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

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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 pH; 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 pH; 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 passed 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 pH for each type of RBC was that at which the lowest concentration of antigen produced complete HA. This was determined by making titrations at pH values of 5.8 to 7.2 at 0.2 intervals and then testing in the indicated pH 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 Arbauad antigen, comparing goose and dog RBC

| Antigen 1:8 | pH 5.8 (4 units) | pH 6.0 (2 units) |
|-------------|------------------|------------------|
|              | Goose RBC | Dog RBC | Goose RBC | Dog RBC |
| Grand Arbaud  | 80<sup>a</sup> | 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 |

<sup>a</sup> Reciprocal of serum titer; 0 = <1:20, lowest dilution used.

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

| Antigen 1:4, pH 6.5 (4 units) | Goose RBC | Hamster RBC | Guinea pig RBC |
|-----------------------------|-----------|-------------|---------------|
| Hazara, serum               | 160<sup>a</sup> | 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 |

<sup>a</sup> CHF, Crimean hemorrhagic fever; EHD, epizootic hemorrhagic disease of deer.

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

| Antigen 1:4, pH 6.3 (4 units) | Goose RBC | Hamster RBC |
|-----------------------------|-----------|-------------|
| California, guinea pig serum | 20<sup>a</sup> | 20 |
| Keystone, guinea pig serum   | 80 | 80 |
| Jamestown Canyon, mouse      | 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 | 0 |
|                            | Group B, mouse ascitic fluid | 0 | 0 | 0 |

<sup>a</sup> 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

| Antigen, pH 6.4 (8 units) | Goose RBC | Human RBC | Guinea pig RBC |
|---------------------------|-----------|-----------|---------------|
| Mouse 1                   | 640<sup>a</sup> | 160 | 320 |
| Mouse 2                   | 80 | 40 | 40 |
| Mouse 3                   | 1,280 | 640 | 640 |

| Antigen, pH 6.6 (8 units) | Goose RBC | Human RBC | Guinea pig RBC |
|---------------------------|-----------|-----------|---------------|
| SLE                       | 1,280 | 160 | 320 |
| Rabbit 1                  | 80 | 40 | 40 |
| Rabbit 2                  | 80 | 80 | 80 |
| Rabbit 3                  | 80 | 160 | 80 |

| Antigen, pH 6.4 (8 units) | Goose RBC | Human RBC | Guinea pig RBC |
|---------------------------|-----------|-----------|---------------|
| Guinea pig 1              | 160 | 60 | 160 |
| Guinea pig 2              | 160 | 60 | 160 |
| Guinea pig 3              | 160 | 60 | 160 |

| Antigen, pH 6.6 (8 units) | Goose RBC | Human RBC | Guinea pig RBC |
|---------------------------|-----------|-----------|---------------|
| SLE                       | 320 | 160 | 80 |
| Human 1                   | 160 | 60 | 160 |
| Human 2                   | 160 | 60 | 160 |
| Human 3                   | 160 | 60 | 160 |
| Human 4                   | 160 | 60 | 160 |
| Human 5                   | 160 | 60 | 160 |

| Antigen, pH 6.4 (8 units) | Goose RBC | Human RBC | Guinea pig RBC |
|---------------------------|-----------|-----------|---------------|
| Control Tahyna, mouse     | 0 | 0 | 0 |

<sup>a</sup> 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

| Antigen, pH 6.3 (8 units) | Goose RBC | Hamster RBC | Goose RBC | Hamster RBC |
|---------------------------|-----------|-------------|-----------|-------------|
| EEE (vaccine)             | 40<sup>a</sup> | 20 | 20 | 20 |
| Human 1                   | 40 | 20 | 20 | 20 |
| EEE (prototype)           | 80 | 40 | 40 | 40 |
| Rabbit 2                  | 80 | 80 | 40 | 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 T(acaribe, mouse  | 0 | 0 | 0 | 0 |

<sup>a</sup> Reciprocal of serum titer; 0 = < 1:20, lowest dilution used.
and guinea pig, or hamster to the same HA titer at the same pH. 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 the 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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