Neuraminidase Content of Strains of Newcastle Disease Virus which Differ in Virulence

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SUMMARY

The neuraminidase content of purified virus particles of three velogenic strains of Newcastle disease virus was compared with that of three lentogenic strains. Velogenic strains possessed approximately three times as much enzyme as lentogenic strains.

Many strains of Newcastle disease virus (NDV) have been isolated; these show a continuous spectrum of virulence for chickens and chicken embryos (Waterson, Pennington & Allan, 1967). Velogenic strains, i.e. those highly virulent for chickens, cause an extensive c.p.e. in chick embryo fibroblasts and produce large plaques, whereas lentogenic or avirulent strains cause little or no c.p.e. and if plaques are produced they are very small (Reeve & Poste, 1971). Furthermore, chick embryo cells infected with virulent strains of NDV show a greater cytoplasmic accumulation of virus material, including virus neuraminidase, than do cells infected with avirulent strains (Reeve, Rosenblum & Alexander, 1970). In this connection, it is interesting that a marked reduction in the size of plaques produced by NDV in chick embryo fibroblasts can be obtained by the use of 2-deoxy-2,3-dehydro-N-trifluoroacetyl-neuraminic acid, a specific inhibitor of neuraminidase (Palese et al. 1974). These results suggest that neuraminidase might play a key role in the development of cytopathic changes, and raise the question whether strains of NDV which differ in virulence might also differ in neuraminidase content. In the experiments reported here, the neuraminidase content of six strains of NDV was compared, and the results show that virus particles of velogenic strains possess approx. three times the amount of enzyme as that associated with virus particles of lentogenic strains.

The six strains of NDV used were: Herts 33, Italien, Lurgan, B1, F and Ulster. Except for Herts 33, which was obtained from the Central Veterinary Laboratories, Weybridge, virus strains were kindly supplied by Dr J. B. McFerran, Veterinary Research Laboratories, Stormont, Belfast. Herts 33, Italien and Lurgan are velogenic strains, while B1, F and Ulster are lentogenic strains (Alexander, Reeve & Allan, 1970; J. B. McFerran, personal communication). The neuraminidase content of each strain was obtained by measuring the sp. act. of the enzyme in preparations of purified virus.

Virus was grown in the allantois of 9-day-old fertile chickens' eggs. Allantoic fluids were harvested 48 h later, and were used immediately for virus purification. All subsequent operations were carried out at 4 °C. Infected allantoic fluids were clarified by centrifuging for 20 min at 75000 g. The virus was pelleted at 50000 g, for 1 h and was resuspended in Dulbecco's phosphate-buffered saline at 0.02% of the original vol. of allantoic fluid. This suspension was vigorously mixed for 1 min with an equal vol. of Halocarbon 113, obtained from BDH, and centrifuged at 75000 g for 10 min. The upper aqueous phase was removed and layered on to a pre-formed linear gradient of 15 to 40% (w/w) potassium tartrate in 0.01 M-phosphate buffer (pH 7.2), and centrifuged at 284000 g for 2.5 h. The visible virus band was collected and dialysed against 0.01 M-phosphate buffer (pH 7.2). The virus was then subjected to a second cycle of potassium tartrate gradient sedimentation and dialysed as above. This
material was used immediately for neuraminidase assays, protein determinations and polyacrylamide gel electrophoresis.

The polypeptides present in the preparations of purified virus were examined by polyacrylamide gel electrophoresis to determine whether this material was contaminated with non-virus protein. A solution containing 10% (w/v) acrylamide, 0.3% N,N'-methylenebisacrylamide, 0.375 M-tris-HCl buffer (pH 8.9), 5 M-urea and 0.075% N,N',N'-tetramethylethylenediamine was polymerised by the addition of 0.15% ammonium persulphate to form gels of 5 mm diam. and approx. 6 cm length. Proteins were solubilized prior to electrophoresis by the addition of a solution containing 1% SDS, 0.1% 2-mercaptoethanol and 6 M-urea to virus preparations; this mixture was incubated at 70 °C for 30 min, then at 100 °C for 1 min. Electrophoresis was performed for 2-0 to 2-5 h at 2 mA/gel. A discontinuous buffer system was used, 0.05 M-tris-glycine, pH 8.9, in the upper tray and 0.1 M-tris-HCl, pH 8.1, in the lower tray. Both buffers contained 0.1% SDS. Bromophenol blue was used as tracker dye. Gels were stained for at least 2 h using 0.25% Coomassie brilliant blue in methanol:acetic acid:water (5:1:5), and were destained in methanol:acetic acid:water. Stained gels were scanned at 580 nm using a Unicam SP500 spectrophotometer with a Gilford Model 222 Photometer and Model 2410-S Linear Transport gel scanning accessory.

