Isolation and Characterization of Monoclonal Antibody-resistant Mutants of Newcastle Disease Virus

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(Accepted 24 October 1984)

SUMMARY

Neutralizing monoclonal antibodies incorporated into plaque assay overlay medium were used to select antibody-resistant (AbR) mutants of both the Herts (using anti-fusion protein monoclonal 481) and Beaudette C (using anti-haemagglutinin-neuraminidase protein monoclonal 445) strains of Newcastle disease virus at the permissive temperature of 34 °C. Certain of the Herts, but none of the Beaudette C, AbR mutants were also temperature-sensitive (ts-) and failed to form plaques at the non-permissive temperature of 41.5 °C. [35S]Methionine-labelled proteins from chick embryo fibroblasts infected with wild-type, ts+ AbR and ts- AbR virus when separated by two-dimensional polyacrylamide gel electrophoresis revealed a variety of changes in the isoelectric point of the fusion protein F (using monoclonal 481) and the haemagglutinin-neuraminidase protein HN (using monoclonal 445). The ts+ ‘revertants’ of ts- AbR mutants remained AbR and also showed changed isoelectric points in the F protein.

Monoclonal antibodies raised against viruses are of value in identifying virus antigens in virus-infected cells, in aiding purification of virus proteins by affinity chromatography (Varsanyi et al., 1984), and in investigating site(s) on virus surface antigens that are important in virus neutralization (Wiley et al., 1981; Emini et al., 1983; Dimmock, 1984). This study investigates the utility of neutralizing monoclonal antibodies directed against the fusion (F) and haemagglutinin-neuraminidase (HN) surface glycoproteins of Newcastle disease virus (NDV) to select temperature-sensitive (ts-) antibody-resistant (AbR) mutants. Such mutants would be expected to have lesion(s) within one or the other glycoprotein and ts+ ‘revertants’ of these mutants should shed light on interactions of the antibody-binding sites with other sites in the same or indeed other virus proteins.

The monoclonal antibodies 481 (Ab481, anti-F) and 445 (Ab445, anti-HN-2) used in this study, and isolated and characterized earlier by one of us (Russell et al., 1983), were raised against the Ulster strain of NDV. This strain requires the presence of trypsin in the overlay medium to allow plaque formation; therefore, for convenience the Herts (sensitive to Ab481) and Beaudette C (sensitive to Ab445) strains of NDV were used in this study, as these strains do not require the addition of trypsin in the plaque overlay medium.

Serial dilutions of chorioallantoic fluids from eggs infected with plaque-purified Herts and Beaudette C virus were plated on chick embryo fibroblast (CEF) secondary monolayers in 50 mm diam. Petri dishes (Flow Laboratories) and overlaid with 5 ml HEPES-buffered Medium 199 containing 5% calf serum and 1% agar to which was added unheated ascites fluid (5 μl per monolayer) prepared from the monoclonal antibody-producing cells. Selection and control plates were incubated at 34 ± 0.1 °C in sealable plastic boxes which were completely immersed in a water-bath for 4 days. Plaques formed at 34 °C in the presence of monoclonal antibody were picked using Pasteur pipettes and the agar plugs were incubated overnight at 4 °C in 0.5 ml Tris–saline buffer pH 7.4.
Fig. 1. Common regions from fluorograms of 2D gels of $[^{35}S]$methionine-labelled proteins from (a) mock-infected or (b to d) NDV Herts-infected CEF cells; they had been incubated at 34 °C for 8 h and radiolabelled for 2 h at 34 °C. Ab* mutants can be classified: (b) class II (A10), (c) class III (A7) or (d) class I (B6). Proteins identified are: A, actin; HN, haemagglutinin–neuraminidase; $F_0$, uncleaved fusion protein; NAP, nucleocapsid-associated protein. Isoelectric focusing was from left (+) to right (−).
Table 1. Classes of $F_0$ charge changes

<table>
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<tr>
<th>NDV Herts</th>
<th>Antibody</th>
<th>Electrophoretic type of F protein</th>
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<tbody>
<tr>
<td>A, wild-type</td>
<td>481†</td>
<td>Anode III</td>
</tr>
<tr>
<td>B₂</td>
<td>R</td>
<td>-</td>
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<tr>
<td>A₁₀</td>
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<td>B₉</td>
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<td>B₁₁₀</td>
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† S, Sensitive; R, resistant to monoclonal antibody 481.
‡ +, Temperature-resistant; -, temperature-sensitive.

