Archives of Virology 81, 53—65 (1984)

Archives of Virology
© by Springer-Verlag 1984

Biological Functions of Monospecific Antibodies to Envelope Glycoproteins of Newcastle Disease Virus

By

Y. UMINO[1], T. KOHAMA[1], M. KOHASE[1], A. SUGIURA[1], H.-D. KLENK[2], and R. ROTT[2]

[1] Department of Measles Virus, National Institute of Health, Gakuen, Musashimurayama, Tokyo, Japan

[2] Institut für Virologie, Justus-Liebig-Universität Giessen, Giessen, Federal Republic of Germany

With 8 Figures

Accepted January 20, 1984

Summary

Monospecific antisera to HN and F glycoproteins of Newcastle disease virus were prepared, and their effects on the biological activities of the virus were investigated. Anti-HN serum inhibited hemagglutinating and neuraminidase activity, as well as hemolysis. Anti-F serum had no effect on hemagglutination or neuraminidase but inhibited hemolysis and virus-induced cell fusion.

Anti-HN serum was highly neutralizing, while neutralization by anti-F serum was very inefficient in conventional plaque reduction tests, although both sera were estimated to contain comparable amounts of antibody reacting with the virus as indicated by complement fixation and immunodiffusion tests. The neutralizing activity of anti-F serum was greatly enhanced by the addition of anti-IgG serum or fresh guinea pig serum, whereas that of anti-HN serum was little enhanced.

Anti-HN serum incorporated in the agar overlay suppressed the development of plaques to some degree, while anti-F serum had little effect. The combination of anti-HN and anti-F sera resulted in a marked decrease in the number and size of plaques, demonstrating the synergistic effect of the two species of antibody in the containment of the spread of viral infection.

Introduction

Two species of glycoproteins are present on the surface of paramyxovirus; HN has hemagglutinating and neuraminidase activities and mediates virus adsorption to the cells, while F is responsible for hemolytic and cell fusing activities and virus penetration (for review see 3).

Immunological properties of the glycoproteins are extensively studied for several members of the paramyxovirus family, Newcastle diesease virus (NDV) (1, 19), Sendai virus (15, 16, 20) and SV 5 (11, 12).

It is generally agreed upon that the antibodies to HN glycoprotein inhibit hemagglutinating and neuraminidase activities and neutralize viral infectivity. In contrast, the role of antibodies to F glycoprotein in neutralization has not been definitively established, although they are known to inhibit both the virus-induced hemolysis and cell fusion.

In the present communication we report the effects of monospecific antibodies to HN and F glycoproteins of NDV on biological functions of different strains of NDV. The results have provided further information on the mode of action of the specific antibodies against the two envelope components.

Materials and Methods

Cells and Viruses

MDBK and BHK cells were grown in reinforced Eagle's medium (REM) containing 10 per cent fetal calf serum. Eleven-day-old embryonated eggs were used for the preparation of monolayer culture of chick embryo (CE) cells.

Virus Strains

Italien and Ulster strains of NDV were used. Virus stocks were prepared in the allantoic cavity of embryonated eggs. Infectivity of Italien strain was determined by plaque assay in CE cells. Agar overlay medium consisted of MEM, 0.8 per cent Noble agar, and 2 per cent calf serum. Virus to be purified, either radiolabeled or unlabeled, was grown in MDBK cells in the presence of trypsin (Serva, Heidelberg, Germany; 10 μg/ml medium). Purification procedures were reported previously (6).

Separation of Glycoproteins

The method described previously was followed (6). Briefly, virus preparations were treated with 2 per cent octylglucoside. The viral cores were pelleted by centrifugation at $200{,}000 \times g$ for 45 minutes. The supernatant containing solubilized glycoproteins was subjected to preparative isoelectric focusing in a LKB 8101 column (110 ml, LKB Produkter, Bromma, Sweden) containing approximately 1 per cent Ampholine and 1 per cent octylglucoside in a 0 to 40 per cent (w/v) discontinuous gradient.

Preparation of Antisera Against Individual Glycoproteins

Approximately 500 μg/ml of purified HN or F glycoproteins in PBS were emulsified by forced mixing with complete Freund's adjuvant. The mixture was injected intramuscularly into rabbits at several sites. Four weeks later the rabbits received a booster injection. The rabbits were bled one week later.

