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STUDIES ON THE MECHANISM OF ANTIBODY-MEDIATED ENHANCEMENT OF GETAH VIRUS INFECTIVITY

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SUMMARY Inoculation on BHK-21 cells with Getah virus sensitized with hyperimmune homologous mouse antiserum resulted in higher infective titers than those obtained with non-sensitized control virus. This phenomenon was not observed with Vero cells. Experiments were carried out on the mechanism of this enhancement with the following results. When BHK-21 cells were pretreated with a mixture of UV-irradiated virus and antiserum before inoculation, the enhancement of infectivity of Getah virus was markedly decreased. IgG antibody against Getah virus which had been digested with 1–2% of pepsin did not show any enhancing activity. Sensitized sheep erythrocytes adhered to monolayered cultures of BHK-21 cells. These results indicate that the appearance of enhancing activity of a complex of the virus and antibody is closely related with the existence of a receptor for the Fc part of IgG on ordinary tissue culture cells, BHK-21 cells.

INTRODUCTION

Enhanced viral infectivity as a result of treating virus with specific antiserum has been observed in some virus-antiserum-cell systems (Hawkes, 1964; Halstead and Orourke, 1977a; Clerx et al., 1978; Kimura and Ueba, 1978). This enhancing activity was shown to reside in the IgG fraction of the antiserum. Enhancement was seen only when the cells of the assay system were from the species of animal used to produce the antiserum (Hawkes, 1964; Hawkes and Lafferty, 1967). The mechanism of this infectivity-enhancement is unknown, but recently it was shown that pepsin-digested anti-Dengue virus IgG did not enhance the infectivity of Dengue 2 virus when a mixture of the virus and antiserum was assayed on peripheral blood leukocytes (Halstead and Orourke, 1977b; Halstead et al., 1978), and that, the blocking effect of pepsin-digested IgG on enhancement of virus infectivity was not observed when ordinary tissue culture cells were used.

Watkins (1964) found that herpes simplex virus-infected cells form rosettes with sheep erythrocytes sensitized with rabbit anti-sheep erythrocytes serum and that this phenomenon is due to the induction of a receptor for the Fc part of IgG. Recently, a receptor for the Fc portion of IgG was detected in normal human tissues by adherence using a " closed

chamber technique" (Braathen et al., 1979). We also tested for the existence of this receptor on normal BHK-21 cells.

This paper reports that the enhanced infectivity of a complex of Getah virus and a specific antibody in BHK-21 tissue culture cells is due to the activity of the Fc portion of IgG bound to the virus.

MATERIALS AND METHODS

1. *Viruses*

The source and preparation of Getah virus were described previously (Kimura and Ueba, 1978). The virus was further passaged two to three times in suckling mouse brain. Japanese encephalitis virus, JaGAr-01 strain, obtained from the National Institute of Health of Japan, was used at the 9th passage in suckling mouse brain. Sindbis virus from the National Institute of Health of Japan was used after three passages in suckling mouse brain. Pooled brains of suckling mice infected with these viruses were homogenized to give a 10% emulsion in Tris-buffered saline, pH 7.4, containing 0.28% bovine plasma albumin. The emulsion was centrifuged at 10,000 rpm for 60 min at 4 C and the supernatant was used for experiments.

2. *Antisera*

For preparation of hyperimmune sera, mice were immunized by five successive i.p. injections of partially purified virus at weekly intervals. Mice were bled 10 days after the last injection.

3. *Cell culture and plaque assay*

BHK-21 and Vero cells were used.
Virus was titrated by the plaque method in petri dishes, as described previously (Kimura and Ueba, 1978).

4. *Antibody measurement*

The hemagglutination-inhibition (HI) test was carried out by a modification of the method of Clarke and Casals (1958) in plastic trays. The complement fixation (CF) test was performed by a checker-board procedure with serial dilutions of serum and antigen. Details of the neutralization (NT) method were reported previously (Kimura and Ueba, 1978).

5. *Pepsin-digestion of IgG*

The IgG fraction was prepared from hyperimmune mouse serum precipitated with 33% saturated ammonium sulfate. The precipitate was dissolved in phosphate-buffered saline (PBS), pH 7.2, and dialyzed overnight against PBS at room temperature. IgG was digested with pepsin (Sigma Chemical Co.) by a modification of the method of Nisonoff et al. (1960).

