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EFFECT OF NEUTRALIZING MONOCLONAL ANTIBODIES ON HANTAAN VIRUS INFECTION OF THE MACROPHAGE P388D1 CELL LINE

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ABSTRACT

The effect of neutralizing monoclonal antibodies (N MAbs) on Hantaan virus infection of macrophages was investigated using P388D1 cells, a murine macrophage cell line. MAbs to the G1 protein (G1b) and the G2 protein (G2a and G2c) neutralized viral infectivity in P388D1 cells. N MAbs to G1b showed much higher neutralizing potency than those to G2a and G2c. With each N MAbs, two distinct effects were observed: neutralization of viral infectivity occurring at high concentrations and enhancement of that at low concentrations. Non-neutralizing MAbs, on the other hand, showed only enhancement of viral infectivity even at high concentrations without any inhibitory effects. The Fab fragments of N MAbs showed neither neutralizing nor enhancing activities. However, when the virus-Fab complexes were reacted with the anti-Fab antibodies, both neutralization and enhancement of viral infectivity were restored depending on the dose of Fab fragments. These results indicate that Hantaan virus infection of P388D1 cells is mediated by the Fc portion of the antibodies and neutralization is dependent on the concentration of N antibodies bound bivalently to the neutralization site on the virion.

Key words: Hantaan virus, Neutralizing antibody, Macrophage, Monoclonal antibody

INTRODUCTION

Hantaan virus is a prototype virus of the *Hantavirus* genus of the *Bunyaviridae* family and is a causative agent of hemorrhagic fever with renal syndrome (HFRS) that is a typical zoonosis transmitted from rodents[16]. In nature, the virus is maintained in small wild mammals and isolated easily from aged animals with a relatively high

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neutralizing antibody titer[2].

Macrophages or monocytes have been reported as target cells for hantavirus infection and are considered to contribute to the spread of hantavirus infection *in vivo*[12]. Previous studies showed that infected macrophages or monocytes carried the virus for a long time despite the existence of neutralizing antibody[11]. Therefore, it was doubtful whether the antibody could neutralize the virus effectively in macrophages or monocytes *in vivo*. Recently, it has been shown that antibody dependent enhancement (ADE) of viral infection of murine macrophage cell lines, J774.1 or P388D1, occurred with hantavirus *in vitro*[17]. The addition of non-neutralizing antibodies efficiently enhanced the infectivity of the virus in these cell lines, originally exhibiting low susceptibility to the virus. The mechanism of neutralization of hantavirus in macrophages, however, is still poorly understood. In this study, the effect of neutralizing monoclonal antibodies (N MAbs) to different antigenic sites defined previously on hantavirus infection of macrophages was analysed using P388D1 cells. In addition, the effect of the Fab fragments of N MAb on the infection and neutralization was also investigated to provide information on the mechanism of neutralization of hantavirus by antibodies.

MATERIALS AND METHODS

Cells

The murine macrophage cell line P388D1 (kindly provided by Dr. J. KIMURA-KURODA of the Tokyo Metropolitan Institute for Neuroscience), and Vero E6 cells were used. The P388D1 cells were grown in Dulbecco's modified Eagle medium (DMEM, Gibco, Grand Island, N. Y. U. S. A.) containing 10% fetal calf serum (FCS) and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin). Vero E6 cells were grown in Eagle's minimum essential medium (MEM, Nissui Co., Tokyo, Japan) supplemented with L-glutamine (0.292 *g/l*) and 5% FCS[17].

Virus

Hantaan virus strain Hantaan 76–118, which was isolated from *Apodemus agrarius*[8], was propagated in a monolayer of Vero E6 cells and the infected culture fluid was stored at −80°C as a stock virus until used.

Antibodies

The MAbs directed envelope glycoproteins of strain Hantaan 76–118 were prepared and characterized previously[1]. The properties of the MAbs used in this study are shown in Table 1. Mouse ascitic fluids containing MAbs were used as the source of MAb. The focus reduction neutralization test (FRNT) in Vero E6 cells was done as described previously[15].

