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Protective Effect of Antibodies to Two Viral Envelope Glycoproteins on Lethal Infection with Newcastle Disease Virus

By

Y. Umino[1], T. Kohama[1], M. Kohase[2], A. Sugiura[1], H.-D. Klenk[3], and R. Rott[4]

[1] Department of Measles Virus, National Institute of Health, Tokyo, Japan
[2] Central Virus Diagnostic Laboratory, National Institute of Health, Tokyo, Japan
[3] Institut für Virologie, Philipps-Universität Marburg, Federal Republic of Germany
[4] Institut für Virologie, Justus-Liebig-Universität Giessen, Federal Republic of Germany

With 3 Figures

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Summary

The protective effect of humoral immunity against lethal infection of chickens with Newcastle disease virus was studied.

Chickens hatched from eggs laid by hens vaccinated with live attenuated Newcastle disease virus vaccine possessed antibody to various components of the virus, and were resistant to a challenge with a virulent strain of Newcastle disease virus which was 100 per cent fatal for the offspring of non-vaccinated hens.

Passive administration of antiserum raised against whole virions provided susceptible chickens protection comparable to that seen in the birds with maternal antibody.

When administered passively, both anti-HN serum with virus neutralizing activity, and anti-F serum with only marginal virus neutralizing activity significantly prolonged the survival of infected birds but failed to achieve the level of protection as afforded by the anti-whole NDV serum.

The protection provided by the simultaneous presence of anti-HN and anti-F serum was significantly greater than that afforded by either alone and comparable to that of anti-whole NDV serum, indicating the complementary effect of anti-HN and anti-F antibodies not only in cell cultures as reported previously (19), but also in a natural host.

Introduction

Newcastle disease is a disease of domestic birds with high morbidity and mortality. The grave economic consequence of the epizootics has prompted an intensive study of the disease and its causative agent (2, 3, 9, 10, 12).

The problem has been practically solved, however, by the introduction of live attenuated vaccine during 1950's (for review, see HANSON *et al.*, 10). Since then, little attention has been paid to the detailed mechanism of protection provided by vaccine-induced immunity against Newcastle disease virus (NDV). It became known subsequently that NDV is a member of paramyxovirus family and possesses two surface glycoproteins, HN, the hemagglutinin-neuraminidase, and F, the fusion protein on its envelope.

The role of the antibodies to the two envelope glycoproteins in protection against lethal infection in experimental animals has been investigated using monoclonal antibodies directed to the HN or F of several viruses of paramyxovirus group (8, 14, 22).

It is generally agreed that the protective effect of the antibodies to HN glycoprotein on viral infection is due to their virus-neutralizing activity (8, 22). On the other hand, the role of antibodies to F glycoprotein in protection has not been definitively established. MERZ *et al.* (15) proposed that the antibody to F is important in the host defence against paramyxovirus infection, based on their finding that the antiserum to F protein of SV 5 not only neutralized viral infectivity but also inhibited the spread of infection even after it had been initiated (16).

In contrast, some monoclonal antibodies directed to various epitopes of F protein were neither neutralizing nor protective (22), whereas others were protective despite the absence of virus-neutralizing activity (14).

We studied previously the effect of rabbit polyclonal antibodies specific to HN and F proteins of NDV on the infection of cultured cells. While anti-HN serum was highly neutralizing, anti-F serum was barely so. Nevertheless, the coexistence of anti-F serum markedly enhanced the effect of anti-HN serum in restricting the spread of infection (19). In the present study, we assessed the relative contribution of anti-HN and anti-F antibodies and their cooperative effect for the protection of baby chickens from lethal infection with virulent NDV.

Materials and Methods

Embryonated Eggs and Chickens

Embryonated eggs laid by white leghorn hens were used for preparation of chicken embryo cell cultures and egg-grown NDV. The hens had been vaccinated against NDV and maintained for breeding in Chiba Prefectural Livestock Experimental Station, Chiba, Japan. The eggs laid by unvaccinated specified pathogen-free (SPF) white leghorn hens were purchased from SPF Poultry Unit, Nippon Institute of Biological Sciences, Tokyo,

Japan. Eggs were incubated at 37° C and newborn chickens hatched from the eggs were kept in flexible plastic film poultry isolators. Eight-week-old SPF chickens used for hyperimmunization with NDV or its components were purchased also from SPF Poultry Unit described above.

Cells and Virus

Madin-Darby bovine kidney (MDBK) cells were grown in Eagle's minimum essential medium (MEM) containing 5 per cent fetal calf serum and 10 per cent tryptose phosphate broth. Eleven-day-old embryonated hens' eggs were used for the preparation of monolayer cultures of chick embryo (CE) cells.

