Influence of Viral Envelope on Newcastle Disease Virus Infection¹

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The adsorption characteristics of Newcastle disease virus (NDV) propagated in chicken cells (NDV-C) and in human cells (NDV-H) were examined. Adsorption experiments performed at different temperatures indicated that virus propagated in a particular cell infected that cell type more readily than did virus propagated in a different host. For example, NDV-C was more efficient in initiating infection of chicken cells at 22°C than was NDV-H; the reverse was true when human cells were employed. The results indicate that infection of susceptible cells by NDV is influenced by the host cell in which the virus was propagated. The data also suggest that NDV may be useful in studies on homologous and heterologous membrane-membrane interactions.

Newcastle disease virus (NDV), a paramyxovirus, possesses an inner nucleocapsid surrounded by a lipoprotein envelope derived, in part, from the host cell. That NDV and related myxoviruses acquire structural material from the host cells in which they are propagated is well established from serological studies (11, 21), electron microscope observations (17), and chemical analyses of similarities in host membrane and viral envelope material (5, 8, 9, 10). This intimate association of host membrane components with virus has been suggested as an explanation for various changes (e.g., plaque morphology, density) observed in NDV when grown in different host cells (2, 3, 22).

We have shown that host components in the NDV envelope influence the induction of early polykaryocytosis, a phenomenon related to the initial stages of NDV infection (24). In the present study, an attempt was made to examine the early interaction of NDV propagated in chicken and human cells with their respective host cells, and to determine whether the previous virus host influenced the infectious process.

MATERIALS AND METHODS

Cells. HeLa cells, obtained from W. A. Cassel, were grown in Eagle minimal essential medium (MEM) containing 5% calf serum. Chicken embryo fibroblasts (CEF) were prepared from 10-day-old embryos by conventional procedures (18); MEM was used as growth medium. For the experiments and assays to be described, secondary cultures of CEF were used.

Viruses. The OS strain of NDV (1) was used. The original virus suspension was obtained as an allantoic fluid suspension, and from this two virus stocks were prepared as follows. (i) Ten-day embryonated eggs were inoculated with 200 to 500 hemadsorption focal units (HFU) per egg. Infected eggs were incubated at 37°C for 40 to 44 h prior to collection of allantoic fluid. Virus so prepared was designated NDV-C. (ii) HeLa cells were infected with virus at an input multiplicity of 1 HFU/cell. Virus harvested after 24 h at 37°C was designated NDV-H. The ratio of infectivity to hemagglutinating units was the same for both virus stocks. The density of NDV-C and NDV-H was determined by equilibrium sedimentation in CsCl and found to be 1.18 and 1.25 g/ml, respectively (24).

Virus suspensions utilized in certain experiments were dialyzed against several changes of distilled water at 4°C for 24 h. This procedure effectively removed most of the ions present in the original suspension as indicated in Results. There was no decrease in virus infectivity after dialysis.

Virus purification. Virus was precipitated from fluids by the addition of solid polyethylene glycol (Carbowax 6000) to a final concentration of 6% (wt/vol) (14). The virus was suspended in water and banded in a preformed gradient of 2 to 45% potassium tartrate (13). The gradient was centrifuged in an SW65 rotor in a model L2-65B Spinco ultracentrifuge at 42,500 rpm for 105 min at 4°C. Virus was collected from a sharp band and dialyzed against several changes of phosphate-buffered saline (PBS, pH 7.2) prior to use. This procedure was used for both NDV-C and NDV-H for some experiments (see Results).

Infectivity assays. NDV suspensions were assayed on either HeLa or CEF cells as required. Virus titers were determined by a hemadsorption focus assay (1). For the assay on HeLa cells, a fluid overlay was utilized and plates were incubated at 37°C for 24 h.
before the number of hemadsorption foci were counted. A solid overlay consisting of equal parts of 1.8% agar (Difco) and twice-concentrated MEM with 5% calf serum (final concentration in overlay mixture) was used in assays on CEF. Titrations on CEF were incubated at 37 C for 48 h prior to determining the number of HFU. A 60-min adsorption period was used in both assay procedures. The hemadsorption method allows better definition of an infectious center or plaque than do conventional staining procedures. All virus titers are expressed in terms of HFU.

