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More than one component of the Newcastle disease virus particle is capable of interferon induction

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Abstract

The interferon (IFN)-inducing capacities of intact NDV virions, β -propiolactone-inactivated particles and several structural components were compared, using human PBML as the IFN producing cells. Intact and inactivated virions as well as the nucleocapsid fraction did not differ significantly in their IFN-inducing capacity. In contrast, genomic RNA as well as M protein fraction and envelopes induced IFN titres to a level of about 10% of those achieved with virions. NDV-induced IFN production could be blocked specifically by incubation with polyclonal anti-NDV-mono-clonal antibodies (mAbs) and with two of three anti-HN-mAbs, but not with anti-NDV-mAbs directed against the F, M or NP protein. In addition, IFN induction by fixed MDBK cells, expressing NDV surface proteins after infection with NDV Ulster, was inhibited by one of two anti-F-mAbs. The results suggest that the induction of IFN synthesis in human PBML is a complex process involving not only the HN protein but also the uncleaved F protein precursor, a component of the M protein fraction and – once having entered the cell – the genomic RNA.

Key words: Newcastle disease virus; Interferon induction; Poultry

1. Introduction

Newcastle disease virus (NDV) is a member of the virus family Paramyxoviridae. NDV is an important pathogen in poultry, causing respiratory and central nervous symptoms with a potentially high mortality rate. The species NDV comprises many strains with a wide range of virulence. The molecular basis thereof was found to be the cleavability of the two surface glycoproteins, the hemagglutinin-neuraminidase (HN) and especially the fusion

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(F) protein (Nagai et al., 1976, Kawahara et al., 1992). The latter is cleaved in many cell systems and thereby activated in velogenic and mesogenic strains, but not in lentogenic ones (Nagai et al., 1976). Extremely lentogenic strains, such as NDV Ulster, contain both glycoproteins in an uncleaved form (Millar et al., 1988, Nagai et al., 1977, Sato et al., 1987). The third envelope protein, the matrix (M) protein, is located on the inner side of the lipid membrane, from where it mediates the binding of the nucleocapsid to those areas of the cell membrane which contain the viral surface glycoproteins. This role in assembly of the virus is the only function known for M protein so far, but as it can be detected in the nucleus of infected cells (Faaberg and Peeples, 1988; Peeples et al., 1992), this protein is probably of further importance.

Beside its role as a pathogen for poultry the virus is well known as an excellent IFN inducer (e.g. Klein et al., 1984). This feature renders NDV a suitable model virus for studying the mechanism of IFN induction. Because of the importance of this cytokine group, especially in protecting from viral infections and tumor diseases, many attempts have been made to define the mechanism of IFN induction. However the intrinsic IFN inducer of NDV has not yet been identified. Studies published so far have been, for the most part, indirectly designed and have produced conflicting results. Meager and Burke (1972), as well as Lomniczi (1973), discuss the hypothesis that a subtle activity of the virion-associated RNA-Polymerase is responsible for IFN induction. Other authors assume that primary transcription followed by the formation of double-stranded (ds) RNA is necessary for IFN induction (Sheaff et al., 1972) or that the genomic RNA is the intrinsic inducer molecule (Gandhi and Burke, 1970, Gandhi et al., 1970, Lomniczi, 1973).

For lymphoid cells it has been shown that simply the contact between cell and virion surface is sufficient to induce IFN production, whereas in fibroblasts the virus has to enter the cell (Ito et al., 1982, Ito and Hosaka, 1983). To the best of our knowledge there is only one description of Paramyxovirus surface proteins as IFN inducers in lymphoid cells (Ito et al., 1983). The authors found that isolated Sendai virus HN protein is able to stimulate the release of low IFN titres in the supernatant of mouse spleen cells.

In this report we show that not only the HN protein, but also other NDV components are capable of inducing IFN release by mononuclear cells.