When necessary, the antibody against heterologous antigens was removed by passing about 5 ml of serum through a column of Sepharose 4B (Pharmacia Fine Chemicals, Sweden) to which the heterologous antigens had been conjugated (18). Approximately 500 μg of the antigen was conjugated to cyanogen bromide-activated Sepharose 4B. The conjugate was washed extensively with PBS, followed by 0.2 M ethanolamine-glycine buffer (pH 8.5) and finally PBS. After the sera had been passed through, the immunoadsorbent was regenerated by dissociating the bound antibodies with 3 M NH_4 SCN in PBS. The antisera were heat-inactivated at 56° C for 30 minutes before use.

Immunoprecipitation and SDS-Polyacrylamide Gel Electrophoresis

Immunoprecipitation was performed in Eppendorf microtest tubes (Eppendorf, Hamburg, Germany). To 50 to 100 μl of labeled, RIPA buffer (8) disrupted virus preparation (2—5×10^5 cpm), 25 to 50 μl of appropriate antiserum was added. The mixture was kept overnight at 4° C and then received 25 μl of 50 per cent (w/w) protein A-Sepharose. After 2 hours at 4° C, the precipitate was pelleted, washed three times with 0.5 ml of the RIPA buffer, once with 1 ml of Tris-HCl buffer. The precipitate was resuspended in Laemmli sample buffer (7) containing 1 per cent 2-mercaptoethanol, boiled for 2 minutes and then pelleted. The supernatant was applied to a 10 per cent polyacrylamide cylindrical gel (7). After electrophoresis for 4 hours at 3 mA/gel, the gels were sliced in 1 mm thickness and were counted for radioactivity in a liquid scintillation counter (Packard Tricarb model 3380) (13).

Hemagglutination Inhibition (HI) Test

HI test was performed by the standard method, using 4 hemagglutinating unit (HAU) antigen.

Neuraminidase Inhibition (NI) Test

The standard procedure described by SETO and ROTT (17) was used. Antibody titer was expressed as the highest dilution which inhibits 50 per cent of the enzyme activity. Fetuin was used as substrate for viral neuraminidase assays.

Hemolysis Inhibition (HLI) Test

Inhibition of virus-induced hemolysis was assayed as follows: To 0.1 ml of virus (approximately 50 HAU/0.1 ml), 0.5 ml of five-fold serial dilution of serum was added. The mixture was kept for 30 minutes at room temperature, and 0.4 ml of 5 per cent chicken erythrocyte suspension was added. After incubation for 60 minutes at 37° C the unlysed erythrocytes were pelleted by centrifugation at 3,000 rpm for 2 minutes. The optical density of the supernatant was measured at 540 nm. Fifty-per cent inhibition of hemolysis was taken as the end point. In the absence of serum, the virus gave the OD_{540} value of 1.5—2.5.

Inhibition of Cell Fusion (FI)

Confluent monolayers of BHK 21-F cells were infected with NDV-Italien at a multiplicity of about 10 PFU/cell. After adsorption at 37° C for 1 hour, the inoculum was removed and the medium containing five-fold serial dilution of serum was added. After incubation at 37° C for about 10 hours, the cultures were examined microscopically for the percentage of fused cells.

Complement Fixation (CF) Test

CF test was performed by box titration in microtiter method using purified virus as antigen and five 50 per cent hemolytic units of complement.

Virus Neutralization Test

Serum was mixed with the equal volume of NDV-Italien containing 30—50 PFU/ 0.05 ml. The mixture was kept at 37° C for 60 minutes and 0.1 ml was inoculated onto CE cells in 35-mm wells on Costar 6-well culture plates No. 3506 (Costar, Cambridge, MA, U.S.A.). After a 60-minute adsorption at room temperature, the inoculum was removed. Cultures received 3 ml of agar overlay medium consisting of MEM, 0.8 per cent Noble agar, and 2 per cent calf serum and, 2 days later, 2 ml of the agar medium containing 0.008 per cent neutral red. Plaques were counted on the following day. The serum dilution causing 50 per cent plaque reduction was taken as the neutralizing titer.

In enhanced neutralization, either goat anti-rabbit IgG serum (Miles-Yeda, Rehovot, Israel) at the final concentration of 1:23 or fresh guinea pig serum at the final concentration of 1:180 was added to the preincubated virus-serum mixture. The final mixture was kept for additional 30 minutes at 37° C before inoculation.