MAb clones 16D2 and 1G8 were purified through a protein A-sepharose column (Pharmacia, Uppsala, Sweden). The Fab fragments were prepared by papain digestion according to the methods reported by CASALE et al.[4]. Briefly, purified IgG was

digested with papain (enzyme : protein ratio of 1 : 400), and Fab fragments were obtained by passing the mixture through a protein A-sepharose column. Protein concentrations of IgG or Fab fragments were estimated spectro-photometrically by absorbance at 280 nm. The binding activity of Fab fragments to viral antigen was determined using acetone-fixed infected Vero E6 cells by indirect immunofluorescence antibody (IFA) test with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-Fab of mouse IgG (Wako, Co., Osaka, Japan).

Procedure for Hantaan virus infection of P388D1 cells in the presence of antibodies

Infection of P388D1 cells by Hantaan virus in the presence of antibodies was carried out as described previously[17]. Briefly, strain Hantaan 76–118 ($2x10^3$ FFU) was mixed with an equal volume of MAbs serially diluted ten-fold in the diluent (MEM with 5% FCS, pH 7.2) at 37°C for 30 min. The mixtures were then inoculated onto the monolayers of P388D1 cells in 96–well plates (Falcon, Lincoln Park, New Jersey, U. S. A.) at a multiplicity of infection (m. o. i.) of approximately 0.01. Normal mouse ascitic fluid was used as a control. After incubation for 1h at 37°C, the cells were washed with MEM three times to remove unadsorbed viruses in the inoculum and cultivated with DMEM containing 10% FCS at 37°C in a CO_2 incubator. The viral infectivity in culture fluid at 48h after incubation was titrated using Vero E6 cells by the peroxidase anti-peroxidase (PAP) method as described previously[15].

To examine the effect of Fab fragments on the viral infection of P388D1 cells, strain Hantaan 76–118 was mixed with an equal volume of purified IgG (1mg/ml) or Fab fragments (1mg/ml) of MAb serially diluted ten-fold in the diluent at 37°C for 30 min. To the mixtures of virus-Fab fragments, one 20th of the volume of rabbit anti-Fab antibody (final concentration 50 μg/ml, Wako, Co., Osaka, Japan) was further added and maintained for 30 min at 37°C. The mixtures were inoculated onto P388D1 cell monolayers at a m. o. i. of 0.01 and incubated for 1h at 37°C. The monolayer cells were then washed three times with MEM to remove the unadsorbed complexes, fed with DMEM containing 10% FCS and incubated at 37°C in a CO_2 incubator. After 48h of incubation, the infectivity titers of the virus in the culture fluids were measured using Vero E6 cells by the PAP method. Purified IgG from normal mouse ascitic fluid was as a control.

The effect of the N MAbs on the infectivity of virus pre-bound on P388D1 cells was measured by the method as reported by GOLLINS and PORTERFIELD[6]. In brief, nonneutralizing MAb 23G10–1 diluted at 10^{-2} in the diluent was mixed with an equal volume of strain Hantaan 76–118 at 37°C for 30 min. The mixtures were then cooled to 4°C and inoculated to P388D1 cell monolayers grown in 96–well plates at 4°C at a m. o. i. of 0.01. After incubation at 4°C for 2h, the inoculum was aspirated and the cells were washed four times with ice-cold MEM to remove unbound virus. N MAbs serially diluted ten-fold in the ice-cold diluent were added to the infected cells and incubated at 4°C for 2h. Normal mouse ascitic fluid was used as a control. After

incubation, the inoculum was removed by aspiration, and the cells were washed four times to eliminate the free antibody and cultivated in DMEM with 10% FCS at 37°C. At 16–18h after incubation, the monolayer cells were washed with PBS and dispersed in trypsin solution. The cell suspension was centrifugated at low speed (500 g) for 5 min, and the cells were resuspended in MEM with 5% FCS and inoculated to Vero E6 cell monolayers grown in 24–well plates (Falcon, Lincoln Park, New Jersey, U. S. A.). After overnight incubation at 37°C in a CO_2 incubator, the inoculum was removed and the cell monolayers were overlaid with MEM containing 5% FCS and 1.5% carboxymethyl cellulose (overlay medium). Seven days later, the infected cells was measured by infectious center assay as described previously[17]. The culture fluids of P388D1 cells after incubation for 48h post infection were also harvested and the infectivity titers of the virus in the culture fluids were determined using Vero E6 cells by the PAP method.