2. Materials and methods

Viruses

Three NDV strains with various degrees of virulence were used for IFN induction: Montana (velogenic), La Sota (lentogenic) and Ulster (extremely lentogenic). The first was kindly provided by Dr. Dirk Schneeganß, Inst. f. Poultry Diseases, Munich, FRG, and the third by Prof. Dr. Volker Schirmacher, DKFZ, Heidelberg, FRG (Von Hoegen et al., 1988). The virus was propagated in 10 d old embryonated eggs and purified from the allantoic fluid by sucrose gradient centrifugation. Influenza virus (sw/Germ/2/87 H1N1) and Parapoxvirus ovis (vaccine strain D 1701 strain) were used as a control.

NDV-infected, fixed cells

MDBK cells were trypsinized and infected with NDV Ulster at a multiplicity of infection (MOI) of 10 and held in suspension for 24 h at 37°C. After this the cells were washed twice in PBS, fixed on ice with 1% paraformaldehyde for 15 min and washed again twice with PBS to remove the paraformaldehyde. The expression of NDV surface proteins was checked by flow cytometry (FacScan, Becton Dickinson, primary antibody: anti-HN-mAb 10-1 or mouse anti-NDV-immune serum, secondary antibody: anti-mouse-IgG-FITC, Sigma).

NDV inactivation

Purified NDV with a titre of 4×10^8 egg infectious doses 50% (EID₅₀)/ml was incubated with 0.05% β -propiolactone (BPL) in 0.2 M Tris-HCl, pH 8.6, for 30 min at 4°C, for a further 60 min at 37°C on a magnetic stirrer and overnight without stirring at 4°C. The inactivation of the virus batch was controlled by three passages in embryonated eggs.

Isolation of NDV components

The viral envelope, the M protein fraction and the nucleocapsid were separated by the method of Scheid and Chopin (1973) with some modifications. Briefly, purified NDV was pelleted by ultracentrifugation and resuspended in 0.01 M sodium-phosphate, pH 7.4, with or without 1 M NaCl (depending on whether the M protein should detach from the nucleocapsid or not). Triton X-100 or sodium-deoxycholate (20% in the corresponding buffer) were added at a ratio of 4:1 to the amount of virus protein employed. After 1 h of incubation at room temperature, uncleaved clumped virus was sedimented by low-speed centrifugation (20 min, 10 000 g, 4°C). The supernatant was centrifuged for 1 h and 10 000 g at 4°C in order to pellet the nucleocapsids. These were resuspended in TEN buffer (10 mM Tris, pH 7.4, 50 mM EDTA, 0.1 M NaCl). The detergent was removed by incubation with SM2 beads (Biorad) from the nucleocapsid fraction and by the SM2 dialysis method (Volsky and Loyter, 1978) from the supernatant. The supernatant became turbid on formation of reaggregated envelopes, which were then collected by ultracentrifugation (1 h, 100 000 g) and resuspended in TEN buffer.

In order to obtain the M protein fraction, NDV was cleaved with Triton X-100 (2% final concentration) in high salt buffer (0.01 M sodium-phosphate pH 7.5, 1 M NaCl), and after the centrifugation steps the supernatant was dialysed against low salt buffer (0.01 M sodium-phosphate, pH 7.5). The insoluble M protein could then be sedimented at 10 000 g for 20 min.

For isolation of individual envelope proteins, envelope fractions were subjected to immune affinity chromatography. MAbs 10-1, LA 15-7 and 45, respectively, were coupled to Sepharose CL-4B according to the manufacturer's instructions. The samples were loaded in Ca and Mg-free PBS with 0.5 M NaCl. The column was washed with 6 column volumes of the same buffer. The proteins were eluted with 3 M KSCN-0.01 M Tris-HCl, pH 7.4. The chaotropic agent was removed by ultrafiltration in an Amicon cell (SM 14539 filter, MWCO 10 kDa, Sartorius).

Liposomes were prepared (Loh et al., 1979) with cholesterol, L- α -dipalmitoyl-phosphatidylcholine and L- α -phosphatidylethanolamine (Sigma, molar ratio 1.5:2:0.2, Faaberg and Peeples (1988)). Immune affinity-purified NDV envelope proteins were added to the lipid mixture in every possible combination.

The genomic RNA of NDV was isolated by phenol extraction (Sambrook et al., 1989). Lipofectin (BRL) was used to introduce the genomic RNA into peripheral blood mononuclear leukocytes (PBML) according to the manufacturer's instructions.