Results

Specificity of Antisera Against HN and F Glycoproteins of NDV

HN and F glycoproteins of Ulster strain were prepared from octylglucoside-solubilized envelopes by isoelectric focusing (6). This procedure enabled us to prepare highly purified glycoproteins in quantities sufficient to immunize rabbits. Anti-HN sera thus obtained were specific to the homologous antigen. However, animals immunized with F glycoprotein always produced variable amounts of anti-HN antibody, ranging from 1:150 to 1:300 in HI titer. Exhaustive absorption with purified HN glycoprotein covalently attached to Sepharose beads reduced the HI titer of anti-F to the undetectable level. Anti-F sera absorbed with HN were used throughout this study. Immunodiffusion test on agarose gel demonstrates that anti-HN and anti-F sera are specific to the respective antigens (Fig. 1). It should be noted that anti-F serum diluted 1:40 was used, while anti-HN

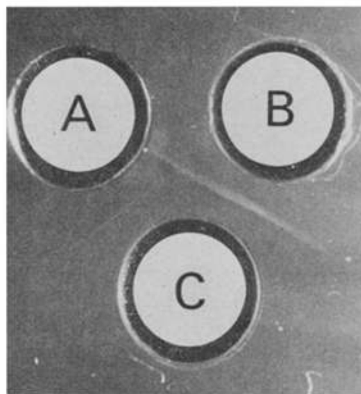

Fig. 1. Immunodiffusion analysis of hyperimmune rabbit antisera against HN and F glycoprotein of Ulster strain. Anti-F (1:40) *(A)* and anti-HN (1:5) *(B)* were tested against octylglucoside-solubilized NDV-Ulster strain (1.0 mg/ml) *(C)*

serum was used at 1:5, in order to produce precipitation bands approximately at the middle between antibody and antigen wells. Neither anti-HN, nor anti-F sera cross-reacted with lysates of MDBK cells or fetal calf serum in gel diffusion test (data not shown).

Immunoprecipitation of [^{3}H] glucosamine-labeled virions of the Ulster strain with anti-HN and anti-F sera confirms the specificity of the antisera (Fig. 2).

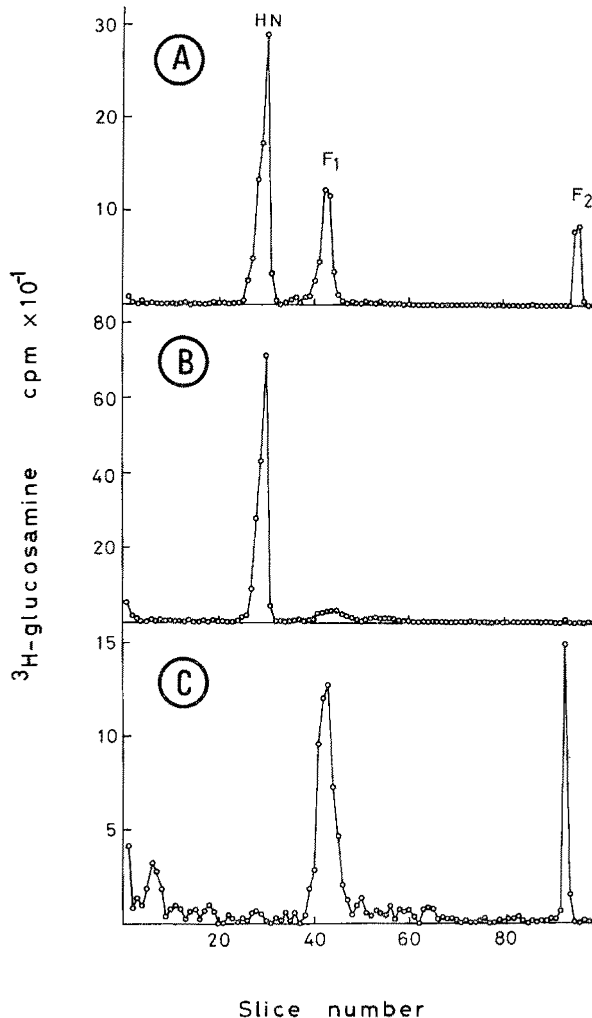

Fig. 2. Electrophoresis of NDV-Ulster glycoprotein immunoprecipitated by anti-HN and anti-F. Virus was metabolically labeled with [^{3}H] glucosamine during the growth in MDBK cells and subsequently purified. Immunoprecipitation and SDS-polyacrylamide gel electrophoresis were carried out by the method in Materials and Methods. Electrophoretic profiles of *(A)* [^{3}H] glucosamine-labeled virions, *(B)* immunoprecipitate with anti-HN, *(C)* immunoprecipitate with anti-F

Table 1. *Effect of antisera against glycoproteins of NDV-Ulster on different strains of NDV*[a]

