Studies on the Cytopathogenicity of Newcastle Disease Virus: Relation Between Virulence, Polykaryocytosis and Plaque Size

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(Accepted 17 December 1970)

SUMMARY

The cytopathic effects produced by seven strains of Newcastle disease virus grown in chick embryo cell culture were examined. The principal form of cytopathic effect involved the formation of multinucleate cells (polykaryocytes) by cell fusion. The capacity of the different Newcastle disease virus strains to induce cell fusion was related directly to their virulence for chicks and fertile eggs. The virulent (velogenic) strains HERTS, WARWICK and TEXAS produced significantly greater polykaryocytosis than the mesogenic strain BEAUDETTE C which, in turn, produced greater polykaryocytosis than the avirulent (lentogenic) vaccine strain F. The lentogenic strains QUEENSLAND and ULSTER failed to produce detectable cytopathic effects. Distinct morphological differences were noted in the polykaryocytes produced by the different strains. The ability to form plaques and plaque size in chick embryo monolayers was also related to the virulence of the virus strains.

INTRODUCTION

Newcastle disease virus (NDV) provides a good model for the investigation of virus virulence because of the many stable and well-characterized strains available for study (Waterson, Pennington & Allan, 1967). Another useful feature of this virus is that the virulence of strains in vivo appears to be paralleled by the ability of the same strains to destroy and damage cells when grown in vitro. Similarly, strains which are avirulent in vivo generally fail to produce cytopathic effects (CPE) in vitro. Schloer & Hanson (1968) found that the ability of NDV strains to destroy cells and produce plaques was confined to strains which were virulent for chicks and embryonated eggs. Reeve & Alexander (1970) confirmed this finding, and also showed that the ability to induce polykaryocytosis was confined to their more virulent strains. Thus, to some extent, factors associated with NDV virulence can be examined using in vitro systems (Waterson et al. 1967; Reeve et al. 1970b).

Polykaryocytosis is the initial principal form of CPE produced by NDV strains grown in cell culture (Bankowski, 1964; Johnson & Scott, 1964; Kohn & Fuchs, 1969; Reeve & Alexander, 1970). However, it is important to distinguish between the two forms of polykaryocytosis induced by NDV. The first, called ‘fusion from without’ (Bratt & Gallaher, 1969), is independent of virus multiplication, can be induced by non-infective virus and involves the formation of polykaryocytes only two to three hr after infection at high multiplicity. This type of polykaryocytosis is a laboratory phenomenon and is similar to the rapid polykaryocytosis induced by high multiplicities of Sendai virus, SV5 and measles virus (Poste, 1970). The second type of polykaryocytosis, or ‘fusion from within’, is characterized
by cell fusion beginning some hr after infection at moderate or low multiplicity, and fusion is related to the intracellular growth of the virus. This paper is concerned only with the second type of NDV-induced cell fusion.

It has been found that the ability of various NDV strains to induce polykaryocytosis is related directly to their virulence. It is suggested that polykaryocytosis may provide a useful in vitro marker for virulent virus strains. In contrast, the avirulent strains examined failed to produce a detectable CPE.

METHODS

Virus strains and cell culture. Cell culture techniques and most of the virus strains have been described (Reeve & Waterson, 1970; Reeve, Rosenblum & Alexander, 1970). Strain WARWICK was obtained from W. H. Allan, Central Veterinary Laboratory, Weybridge, Surrey. Plaque purified stock virus strains were grown in the allantois of 10-day-old fertile hens’ eggs. The allantoic fluid was harvested, centrifuged at low speed to remove cell debris and the virus sedimented at 45,000 g for 45 min. Chick embryo cell monolayers were inoculated with 0.5 ml of virus-infected allantoic fluid diluted in maintenance medium (Eagle’s BHK supplemented with 2% heated foetal calf serum) to provide a multiplicity of 50 infective particles/cell. After adsorption at 37° for 1 hr the inoculum was removed, the monolayer washed three times with pre-warmed phosphate-buffered saline to remove unadsorbed virus, and 1.0 ml fresh maintenance medium was added. Cultures were incubated at 37° in the stationary position. All experiments were done using cells grown on coverslips, except for plaque assays which were done in 50 mm. plastic Petri dishes (Sterilin Ltd., Richmond, Surrey).