Antibodies

Polyvalent anti-NDV-antibodies were obtained by immunizing rabbits and mice two times with 170 and 50 μg of purified NDV La Sota respectively with a 4 week interval. A third NDV dose was applied with half the antigen amount. The first time Freund's complete adjuvant was added to the antigen, whereas the virus for the boosters was mixed with incomplete Freund's adjuvant. The animals were bled three weeks after the last booster.

Monoclonal antibodies directed against NDV were generously provided by Liu Xiufan (Xiulong and Xiufan, 1988, Weisong and Xiufan, 1991) (mAbs 10-1, L15-8, LA15-7, B4), Tomoaki Kohama (Umino et al., 1990a and b) (mAbs 142, 83, 45, 126) and Yoshiyuki Nagai (Nishikawa et al., 1987) (mAbs 201, 208).

ELISA

Microtiter-plates (nunc) were coated with 1 μg of purified virus. After blocking with 10% FCS and 0.05% Tween 20, serial twofold dilutions of antibody samples were incubated for 1 h at 37°C. The peroxidase-conjugated anti-species antibody (Sigma) was adsorbed to the first antibody during a further 30 min at 37°C. All reaction steps were followed by four washings. Tetramethylbenzidine was used to visualize the antigen-antibody-reactions. The OD was measured at 450 nm (Multiscan-photometer, Flow Laboratories).

HI-assay

The inhibition of hemagglutination was determined as described previously (Mayr et al., 1977).

Peripheral blood mononuclear leukocytes

Human PBML were separated from blood (supplied with sodium-citrate) by Ficoll-Hypaque density gradient centrifugation (Böyum, 1968) and cultured as a suspension of 2×10^6 cells/ml in RPMI with 5% FCS at 37°C and 5% CO₂.

Interferon induction

The PBML were incubated with samples of virions, virion components or virion antibody mixtures at 37°C and 5% CO₂. In the latter case serial dilutions (from 1 : 500 to 1 : 6.4×10^6) of the respective antibodies were incubated with NDV (MOI 1) for 1 h at room temperature before the mixture was added to PBML. After 24 h the supernatants were collected, UV-irradiated for 15 min for inactivation of the inducer virus, and tested for antiviral activity.

IFN assay

The IFN contents of the supernatants were determined by their antiviral activity on MDBK cells (ATCC CCL-22) against a lytic infection by vesicular stomatitis virus in a cpE inhibition test (Rubinstein et al., 1981). The NIH rhuIFN_{α11} reference preparation (Catalog-No. Gxa01-901-535, NIH-Research Reference Reagent Note No. 31, 1984) was included in each assay. The antiviral activity was shown to be IFN, as the effect of the supernatant

was blocked by incubation with an anti-IFN-serum (kindly provided by Prof. Kari Cantell, National Public Health Institute, Helsinki). In order to control whether NDV which eventually was not inactivated sufficiently by the irradiation procedure (Jacobsen et al., 1988), irradiated virus samples were tested for their protection effect in the IFN assay. Only NDV doses tenfold higher than used in the induction protocols protected the cells from cell lysis with the exception of NDV strain Montana which causes a cpE on MDBK cells, if not inactivated completely.

Gel electrophoresis

The protein types of the NDV fractions were determined in SDS-PAGE (Laemmli, 1970) followed by silver staining of the gel (Merril et al., 1981) or by Western blotting (Towbin et al., 1979). The integrity of the genomic RNA was controlled by Agarose gel electrophoresis (Sambrook et al., 1989).

3. Results

IFN induction by replicating and by non-replicating NDV

The dose-response-relationship for IFN induction of intact NDV and of BPL-inactivated NDV showed that the multiplication of NDV was not essential for triggering the IFN response of human PBML, as the titre levels did not show much variation (Table 1). The titres reached their maximal level at a virus dose of about an MOI of 1 with an IFN titre in the order of magnitude of 2000 I.U./ml. A further increase of the virus dose did not result in a higher IFN production. The IFN release dropped to background levels at virus doses between 0.01 and 0.001 MOI. The virulence of the NDV strain did not influence IFN induction significantly.