Antisera raised against	HI		NI		HLI		FI		CF	
	Ul[b]	It[c]	Ul	It	Ul	It	Ul	It	Ul	It
HN	2560	2560	230	230	320	650	—[d]	4	128	—
F	<16	<16	<1	<1	28	300	—	10	512	—

[a] Values represent titers of serum dilution
[b] Ulster
[c] Italien
[d] —, not tested

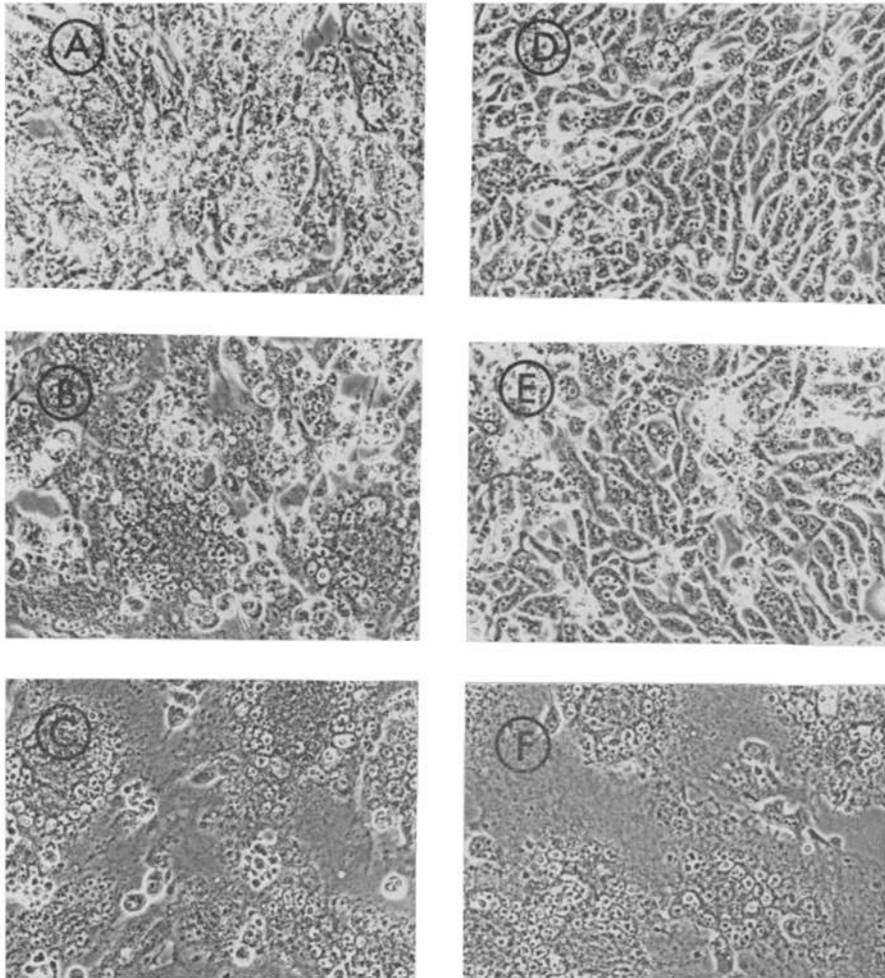

Fig. 3. Inhibition of virus-induced fusion of BHK cells by anti-HN and anti-F. Cells were infected with Italien strain at a MOI of 10 PFU/cell, 60 minutes prior to addition of antisera. Photographs were taken 11 hours p.i. Panels *(A)*, *(B)*, and *(C)*; anti-HN. Panels *(D)*, *(E)*, and *(F)*; anti-F. Serum dilutions (A) and (D); 1:2, (B) and (E); 1:10, (C) and (F); 1:50

Biological Properties of Antisera Against HN and F Glycoproteins

The antisera were tested for inhibitory effects on various biological activities of NDV (Table 1). Anti-HN serum was found to possess HI, NI, as well as HLI activity. In contrast, anti-F serum showed HLI activity, but not HI or NI activity.

Cell fusion inhibition by antisera was tested on Italien-infected BHK cells, because of the unique susceptibility of this cell line to NDV-induced cell fusion (5). Anti-F serum inhibited syncytium formation completely at the dilution of 1:2 and by more than 50 per cent at 1:10 (Fig. 3). Anti-HN serum was less inhibitory, permitting about 20 per cent of cells to fuse at 1:2, and more than 80 per cent at 1:10. It should be noted that the test was carried out between antisera directed to glycoprotein antigens from the Ulster strain and a heterologous strain (Italien) as fusing agent, because Ulster does not induce fusion. Antiserum against HN glycoprotein from Ulster strain reacted to a nearly equal titer with Ulster and Italien strains. Similarly antiserum immunized with HN glycoprotein from Italien strain reacted equally well with the two strains (data not shown). The results suggested the absence of noticeable antigenic difference between Ulster and Italien HN glycoproteins. On the other hand, the hemolysis induced by the Italien strain as compared with the Ulster strain was consistently more susceptible to inhibition by antiserum against F glycoprotein of either strain. This may result from the different spatial arrangement of F glycoproteins on the virion surface of two strains but has not further been investigated.