RESULTS

Effect of neutralizing or non-neutralizing MAbs on Hantaan virus infection of P388D1 cells

The N MAbs directed to different antigenic sites on the glycoproteins were reacted with homologous strain Hantaan 76–118 virus ($2x10^3$ FFU) and the dose response of the N MAbs in the neutralization of viral infection to P388D1 cells was examined (Fig. 1). The properties of MAbs used are described in Table 1. No viral growth was observed when the virus was reacted with normal mouse ascitic fluid (< 20 FFU/ml). In the presence of a non-neutralizing MAb 23G10–1, however, marked enhancement of infection was observed with dilutions from 10^{-1} to 10^{-3}. The enhancing effect decreased gradually depending on the dilution until 10^{-5} and disappeared at a dilution of 10^{-6}. On the other hand, in the presence of N MAbs, no or very low infectivity titer of the virus was seen at high concentrations of antibody. At 10^{-1} dilution of the N MAbs, both 16D2 to the antigenic site G1b and HCO2 to the site G2a completely inhibited the virus growth in the culture fluids. Particularly with 16D2, no virus was detected in culture fluid even at 10^{-3} dilution. With further dilution of 16D2, the ADE appeared and reached peak enhancement at 10^{-6} dilution. N MAb HCO2 produced maximum counts up to 10^{-6} dilution, however the neutralizing effect was much weaker than that of 16D2. The reactivity of 11E10 to the site G2c was low although its FRNT and IFA titers were very similar to those of HCO2 (Table 1). The peak enhancement of 11E10 appeared between dilutions of 10^{-2} and 10^{-3} and the inhibition of viral infection, which was not as efficient as that of 16D2 and HCO2, was shown only with 10^{-1} dilution.

Effect of Fab fragments of MAbs on Hantaan virus infection of P388D1 cells

To confirm whether antibody-dependent infection with Hantaan virus was Fc receptor-mediated or not, the purified intact IgG molecules or Fab fragments of 16D2

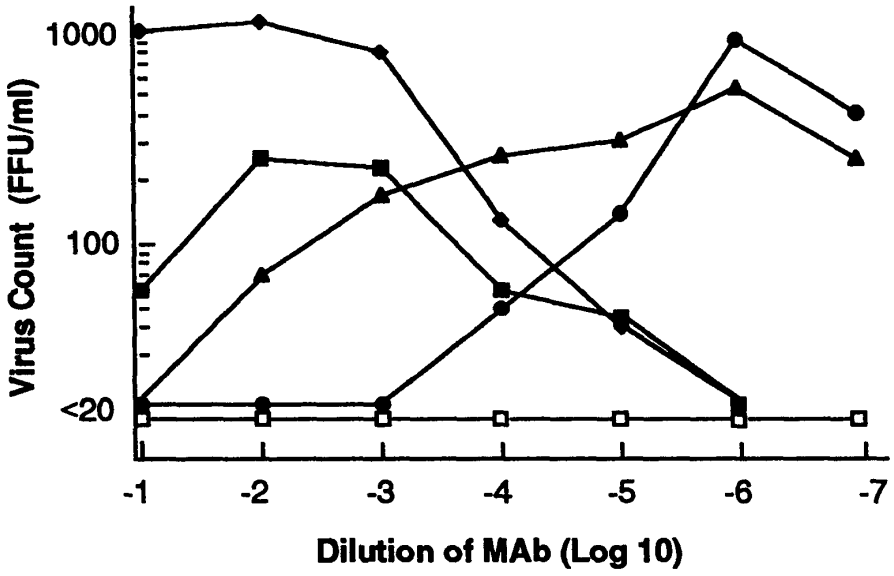

Fig. 1. Infection of P388D1 cells in the presence of MAbs. P388D1 cells were infected with strain Hantaan 76–118 which was incubated with serial dilutions of ascitic fluid of MAbs; 16D2 (●), HCO2 (▲), 11E10 (■), 23G10–1 (◆) and normal mouse ascitic fluid (□). The viral titer in culture fluid after 48h of incubation was determined using Vero E6 cells. Each value was calculated as the average of three titrations.