Specific inhibition of NDV-induced IFN production by anti-NDV-antibodies

Using specific blocking protocols we attempted to determine whether surface structures of NDV are involved in IFN induction. The influence of several poly- and monoclonal anti-NDV-antibodies as well as control sera and mouse ascitic fluids on IFN induction by NDV

Table 1
Ig2 IFN titres in supernatants of human PBML collected 24 h after stimulation with NDV

| NDV strain | Virus dose (MOI) | | | | |
|---------------------|------------------|-------|------|----------------|-------|
| | 10 | 1 | 0.1 | 0.01 | 0.001 |
| La Sota | 10.55 | 10.87 | 5.74 | < ¹ | < |
| Montana | 12.35 | 10.49 | 8.55 | 2.94 | < |
| Ulster | 11.13 | 8.61 | 1.28 | < | n.d. |
| La Sota, BPL-inact. | 12.28 | 9.82 | 6.65 | 0.73 | n.d. |
| Montana, BPL-inact. | 12.11 | 9.61 | 5.43 | < | < |
| Ulster, BPL-inact. | 11.71 | 8.89 | 6.16 | < | < |

¹Titre below detection limit.

n.d. = not done.

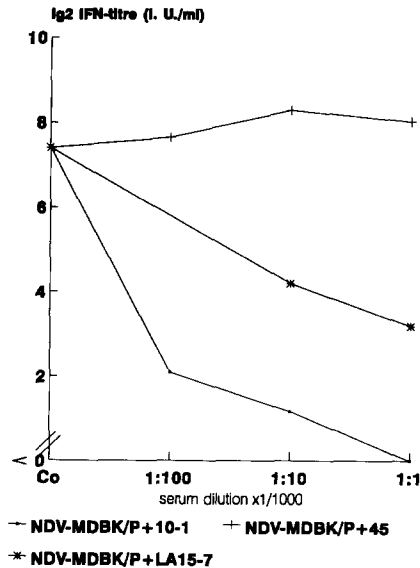


Fig. 1. Blocking capacity of the anti-NDV-mAbs on IFN induction by paraformaldehyde-fixed NDV Ulster-infected MDBK cells. NDV-MDBK/P = NDV Ulster-infected, paraformaldehyde-fixed MDBK cells. 10-1 = anti-HN-mAb. LA 15-7 = anti-F-mAb. 45 = anti-M-mAb.

was tested. The polyclonal anti-NDV-antibodies and two of three anti-HN-antibodies significantly reduced the IFN titre reached in the supernatants when compared to mock-treated virions (Table 2). On the other hand, mAbs against the F protein and against inner NDV proteins did not influence the IFN release by PBML nor did control sera or ascitic fluid. Analogous experiments were done with the control viruses influenza virus and parapoxvirus ovis (PPV). Influenzavirus was chosen as a hemagglutinin containing virus because of the effect of anti-HN-mAbs on IFN induction by NDV. PPV was included for its increased IFN induction capacity after having been incubated with anti-PPV-antibodies (Büttner, M. and Czerny, C.-P., pers. comm.). Neither anti-NDV- nor anti-PPV-antibodies nor control serum or mouse ascitic fluid reduced the IFN induction by PPV ovis. In contrast to this, IFN induction by influenza virus was blocked by the same anti-NDV-antibodies as was NDV. Although anti-NDV-antibodies inhibited IFN induction by influenzavirus only up to an antibody dilution of 1:500 significantly, whereas the IFN response after stimulation with NDV was impaired up to an antibody dilution of 1:640 000. The influence of anti-NDV-antibodies on IFN induction by influenza virus was paralleled by the crossreactivity of this virus with the anti-NDV-antibodies in ELISA and HI-assay (Table 3).