The Italien strain of NDV was used throughout the study. Seed stocks of the virus were prepared in the allantoic cavity of embryonated hens' eggs (17). Infectivity of the virus was determined by plaque assay in CE cells. Virus to be purified, either radiolabeled or unlabeled, was grown in MDBK cells. For enzyme-linked immunosorbent assay (ELISA), virus from embryonated eggs was used as antigen. Purification of virus and separation of HN and F proteins were reported previously (11).

Preparation of Antisera

Hyperimmune sera to purified Newcastle disease virions and to viral glycoproteins were prepared as follows; approximately 500 μg of u. v.-irradiated virus, HN, F, or a mixture of HN and F (HN + F) in PBS were emulsified in Freund's complete adjuvant by forced mixing. The mixture was injected intramuscularly (i. m.) into eight-week-old SPF chickens at several sites. Six weeks later the chickens received the booster injection of the respective antigens mixed with Freund's incomplete adjuvant. They were bled two weeks later.

Since chickens immunized with F protein produced low levels of anti-HN antibody, it was removed by passing about 5 ml of the serum through a column of Sepharose 4 B (Pharmacia, Uppsala, Sweden) to which HN protein had been conjugated (19). The absorption procedure reduced the hemagglutination inhibition (HI) titer to an undetectable level. The antisera were heat-inactivated at 56° C for 30 minutes before use.

Radioimmunoprecipitation and SDS-Polyacrylamide Gel Electrophoresis (PAGE)

The procedure previously described was followed (19).

HI Test

HI test was performed on microtiter trays with 0.5 per cent chicken erythrocytes and 4 hemagglutinating units (HAU) of Italien strain as antigen.

ELISA

Purified virions of egg-grown NDV at a concentration of 0.5 μg/ml was attached to the solid phase as antigen. The method described previously was followed (20) except for the use of peroxidase-conjugated IgG fraction of rabbit anti-chicken IgG serum (Cappel Laboratories, Cochranville, PA, U.S.A.). ELISA titer was expressed by the highest dilution of serum which gave an optical density (O. D.) value of 0.1.

Virus Neutralization (NT) Test

The method had been described previously (19). Neutralization titer was expressed as the highest dilution of serum that reduced the number of plaques in CE cells by 50 per cent.

Challenge of Newborn Chickens with Virulent NDV

Immunity afforded to a newborn chicken was assessed by the time interval required for the virus to kill the bird. The method had been developed originally for the comparison of virulence of various NDV strains (21), but was adapted here to the above purpose. A group

of four to five 10-day-old newborn chickens received i. m. injection of 0.1 ml each of NDV-Italien strain of appropriate dilution, and were housed in one flexible plastic film poultry isolator. The chickens were observed for signs of disease. Death usually followed the onset of the disease within 18 hours. Observation was terminated on day 10 (240 hours post infection) before the possible appearance of illness among cagemates resulting from secondary spread of infection. Mean death time (MDT) is the average time in hours.

Passive Immunization

Nine-day-old chickens were injected intravenously with 0.1 ml of the appropriate immune serum or control serum free of NDV antibody. Antisera with NT activity were injected at a dose expected to make the serum NT antibody level in the recipient birds comparable to that in immune chickens.

Antisera or normal chicken serum with little or no NT activity were used at the same dilution with anti-HN serum. Twenty-four hours later the chickens were challenged i. m. with 0.1 ml of virus containing 10 PFU/dose. The deaths of chickens were scored and the MDT was determined. The statistical significance of the difference of death time between control and test groups and between two test groups was assessed by Student's *t*-test.

Results

Maternal Antibodies in the Sera from Newborn Chickens Derived from Vaccinated Hens

Antibodies are known to be transmitted from hens to chicken embryos, and hence, to newborn chickens via egg yolk (4, 6). The degree and duration of immunity to NDV in newborn chickens hatched from fertilized eggs laid by vaccinated hens were assessed by serum antibody levels in newborn