The substrate used in the neuraminidase assays was α1 glycoprotein (orosomucoid) concentrate from human plasma obtained from the Scottish National Blood Transfusion Association. Triplicate samples of virus were diluted with 0.2 M-sodium acetate buffer, pH 5.1, and were added to 0.25 ml of orosomucoid (8 mg/ml) in the same buffer. The reaction mixture was incubated at 37 °C for 15 min. Liberated N-acetylneuraminic acid (NANA) was measured by the thiobarbituric acid procedure of Aminoff (1961). One unit of enzyme is defined as the amount releasing 1 nmol of NANA per min under standard assay conditions. In most experiments the sp. act. was measured using two different dilutions of virus, where this was done, the mean of the two results was taken.

Protein determinations were done as described by Lowry et al. (1951) using bovine serum albumin as standard.

The polypeptides present in preparations of purified virus are shown in Fig. 1. The pattern obtained closely resembles that reported by others for NDV (Mountcastle, Compans & Choppin, 1971; Meager & Burke, 1973). The peak near the origin corresponds to VP1 described by Meager & Burke (1973), and the numbering system of Mountcastle et al. (1971) has been applied to the remaining polypeptides. Using bovine serum albumin, deoxyribonuclease I, ovalbumin and chymotrypsinogen as standards, our estimates for the mol. wt. of VP1, VP3 and VP6 are 78000, 60000 and 40000 respectively. All six strains examined gave a similar electrophoretic pattern, although VP5 of Herts and Italien consistently migrated slightly faster than VP5 of the other four strains. As no other protein bands were detected, we concluded that our virus preparations were substantially free from contaminating non-virus material.

The neuraminidase content of these virus preparations was measured, and the results (Table 1) indicate that the neuraminidase content of the velogenic strains is higher than that of the lentogenic strains. Statistical analysis of these results show that the strain differences in neuraminidase content are indeed highly significant (P < 0.001). Furthermore, comparison of the velogenic strains as a group with the lentogenic strains shows that the neuraminidase content of the two groups is different (P < 0.001), however, differences between strains belonging to each group are not significant. The ratio of the estimated means of the sp. act. of the velogenic and lentogenic groups is 3.6.
Short communications

Electrophoretic migration

Fig. 1. Electrophoretic pattern of polypeptides in preparations of purified NDV (strain B1).

Table 1. Comparison of neuraminidase content of different NDV strains

<table>
<thead>
<tr>
<th>NDV strain</th>
<th>Pathogenicity</th>
<th>Number of experiments</th>
<th>Neuraminidase sp. act.* (units enzyme/µg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Lentogenic</td>
<td>6</td>
<td>0.17 ± 0.059†</td>
</tr>
<tr>
<td>F</td>
<td>Lentogenic</td>
<td>5</td>
<td>0.20 ± 0.038</td>
</tr>
<tr>
<td>Ulster</td>
<td>Lentogenic</td>
<td>5</td>
<td>0.23 ± 0.074</td>
</tr>
<tr>
<td>Herts 33</td>
<td>Velogenic</td>
<td>5</td>
<td>0.89 ± 0.271‡</td>
</tr>
<tr>
<td>Italian</td>
<td>Velogenic</td>
<td>5</td>
<td>0.68 ± 0.185</td>
</tr>
<tr>
<td>Lurgan</td>
<td>Velogenic</td>
<td>3</td>
<td>0.64 ± 0.121</td>
</tr>
</tbody>
</table>

* Expressed as mean value ± one standard deviation.
† Estimated mean for lentogenic strains = 0.19.
‡ Estimated mean for velogenic strains = 0.69.

The results obtained in these experiments are in apparent disagreement with those of Alexander et al. (1970), who did not observe differences in neuraminidase content in ten NDV strains of differing virulence. Neuramine lactose was used as substrate in their experiments. However, when we assayed Herts 33 and B1 using neuramine lactose (200 µg per test, reaction mixture pH 5.5), the ratio of the sp. act. was unchanged although enzyme activity was slightly increased. Therefore the differences between our results and those of Alexander et al. (1970) cannot be explained on this basis. However, it is possible that our measurement of neuraminidase content by relating enzyme activity to protein concentration has revealed differences which could not be detected by relating enzyme activity to a haemagglutinin titre, as described by Alexander et al. (1970). In our experiments, if enzyme activity was
related to a haemagglutinin titre rather than to protein concentration, the results showed much greater variation. This is probably because a protein determination gives a more precise measurement of the quantity of virus in purified preparations than a haemagglutination titration. We did not observe statistically significant differences between strains in mean haemagglutinin titre/protein ratios, but the standard deviations obtained were too large for meaningful conclusions to be drawn.

Our finding that velogenic strains of NDV possess more than three times the neuraminidase activity per virus particle of lentogenic strains lends support to the view that neuraminidase plays a part in the development of cytopathic changes in infected cells, but further experiments are needed to test this hypothesis more fully.

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