Studies on Hemagglutination with Herpes Simplex Virus: I. Adsorption Studies As Evidence That the Antigen-Antibody Reaction Is Specific and the Neutralizing and Hemagglutinating Antibodies Are Identical

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STUDIES ON HEMAGGLUTINATION WITH HERPES SIMPLEX VIRUS

I. ADSORPTION STUDIES AS EVIDENCE THAT THE ANTIGEN-ANTIBODY REACTION IS SPECIFIC AND THE NEUTRALIZING AND HEMAGGLUTINATING ANTIBODIES ARE IDENTICAL

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It has been shown by Scott et al. (1) in a previous paper that tannic acid treated sheep erythrocytes sensitized with herpes simplex virus will agglutinate when exposed to specific immune serum. Later, Friedman and Bennett (2) demonstrated hemagglutination using a similar technique with adenoviruses. The techniques used were modified from the methods of Boyden (3) and Stavitsky (4).

Whether this hemagglutination is a true antigen-antibody reaction and if so, whether the antibody is identical with the virus neutralizing antibody is the subject of investigation in this paper.

MATERIAL AND METHODS

**Viral antigen.** The HF strain of herpes simplex virus, obtained from the Communicable Disease Center, Montgomery, Alabama, was used as seed inoculum. It has been propagated 60 times on the chorioallantoic membranes (CAM), 9 times in the yolk sacs (YS) of developing chick embryos, 3 times in the brains of white Swiss mice and twice on the corneas of rabbits. Seed virus was inoculated into the YS of the chick embryo and the infected allantoic fluid was harvested upon death of the embryo. The allantoic fluid virus was partially purified and concentrated by differential centrifugation at 450 × G for 15 min followed by 24,500 × G for 2 hr. This cycle was repeated 4 times. After each high speed centrifugation the sediment was resuspended in pH 7.2 buffered saline, and after the last centrifugation the virus was resuspended in buffered saline to make a 10-fold concentration.

**Antisera.** Preimmunization sera were obtained from rabbits for use as controls. Herpes simplex virus antisera were produced in rabbits in response to a series of 3 injections each week over a period of 4 weeks. Mouse brain adapted herpes simplex virus which had been inactivated with formalin was used as the inoculum during the first 2 weeks of the immunization period and active mouse brain virus was used during the last 2 weeks. Postimmunization sera were obtained 1 week after cessation of inoculations. All sera were inactivated by heating at 56°C for 30 min and were adsorbed with tannic acid treated sheep erythrocytes to remove nonspecific agglutinins.

**Preparation of tannic acid treated and virus sensitized cells.** Whole sheep blood was collected at a local abattoir and stored in equal volumes of sterile Alsever's solution. The erythrocytes were washed 3 times in buffered saline and packed by centrifuging at 450 × G for 15 min. A 2.5% suspension of these cells was treated with a 1:20,000 dilution of Mallinckrodt reagent grade tannic acid in physiologic saline. After this, the virus was adsorbed onto these cells.

**Adsorption of immune serum with virus preparation.** Tannic acid treated and virus sensitized sheep erythrocytes were mixed in equal amounts with herpes simplex immune serum and allowed to incubate overnight at 25°C. The serum was removed, mixed with fresh tannic acid treated and virus sensitized sheep erythrocytes, and again allowed to incubate overnight at 25°C. The virus that was adsorbed onto the tannic acid treated erythrocytes had been inactivated prior to use. The serum was removed from the cells by low speed centrifugation. It was then centrifuged at 24,500 × G for 2 hr and then sterilized by passing through a Swinny filter. This serum was used in the experiments as tannic acid virus cell adsorbed serum (TVCS).

**Preparation of control serum.** The above procedure was repeated with another aliquot of the

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same herpes simplex immune serum except that the tannic acid treated sheep erythrocytes were not sensitized with the virus. This serum was used in the experiments as tannic acid cell adsorbed serum (TCS).

**Immunological tests.** The herpes simplex virus hemagglutination procedure previously described (1) was performed using the 2 sera, TVCS and TCS, in 2-fold dilutions in serum diluent composed of 1:100 dilution of preimmunization rabbit serum and buffered saline.

Ten fold dilutions of $10^{-3}$ through $10^{-7}$ of the HF strain of herpes simplex virus were made in serum diluent. Each dilution of this virus was mixed with an equal volume of each of the undiluted sera (TVCS and TCS). The virus-serum mixtures were incubated in an icewater bath at $8^\circ$C for 1 hr. After incubation, 0.05 ml of each mixture was inoculated onto the CAM of 12-day-old developing chick embryos by the method described by Beveridge and Burnet (5). These were incubated for 72 hr, the CAM were harvested and infectivity of the membranes was determined.

The controls included: a) saline plus TVCS, b) saline plus TCS, and c) each virus dilution plus saline.

**RESULTS**

Typical negative hemagglutination reactions were obtained with titrations of TVCS. All demonstrable specific antibodies which react in the hemagglutination test were removed upon adsorption with tannic acid treated erythrocytes which had herpes simplex virus adsorbed on them. Positive hemagglutination reactions were obtained with titrations of TCS demonstrating that the specific antibodies had not been removed.

Typical results of repeated virus titrations with saline, TVCS and TCS, are shown in Table 1. The presence of herpetic lesions on the membrane is recorded as positive and the absence as negative. In the table, the numerator is the number of positive membranes and the denominator is the total number of embryos used for that particular mixture of virus and serum. It is demonstrated that the specific neutralizing antibody was removed by adsorption with treated erythrocytes which had virus adsorbed on them.

**DISCUSSION**

The negative results obtained in the hemagglutination test with immune serum which had been adsorbed with viral antigen demonstrates that specific antibodies were removed from that serum. The effect of adsorption of the sera on the infectivity of the virus also demonstrates the removal of specific neutralizing antibodies from the immune sera. The virus adsorbed serum (TVCS) did not neutralize the various dilutions of virus. The 100% infectivity of the CAM inoculated with the virus and serum (TVCS) mixture containing virus diluted through $10^{-4}$ showed that no demonstrable neutralizing antibodies were present.

As a comparison, the immune serum adsorbed with tannic acid treated cells without virus (TCS) neutralized the dilutions of virus. The results of these adsorption studies on the hemagglutination reaction and virus infectivity add evidence to support the fact that a specific antigen-antibody reaction is involved in the hemagglutination test with herpes simplex virus.

Since the antibodies which react in the hemagglutination and neutralization reactions are both removed by the same adsorption procedure, it appears that the neutralizing and hemagglutinating antibodies in the immune serum may be identical.

**SUMMARY**

The antibody in herpes simplex immune serum can be removed by adsorption with tannic acid treated and virus sensitized sheep erythrocytes.

The removal of this antibody as demonstrated by both hemagglutination and infectivity.
techniques indicate that the herpes simplex neutralizing and hemagglutinating antibodies are probably identical and that a specific antigen-antibody reaction is involved in the hemagglutination test with herpes simplex virus.

REFERENCES