6. *Pretreatment of BHK-21 cells with UV-virus-serum complex*

After UV-irradiation, the 20% emulsion (ca., 10^9 PFU/ml) of mouse brain infected with either Getah or JaGAr-01 virus was mixed with an equal volume of antiserum diluted 1: 40 or 1: 320. The mixture was incubated for 60 min at 37 C and inoculated onto BHK-21 cells (1 ml/petri dish). After further incubation for 1.5 h at 37 C, the cells were washed three times with Hanks' BSS and used for assay of the blocking effect on the enhancement of infectivity of a complex of Getah virus (20–30 PFU/0.4 ml) and antiserum (at 1: 320 dilution).

7. *Hemadsorption assay*

Sheep erythrocytes were washed four times with veronal-buffered saline (VBS), pH 7.2, and suspended at 1% in VBS. They were then sensitized with 4, 2, 1, 0.5 or 0.25 agglutinating concentration of rabbit antiserum against sheep erythrocytes for 30 min at room temperature. BHK-21 cells prepared in glass tissue culture bottles were washed with Hanks' solution and mixed with 1% sensitized sheep erythrocytes. After standing for 30 min at room temperature, they were washed gently four times with Hanks' solution and examined microscopically.

RESULTS

1. *Specificity of the enhancing activity*

The specificity of the enhancing effect of Getah virus infectivity was examined using various virus-antiserum combinations (Table 1, 2). Enhancement was observed when Getah virus was mixed with homologous or anti-Sagiyama (Getah group) mouse antiserum. No significant enhancement was demonstrated in heterologous systems; i.e., combinations of either Getah virus and normal

TABLE 1. *Dose-response relationship between the dilution of anti-Getah serum and the titer of homologous or heterologous viruses*

Serum dilution	Viruses		
	Getah virus	JaGAr-01 virus	Sindbis virus
1 : 40		1.0	0.9
1 : 160	37.7[a]	1.1	1.1
1 : 640	51.4	1.0	1.1
1 : 2560	5.4	1.0	1.1
1 : 10240	0.4		

[a] Ratio of number of plaques produced after mixing virus and antibody to the control plaque count.

mouse serum or anti-JaGAr-01 antiserum, or anti-Getah virus antiserum and JaGAr-01 or Sindbis virus. In this experiment, the virus infectivity in the homologous system was 51.4 or 62.9 times higher than that of the control virus on BHK-21 cells. Maximum enhancement of infectivity was obtained at a dilution of antiserum of 1/640 or 1/320.

2. *Host-specificity of the enhancement*

Figure 1 shows the results of comparative experiments with different host cell systems. In BHK-21 cells, enhancement of infectivity was detected with serum dilutions of 1/40 to 1/2560, and maximum enhancement (v/vo= 14.9) was attained at a dilution of 1/320. In contrast, when assayed in Vero cells with the same virus-serum complex no enhancement was observed at any serum dilution.

TABLE 2. *Dose-response relationship between dilution of various antisera and titer of Getah virus*

Antiserum	Serum dilution				
	1/20	1/80	1/320	1/1280	1/5120
Anti-JaGAr-01	1.6[a]	1.6	1.5	1.2	1.1
Normal mouse serum	0.9	1.0	1.1	1.0	0.9
Anti-Sagiyama	3.4		28.9		1.8
Anti-Getah (AMM-2021)		49.1	62.9		

[a] Ratio of number of plaques produced after mixing virus and antibody to the control plaque count.

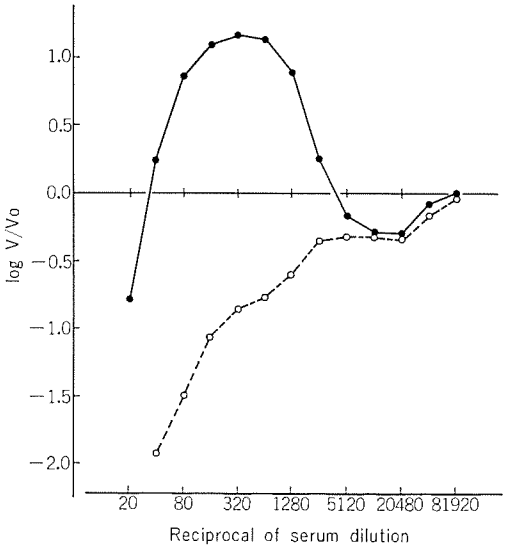

FIGURE 1. Neutralization curves of Getah virus by homologous mouse antiserum assayed on BHK-21 and Vero cells. Mixtures of Getah virus and various dilutions of mouse antiserum against Getah virus were assayed simultaneously on monolayers of either BHK-21 or Vero cells. V: number of plaques with a mixture of virus and antiserum, Vo: control number of plaques. symbols: ●, BHK-21 cells; ○, Vero cells.