Neutralization of Infectivity of NDV by Antisera Against HN and F Glycoproteins

Anti-HN and anti-F sera were tested for neutralizing activity by plaque reduction assays in CE cells. Only neutralization of Italien strain was studied because Ulster strain did not form plaques in the absence of trypsin in medium (13). Neutralization profiles show the marked difference in the neutralizing activity between anti-HN and anti-F sera (Fig. 4). The neutralizing titer was $10^{3.8}$ for antiHN and $10^{1.6}$ for anti-F sera. From the gentle slope of the curve for anti-F serum, complete inhibition would not be expected even by the undiluted serum.

According to MERZ *et al.* (12), antiserum against F glycoprotein of SV 5 efficiently neutralized the infectivity of SV 5, most likely by interfering with the fusion of viral envelope with the cell membrane. Inefficiency of anti-NDV F glycoprotein in neutralization compared with anti-SV 5 F glycoprotein could be due to either inefficient binding of the former to the virus or inability of the attached antibody to interfere with the subsequent steps.

To resolve the question, we studied whether anti-IgG serum (10, 14) (Fig. 5) or fresh guinea pig serum (Fig. 6) added to preincubated virus-antiserum mixture enhances the neutralization. When anti-rabbit IgG

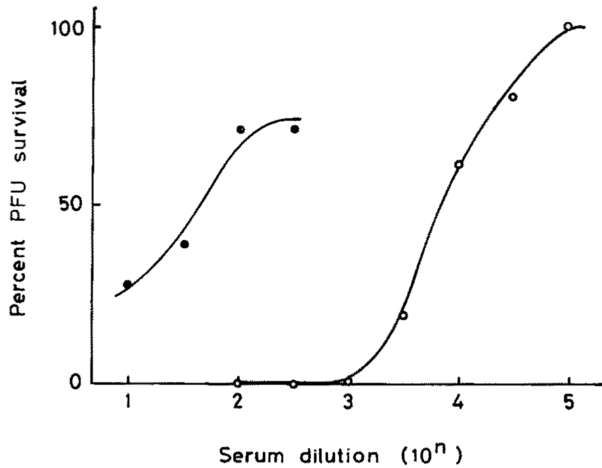

Fig. 4. Neutralization of NDV-Italien strain by anti-HN (○) and anti-F (●)

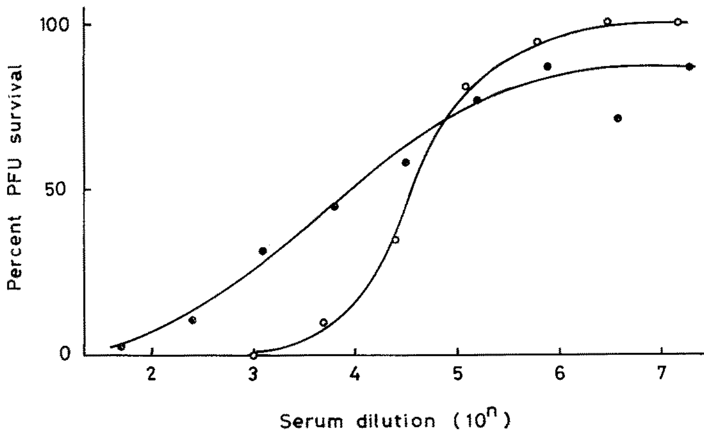

Fig. 5. Enhanced neutralization of NDV-Italien strain by anti-HN (○) and anti-F (●) in the presence of goat anti-rabbit gamma globulin serum (1:23)

serum was added, the neutralizing titer of anti-HN serum was $10^{4.6}$, while that of anti-F serum $10^{4.0}$, the enhancement being 6-fold and 250-fold, respectively.

A more pronounced effect was seen with fresh guinea pig serum. The complement has been reported to enhance the neutralization of NDV (9). When fresh guinea pig serum was added to a final concentration of 1:180,

neutralization by anti-F serum was enhanced 800-fold. Because the fresh guinea pig serum alone reduced the infectivity by 10 per cent at the same concentration, in agreement with the previous finding (4), the neutralization profile in Fig. 6B was redrawn taking the effect into consideration.

