NEUTRALIZATION OF AN INFECTIOUS HERPES SIMPLEX VIRUS-ANTIBODY COMPLEX BY ANTI-γ-GLOBULIN

By Warren K. Ashe and Abner Louis Notkins

LABORATORY OF MICROBIOLOGY, NATIONAL INSTITUTE OF DENTAL RESEARCH, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

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Recently it was shown that incubation of lactic dehydrogenase virus (LDV) with anti-LDV resulted in the formation of an infectious virus-antibody complex.[1] The presence of such a complex was demonstrated by the fact that the virus fraction which persisted after *in vivo* or *in vitro* exposure to mouse anti-LDV was readily neutralized by the serum of goats which had been immunized with normal mouse γ-globulin (goat antimouse γ-globulin), whereas virus that had not been previously exposed to mouse anti-LDV was completely resistant to neutralization by goat antimouse γ-globulin.[1] The present study was undertaken to see whether these observations could be extended to another virus-antibody system and in particular to one which was more amenable to quantitation than the LDV-animal infectivity assay. Herpes simplex virus (HSV) was chosen for study and the tissue culture plaque-counting technique was employed for infectivity titrations.

Materials and Methods.—Virus: HSV, strain 11123, was isolated from a patient with recurrent *herpes labialis*.[2] The virus has been passed 19 times in primary rabbit kidney cells and has an infectivity titer of $10^{6.7}$ to $10^{6.9}$ plaque-forming units (PFU) per ml.

Cell culture: Primary rabbit kidney cell cultures were prepared from trypsinized kidneys of 8- to 10-week-old New Zealand white (NIH strain) rabbits. The growth media consisted of lactalbumin hydrolysate medium supplemented with 8% inactivated calf serum and containing penicillin, streptomycin, tetracycline, and nystatin. Details concerning the preparation and maintenance of the monolayers will be found in an earlier report.[2]

Virus assay: Virus was diluted in phosphate-buffered saline (PBS) containing 5% inactivated horse serum and aliquots of the appropriate dilutions were inoculated in duplicate onto monolayers of rabbit kidney cells. After a 2.5-hr adsorption period the cultures were overlaid with a nutrient agar medium supplemented with 4% inactivated calf serum and antibiotics.[2] The plates were incubated in a humidified atmosphere of 5% CO_2 and air. Plaques were counted on the fifth and sixth days following inoculation and the virus titer was expressed in terms of PFU/ml.

Antiserum: Antibody to HSV (strain 11123) was prepared in an earlier experiment[2] by injecting a rabbit intraperitoneally with approximately $10^{6.7}$ PFU. Ten such injections were given over a 2-week period. A booster was given 6 days after the last injection and serum was collected 7 and 18 days after the booster. Except where otherwise indicated, the 18-day serum was employed in the experiments described below. Control serum was obtained prior to immunization. All sera were stored at −55° until used.

Normal goat serum was obtained from Pentex Laboratories, Kankakee, Illinois. Goat antiserum to normal rabbit serum (goat antirabbit serum) or normal rabbit γ-globulin (goat antirabbit γ-globulin) were obtained from Hyland Laboratories, Los Angeles, California. Prior to use, all sera were heated at 56° for 30 min and appropriately diluted in PBS.

Results.—To see if exposure of HSV to anti-HSV resulted in the formation of an infectious virus-antibody complex (sensitized virus)[3] the following experiment was performed. Approximately $10^{6.0}$ PFU/ml of HSV were incubated with an equal volume of a 1:50 dilution of normal rabbit serum or rabbit anti-HSV at 37° and, at various times thereafter, the virus titer was determined and the surviving fraction calculated. At the end of 2 hr, as seen in Figure 1, anti-HSV had reduced the virus titer by 2.0 log units, whereas normal rabbit serum failed to produce any substantial

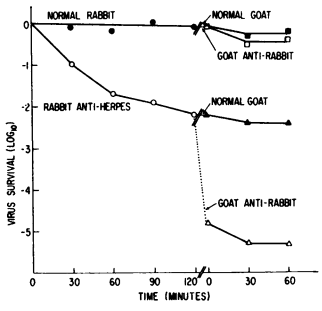

FIG. 1.—Approximately $10^{6.0}$ PFU/ml of HSV were incubated at 37° with a 1:50 dilution of normal rabbit serum or rabbit anti-HSV. At the end of 2 hr, aliquots from each reaction mixture were removed and mixed with a 1:25 dilution of normal goat serum or goat antirabbit serum. Within 15 sec after mixing (*dotted line*), samples were removed and the virus titer was determined. All reaction mixtures were then incubated for an additional hour at 37°.