Estimation of cell fusion as % polykaryocytosis. Coverslip cell cultures were fixed with methyl alcohol 15 hr after infection, stained with 10 % (v/v) Giemsa and 10 % (v/v) May-Grünwald at pH 6.8 and examined microscopically. The extent of cell fusion was estimated by counting the number of nuclei present in polykaryocytes and expressing this as a percentage of the total number of nuclei present in the same microscope field. At least ten fields were counted from each coverslip cultures, and four replicate coverslips were examined for each sample.

Measurement and definition of virus virulence

The virulence of NDV strains is divided arbitrarily into the following three groups which are based on the time taken to kill chicks and embryos inoculated with a minimum lethal dose of a given strain virus (Hanson & Brandley, 1955): (1) lentogenic strains which take 90 to 150 hr to kill embryos but are rarely pathogenic for chicks; (2) mesogenic strains which take 60 to 90 hr to kill embryos but show some pathogenicity for chicks; (3) velogenic strains which kill embryos in 40 to 60 hr and have a high pathogenicity for chicks. The intracerebral pathogenicity index (ICPI) in chickens and the mean death time in eggs of the various NDV strains were estimated by standard techniques (Methods for the Examination of Poultry Biologics, 1963).

RESULTS

Relationship between polykaryocytosis and virus virulence

Microscopic examination of cell cultures infected with the various NDV strains revealed that polykaryocytosis was the principal cytopathogenic effect. Polykaryocytes were seen in cultures infected with the virulent (velogenic) strains HERTS, WARWICK and TEXAS, the
Table 1. A summary of the properties of seven NDV strains showing their ability to cause polykaryocytosis and plaque formation in chick embryo cell cultures and their virulence for chick embryos and chicks

<table>
<thead>
<tr>
<th>Strain</th>
<th>Virulence rating</th>
<th>Mean % polykaryocytosis* ± S.D.</th>
<th>Mean no. nuclei/ polykaryocyte*</th>
<th>Plaque size ± S.E. † (mm.)</th>
<th>Mean death time for embryonated eggs ‡ (hr)</th>
<th>Intracerebral pathogenicity index ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERTS 33</td>
<td>Velogenic</td>
<td>70 ± 11</td>
<td>40 ± 13</td>
<td>1.67 ± 0.13</td>
<td>49</td>
<td>1.88</td>
</tr>
<tr>
<td>TEXAS</td>
<td>Velogenic</td>
<td>63 ± 9</td>
<td>41 ± 18</td>
<td>1.64 ± 0.16</td>
<td>55</td>
<td>1.75</td>
</tr>
<tr>
<td>WARWICK</td>
<td>Velogenic</td>
<td>71 ± 10</td>
<td>49 ± 12</td>
<td>1.62 ± 0.09</td>
<td>62</td>
<td>1.75</td>
</tr>
<tr>
<td>BEAUDETTE C</td>
<td>Mesogenic</td>
<td>41 ± 9</td>
<td>27 ± 14</td>
<td>0.37 ± 0.08</td>
<td>78</td>
<td>1.46</td>
</tr>
<tr>
<td>F</td>
<td>Vaccinal</td>
<td>10 ± 5</td>
<td>9 ± 5</td>
<td>No plaques</td>
<td>119</td>
<td>0.25</td>
</tr>
<tr>
<td>QUEENSLAND</td>
<td>Lentogenic</td>
<td>0</td>
<td>0</td>
<td>No plaques</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>ULSTER</td>
<td>Lentogenic</td>
<td>0</td>
<td>0</td>
<td>No plaques</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean from counts on 60 polykaryocytes at 15 hr after infection.
† Mean from measurements of 50 plaques at 72 hr after infection.
‡ Data provided by W. H. Allan, Central Veterinary Laboratory.
mesogenic strain BEAUDETTE C and the avirulent (lentogenic) vaccine strain F. The lentogenic strains ULSTER and QUEENSLAND were acytopathogenic. The polykaryocytes induced by the different strains were all formed by the fusion of previously separate cells rather than from single cells by successive abnormal cycles of nuclear division. This conclusion was based on: (1) the lack of demonstrable increase in the number of nuclei in cultures showing polykaryocytosis as compared with the uninfected control cultures; (2) the failure to find mitotic and/or amitotic nuclear division in any of the polykaryocytes.