Table 1. Characteristics of MAbs to Hantaan 76-118 virus

MAb[a]	IgG Subclass	Antigenic Site	IFA[b] Titer	FRNT[c] Titer
16D2	IgG1	G1b	6400	2560
HCO2	IgG2b	G2a	25600	160
11E10	IgG1	G2c	12800	160
23G10-1	IgG1	G2f	12800	<10
1G8	IgG1	G2f	12800	<10

[a] The characterization of individual MAbs including isotype and antigenic site has been described previously [1].

[b] IFA titer was expressed as the reciprocal of the highest MAb dilution giving specific fluorescence in the infected Vero E6 cell cytoplasm.

[c] FRNT titer was tested by Vero E6 cells and was expressed as the reciprocal of the highest dilution giving a reduction of the number of foci by 80% compared with that of the control.

which exhibited both strong neutralizing and enhancing activities were used. A non-neutralizing MAb 1G8 and normal mouse IgG molecules were used as the control. Hantaan 76–118 virus ($2x10^3$ FFU) was mixed with an equal volume of purified IgG or Fab fragments diluted ten-fold and then inoculated onto P388D1 cells. As shown in Fig. 2, in the presence of purified intact IgG of 16D2, inhibition of viral infection was seen at a low dilution (10^{-1}) and enhancement of infection at high dilutions (10^{-2} to 10^{-5}) with the same reaction pattern as observed in Fig. 1. As a control, non-neutralizing MAb 1G8 did not show any inhibition of viral infection even at low antibody dilutions (10^{-1} to 10^{-2}). Neither enhancement nor inhibition of viral growth was observed in the presence of Fab fragments of 16D2 or 1G8 (<20 FFU/ml), although the Fab fragments still retained reactivity to viral antigen in the infected Vero E6 cells by IFA (Table 2). However, when the virus-Fab complexes were reacted with anti-Fab antibodies, both enhancement and inhibition of the viral infection with the Fab of 16D2 reappeared depending on the dilution of the Fab fragments, while only enhancement was seen with 1G8. These results indicate that the infection of P388D1 cells with virus-Fab complexes was mediated by the Fc portion of the anti-Fab antibodies. In addition, the anti-Fab antibodies restored the neutralizing activity of Fab fragments of 16D2, since no virus was detected in the culture fluids at high concentrations of 16D2 Fab fragments with addition of the anti-Fab antibodies.

To confirm the above results, Vero E6 cells were infected with the virus in the presence of Fab fragments. The infection method was same as that for P388D1 cells described in the text except that 100 FFU of virus was used. As shown in Table 2, the Fab fragments of 16D2 failed to neutralize the viral infectivity (FRNT titer; <1 : 40). However, the infectivity of the virus incubated with the Fab fragments of 16D2 was again neutralized by addition of anti-Fab antibodies. In contrast, no neutralization was observed either with high concentrations of Fab fragments of 1G8 or after addition of anti-Fab antibodies to the virus-Fab complexes. Thus, the Fab fragments of 16D2 could not neutralize the viral infectivity but their neutralizing activity was restored after the addition of anti-Fab antibodies.