change. The reaction mixtures were then placed in ice, and aliquots from each were mixed with an equal volume of a 1:25 dilution of normal goat serum or goat antirabbit serum. At intervals thereafter, samples were removed and the virus titers were determined. Figure 1 shows that within 15 sec after the addition of goat antirabbit serum to virus that had been preincubated with anti-HSV, the surviving fraction was reduced by an additional 2.6 log units. Continuation of the incubation at 37° for 60 min resulted in a further reduction of only 0.5 log units. Results very similar to that seen in Figure 1 were obtained with goat antirabbit γ-globulin.[4] In contrast, addition of goat antirabbit γ-globulin or goat antirabbit serum to virus that had been preincubated with normal rabbit serum resulted in little if any reduction in virus titer. Addition of normal goat serum to virus that had been preincubated with either normal rabbit serum or rabbit anti-HSV also failed to affect the virus titer. From these experiments we conclude that the residual infectious virus which persisted after exposure of HSV to rabbit anti-HSV existed in the form of a virus-antibody complex.[5]

To study the effect of antibody concentration on virus sensitization, an experiment similar in design to that in Figure 1 was performed, except different concentrations of rabbit anti-HSV ranging from 1:1600 to 1:25 were employed to sensitize the virus. Table 1 shows that as the concentration of neutralizing antibody was increased (column 1), the fraction of surviving virus decreased (column 2). Aliquots from incubation I then were removed and incubated with a 1:25 dilution of normal goat serum or goat antirabbit serum. Virus survival (column 3) was calculated by subtracting the log of the titer remaining after incubation with goat antirabbit serum from the log of the titer remaining after incubation with normal goat serum. The data show that at concentrations of anti-HSV (1:1600–1:400) which produced little if any viral neutralization ($\lesssim$ 0.5 log units), incubation with goat antirabbit serum reduced the surviving fraction by as much as 1.8 log units indicating that sensitization had occurred. At a higher concentration of anti-HSV (1:100) which resulted in considerable neutralization (2.0 log units), incubation with goat antirabbit serum reduced the surviving fraction by an additional 3.6 log units. However, as the concentration of anti-HSV was increased further (1:50–1:25), the extent of the neutralization produced by the goat antirabbit serum decreased. A possible explanation for this inhibition comes from experiments which showed that the addition of a 1:25 dilution of normal rabbit serum to sensitized virus, prior to incubation with goat antirabbit serum or goat antirabbit γ-globulin, substantially inhibited neutralization of the sensitized virus. A similar inhibition was noted following the addition

TABLE 1

EFFECT OF DIFFERENT CONCENTRATIONS OF ANTI-HSV ON VIRUS SENSITIZATION*

Dilution of rabbit anti-HSV	Incubation I Rabbit anti-HSV (virus survival; log 10)	Incubation II Goat antirabbit serum (virus survival; log 10)	Virus survival after incubations I and II (log 10)
1:1600	−0.1	−0.4	−0.5
1:800	−0.3	−1.0	−1.3
1:400	−0.5	−1.8	−2.3
1:200	−0.9	−3.0	−3.9
1:100	−2.0	−3.6	−5.6
1:50	−2.2	−2.9	−5.1
1:25	−3.0	−0.9	−3.9
NRS(1:25)	0.0	−0.1	−0.1

* The experimental design was similar to that in Fig. 1 except that different concentrations of rabbit anti-HSV were incubated with the virus (column 1). Normal rabbit serum (NRS) served as the control. The data in column 2 represent the surviving fraction at the end of 2 hr. Aliquots from each reaction mixture were then removed and incubated at 37° for 1 hr with a 1:25 dilution of goat antirabbit serum or normal goat serum. Virus survival (column 3) was calculated by subtracting the log of the titer remaining after incubation with goat antirabbit serum from the log of the titer remaining after incubation with normal goat serum. Column 4 represents the surviving fraction after incubations I and II.

of normal mouse serum to the LDV system.[6] These observations suggest that an excess of unbound γ-globulin in the reaction mixture can prevent the anti-γ-globulin from reacting with the sensitized virus. To study the effect of the concentration of goat antirabbit serum on the neutralization of sensitized virus the following experiment was performed. HSV was sensitized with anti-HSV as in Figure 1, except that antiserum from an earlier bleeding was employed (see *Materials and Methods*). Aliquots of the sensitized virus were then incubated with different dilutions of goat antirabbit serum, and virus survivals were determined. Table 2 shows that neutralization of the sensitized virus was substantially greater at the higher concentrations of goat antirabbit serum. Thus, the ability to neutralize sensitized virus depends not only on the degree of sensitization but on the concentration of free γ-globulin and anti-γ-globulin in the reaction mixture. In addition, it appears that the type of antiviral antibody (early or late) used to sensitize the virus and the type of anti-γ-globulin (viz., anti-γG vs. anti-γM) used to neutralize the sensitized particle also affect the extent of neutralization.[7]