IFN induction by NDV Ulster-infected, fixed cells

The experiments described above could not exclude the possibility that IFN induction was blocked because the antibodies prevented the entry of the virus into the cell. Therefore, a corresponding approach was used with fixed MDBK cells expressing NDV Ulster surface glycoproteins. At a ratio of 0.1 infected MDBK cells to PBML the latter released IFN titres of $2^{9.67}$ I.U. IFN/ml. Non-infected fixed MDBK cells induced no IFN response. Again, the

Table 2

Specific inhibition of IFN induction by NDV, PPV ovis and influenza virus with antibodies

| Antibody | Specificity | LS | LS-BPL | M | M-BPL | U | U-BPL | Flu | PPV |
|----------------|-------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------|
| K1 | NDV | -0.691^a | n.d. | -0.979^a | n.d. | -0.725^c | n.d. | n.d. | -0.017 |
| K2 | NDV | -0.620^a | -0.866^a | -0.504 | -0.682^c | -0.856^b | -0.816^b | -0.870^a | -0.126 |
| IgG/K2 | NDV | -0.490^b | n.d. | n.d. | n.d. | n.d. | n.d. | -0.927^b | -0.171 |
| K3 | NDV | -0.645^b | -0.521 | -0.450 | -0.636^c | n.d. | n.d. | n.d. | 0 |
| Ms | NDV | -0.763^a | n.d. | -0.502^a | -0.967^c | -0.784 | -0.419 | -0.889^b | n.d. |
| PPV-KS | PPV ovis | 0.073 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.125 |
| IgG/ PPV-KS | PPV ovis | 0 | 0.116 | -0.588 | 0.351 | -0.888 | n.d. | n.d. | n.d. |
| Flu-FS | Flu H1N1 | -0.626 | 0.357 | -0.180 | -0.383 | -0.394 | n.d. | -0.657 | 0.504 |
| Flu-SS | Flu H1N1 | -0.054 | n.d. | n.d. | n.d. | n.d. | n.d. | -0.054 | n.d. |
| 10-1 | HN | -0.635^a | -0.621^b | -0.645^a | -0.121 | -0.569^b | -0.773^b | -0.982^a | -0.176 |
| 142 | HN | -0.390^b | -0.704^b | -0.395^c | -0.286 | -0.714^a | -0.484^b | -0.907^c | n.d. |
| L15-8 | HN | 0.016 | -0.028 | -0.098 | 0.052 | -0.032 | -0.386 | -0.084 | n.d. |
| LA 15-7 | F | 0.195 | -0.105 | -0.028 | 0.282 | 0.222 | -0.209 | -0.326 | n.d. |
| 83 | F | -0.218 | -0.281 | n.d. | -0.607 | -0.098 | -0.031 | 0.116 | n.d. |
| B4 | NT | -0.149 | -0.223 | -0.045 | 0.066 | -0.169 | n.d. | -0.222 | n.d. |
| 45 | M | 0.117 | 0.050 | -0.225 | n.d. | -0.092 | 0.013 | n.d. | -0.336 |
| 126 | M | 0.042 | -0.047 | 0.030 | 0.135 | 0.128 | -0.111 | -1.00 | 0.384 |
| 201 | M | -0.085 | 0.154 | -0.006 | -0.012 | -0.161 | 0.019 | -0.309 | n.d. |
| 208 | M | 0.144 | 0.126 | 0.032 | 0.086 | 0.088 | 0 | 0.106 | -0.156 |
| 5B4/2F2 | MVA | 0.336 | 0.120 | 0.406 | -0.700 | 0.322 | 0.514 | 0.728 | -0.588 |
| 0-Asc.- | | -0.097 | -0.199 | n.d. | n.d. | 0.479 | n.d. | n.d. | 0.578 |

Criteria for a blocking of IFN induction:

1. Reduction of the IFN titre after virus incubation with 1 : 500 diluted antibody with respect to the control by more than two dilution steps (indicated as bold type numbers).
2. Significant relation between log IFN titre and antibody dilution in F-test. a = $P < 0.001$. b = $P < 0.01$. c = $P < 0.05$.
3. Degree of the relation expressed as correlation coefficient r. The closer the value of r is to -1 the stronger is the reduction of the virus-stimulated IFN release by the respective antibody.

LS = La Sota; M = Montana; U = Ulster; Flu = influenza virus; PPV = parapoxvirus ovis.