Experimental procedure. Two methods were employed to study infection of cell monolayers by NDV.

(i) In the first approach the appearance of hemadsorption foci after various intervals of contact between virus and cells was studied. Virus (0.2 ml) was added to cell monolayers in 60-mm plastic dishes and allowed to adsorb for a suitable period of time. The interaction was stopped by the addition of 2.0 ml of MEM to each dish. This medium was quickly removed and replaced with 5.0 ml of fresh MEM (for HeLa cells) or 5.0 ml of agar overlay mixture (for CEF). The cultures were incubated at 37 C, and the number of HFU was counted at 24 h (HeLa cells) or 48 h (CEF). (ii) The disappearance of virus from the inoculum was used as a measure of virus attachment to cell monolayers. Virus (0.2 ml) was added to each culture. At various intervals, 0.1 ml of the supernatant fluid was removed from the dish and then diluted appropriately. Unattached virus in the supernatant fluid was assayed on a second set of cultures as described above.

RESULTS

Adsorption of NDV in various media. Experiments were performed to compare the adsorption of NDV to HeLa cells in several media. Dialyzed virus suspensions were diluted in MEM, 0.25 M sucrose, and 0.25 M sucrose containing NaCl or CaCl2. The diluted virus was allowed to adsorb to cell monolayers previously washed with the above diluents. After a 60-min adsorption period at 37 C the unadsorbed virus was removed and fresh MEM was added to all dishes. The results are shown in Table 1. It is evident that efficient adsorption of NDV did not occur in sucrose. Addition of CaCl2 and NaCl greatly facilitated attachment, confirming the observation that ions are necessary for the initial interaction of NDV with cells (12). In our experiments, MEM was found to be a better adsorption medium than the other diluents. Both NDV-C and NDV-H behaved similarly with respect to adsorption in the various diluents. The MEM was also found to be superior to PBS as an adsorption medium (unpublished observations).

Effect of adsorption temperature on NDV infection of HeLa cells. Infection of either HeLa cell or CEF monolayers with NDV results in the formation of discrete foci of hemadsorbing cells. The number of such foci in HeLa cells as a function of adsorption temperature at various time periods is presented in Fig. 1. All data were normalized to the 60-min point, i.e., the 60-min value was taken as 100%. The absolute amounts of virus added to cell monolayers were similar for all experiments and usually fell within the range of 100 to 200 HFU per dish. The ability of NDV-C and NDV-H to initiate focus formation on HeLa cells preceded at the same rate at 37 C. At lower temperatures NDV-H infected HeLa cells more readily than did NDV-C. The relationship between adsorption temperature and HFU is illustrated in Fig. 2 in which 60-min values from several experiments are plotted. It is evident that NDV-C initiates infection of HeLa cells poorly at the lower adsorption temperatures.

The relationship between the appearance of hemadsorption foci in HeLa cells and the disappearance of virus from the supernatant at 37 C is presented in Fig. 3. As expected, the amount

| Expt | Medium* | NDV-C | NDV-H |
|------|---------|-------|-------|
|      | HFU<sup>a</sup> | Maximum (%) | HFU<sup>a</sup> | Maximum (%) |
| 1    | MEM     | 143   | 100   | 100   | 100   |
|      | CaCl₂   | 92    | 64    | 78    | 78    |
|      | Sucrose | 7     | 5     | 14    | 14    |
| 2    | MEM     | 201   | 100   | 428   | 100   |
|      | NaCl    | 100   | 50    | 270   | 63    |
|      | Sucrose | 6     | 3     | 28    | 7     |

<sup>a</sup>The sucrose concentration was 0.25 M; CaCl₂ and NaCl were added to the sucrose solution in a final concentration of 0.1 M.

* Average number of plaques per triplicate dishes.

Fig. 1. Effect of adsorption temperature on the appearance of HFU in HeLa cells. (Left) NDV-H; (right) NDV-C.
of unattached virus decreased with time, and the number of HFU increased in a nearly reciprocal fashion. Further, NDV-C and NDV-H did not differ at this temperature. Since the number of hemadsorption foci formed by NDV-C at an adsorption temperature of 22°C was much less than for NDV-H, we examined the rate of removal of virus from the supernatant at this temperature (Fig. 4, left). It is clear that the rate of virus attachment to HeLa cells at 22°C is the same for NDV-C and NDV-H. It is also noteworthy that the rate of attachment of NDV-C and NDV-H at 22 or 37°C is not significantly different (Fig. 4, right). It would appear, therefore, that NDV-C and NDV-H attach to HeLa cells at the same rate but the ability of NDV-C to complete the infectious cycle is impaired.