Half of each suspension was used to inoculate CEF primary monolayers in 30 mm diam. Petri dishes and incubated for a further 3 to 4 days under 2 ml of HEPES-based liquid Medium 199 containing 5% calf serum at 34 °C in an incubator. Media from these plates were diluted 10⁴-fold and 100 μl aliquots plated on CEF primary monolayers in 30 mm dishes overlaid with agar medium as before, with or without antibody, and incubated at 34 ± 0·1 °C or 41·5 ± 0·1 °C in water-baths for 3 or 4 days. Plaque-purified virus that remained Ab R was again plaque-purified, retested, and used to inoculate 10-day-old embryonated hens' eggs. Eggs were incubated at 34 °C for 4 days and the chorioallantoic fluid was harvested and used without further purification.

Radioactively labelled proteins from virus-infected CEF secondary monolayers incubated at 34 °C for all Herts analyses and at 37 °C for all Beaudette C analyses were analysed by isoelectric focusing: SDS–PAGE (2D-PAGE) as described previously (Samson et al., 1981) except that focusing was for 4800 Vh and the radioactive amino acid used was [35S]methionine (Amersham) at 4 μCi/dish.

Ab481 R mutants were cloned from the Herts wild-type stock and approximately half of these were also ts −. Four ts + and three ts − Ab R mutants were chosen for analysis by 2D-PAGE and the patterns of isoelectric points of the $F_0$ (uncleaved) glycoprotein were found to fall into three classes (Fig. 1 and Table 1). Two of the three ts − Ab R mutants (A₁₀ and B₉) showed virus protein spot patterns identical to that of wild-type (Fig. 1 b) and therefore from these patterns it could not be concluded that the lesion(s) lay within the $F_0$ (or any other particular virus gene); however, all four ts + Ab R mutants analysed showed the same charge change, in that the $F_0$ spot took up a position nearer the cathode (Fig. 1 c). Only one of the ts − Ab R mutants analysed (B₆) showed any charge change: this again affected the isoelectric point of the $F_0$ protein which focused nearer the anode than the wild-type (Fig. 1 d). In no other virus protein which focuses in this gel system was there any reproducible isoelectric point change, and the changes in $F_0$ protein have subsequently been confirmed by mixed (e.g. class I + II) gel loading analysis (not shown).

In contrast, none of the 30 independently isolated Ab445 R mutants cloned from the Beaudette C strain was ts − even at 42·5 °C. Moreover when five of these Ab445 R mutants were chosen at random for examination by 2D-PAGE they all revealed the same HN protein isoelectric point change (moved towards the cathode) with respect to the wild-type (Fig. 2 b, c). Again, no other virus protein charge changes were seen in proteins which focus, and the HN charge changes have been confirmed by mixed loading analysis (not shown). The analysis is slightly complicated by the fact that unlike the Herts HN protein the Beaudette C HN protein shows charge heterogeneity (Chambers & Samson, 1982).