3. *Blocking effect of prior treatment of BHK-21 cells with a complex of UV-irradiated virus and serum on the enhancement with the virus-antibody complex*

Next we tested whether pretreatment of

TABLE 3. *Effect of pretreatment of BHK-21 cells with a complex of serum and UV-irradiatea viruses on infectivity enhancement*

Pretreatment	V/Vo[a]	
	Expt. I	Expt. II
None	19.0	6.5
Anti-Getah (1 : 40)+Getah virus (UV-irradiated)	0.02	0.5
Anti-Getah (1 : 320)+Getah virus (UV-irradiated)	2.4	1.0
Anti-Getah (1 : 40)	18.3	7.6
None	5.7	12.7
Anti-JaGAr-01 (1 : 40)+JaGAr-01 virus (UV-irradiated)	1.2	2.2
Anti-JaGAr-01 (1 : 40)	5.0	12.8
Normal mouse serum (1 : 40)+JaGAr-01 virus (UV-irradiated)	5.3	8.2

[a] Ratio of number of plaques produced after mixing Getah virus and antiserum to the control plaque count.

BHK-21 cells with a UV-virus-serum complex could block the enhancing activity. As shown in Table 3, results on BHK-21 cells pretreated with either antiserum alone or a mixture of normal mouse serum and UV-inactivated virus, were similar to those on untreated BHK-21 cells. However, the infectivity of the sensitized virus was not enhanced in cells that had been pretreated with a complex of a suitable concentration of antiserum and UV-inactivated virus. In control BHK-21 cells pretreated with UV-irradiated virus or with a complex of serum and UV-irradiated virus, the plaque counts were similar to those in untreated BHK-21 cells. In this experiment, the infectivity of the sensitized virus was 6.5 to 19.5 times higher than that of the control virus in untreated BHK-21 cells, whereas it was 0.5 or 0.02 times higher with pretreated cells. These results suggest that the enhancement of infectivity was associated with the activity of the Fc portion of IgG to which the virus was bound.

4. *Effect of pepsin-digestion of IgG*

The enhancing activity of immune IgG against Getah virus was examined after digestion with pepsin. Figure 2 shows that the enhancement of infectivity was inversely proportional to the concentrations of pepsin: the

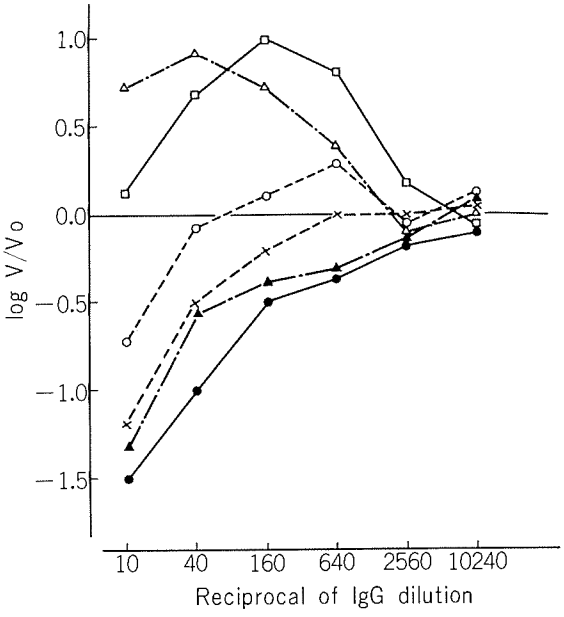

FIGURE 2. Effect of IgG digested with various concentrations of pepsin on infectivity-enhancing activity. Pepsin-digested IgG was mixed with 50–70 PFU of Getah virus and incubated for 1.5 h at 37 C. Then 0.4 ml aliquots of the mixtures were inoculated onto three or four monolayer cultures of BHK-21 cells. The ordinate indicates the titer of infectivity. Infectivity was determined as described in the legend to Fig. 1. symbols: Digestion with ●, 2% of pepsin; ▲, 1% pepsin; ×, 0.5% pepsin; ○, 0.25% pepsin; △, 0.125% pepsin; □, Undigested.

enhancement decreased with increase in the concentration of pepsin, and no enhancing activity was seen when 1–2% pepsin was used.