In contrast, the neutralization by anti-HN serum was very little affected by the addition of the fresh guinea pig serum. These findings suggest that both antibodies to HN and F glycoproteins bind to the virus and while the attachment of antibody to HN glycoprotein is sufficient to render the virus

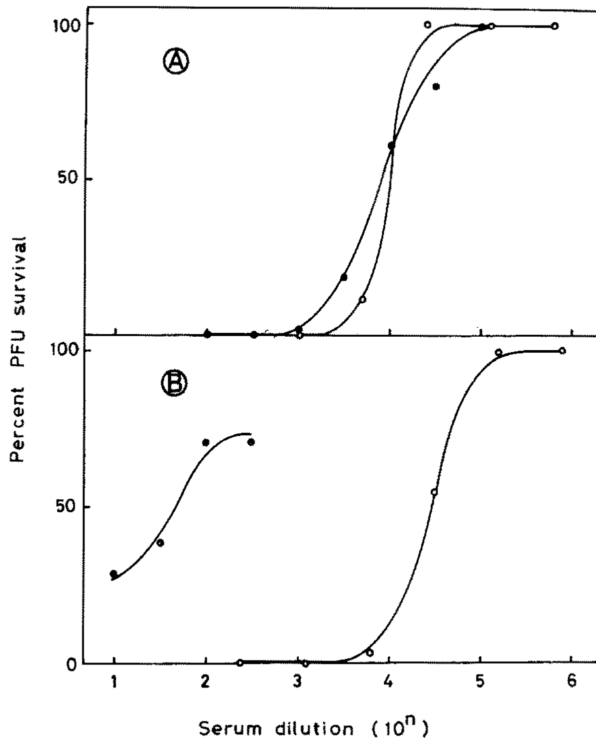

Fig. 6. Enhanced neutralization of NDV-Italien strain by *(A)* anti-HN and *(B)* anti-F in the absence (•) or presence of fresh normal guinea pig serum at a dilution of 1:180 (○)

noninfectious, the mere binding of antibody to F glycoprotein does not prevent NDV, unlike SV 5, from initiating infection. Either cross-linking of attached anti-F antibody by the secondary antibody or the irreversible damage inflicted by activated complement systems was required to prevent the virus to attach to or enter the cell.

Effect of Antisera Against HN and F Glycoproteins on the Spread of Infection

The effect of antiserum on the spread of infection was studied by comparing plaque development under the agar overlay containing various

concentrations of antisera. Anti-HN serum did exert inhibitory effect upon plaque development to some extent, but the effect was less pronounced than expected from its neutralizing activity shown in Fig. 4. Even at the dilution of 1:100, which reduced the infectivity by more than 99 per cent, 40 per cent of plaques, though diminished in size, appeared (data not shown).

Anti-F serum incorporated in the agar overlay had less effect. The dilution of 1:10 allowed the formation of 40 per cent plaques, and these were only slightly diminished in size (data not shown). This is not surprising, since anti-F serum had virtually no neutralizing effect on released virus.

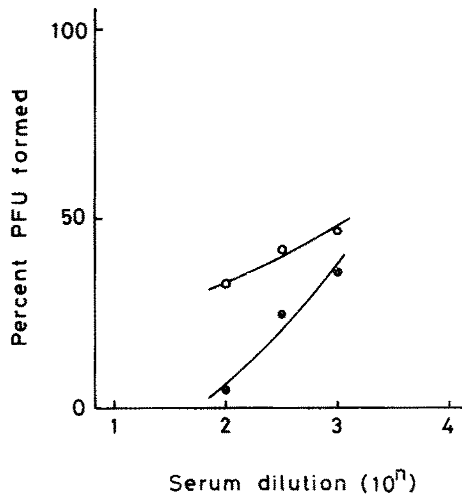

Fig. 7. Effect of anti-HN on the development of NDV plaques in the presence or absence of anti-F. Anti-HN at the final concentration indicated on the abscissa alone (○) or together with the constant amount of anti-F (final concentration 1:30) (•) were incorporated into the agar overlay medium