The medium in which NDV was frozen had no effect on the behavior of the virus. When NDV-C was frozen in HeLa cell extracts the number of HFU was not increased on HeLa cell monolayers at a 22°C adsorption temperature (Table 2). Similarly, NDV-H frozen in allantoic fluid did not display a decrease in HFU under the same adsorption conditions. If the suspending media were influencing the differences in HFU observed at 22°C, this experiment should have revealed this. Further support that the initial suspending medium is not involved is presented later.

**Effect of adsorption temperature on NDV infection of CEF.** The data presented thus far suggest that NDV-H infects HeLa cells more

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**TABLE 2. Effect of suspending medium on the number of HFU**

| Virus   | Suspending medium* | HFU/dish* |
|---------|--------------------|-----------|
| NDV-H   | MEM                | 188       |
| NDV-C   | Allantoic fluid    | 192       |
|         | MEM                | 113       |
|         | HeLa cell extract  | 100       |

*NDV-H was mixed with an equal volume of allantoic fluid and frozen at −20°C for 24 h before dilution and plating on cell monolayers. The same procedure was employed for NDV-C except that HeLa cell extract prepared by freeze-thaw (2 ×) of culture was substituted for allantoic fluid.

*Adsorption at 22°C, average of triplicate dishes.
readily at 22 C than does NDV propagated in chick cells. Reciprocal experiments were performed on chick cells to test whether NDV-C behaves similarly in its homologous host cell. The relationship between adsorption temperature and HFU on CEF is plotted in Fig. 5. Again, 60-min values are plotted with the 37 C values taken as 100%. The results indicate a relationship identical to that shown in Fig. 2 in that the homologous virus infects its host cell more readily than the heterologous virus. In this case, NDV-C was able to initiate focus formation more readily than NDV-H. In an attempt to eliminate the possibility that components in the medium were responsible for the effects described, the same experiment was performed with purified NDV-C and NDV-H. The results shown in Fig. 6 are identical with those for unpurified stock virus.

**DISCUSSION**

The entry of enveloped viruses into susceptible cells is thought to occur either by an engulfment process ("viropexis") in which the virus is enclosed in a portion of the cell membrane and is subsequently transported to the interior of the cell (4, 19, 20), or by a process of membrane interaction in which the envelope of the virus fuses into the cellular membrane with resulting liberation of the nucleocapsid (6, 7, 15, 16). It is probable that both processes are operative. Homologous or heterologous cell components in the viral envelope could influence these entry mechanisms.

The data suggest that the cell on which NDV is grown can influence the early stages of virus infection. NDV suspensions from different hosts appear to infect cells with the same facility at 37 C. When virus attaches at lower temperatures, however, subsequent events leading to fruitful infection do not occur as readily with virus propagated on a heterologous host cell. The results are apparently not due to differences in attachment of virus to cells since the amount of NDV removed from the supernatant was the same for both viruses at 37 C or 22 C. It is known that attachment of NDV to cells is not temperature dependent (12). It is unlikely that events after entry are involved since all cultures were incubated at 37 C after the adsorption period.

If the uptake of NDV by cells occurs only by phagocytosis, the kinetics of entry of NDV-C and NDV-H should be similar at a given temperature. Less uptake of both viruses would be expected at 22 C, a temperature at which phagocytosis is decreased. The inefficiency of "heterologous" virus entry at the lower adsorption temperature (22 C) suggests that phagocytosis may not be the only process operative. It is possible that the interaction of NDV with its homologous host cell may be more favorable for fusion of the viral envelope with the cell membrane because of similarities in lipid composition (9, 23). When homologous NDV interacts

**Fig. 6. Relationship between adsorption temperature and HFU by purified NDV in CEF. Circles, NDV-C; squares, NDV-H.**
with its host cell, the complementary nature of the lipids may facilitate integration of the envelope with the cellular membrane.