Effect of neutralizing MAbs on Hantaan virus bound to P388D1 cells

The experiments were carried out to determine if N MAbs neutralized the virus at the later stage after the attachment of the virus-antibody complex to P388D1 cells. The virus was reacted with non-neutralizing MAb 23G10–1, and the virus-antibody complex was inoculated onto the monolayer cells and incubated at 4°C for 2h to retain the complex on the cell surface. After washing, the virus-antibody complex on the cells was further reacted with N MAbs at 4°C for 2h and then the monolayer cells were further washed to remove free antibodies and cultivated at 37°C. At 16–18h after incubation, the infected cells were harvested and inoculated onto Vero E6 cell monolayers for the infectious center assay. As shown in Fig. 3, the number of infected cells was reduced with the increasing concentrations of N MAbs added, as

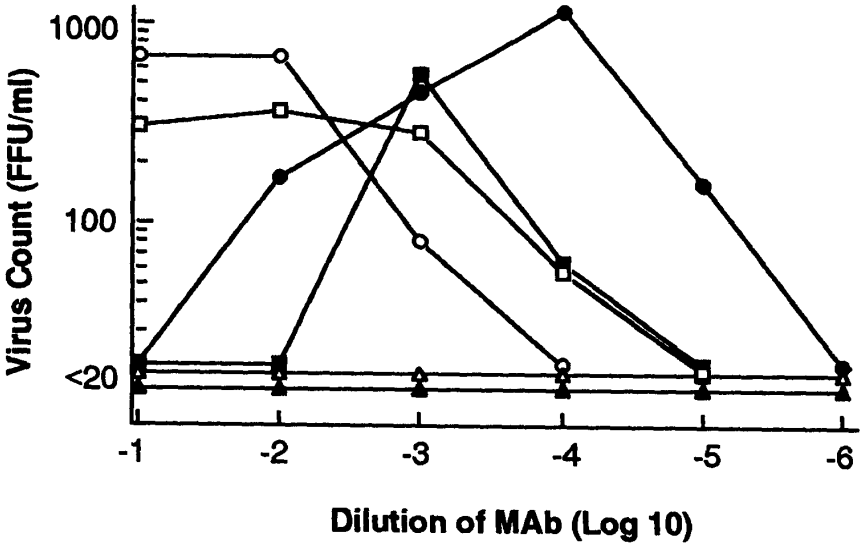

Fig. 2. Infection of P388D1 cells in the presence of purified IgG and Fab fragments. Purified IgG and Fab fragments were adjusted to protein concentration of 1 mg/ml. Ten-fold dilutions of IgG or Fab fragments were mixed with an equal volume of strain Hantaan 76–118 at 37°C for 30 min. Rabbit anti-mouse Fab antibody (final concentration 50 μg/ml) was added to the virus-Fab mixtures prepared as above and further incubated at 37°C for 30 min. The virus titer in culture fluid after 48h of incubation was determined using Vero E6 cells. Each value was calculated as the average of three titrations. 16D2 IgG (●), Fab (▲), Fab+anti-Fab antibody (■) and 1G8 IgG (○), Fab (△) and Fab+ anti-Fab antibody (□).

compared to normal mouse ascitic fluid. A similar result was also obtained by titration of the virus in the culture fluids after incubation at 37°C for 48h post-infection (data not shown). Of the three N MAbs employed, 16D2 directed to site G1b exhibited the highest neutralizing effect compared with HCO2 to G2a and 11E10 to G2c. The number of infected cells was significantly reduced with 16D2 even at 10^{-3} dilution which was comparable to that shown in Fig. 1. These results indicate that the N MAb could neutralize the infectivity of the virus absorbed on the P388D1 cell surface by inhibiting the intracellular infection process.

Table 2. Effect of Fab fragments on Hantaan 76-118 virus infectivity in Vero E6 cells

MAb	IFA titer[a]	FRNT titer[b]
16D2 Fab fragment	640	<40
Fab+anti−Fab[c]		1280
1G8 Fab fragment	640	<20
Fab+anti−Fab		<20

[a] IFA titer was expressed as the reciprocal of the highest dilution of Fab fragments giving specific fluorescence in the infected cell cytoplasm.

