or showed only a twofold difference, whereas the divergence in the respective antigen dilutions was eightfold.

The results with antigens for strains M and L of EEE virus are given in Table 5. With strain L antigen, there was agreement in HI titers although four times as much antigen was used with hamster as with goose RBC. In the case of strain M antigen, despite the use of four times as much antigen with hamster RBC, HI titers with goose RBC were generally twofold higher.

DISCUSSION

Our experience in the course of this study confirms Porterfield's (4) finding that viruses able to agglutinate goose RBC may give equally high, but not higher, HA titers with other types of RBC. The observed tendency for HA titers to be generally lower with the mammalian RBC likewise supports earlier studies (1), in which, however, nothing was said about HI reactivity. It has been suggested (1) that RBC from goose are more sensitive to viral HA than those from mammalian species owing to the fact that, the goose cells being larger, fewer will pack in a given volume. That RBC size per se is not responsible for increased sensitivity is shown by the observation that red cells from turtle (300 nm^3) and frog (670 nm^3) were definitely inferior to goose RBC (160 nm^3).

The present evidence indicates that HI titer is not necessarily directly related to the dilution of antigen used with different types of RBC (Tables 4, 5), but the reasons for this are not yet known.

The fact that HI titers were slightly higher with mammalian RBC than with goose RBC when both types of cells were equally agglutinated (Tables 1 to 3) opens up the possibility of developing more sensitive HI systems for Grand Arbaud, Hazara, and California antigens.

In tests with the EEE antigens with goose RBC, the titers of the two immune mouse sera, both highly specific, were 1:160 with strain M antigen but only 1:40 with strain L antigen (Table 5); these results indicate not only that strain M is the more sensitive antigen in HI tests but also that goose RBC may give a somewhat more sensitive HI test than hamster RBC for the study of antigenic differences among strains of EEE virus.

As Hazara antigen was inhibited not only by its homologous serum but also by sera for Congo and Crimean hemorrhagic fever viruses (Table 2), it may be useful in serological surveys until such time as a Congo hemagglutinin becomes available.

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LITERATURE CITED

1. Banerjee, K. 1965. Comparative agglutinability of erythrocytes of different species of animals with arboviruses. Indian J. Med. Res. 53:199–203.
2. Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. 7:561–573.
3. Mettler, N. E. 1962. Identificacion de cepas de encefalitis equinas aisladas en la Republica Argentina. Rev. Soc. Argent. Biol. 48:55–62.
4. Porterfield, J. S. 1957. Use of goose cells in haemagglutination test with arthropod-borne viruses. Nature (London) 180:1201–1202.
5. Sartorelli, A. C., D. S. Fischer, and W. G. Downs. 1966. Use of sarcoma 180/TG to prepare hyperimmune ascitic fluid in the mouse. J. Immunol. 96:676–682.
6. Sever, J. L. 1962. Application of a microtechnique to viral serological investigations. J. Immunol. 88:320–329.