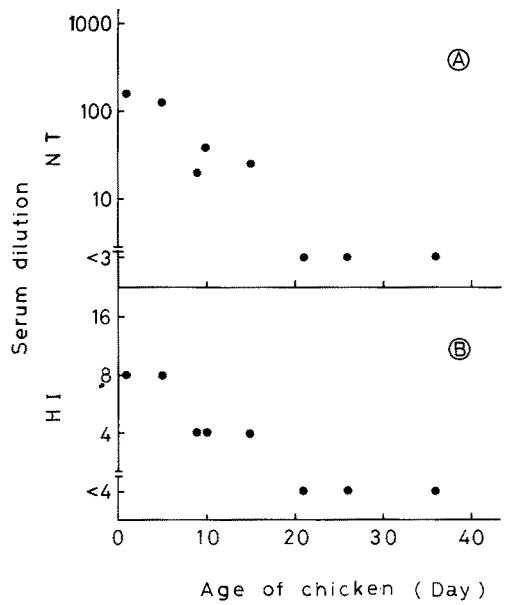

Fig. 1. Antibody levels against Newcastle disease virus in the offspring of vaccinated hens. Five to seven newborn chickens were bled at indicated times after hatching. The sera were combined and tested for neutralizing *(A)* and hemagglutination inhibiting activity *(B)*

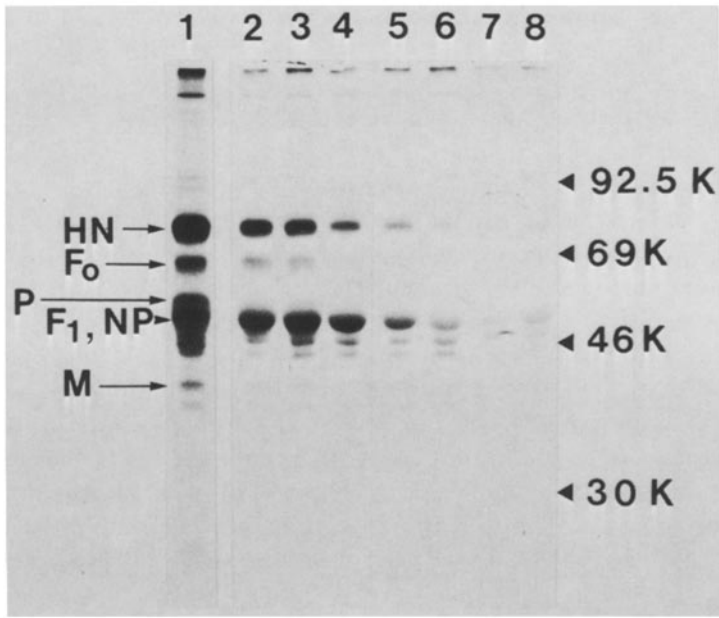

Fig. 2. SDS-PAGE of immunoprecipitates with sera from offspring of vaccinated hens. MDBK cells infected with NDV were labeled with [^{35}S] methionine from 12 to 42 hours postinfection. At the end of labeling, cells were lysed in RIPA buffer. The lysate was mixed with sera from the chickens shown in Fig. 1. Immunoprecipitates were analyzed by SDS-PAGE. *1* anti-NDV hyperimmune serum; *2* to *8* newborn chicken sera; *2* day 1; *3* day 5; *4* day 10; *5* day 15; *6* day 21; *7* day 28; *8* day 36

chickens. Fig. 1 shows the profile of NT and HI antibodies in the sera collected from chickens of various ages. Both antibodies decreased in parallel and were no longer detectable at 3 weeks of age.

The composition of maternal antibodies transmitted to the offspring in terms of target antigens was examined. Radiolabeled viral proteins were immunoprecipitated by newborn chicken sera and analyzed by SDS-PAGE (Fig. 2). All major structural proteins, HN, F, P, NP, and M, were identified in the immunoprecipitates with sera from chickens early in life. The relative intensities of viral protein bands showed that the antibody reacting with NP was most abundant, followed by that reacting with HN and F in decreasing order. Those reacting with P and M appeared to be present in much smaller amounts. The amount of HN protein in the immunoprecipitates roughly paralleled NT and HI antibody level shown in Fig. 1.

Susceptibility of Offspring of Unvaccinated and Vaccinated Hens to Newcastle Disease Virus Infection

Newborn chickens with and without maternal antibody were compared with regard to susceptibility to infection by NDV (Table 1). Since the Italien

strain was highly virulent, a dose as low as 1 PFU was sufficient to kill all SPF chickens within 4 days. The MDT was shortened with increasing dose of challenge virus. By contrast, the resistance of chickens derived from vaccinated hens was remarkable. A challenge dose nearly five orders of magnitude higher was required to give rise to an outcome comparable to that in unprotected chickens. The resistance of offspring of vaccinated hens was reproduced by passive immunization of susceptible chickens. The serum from a SPF chicken immunized with u. v.-irradiated NDV (Table 2) was administered intravenously to newborn SPF chickens in a dose that would make the serum NT antibody titer in the recipient birds equivalent to maternal NT antibody level in the offspring of vaccinated hens. That passive immunization had achieved similar levels of circulating antibody, corresponding to NT antibody titer of 1 : 40, was verified by ELISA titration of pooled sera from 5 SPF chickens which received the same dose of antiserum. Table 1 shows that a similar degree of protection was afforded by passive immunization as that due to maternal vaccination. Serum NT antibody at a titer of 1 : 40, irrespective of whether it is maternal or passively acquired, protected the chickens from otherwise lethal challenge with 10 PFU of NDV.