K1, 2, 3 = rabbit anti-NDV immune sera; IgG/K2 = IgG, purified from K2; Ms = mouse anti-NDV immune serum; PPV-KS = rabbit anti-PPV immune serum; Flu-FS = ferret anti-influenza virus immune serum; Flu-SS = pig anti-influenza virus immune serum; 10-1, 142, L 15-8 = monoclonal antibodies against HN protein; 83, LA 15-7 = monoclonal antibodies against F protein; 45, 126, 201, 208 = monoclonal antibodies against M protein; B4 = monoclonal antibodies against NP protein; 5B4/2F2 = monoclonal antibodies against vaccinia-virus strain MVA (Czerny and Mahnel, 1990). Kindly provided by Claus-Peter Czerny. O-Asc. = ascitic fluid of nonimmunized Balb/c mice.

Table 3

Crossreactivity of anti-NDV- and anti-influenza virus antibodies in ELISA and HI-assay. Anti-PPV-antibodies and control ascitic fluid did not bind to either virus

| ELISA-Titer | Antibody | |
|-------------|-------------------------|-----------------|
| | NDV La Sota × 1/1000 | influenza virus |
| K1 | 1:640 | 1:2560 |
| K2 | 1:2560 | 1:905 |
| IgG/K2 | 1:1810 | 1:1280 |
| K3 | 1:640 | 1:2560 |
| Ms | 1:640 | 1:640 |
| 10-1 | 1:5120 | 1:2560 |
| 142 | 1:2560 | 1:2560 |
| HI-Titer | | |
| K1 | 1:1280 | 1:20 |
| K2 | 1:320 | 1:80 |
| K3 | 1:160 | 1:80 |
| Flu-FS | 1:80 | 1:160 |
| Flu-SS | 1:80 | 1:40 |

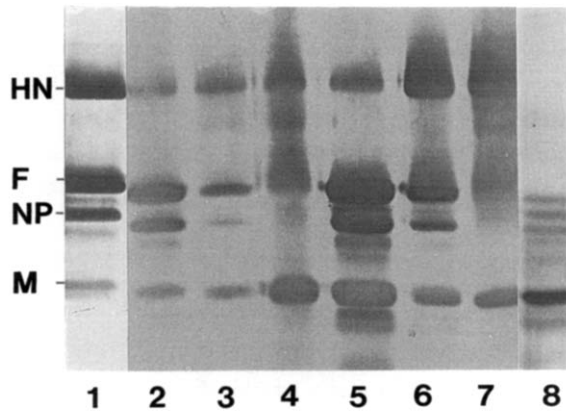


Fig. 2. Protein types in the different NDV fractions. Lane 1–7 (Western blot, proteins detected by anti-NDV-rabbit immune serum, secondary antibody: anti-rabbit-IgG-HRP, substrate: α -Chloro-naphthole). Lane 1: NDV Virus disruption with deoxycholate; Lane 2: uncleaved, clumped virus (pellet after low speed centrifugation of detergent-treated virus). Lane 3: nucleocapsid fraction (pellet after ultracentrifugation). Lane 4: envelope fraction. Virus disruption with Triton X-100: Lane 5: uncleaved, clumped virus. Lane 6: nucleocapsid fraction. Lane 7: envelope fraction. Lane 8 (silver stained gel): M protein fraction.

IFN induction was reduced to undetectable levels by the anti-HN-mAb 10-1, but was, in contrast to the results with virions, also blocked by the anti-F-mAb LA 15-7 (Table 2). The anti-M-mAb 45 did again not influence IFN production. As MDBK cells don't cleave NDV

Table 4

Ig2 IFN titres in supernatants of human PBML collected 24 h after stimulation with NDV components. All stimulation protocols were repeated at least three times unless otherwise indicated