The extent of the polykaryocytosis induced by different NDV strains varied considerably (Table I). Polykaryocytosis was greater with the virulent strains. The correlation between ICPI in chicks and the % polykaryocytosis was statistically significant ($r = 0.98$ and $P = 0.00005$ in a one-tail test of significance). A similar statistically significant correlation was found between mean death time in eggs and % polykaryocytosis ($r = -0.97$ and $P = 0.004$ in a one tail test of significance).

**Morphology of NDV-induced polykaryocytes**

The size and morphology of the polykaryocytes induced by different strains also showed considerable variation. The largest polykaryocytes were produced consistently by the virulent strains WARWICK, TEXAS and HERTS. The mean number of nuclei in individual polykaryocytes produced by these strains was greater than that in the polykaryocytes induced by the mesogenic BEAUDETTE C strain but this, in turn, was significantly greater than that of the polykaryocytes produced by strain F (Table 1).

Morphologically, the large polykaryocytes induced by the virulent and mesogenic strains were characterized by a broad 'spreading' cytoplasm in which the nuclei were arranged...
NDV-induced polykaryocytosis and virulence

randomly (Fig. 1). The polykaryocytes produced by strain F were very different from those produced by the other strains. Typically, they were small, slender, elongated structures in which the nuclei were arranged in a line (Fig. 2, 3). Morphologically they resembled the ‘strap’ multinucleate cells seen in muscle cell cultures (Yaffe, 1969).

![Chick embryo cell culture 15 hr after infection with strain F showing hyperchromatic 'strap' polykaryocyte. May-Grünwald Giemsa × 135.](image)

**Time of formation of polykaryocytes after infection**

Polykaryocytosis was first detected 6 hr after infection with strains HERTS and TEXAS, 8 hr after with strain WARWICK, and 10 hr after with strains BEAUFETTE C and F. Maximum polykaryocytosis was observed 15 and 18 hr after infection with strains HERTS, WARWICK and TEXAS and 20 hr after infection with BEAUFETTE C and F.

In cultures infected with virulent strains, degeneration and lysis of polykaryocytes commenced 24 hr after infection and few recognizable polykaryocytes remained at 72 hr. In contrast, cultures infected with the less virulent strains BEAUFETTE C and F showed less degeneration and at 72 hr after infection viable polykaryocytes and single cells were still common, and much of the general architecture of the cell culture remained.

**Relationship between virus virulence and plaque size**

Schloer & Hanson (1968) found that the ability of NDV strains to produce plaques in chick embryo cell cultures was related directly to their virulence. Since our observations also link polykaryocytosis with virulence, the relationship between plaquing ability and polykaryocyte formation was examined.

It was found that there was a direct correlation between the ability of strains to produce plaques and their capacity to induce polykaryocytosis (Table 1).
The size of the plaques produced by different strains varied significantly (Table I) and was related directly to virulence. The avirulent strains ULSTER and QUEENSLAND failed to produce plaques.