Table 1. *Susceptibility of baby chickens of varying immune status to challenge with virulent NDV*

Virus dose inoculated (PFU/chicken)	Mean death time[a]		
	Chickens with maternal antibody[b]	SPF chickens without antibody	SPF chickens with passively administered antibody[c]
1	–[d]	94	–
10	–	74	>240
10^2	–	70	–
10^3	>240	66	>240
10^4	>240	51	>240
10^5	98	46	107
$10^{5.8}$	90	41	–
$10^{6.8}$	66	41	–
$10^{7.8}$	45	–	–
$10^{8.8}$	27	–	–

[a] A group of four to five 10-day-old newborn chickens received intramuscular injection of NDV Italien strain and were kept for observation for 10 days (240 hours). Mean death time was calculated as described in Materials and Methods

[b] Serum neutralizing antibody titer was assumed to be approximately 1 : 40 at the time of virus challenge from the results shown in Fig. 1

[c] On the day before challenge, chickens received an intravenous injection of the serum from a chicken immunized with whole NDV in a dose which would make the serum neutralizing antibody titer in recipient birds 1 : 40

[d] Not tested

Biological Properties of Sera from Chickens Immunized with Newcastle Disease Virus and Its Glycoproteins

SPF chickens were immunized with inactivated NDV or viral glycoproteins. The sera were tested for their effects on biological properties of the virus (Table 2). Anti-HN antibody had HI and NT activity. Antiserum to F protein had no HI and very little NT activity, despite its higher antibody titer, as determined by ELISA, than that of anti-HN serum. Antiserum against the mixture of HN and F also had HI, NT, and ELISA antibody titers.

It had been shown that a low NT activity of rabbit anti-F serum was markedly enhanced by the addition of fresh guinea pig serum (19). For the chicken anti-F serum, fresh guinea pig serum had no effect, while the presence of fresh chicken serum did cause a fourfold increment in NT titer. The activation of the chicken complement system, although less prominent than in rabbit antiserum, may contribute to the life-prolonging effect of anti-F antibody *in vivo* as described below.

Table 2. *Antibody titer of chicken hyperimmune sera*

Immunizing[a] materials	Antibody titer[b]			
	HI[c]	NT[d]	CNT[e]	ELISA[f]
HN	128	3,980	3,980	63,100
F	4	13	50	316,200
HN + F	128	1,590	1,590	125,900
U.V.-irradiated NDV	256	3,980	6,310	125,900

[a] Each serum was obtained from a single chicken
[b] Reciprocals of highest serum dilution
[c] Hemagglutination inhibition test
[d] Neutralization test
[e] Enhanced neutralization test. The neutralization test was performed in the presence of fresh SPF-chicken serum at a final concentration of 1 : 342
[f] Enzyme-linked immunosorbent assay

Protection of Susceptible Newborn Chickens by Antisera Against NDV Glycoproteins

Susceptible newborn chickens were passively immunized with the hyperimmune chicken sera shown in Table 2 and then challenged by otherwise lethal NDV infection (Fig. 3). Administration of the anti-whole NDV serum provided a complete protection (see also Table 1). At a comparable NT antibody level, the antiserum against HN protein failed to protect the recipients but significantly prolonged the survival period compared with normal chicken serum (MDT 170 hours over 94 hours, $P < 0.001$). Anti-F serum, given at the same dilution as anti-HN serum, was similarly effective (MDT