[b] 100 FFU of strain Hantaan 76-118 were incubated with serially diluted Fab fragments at 37℃ for 30 min. One 20th of the volume of rabbit anti−Fab antibody was added to the virus−Fab complexes prepared as above and further incubated at 37℃ for 30 min. These mixtures were then inoculated onto Vero E6 cells and incubated at 37℃ for 60 min. Then, the inoculum was removed and the monolayer cells were overlaid with overlay medium. seven days later, the number offoci were counted. FRNT titer was expressed as the reciprocal of the highest dilution of Fab fragments giving a reduction of the number of foci by 80% compared with that of normal mouse lgG used as the control.

[c] Rabbit anti-mouse Fab fragment antibody was added at final concentration of 50 μg/ml.

DISCUSSION

P388D1 cells originally were of low susceptibility to Hantaan virus infection and addition of the non-neutralizing antibody was essential for the efficient infection of cells by the virus as decribed previously[17]. In this study, the effect of N MAbs was estimated by titration of the virus released into culture fluid from P388D1 cells after inoculation of the virus-N MAb mixtures. Using this assay system, the present data demonstrates that the N MAbs to different antigenic sites defined previously could neutralize Hantaan virus infectivity in P388D1 cells if the virus was reacted with high concentrations of the antibody. Furthermore, it was found that Hantaan virus infection of P388D1 cells was markedly enhanced with a small amount of N MAbs mediated by the binding of virus-antibody complexes to the cells via Fc receptors as suggested in our previous report[17].

Among N MAbs, 16D2 to site G1b exhibited much higher neutralizing activity than that of HCO2 to G2a and 11E10 to G2c (Fig. 1 and Fig. 3). The high neutralizing potency of 16D2 could not be explained by the difference in avidity for binding to the epitope since both N MAbs, HCO2 and 16D2, had almost the same ADE titers (Fig. 1) and relative binding avidities as measured in an ELISA previously[1]. It is most likely that the G1b site recognized by 16D2 locates nearest or on the critical neutralization domain of Hantaan virus. Furthermore, bivalent binding of the antibody to the site G1b was suggested to be essential for the neutralization, since Fab fragments of 16D2