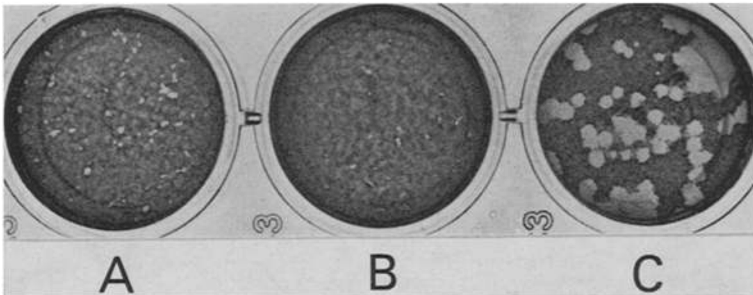

Fig. 8. Cooperative effect of anti-HN and anti-F on the development of NDV plaques. *A* Plaques formed in the presence of anti-HN (1:100) only. *B* Plaques formed in the presence of anti-HN (1:100) and anti-F (1:30). *C* Plaques formed in the absence of the antisera. Inoculum of virus was adjusted to a half that of *A* and *B*

When the constant amount of anti-F serum was combined with various concentrations of anti-HN serum, a marked synergistic effect was obtained (Fig. 7). In combination with anti-HN serum at the concentration of 1:300 or higher, anti-F serum at 1:30, which by itself affected neither plaque number nor plaque size, caused a significant reduction in both plaque number (Fig. 7) and plaque size (Fig. 8). The mixture of anti-HN and anti-F sera was not more effective than anti-HN serum alone in the conventional pre-incubation neutralization test (data not shown). The cooperative effect of the two antisera was, therefore, directed not to the neutralization of infectivity, but to the spread of already initiated infection.

Discussion

Rabbit anti-HN and anti-F sera prepared in this study were specific to respective antigens when tested by immunodiffusion or immunoprecipitation. The anti-HN and anti-F sera appeared to contain a comparable concentration of respective antibody as judged by the intensity of precipitation bands in immunodiffusion and by CF tests.

Anti-HN serum inhibited hemagglutination and neuraminidase activity of NDV as expected. It also inhibited hemolysis, as a secondary effect of hemagglutination inhibition. Anti-F serum had no effect on hemagglutination or neuraminidase, but did inhibit hemolysis and cell-fusion.

There was a marked difference in neutralizing activity between anti-HN and anti-F sera. The antibody to F glycoprotein is vastly inefficient compared with the antibody to HN. These findings are roughly in agreement with two previous studies on NDV (14, 19). On the other hand, one study showed that monospecific serum against F glycoprotein of NDV had neutralizing activity, causing 100 per cent plaque reduction at serum dilution of 1:50 (1). It is not known, however, whether the anti-F serum in this study was free from antibody to HN.

Infection with paramyxovirus is initiated by the adsorption of a virion to a cell, followed by the fusion of viral and cell membranes [reviewed by CHOPPIN and SCHEID (2)]. The first step is mediated by HN glycoprotein while F is involved in the second step. It is therefore surprising that the anti-F serum, capable of inhibiting hemolysis and cell fusion, exerted only a marginal effect on the initiation of infection. Recently, RUSSELL *et al.* (17) reported that a monoclonal antibody to NDV-F had a significant titer of viral neutralization. The reason for the discrepancy between their results and our findings is unknown. One possibility is that the epitopes involved in viral neutralization are inactivated during solubilization of F glycoprotein with detergent.

The addition of either anti-rabbit IgG serum or fresh guinea pig serum augmented the neutralizing activity of anti-F serum to a level comparable

to that of anti-HN serum. This finding indicates that the antibody of F glycoprotein does attach to the virion but fails to prevent the initiation of infection in the absence of anti-IgG or complement system. Whether the cooperation of anti-F serum and either anti-IgG or complement system results in the inhibition of virus adsorption or that of fusion has yet to be determined.

Development of plaque could proceed in two mechanisms; dissemination of released virus to near-by cells, and cell-to-cell spread of infection through cell fusion. Neither anti-HN nor anti-F serum alone suppressed the development of plaques even at low dilution. This is not surprising, because anti-HN serum may neutralize extracellular virus but is unable to inhibit cell fusion, while anti-F serum may inhibit cell fusion but has no effect on released virus. The simultaneous presence of anti-HN and anti-F sera suppressed the development of plaques effectively as shown by a significant reduction in the number of visible plaques.

Based on their study of SV 5, MERZ *et al.* (11) stressed the importance of antibody response to F glycoprotein in the host defence against paramyxo virus infection, because the antibody to F employed in their study was far more efficient in neutralization than our anti-NDV F serum (12). As a result, the anti-SV 5 F serum alone effectively inhibited the spread of infection. The contribution of the antibody to F glycoprotein relative to that to HN glycoprotein in the defense presumably varies depending upon individual members of paramyxovirus.