On the basis of these results showing that 1% of pepsin was suitable for digestion of IgG, the dose-responses of reaction mixtures with 1% pepsin in the presence and absence of fresh guinea pig serum were compared. As shown in Fig. 3, when the virus was sensitized with untreated IgG at a dilution of 1: 160, the infectivity was about ten times that of the control, although the sensitized virus was effectively neutralized by IgG in the presence of complement. In contrast, when pepsin-digested IgG was used the dose-response curves of the reaction mixture were similar irrespective of the presence of complement.

Next the antibody activity of IgG digested with 1% pepsin was examined. The results in Table 4 show that although the HI antibody titer of digested IgG decreased to only 1/4, the CF titer decreased to less than 1/8 of that of untreated IgG. It is noteworthy that the CF activity of pepsin-digested IgG decreased in parallel with loss of infectivity-enhancing activity.

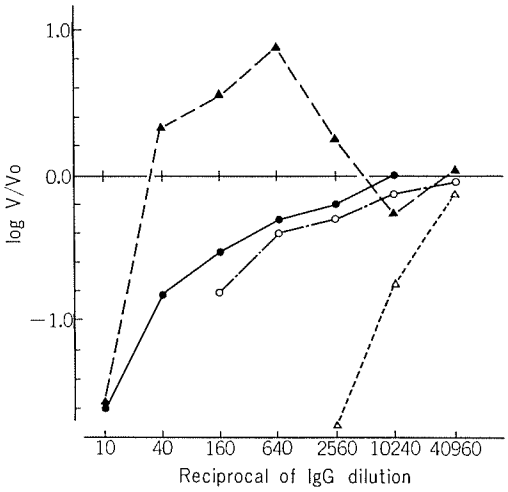

FIGURE 3. Dose-response curves of infectivity of Getah virus sensitized with various concentrations of IgG. Infectivity was determined as described in the legend to Fig. 1. symbols: ●, Pepsin-digested IgG plus virus; ○, Pepsin-digested IgG plus virus plus complement; ▲, IgG plus virus; △, IgG plus virus plus complement.

TABLE 4. *Effect of pepsin digestion of IgG on HI and CF titers and on enhancement of virus infectivity*

	Antibody titer		Enhancement of infection (v/vo)[a] IgG dilution			
	HI	CF	1/40	1/160	1/640	1/2560
Treated with pepsin (1%)	1 : 160	1 : 10	0.2	0.3	0.5	0.6
Untreated	1 : 640	1 : 80	2.2	3.6	7.6	1.8

[a] Ratio of number of plaques produced after mixing virus and antiserum to the control plaque count.

5. *Detection of receptor for the Fc part of IgG*

As shown in Fig. 4, antiserum-coated sheep erythrocytes adhered to normal BHK-21 cells. A preliminary experiment in which sheep erythrocytes were sensitized with 4, 2, 1, 0.5 or 0.25 agglutinating concentrations of rabbit antiserum against sheep erythrocytes showed that 0.5 agglutinating unit was the most suitable concentration of rabbit antiserum.

Neither untreated sheep erythrocytes nor those mixed with normal rabbit serum showed hemadsorption when incubated with BHK-21 cells.