| | $\mu\text{g protein}$ | | | | virus disrupted by | | |
|-----------------------|-----------------------|-------|-------|-------|--------------------|------|-------|
| | 50 | 5 | 0.5 | 0.05 | | | |
| envelope fraction | 5.64 | 7.32 | 3.32 | 3.32 | Deoxycholate | | |
| | 8.41 | 6.38 | 3.36 | 2.35 | Triton X-100 | | |
| M protein fraction | 3.92 | 7.39 | 4.03 | < | Triton X 100 | | |
| | dilution | | | | | | |
| | 1:100 | 1:200 | 1:400 | 1:800 | | | |
| nucleocapsid fraction | 11.19 | 12.45 | 9.92 | 1.25 | | | |
| | $\mu\text{g RNA}$ | | | | | | |
| Exp. 1 | 1 | 0.5 | 0.25 | 0.125 | 0.063 | 0.03 | 0.015 |
| RNA | 7.4 | 8.41 | 7.4 | 6.98 | 7.40 | 7.40 | 3.35 |
| | $\mu\text{g RNA}$ | | | | | | |
| Exp. 2 | 0.4 | 0.2 | 0.1 | 0.05 | | | |
| RNA | 5.86 | 5.86 | 5.37 | 5.86 | | | |

Ulster glycoproteins, these data suggest that in PBML in addition to HN the uncleaved F protein precursor plays a role in IFN induction.

IFN induction by NDV components

Subsequent to the indirect evidence that the NDV surface structures are capable of triggering the IFN response, the different virus components were isolated (Table 5, Fig. 2) and tested for their IFN inducing capacity (Table 4). Reaggregated envelopes and the matrix protein fraction – the first with hemagglutinating activity and both not infectious – were capable of inducing IFN, although the titres reached only 10% of those achieved in stimulation protocols with virions. The non-hemagglutinating, but infectious nucleocapsid fraction induced an IFN response at the same level as intact NDV particles. Electron microscopy of the nucleocapsid fraction showed that this activity was not due to a relevant contamination with intact virions (Table 5). The NDV envelope proteins including M protein were isolated by immune affinity chromatography. The isolated NDV proteins were not able to provoke a detectable IFN release. This was also the case after their incorporation into liposomes. In contrast, the isolated NDV RNA was also capable of stimulating IFN production, provided that it was introduced into the PBML by lipofectin. NDV genomic RNA or lipofectin alone did not induce measurable IFN release, nor did lipofectin enhance IFN induction by NDV.

Table 5
Biological features of three different separated NDV fractions

| procedure for preparation of NDV components | HA | infectivity (EID 50/ml) | morphology (electron microscopy) | protein profile (Western blot) | protein contents ($\mu\text{g/ml}$) |
|--|-------------------------------|-------------------------|---|--------------------------------|---------------------------------------|
| envelope fractions deoxycholate treatment | 1:256 | no | aggregates, ca. 15 nm diameter | HN, F, M | 272 |
| Triton X-100 treatment without final ultracentrifugation | < 1:128, hemolysis up to 1:64 | no | large, polymorph multilamellar vesicles | HN, F, M | 373 |
| M-protein fraction method of Scheid and Choppin (1973) | < 1:2 | no | n.d. | silver staining: mainly M | 287 |
| nucleocapsid fractions deoxycholate- or Triton X-100-treatment | < 1:2 | > 10 ⁵ | nucleocapsids, no intact virions, several empty envelopes | HN, F, NP, P, M | ca. 20 |

HN = hemagglutinin-neuraminidase protein.

F = fusion protein.

M = matrix protein.

EID50 = egg infectious doses 50%.

4. Discussion

In this paper we demonstrate that in PBML cultures infected with NDV neither virulence nor viral replication is essential for the stimulation of IFN α release. The contact between IFN producing cells and IFN-inducing structure(s) is sufficient. One of these IFN inducers is the NDV HN protein. The same was shown for Sendai virus (Ito and Hosaka, 1983). In addition, we found participation of the F protein precursor, probably the M protein and the isolated genomic RNA in IFN induction.

Replicating and inactivated NDV stimulated IFN production by PBML to the same degree irrespective of strain differences. This is in accordance with results obtained with another IFN stimulation protocol using mouse spleen cells (Ito *et al.*, 1982, Ito and Hosaka, 1983). The same authors describe for mouse L cells, however, that only NDV that replicated in these cells induced an IFN synthesis (Ito *et al.*, 1982), suggesting that different mechanisms of IFN induction exist in fibroblasts and in lymphoid cells.

As BPL-inactivation does not destroy the hemagglutinating activity of NDV, it is likely

that BPL-inactivated NDV is capable of entering a host cell. Therefore an RNA synthesis from not alkylated sequences cannot be formally excluded. The resulting RNA, the polymerase activity or the NDV genome itself may theoretically provide enough stimulus to start IFN-synthesis.