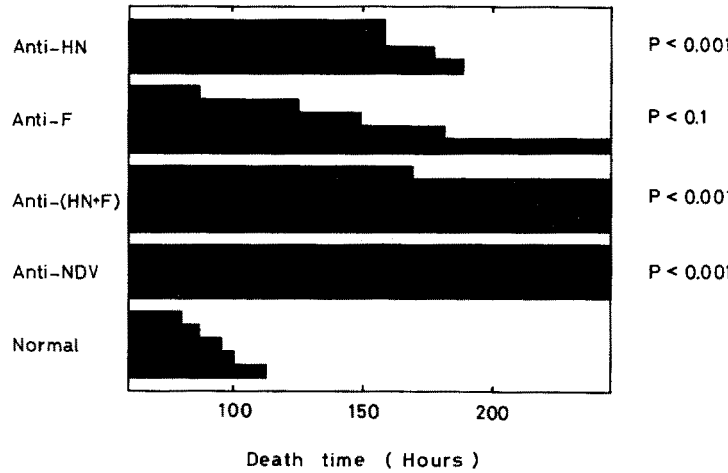

Fig. 3. Effect of passive immunization on death time. Groups of four to five 9-day-old newborn SPF chickens received intravenous injections of 0.1 ml of immune sera as shown in Table 2 or normal chicken serum. Anti-HN and anti-whole NDV sera were diluted 1 : 3, while anti-(HN + F) serum was undiluted so as to make the serum NT antibody level in the recipient birds 1 : 40. The anti-F serum with a very low NT activity and normal chicken serum were given at 1 : 3 dilution. Chickens were challenged 24 hours later with 10 PFU of NDV, Italien strain. Columns show death time of individual birds. The survival time was censored by negative-exponential transformation according to LIDDELL (13), using 0.1 and 240 hours for 0 and T, respectively. Statistical significance of the difference of death time between each test group and the control group assessed by Student's *t*-test is given at the right-hand side

170 hours, $P < 0.1$), in spite of an NT activity 100-fold lower than that of anti-HN serum. When anti-HN and anti-F sera were given simultaneously in the form of antiserum against mixed HN + F, the protection was nearly complete, similar to that seen with anti-whole NDV serum and significantly more effective than either anti-HN ($P < 0.02$) or anti-F serum ($P < 0.05$) alone.

Discussion

The present study showed that the resistance to infection in chickens hatched from vaccinated hen's eggs is reproduced by passive immunization of susceptible birds. It was thus concluded that the resistance of the former chickens is entirely due to the maternal antibody. Both maternal antibodies, transferred to offspring via egg yolk, and passively administered antiserum included anti-HN and anti-F antibodies. The protective effect of anti-HN antibody is most likely to be due to its virus neutralizing activity (8, 14, 22). By contrast, anti-F serum was much less efficient in neutralization. Nevertheless its passive administration significantly delayed the death of sus-

ceptible hosts and enhanced the protective effect of anti-HN serum. The previous (19) and present studies suggest that two mechanisms are involved in the protective effect of non-neutralizing anti-F serum.

The first is the participation of the complement system. The addition of complement enhanced virus neutralizing activity of rabbit anti-F serum markedly (19) and chicken anti-F serum to a lesser extent (Table 2). The life-prolonging effect of chicken anti-F serum (Fig. 3) is also thought to be dependent upon chicken complement. The passive administration of rabbit anti-F serum was ineffective in delaying the death of NDV-infected chickens, despite the fact that rabbit anti-HN serum was as effective as chicken anti-HN serum (data not shown). The difference between chicken and rabbit anti-F sera may be explained by the inability of the latter to activate chicken complement (7). In addition to the enhanced virus neutralization, complement may contribute to the protective effect of anti-F serum by antibody-dependent complement-mediated cytolysis of virus-infected cells. It is worth noting that the virus neutralizing and probably the life-prolonging effects of anti-HN serum, unlike those of anti-F serum, do not appear to depend upon complement.

Recent reports that passively administered non-neutralizing monoclonal antibodies protected mice from lethal alphavirus infections (5, 18) lend support to the role of complement.

The second mechanism by which anti-F serum may contribute to the protection is by restricting infection through inhibition of cell fusion. When incorporated in the agar overlay, anti-F serum, virutally ineffective in reducing plaque number or plaque size by itself, markedly potentiated anti-HN serum in reducing both plaque number and plaque size (19). The chicken anti-F serum was similarly shown to reduce plaque size when combined in an appropriate concentration with the chicken anti-HN serum (data not shown). It is likely that anti-HN and anti-F antibodies cooperate within a bird as in cell cultures toward restricting infection.

The present study showed that the antibody to F protein of NDV, although non-neutralizing, makes a significant contribution to the defence of the host against an infection of virulent NDV.

Recently, ABENES *et al.* (1) prepared monoclonal antibodies directed to F protein of NDV possessing virus-neutralizing activity. Such antibodies would be useful for further elucidation of the mechanism of protection.

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

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