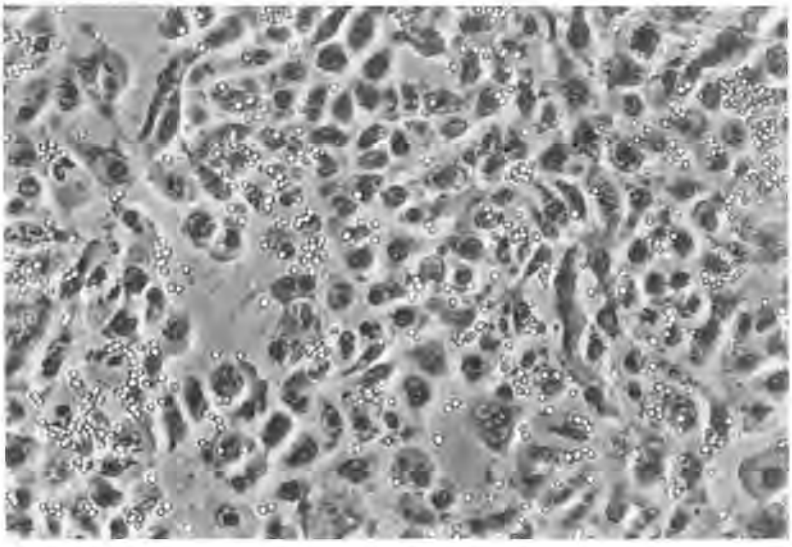

FIGURE 4. Sensitized sheep erythrocytes adhering to normal BHK-21 cells (× 240). Washed sheep erythrocytes were sensitized with 0.5 agglutinating units of rabbit antiserum and allowed to adhere to the cells for 30 min at room temperature.

DISCUSSION

Interaction of anti-Getah virus hyperimmune serum with Getah virus induced enhanced viral infectivity (Kimura and Ueba, 1978). In a preliminary experiment, early immune serum from mice bled 6 days after a single i.p. injection of virus did not enhance the infectivity so effectively as hyperimmune serum. This could be explained as due to a lower antibody titer or a difference in antibody molecules.

Hawkes (1964) reported that the infectivity of Murray Valley encephalitis (MVE) virus assayed on chick embryo cells was enhanced as much as 12 times by mixing the virus with fowl anti-MVE serum, but that no enhancement was observed when it was mixed with mouse or rabbit antiserum to MVE virus. We also demonstrated that the plaque count of Japanese encephalitis virus on chick embryo cells increased with chicken antiserum but not with mouse antiserum (Ueba and Kimura, 1973). The dependency of the infectivity-enhancement on the combination of cells and antiserum is of particular interest.

When BHK-21 cells were pretreated with a complex of UV-irradiated JaGAr-01 virus and anti-JaGAr-01 serum, the blocking effect was lower than that with Getah virus (Table 3). The lower value might reflect insufficient formation of a complex of virus and antiserum. In addition, some variation in the extent of enhancing activity was observed in different experiments, possibly due to differences in culture conditions or the growth state of BHK-21 cells.

The present work showed that IgG antibody digested with 1–2% pepsin did not enhance the infectivity of Getah virus. This finding is in agreement with the report of Halstead et al. (1977b; 1978) that no enhancement of infection of peripheral blood leukocytes by Dengue 2 virus was observed when complexes were prepared with anti-Dengue $F(ab)_2$.

The results in Figs. 1, 2 and 3 show that the extent of enhancement depends on the concentration of antibody. Similar results were obtained by Halstead (1977b) on peripheral blood leukocytes using anti-Dengue sera mixed with Dengue 2 virus, and also by Hawkes (1964; 1967).

From studies of neutralization of the infectivity of viruses by antibody, several hypotheses have been proporsed on the non-neutralizable persistent fraction (Della-Porta and Westaway, 1977). The hypothesis of an

effect of steric hindrance or of a critical site (Rappaport, 1970) suggests that the enhancement of infectivity should depend on the antibody concentration. Thus one explanation of the effect is that when the antibody is bound to a noncritical site on the virion or is bound to the critical site inadequately as a result of steric hindrance at a certain antibody concentration that induces maximum enhancement, virus could show an infective cycle. On the other hand, it has been reported that IgG that is bound to virus can become attached to the cell surface (Yasuda, 1968) and hence that the Fc fragment of the IgG molecule can react with the Fc receptor on the cell surface.

It has been reported that sheep erythrocytes coated with antiserum against them adhere to herpes virus-infected cells (Yasuda, 1968) and to normal tissue sections from humans and animals (Braathen, 1979). These observations indicate the presence of a receptor for the Fc-part of IgG in these tissues. It has been reported that hemadsorption assay can be used to detect these Fc receptors (Watkins, 1964). In this work, we showed that a receptor for the Fc part of IgG could be detected on BHK-21 cells by hemadsorption assay.

From the above considerations and the present results it is concluded that the antibody-sensitized non-neutralized virus is more effectively adsorbed than non-sensitized virus to Fc receptors on BHK-21 cells and consequently results in higher infectivity.

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