TABLE 2

EFFECT OF DIFFERENT CONCENTRATIONS OF GOAT ANTIRABBIT SERUM ON THE NEUTRALIZATION OF SENSITIZED VIRUS*

Dilution of goat antirabbit serum	Virus survival (log 10)
1:100	−0.4
1:50	−0.9
1:25	−1.6
1:5	−3.2

* The experimental design was similar to that in Fig. 1. Approximately $10^{6.0}$ PFU/ml of HSV were incubated at 37° with a 1:50 dilution of rabbit anti-HSV. At the end of 2 hr, aliquots from the reaction mixture (sensitized virus) were removed and incubated at 37° with various concentrations of goat antirabbit serum. Sensitized virus incubated with normal goat serum served as the control. At the end of 1 hr the surviving fraction was calculated by subtracting the log of the titer remaining after incubation with goat antirabbit serum from the log of the titer remaining after incubation with normal goat serum.

Discussion.—The experiments reported herein showed that the infectious virus which persisted after exposure to anti-HSV existed in the form of a virus-antibody complex and that the complex could be neutralized by an antibody which was not directed against the virus particle itself. In addition, we have recently found that

incubation of HSV with papain-digested fragments of rabbit anti-HSV also resulted in the formation of an infectious complex which could be neutralized by an anti-γ-globulin.[7] In this connection, Goodman and Donch[8] recently showed that the neutralization of T1 bacteriophage by fragments and polypeptide chains of rabbit anti-T1 could be enhanced by the use of specific antisera against rabbit γ-globulin. The mechanism by which the sensitized virus is neutralized requires further study. The most likely explanation at the present is that the anti-γ-globulin acts by attaching to and/or forming a bridge between anti-HSV molecules which are bound to the surface of the sensitized particle so as to block "critical sites" (areas which must not be altered or blocked if the virus is to remain infective). However, the anti-γ-globulin might also act by "stabilizing" the attachment of the antiviral antibody to the virion. Aggregation of sensitized particles seems to be a less likely explanation, since relatively low concentrations of sensitized virus (less than 100 ID_{50}/ml) were readily neutralized by anti-γ-globulin.[1] The possibility that the interaction of anti-γ-globulin with the sensitized particle damages the virion with resulting loss of infectivity has not been excluded.

The demonstration that antiviral antibody can actually attach to the virion without loss of infectivity might provide an explanation for the "persistent" virus fraction noted in a number of *in vitro* virus-antibody systems[9-20] and for the persistence of infectious virus *in vivo* in the presence of circulating antibody.[21-24] That is, if neutralization depends on whether antiviral antibody can reach specific sites (critical vs. noncritical) on the virion and/or on the total amount of antibody which ultimately becomes attached to the virion, then the initial sensitization of the virus might sterically hinder the attachment of additional antibody. This would prevent or retard neutralization and result in the formation of a "persistent" fraction. Support for this contention comes from our studies with LDV[1, 6] and recent experiments with HSV[25] which showed that the rate of neutralization of sensitized HSV, as measured by neutralization kinetics,[2] was greatly reduced.

The findings with HSV and LDV suggest that the anti-γ-globulin technique may prove to be a very useful tool in studying the mechanism and kinetics of virus sensitization and that sensitization may play an important role in the susceptibility and resistance of a virus to neutralization.

Summary.—Incubation of herpes simplex virus (HSV) with anti-HSV resulted in the formation of an infectious HSV-anti-HSV complex. The presence of such a complex was demonstrated by the fact that the virus fraction which persisted after incubation with rabbit anti-HSV was rapidly neutralized by goat antirabbit serum or goat antirabbit γ-globulin, whereas virus that had not been previously exposed to anti-HSV was resistant to these goat sera. The titer of the infectious virus-antibody complex (sensitized virus) was reduced by over 99.9 per cent following incubation with the anti-γ-globulin. This technique offers a useful method for (1) detecting sensitized virus, (2) enhancing virus neutralization, (3) demonstrating otherwise undetectable or low levels of antiviral antibody, and (4) neutralizing the "persistent" virus fraction.

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[2] Ashe, W. K., and H. W. Scherp, *J. Immunol.*, **91**, 658 (1963); *ibid.*, **94**, 385 (1965).

[3] The term "virus sensitization" as used throughout this paper refers to the attachment of antiviral antibody to the virion without loss of infectivity.

[4] Similar results also were obtained when HSV (strain 11123) passed 6 times in HeLa cells was employed as the stock virus.

[5] Incubation of HSV with human anti-HSV also resulted in the formation of an infectious virus-antibody complex which was readily neutralized by rabbit antihuman γ-globulin.

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