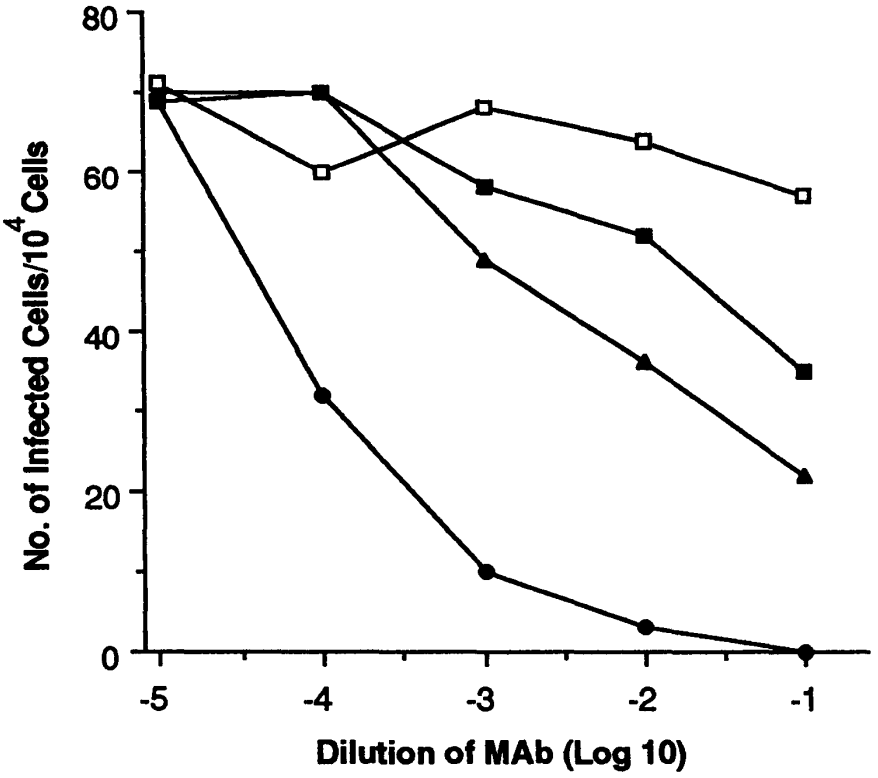

Fig. 3. Neutralization of Hantaan 76–118 virus pre-bound to P388D1 cells. Hantaan 76–118 virus was mixed with 10^{-2} diluted ascitic fluid of a non-neutralizing MAb 23G10–1 and pre-bound to P388D1 monolayer cells at 4°C for 2h. After washing four times to remove unbound virus, the infected cells were added to serially diluted ascitic fluid of N MAbs or normal ascitic fluid and further incubated at 4°C for 2h. The cells were then washed to remove free antibody and incubated at 37°C. At 16–18h after incubation, the monolayer cells were washed again, dispersed and inoculated onto Vero E6 cells for measuring the number of infected cells by the infectious center assay. Each value was calculated as the average of three titrations. 16D2 (●); HCO2 (▲); 11E10 (■) and normal ascitic fluid (□).

failed to neutralize virus infectivity and addition of anti-Fab antibodies to the virus-Fab complexes restored their neutralizing activity in either P388D1 or Vero E6 cells (Fig. 2 and Table 2). Neutralization of alphavirus infection by N MAbs to the critical neutralization domain was demonstrated to require binding of bivalent antibody[9]. Studies with influenza virus by YODEN et al. also indicated that bivalent binding of N MAbs to specific sites on the hemagglutinin protein was needed to neutralize viral infectivity by interference with conformational change of the hemagglutinin molecule[18].

N MAbs showed two distinct effects on the infectivity of the virus: neutralization of viral infectivity at high concentrations and enhancement of that at low concentrations of the antibodies (Fig. 1 and Fig. 2). The inhibiting and enhancing patterns of N MAbs were dose dependent. Similar interaction between N MAbs and viruses has been reported for dengue virus, influenza virus and reovirus infections in P388D1 cells[3, 7, 10, 13, 14]. From the amount of antibodies needed for neutralization and enhancement of viral infectivity, ADE of infection appeared in the presence of at least 1,000 times lower antibody concentration than those showing neutralizing activity. In particular, apparent enhancement was observed with 16D2 at as low as 10^{-5} dilution of purified IgG and 10^{-7} dilution of ascitic fluid. Therefore, only a few IgG molecules would cause ADE of hantavirus infection, whereas neutralization required many more antibody molecules binding of per virion than does enhancement.

Since the neutralization and enhancement of the infection in P388D1 cells with Fab fragments of N MAb 16D2 reappeared after addition of anti-Fab antibodies (Fig. 2), these results offer evidence that the Fc portion of the antibody molecules mediate the binding of the virus-antibody complexes to Fc receptors of the cells. In addition, when the virus was pre-bound to P388D1 cells with a non-neutralizing MAb as a binding antibody, the number of infected cells was apparently reduced by the addition of N MAb to the infected cells (Fig. 3). Neutralization by N antibody after virus attachment to the cells has been described with various viruses[5], and GOLLINS and PORTERFIELD[6] have provided a mechanism for the neutralization of the West Nile virus after pre-binding of the virus to P388D1 cells, in which antiviral antibody inhibits the fusion-mediated viral uncoating at the endosome. Thus, in the case of Hantaan virus, a similar inhibitory mechanism may occur in the neutralization of the virus attached to the cells with a N MAb. Further study of the intracellular infection process of Hantaan virus would be needed to clarify the mechanism of neutralization.

REFERENCES

1) ARIKAWA, J., SCHMALJOHN, A. L., DALRYMPLE, J. M. & SCHMALJOHN, C. S. (1989): Characterization of Hantaan virus envelope glycoprotein antigenic determinants defined by monoclonal antibodies. J. Gen. Virol., **70**, 615–624

2) ARIKAWA, J., TAKASHIMA, I., HASHIMOTO, N., TAKAHASHI, K., YAGI, K. & HATTORI, K. (1986): Epidemiological studies of haemorrhagic fever with renal syndrome (HFRS) related virus infection among urban rats in Hokkaido. Arch. Virol., **88**, 231–240