On a practical level, the results indicate that caution must be exercised in choosing adsorption conditions with parainfluenza viruses propagated on different hosts. Whether other enveloped viruses, e.g., herpesviruses, display the same behavior is not known. The data also suggest that enveloped viruses such as NDV may be useful in studies on homologous and heterologous membrane-membrane interactions.

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LITERATURE CITED

1. Ash, R. J., and H. C. Bubel. 1966. A method for the rapid assay of Newcastle disease virus. Nature (London) 211:891-892.
2. Drake, J. W., and P. A. Lay. 1962. Host-controlled variation in NDV. Virology 17:56-64.
3. Durand, D. P., and A. Eisenstark. 1962. Influence of host cell type on certain properties of NDV in tissue culture. Amer. J. Vet. Res. 23:338-342.
4. Fazekas de St. Groth, S. 1948. Viropexis, the mechanism of influenza virus infection. Nature (London) 159:294-295.
5. Frommhagen, L. H., C. A. Knight, and N. K. Freeman. 1959. The ribonucleic acid, lipid, and polysaccharide constituents of influenza virus preparations. Virology 8:176-197.
6. Heine, J. W., and C. A. Schnaitman. 1969. Fusion of vesicular stomatitis virus with the cytoplasmic membrane of L cells. J. Virol. 3:619-622.
7. Hoyle, L. 1962. The entry of myxoviruses into the cell. Symp. Quant. Biol. 27:113-121.
8. Hoyle, L., and S. P. Davies. 1961. Amino acid composition of protein components of influenza A. Virology 13:53-57.
9. Kates, M., A. C. Allison, D. A. J. Tyrrell, and A. T. James. 1961. Lipids of influenza virus and their relation to those of the host cell. Biochim. Biophys. Acta 52:455-466.
10. Klenk, H.-D., and P. W. Choppin. 1969. Lipids of plasma membranes of monkey and hamster kidney cells and of parainfluenza virions grown in these cells. Virology 38:255-268.
11. Knight, C. A. 1946. Precipitin reactions of highly purified influenza viruses and related materials. J. Exp. Med. 83:281-294.
12. Levine, S., and B. P. Sagik. 1956. The interactions of Newcastle disease virus (NDV) with chick embryo tissue culture cells: attachment and growth. Virology 2:57-68.
13. McCrea, J. F., R. S. Epstein, and W. H. Barry. 1961. Use of potassium tartrate for equilibrium density-gradient centrifugation of animal viruses. Nature (London) 189:220-221.
14. McSharry, J., and R. Benzing. 1970. Concentration and purification of vesicular stomatitis virus by polyethylene glycol "precipitation." Virology 40:745-746.
15. Morgan, C., and H. M. Rose. 1968. Structure and development of viruses as observed in the electron microscope. VIII. Entry of influenza virus. J. Virol. 2:925-936.
16. Morgan, C., H. M. Rose, and B. Mednis. 1968. Electron microscopy of herpes simplex virus. I. Entry. J. Virol. 2:507-516.
17. Morgan, C., H. M. Rose, and D. H. Moore. 1956. Structure and development of viruses as observed in the electron microscope. III. Influenza virus. J. Exp. Med. 104:171-182.
18. Negroni, G. 1964. Tissue culture techniques, p. 328-358. In R. J. C. Harris (ed.), Techniques in experimental virology. Academic Press Inc., New York.
19. Silverstein, S. C., and P. I. Marcus. 1964. Early stages of Newcastle disease virus-HeLa cell interaction: an electron microscopic study. Virology 23:370-380.
20. Simpson, T. E., T. R. Hauke, and S. Dales. 1969. Viropexis of vesicular stomatitis virus by L cells. Virology 37:285-290.
21. Smith, W., G. Belyavin, and F. W. Sheffield. 1955. The host tissue component of influenza viruses. Proc. Roy. Soc. Ser. B 143:504-522.
22. Stenback, W. A., and D. P. Durand. 1963. Host influence on the density of Newcastle disease virus (NDV). Virology 20:545-551.
23. Tiffany, J. M., and H. A. Blough. 1969. Fatty acid composition of three strains of Newcastle disease virus. Virology 37:492-494.
24. Young, N. P., and R. J. Ash. 1970. Polykaryocyte induction by Newcastle disease virus propagated on different hosts. J. Gen. Virol. 7:81-82.