All Ab R mutants tested failed to be neutralized by the respective antibody, as would be expected from the way in which the mutants were selected; this could either be because the virus protein affected is no longer bound by the antibody or that binding still occurs but that this binding fails to cause neutralization. So far, reliable anti-F protein immunoprecipitation has not been realized for the Herts wild-type strain, but HN proteins from the five Ab445 R mutants of
Fig. 2. Common regions from fluorograms of 2D gels of [35S]methionine-labelled proteins from (a) mock-infected or (b, c) NDV Beaudette C-infected CEF cells; they had been incubated at 37 °C for 6 h and radiolabelled for 2 h at 37 °C. (b) Wild-type, (c) AbR mutant (E6) of NDV. Proteins identified are: A, actin; HN, haemagglutinin-neuraminidase; F̄₁, cleaved fusion protein; NAP, nucleocapsid-associated protein. Isoelectric focusing was from left (+) to right (−).

the Beaudette C strain tested, unlike the wild-type, did not precipitate with Ab₄₈₁ (not shown), suggesting that the failure to neutralize these mutants was due to lack of antibody binding.

Two of the Ab₄₈₁₉ts⁻ mutants (A₁₀ which showed no charge change, and B₆ which showed a charge change with respect to wild-type F₀ protein), were plated at the non-permissive temperature of 41.5 °C to select for ts⁺ ‘revertants’. Five of these from each mutant were plaque-
purified and tested for their plaque-forming ability at 34 °C with and without Ab481 in the overlay, and at 41·5 °C. All ten clones were ts+ but remained Ab8 and were therefore presumably suppressed mutants and not revertants. [35S]Methionine-labelled proteins from CEF infected with these presumptive suppressed mutants and incubated at 34 °C were analysed by 2D-PAGE as before. All mutants derived from A10 showed a charge shift in F0 towards the cathode despite the A10 mutant itself showing no change charge in this protein with respect to wild-type. In contrast, revertants derived from B6 exhibited the same F0 isoelectric point as B6.

Selecting for monoclonal antibody 481 (anti-F) resistance at a low (permissive) temperature is a novel way of generating ts− mutants which can show alterations in the F protein. Spontaneous Ab481 resistance was generated at a frequency of 10−4 and approximately half of these mutants turn out to be simultaneously ts−. In contrast, no ts− mutants were obtained from 30 independently isolated spontaneous Ab445R mutants of the Beaudette C strain of NDV. A subsequent study of mutants of Beaudette C resistant to monoclonal antibody 14 (a different anti-HN monoclonal antibody) again showed no ts− phenotypes. Whether these findings reflect properties of the type of target molecule (F or HN), or monoclonal antibody, or the virus strain (Herts or Beaudette C) is unresolved. However, the unusual thermostability of the HN protein of the Beaudette C strain (Granoff, 1959; Harper et al., 1983) may well account for our difficulty in obtaining ts− mutants affected in this protein by using monoclonal antibodies.

Temperature-sensitive mutants are of particular interest because they afford the selection of ts+ 'revertants'. To our surprise all ts+ revertants from each of two ts− Ab481R mutants remained Ab8. The frequency of this genetic suppression (rather than reversion) was 10- to 50-fold that of the forward mutation to ts− Ab8. Presumably there are many more sites in the F gene which can be mutated such that the resultant polypeptide is no longer temperature-sensitive than there are sites for mutation to ts− Ab8 when selecting with a given monoclonal antibody. The fact that ts+ Ab8 suppressed mutants also show charge changes in the F0 protein supports the interpretation that this is an example of intragenic suppression.

It would be particularly interesting to pursue this type of analysis with influenza virus using anti-HA neutralizing monoclonal antibodies. The availability of a three-dimensional structure for the HA molecule would allow a spatial correlation between the site of mutation and the site of suppression to be found. A further use of Ab8 mutants which are selected using neutralizing antibodies, and their suppressed derivatives, is in unravelling the interaction of virion proteins involved in the neutralization process itself. If indeed virus internal proteins such as polymerases are indirectly affected by neutralizing antibodies binding to surface glycoproteins (Dimmock, 1984) then some Ab8 mutants should have altered internal proteins.

Part of this work was supported by a grant from the Wellcome Trust to P. H. Russell.

REFERENCES


(Received 19 September 1984)