3) BURSTIN, S. J., BRANDRISS, M. W. & SCHILESINGER, J. J. (1983): Infection of a macrophage-like cell line, P388D1 with reovirus; effect of immune ascitic fluid and monoclonal antibodies on neutralization and on enhancement of viral growth. J. Immunol., **130**, 2915–2919
4) CASALE, E., WENISCH, E., HE, X. M., RIGHETTI, P. G., SNYDER, R. S., JUNGBAUER, A., TAUER, C., RUKER, F. & CARTER, D. C. (1990): Crystallization of the Fab from a human monoclonal antibody against gp41 of human immunodeficiency virus type 1. J. Mol. Biol., **216**, 511–512
5) DIMMOCK, N. J. (1984): Mechanism of neutralization of animal viruses. J. Gen. Virol. **65**, 1015–1022
6) GOLLINS, S. W. & PORTERFIELD, J. S. (1986): A new mechanism for the neutralization of envelope virus by antiviral antibody. Nature, **321**, 244–246
7) HALSTEAD, S. B., VENKATESHAN, C. N., GENTRY, M. K. & LARSEN, L. K. (1984): Heterogeneity of infection enhancement of dengue 2 strains by monoclonal antibodies. J. Immunol., **132**, 1529–1532
8) LEE, H. W., LEE, P. W. & JOHNSON, K. M. (1978): Isolation of the etiologic agent, Korean haemorrhagic fever. J. Infect. Dis., **137**, 298–308
9) MATHEWS, J. H., ROEHRIG, J. T. & TRENT, D. W. (1985): Role of complement and Fc portion of immunoglobulin G in immunity to Venezuelan equine encephalomyelitis virus infection with glyco-protein-specific monoclonal antibodies. J. Virol., **55**, 594–600
10) MORENS, D. M. & HALSTEAD, S. B. (1990): Measurement of antibody-dependent infection enhancement of four dengue virus serotypes by monoclonal and polyclonal antibodies. J. Gen. Virol., **71**, 2909–2914
11) NAGAI, T. (1987): Pathophysiological studies of hemorrhagic fever with renal syndrome. Fujita-Gakuen Med. Soc., **6**, 212–223 (in Japanese)
12) NAGAI, T., TANISHITA, O., TAKAHASHI, Y., YAMANOUCHI, T., DOMAE, K., KONDO, K., DANTAS, J. R., TAKAHASHI, M. & YAMANISHI, K. (1985): Isolation of hemorrhagic fever with renal syndrome virus from leukocytes of rats and virus replication in culture of rat and human macrophages. J. Gen. Virol., **66**, 1271–1278
13) OCHIAI, H., KUROKAWA, M., HAYASHI, K. & NIWAYAMA, S. (1988): Antibody-mediated growth influenza A NWS virus in macrophage cell line P388D1. J. Virol.,**62**, 20–26
14) PORTERFIELD, J. S. (1986): Antibody-dependent enhancement of viral infectivity. Adv. Virus Res., **31**, 335–355
15) TANISHITA, O., TAKAHASHI, Y., OKUNO, Y., YAMANISHI, K. & TAKAHASHI, M. (1984): Evaluation of focus reduction neutralization test with peroxidase-antiperoxidase staining technique for hemorrhagic fever with renal syndrome virus. J. Clin. Microbiol., **20**, 1213–1215
16) WORLD HEALTH ORGANIZATION (1983): Hemorrhagic fever with renal syndrome: memorandum from a WHO meeting. Bull. WHO., **61**, 269–275
17) YAO, J-S., KARIWA, H., TAKASHIMA, I., YOSHIMATSU, K., ARIKAWA, J. & HASHIMOTO, N. (1992): Antibody-dependent enhancement of hantavirus infection in macrophage cell lines. Arch. Virol., **122**, 107–118
18) YODEN, S., KIDA, H. & YANAGAWA, R. (1985): Is bivalent binding of monoclonal antibodies to different antigenic areas on the hemagglutinin of influenza virus required for neutralization of viral infectivity? Arch. Virol., **85**, 209–216