Acknowledgements

The excellent technical assistance of W. Berk and M. Orlich is gratefully acknowledged. We thank S. Inoue, A. Hasegawa and A. Yamada, National Institute of Health, Tokyo, Y. Yoshikawa, Institute of Medical Science, Tokyo University, for many valuable discussions.

T. Kohama was a recipient of a research fellowship of the Alexander-von-Humboldt-Gesellschaft. This work was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 47).

References

1. AVERY, R. J., NIVEN, J.: Use of antibodies to purified Newcastle disease virus glycoproteins for strain comparisons and characterizations. Infect. Immun. **26**, 795—801 (1979).
2. CHOPPIN, P. W., SCHEID, A.: The role of viral glycoproteins in adsorption, penetration, and pathogenicity of viruses. Rev. Infect. Dis. **2**, 40—61 (1980).
3. COMPANS, R. W., KLENK, H.-D.: Viral membranes. In: FRAENKEL-CONRAT, H., WAGNER, R. R. (eds.), Comprehensive virology, Vol. 13, 293—407. New York-London: Plenum Press 1979.
4. GINSBERG, H. S., HORSFALL, F. L.: A labile component of normal serum which combines with various viruses. Neutralization of infectivity and inhibition of hemagglutination by the component. J. exp. Med. **90**, 475—495 (1949).

5. Klenk, H.-D., Choppin, P. W.: Glycosphingolipids of plasma membranes of cultured cells and an enveloped virus (SV 5) grown in these cells. Proc. Natl. Acad. Sci. U.S.A. **66**, 57—64 (1970).
6. Kohama, T., Garten, W., Klenk, H.-D.: Changes in conformation and charge paralleling proteolytic activation of Newcastle disease virus glycoproteins. Virology **111**, 364—376 (1981).
7. Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**, 680—685 (1970).
8. Lamb, R. A., Etkind, P. R., Choppin, P. W.: Evidence for a ninth influenza viral polypeptide. Virology **91**, 60—78 (1978).
9. Linscott, W. D., Levinson, W. E.: Complement components required for virus neutralization by early immunoglobulin antibody. Proc. Natl. Acad. Sci. U.S.A. **64**, 520—527 (1969).
10. Mayer, M., Link, F.: Sensitization of influenza virus A2/Singapore by anti-neuraminidase. J. gen. Virol. **13**, 355—356 (1971).
11. Merz, D. C., Scheid, A., Choppin, P. W.: Importance of antibodies to the fusion glycoprotein of paramyxoviruses in the prevention of spread of infection. J. exp. Med. **151**, 275—288 (1980).
12. Merz, D. C., Scheid, A., Choppin, P. W.: Immunological studies of the functions of paramyxovirus glycoproteins. Virology **109**, 94—105 (1981).
13. Nagai, Y., Klenk, H.-D., Rott, R.: Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. Virology **72**, 494—508 (1976).
14. Örvell, C.: Identification of paramyxovirus-specific haemolysis-inhibiting antibodies separate from haemagglutinating-inhibiting and neuraminidase-inhibiting antibodies. 2. NDV and mumps virus heamolysis-inhibiting antibodies. Acta Path. Microbiol. Scand. **B 84**, 451—457 (1976).
15. Örvell, C., Norrby, E.: Immunologic properties of purified Sendai virus glycoproteins. J. Immunol. **119**, 1882—1887 (1977).
16. Örvell, C., Grandien, M.: The effect of monoclonal antibodies on biologic activities of structural proteins of Sendai virus. J. Immunol. **129**, 2779—2787 (1982).
17. Russell, P. H., Griffiths, P. C., Goswami, K. K. A., Alexander, D. J., Cannon, M. J., Russell, W. C.: The characterization of monoclonal antibodies to Newcastle disease virus. J. gen. Virol. **64**, 2069—2072 (1983).
18. Seto, J. T., Rott, R.: Functional significance of sialidase during influenza virus multiplication. Virology **30**, 731—737 (1966).
19. Seto, J. T., Becht, H., Rott, R.: Effect of specific antibodies on biological functions of the envelope components of Newcastle disease virus. Virology **61**, 354—360 (1974).
20. Urata, D. M., Seto, J. T.: Glycoproteins of Sendai virus: Purification and antigenic analysis. Intervirology **6**, 108—114 (1976).

Authors' address: Dr. T. Kohama, Department of Measles Virus, National Institute of Health, 4-7-1, Gakuen, Musashimurayama, Tokyo 190-12, Japan.

Received November 15, 1983