DISCUSSION

Our results indicate that the ability of NDV strains to cause polykaryocytosis by cell fusion is related directly to their virulence. Polykaryocytosis is not an all or none phenomenon and the extent of cell fusion is again related to virus virulence. Highly virulent strains produced larger polykaryocytes than strains of intermediate virulence which, in turn, produced larger polykaryocytes than the vaccine strains. The possibility exists that these differences in the extent of polykaryocytosis are not the result of the inherent cytopathic property of the virus strain involved, but reflect differences in the amount of virus manufactured during the first 15 hr. Thus, the ability of a given strain to produce large polykaryocytes may be simply related to faster growth. However, comparison of one-step growth curves of virulent and avirulent NDV strains in chick embryo cells reveals no major differences in the kinetics of virus growth, either in the timing of the growth cycle or in the amount of infective virus released (Drain, 1969; Reeve & Waterson, 1970). We conclude therefore that differences in cytopathogenicity between NDV strains are not related to differences in rates of virus growth but represent valid markers of specific strains. The present results therefore indicate that polykaryocytosis could be used as an in vitro marker for NDV virulence.
The present results contradict those of Kohn & Fuchs (1969), who were unable to relate polykaryocytogenic potential with virulence for eight strains of NDV. This apparent difference can be explained by the very different system used by Kohn & Fuchs, in which very large inocula of NDV induced rapid cell fusion in the absence of multiplication, i.e. fusion from without: large inocula of our avirulent and mesogenic strains may produce more extensive cell fusion and this is under investigation. Our results also differ from the finding by Bratt & Gallaher (1969) that the virulent strain HERTS was unable to induce extensive cell fusion with small inocula. This difference can be explained since, in our experiments, this strain only produced detectable cell fusion 6 hr after infection and it is not surprising that Bratt and Gallaher recorded little cell fusion at 7 hr. The extent of polykaryocytosis induced by strain HERTS at 7 hr was similar to that reported by Bratt & Gallaher, but was extensive at 15 hr.

Examples of the ability of more virulent strains of virus to induce cell fusion more readily are also found with other polykaryocytogenic viruses. Plowright (1962) found that strains of rinderpest virus virulent for cattle induced marked polykaryocytosis in cell cultures, but strains of low virulence produced only cell rounding and ‘stellate’ cell degeneration or only a few very small polykaryocytes. Bodon & Greczi (1966) and Zuffa et al. (1968) found that virulent field strains of pseudorabies virus were polykaryocytogenic in cell cultures, but the avirulent and vaccine strains only produced cell rounding. In the case of herpesvirus hominis, the antigenic type 2 strains induce polykaryocytosis more commonly than the antigenic type 1 strains and the former are more consistently virulent for experimental animals (Nahmias & Dowdle, 1968).

The relationship between virus virulence and polykaryocytosis suggested here can also be linked with recent evidence reviewed by Poste (1970) on the mechanism of virus-induced cell fusion. For polykaryocytosis induced by parainfluenza, canine distemper and measles viruses and certain herpesviruses it has been found that modification of the cell surface glycoproteins is necessary before fusion can occur and that this is achieved by the release of lysosomal enzymes onto the cell surface (Poste, 1970). This dependence of the cell fusion process on lysosomal labilization (Allison, 1967; Poste, 1970) can, in turn, be related to virus virulence since there is evidence that lysosomal labilization is most commonly produced by virulent strains of virus. Thus, for the present NDV system, it is suggested that virulence and cell fusion may be linked through the effects of the various NDV strains on the lysosomal system. The relationship between NDV-induced cell fusion and other virus-induced functions are described by Reeve et al. (1971).

This research was aided by grants from the Wellcome Trust, the Agricultural Research Council and Action for the Crippled Child (Polio research fund). G. P was supported by a grant from the Cancer Research Campaign. We acknowledge the technical assistance of Miss J. Pacey and are most grateful to Professor A. P. Waterson for help and encouragement.

REFERENCES

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(Received 24 July 1970)