To further characterize the surface structures involved in IFN stimulation, the influence of anti-NDV-immune sera and several different anti-NDV-mAbs on IFN induction was determined. Polyclonal anti-NDV-antibodies and two of three anti-HN-mAbs blocked IFN production. As there are neutralizing mAbs without effect on IFN induction (L 15-8 and the anti-F-mAbs, (Weisong and Xiufan, 1991, Umino et al., 1990)) and as NDV glycoproteins expressed on the surface of fixed cells in which the elution of infectious virus is excluded were capable of stimulating IFN production (see also Capobianchi et al., 1988, Lebon, 1985, Jestin and Cherbonnel, 1991, Charley and Laude, 1988), the induction process is not bound to the entry of the virus into the cell. The inhibitory activity of anti-F-mAb which recognizes both cleaved and uncleaved F protein (Xiulong and Xiufan, 1988) is restricted to virus containing an uncleaved F protein. This indicates that an epitope of the F protein precursor which is missing on the mature protein plays a role in IFN induction.

Two lines of control experiments demonstrated that the antibody-mediated inhibition of IFN induction is a specific event. Firstly, control sera or ascitic fluids showed no inhibiting activity. Secondly, anti-NDV-antibodies exerted no inhibitory effect on IFN stimulation mediated by a poxvirus (PPV ovis), whereas they do inhibit IFN production induced by a closely related orthomyxovirus (influenza virus). The latter result was to be expected, if inhibition is due to a specific antigen antibody reaction, as the anti-NDV-antibodies cross-react with influenza virus in an analytic (ELISA) as well as in a functional (HI) assay. We cannot explain, why anti-influenza virus antibodies do not influence IFN induction by NDV. Perhaps the latter recognize epitopes necessary for hemagglutination, but not involved in IFN induction.

The IFN induction protocols with isolated envelopes, matrix protein and nucleocapsid fractions revealed that they all had IFN inducing activity. It is unlikely that there is a single type of inducer molecule which contaminated the different virion fractions, as the properties crucial for IFN induction varied depending on the individual components tested: For efficient stimulation of an IFN response envelope proteins had to be aggregated into complexes and these had to retain hemagglutinating activity.

In contrast, the matrix protein fraction which consisted mainly of aggregated M protein, did not hemagglutinate, but still induced IFN. In this context it is interesting that M protein was found in the nucleus of NDV-infected cells (Faaberg and Peebles, 1988).

The isolated nucleocapsid fraction was still infectious. But the IFN-inducing activity of the nucleocapsid fraction was independent of its infectivity, because nucleocapsids isolated after deoxycholate-disruption of NDV (instead of the treatment with Triton X-100) were infectious, but did not induce IFN. The envelope proteins stained in the immunoblot were derived from empty envelopes in the nucleocapsid fraction. These could not be the sole source of IFN-inducing components, as their concentration in the fraction was lower than the minimal protein amount of envelope fraction required for a measurable IFN release. Besides, the nucleocapsid fraction showed no HA activity.

In order to test the HN, F and M proteins separately, these proteins were isolated by immune affinity chromatography. None of them – alone or integrated into liposomes in

either combination – could provoke a measurable IFN response. A possible explanation could be that the purification procedure destroyed the IFN-inducing activity. Thus, a direct proof of IFN induction by one type of NDV protein could not be obtained.

On the other hand, it was possible to achieve IFN production after introduction of NDV genomic RNA into PBML. As RNA added to PBML without prior incubation with lipofectin did not induce IFN, it was either degraded before reaching the cells or was not able to enter them. This does not necessarily demonstrate that the released IFN was induced by the [–]-RNA of the regular NDV genome. Paramyxovirus stocks have been reported to contain a mixture of minus and plus stranded genomes (Portner and Kingsbury, 1970), indicating that the RNA strands of different polarity can form dsRNA. Also the possibility of intramolecular dsRNA formation is not excluded. This dsRNA might induce IFN. Furthermore, viral proteins synthesized from [+]-genome by cell enzymes could lead to IFN synthesis.

5. References

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