A Neutralizing Monoclonal Antibody to Respiratory Syncytial Virus which Binds to Both F₁ and F₂ Components of the Fusion Protein

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(Received 7 April 1986)

SUMMARY

A virus-neutralizing monoclonal antibody (1E3) specifically immunoprecipitated the 70000 mol. wt. (70K) fusion (F) protein from respiratory syncytial (RS) virus-infected HeLa cells. Western blotting analysis of polypeptides from such cells separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions revealed that 1E3 was peculiar in that it bound to both F₁ (50K) and F₂ (20K) components of the F protein. Antibody subsequently eluted from either the F₁ or the F₂ regions of immunoblots re-bound to both F₁ and F₂ regions of the SDS-PAGE blot. These results show that monoclonal antibody 1E3 reacts with an epitope which is found on both F₁ and F₂ subunits of RS virus fusion protein.

The pneumovirus respiratory syncytial (RS) virus is a member of the Paramyxoviridae (Kingsbury et al., 1978) and is the major cause of lower respiratory tract infection in young infants (Kim et al., 1973). Monoclonal antibodies to a number of RS virus proteins have now been isolated in a variety of laboratories and have been exploited as diagnostic reagents, to prepare purified virus proteins and to provide protection against RS virus infection (Routledge et al., 1985; Walsh et al., 1983, 1984). Among several monoclonal antibodies (MAbs) isolated in this laboratory (Routledge et al., 1985), three react in immunoprecipitation reactions with the fusion (F) glycoprotein. F is made as a precursor F₀ (70K) which is cleaved to two smaller glycoproteins F₁ (50K) and F₂ (20K) which are held together by disulphide bonds, a feature common to paramyxovirus fusion proteins (Lambert & Pons, 1983; Nagai & Klenk, 1977; Samson & Fox, 1973).

One of the F MAbs (1E3) gave an unusual pattern in Western blot (Burnette, 1981) analysis of RS virus-infected HeLa cell lysates in that both F₁ and F₂ components of the F protein showed binding to this antibody (Fig. 1). In addition, ascites fluid containing 1E3 gave very high neutralizing titres in a plaque neutralization test.

A given MAb would be expected to react with a unique epitope within a protein. The unusual behaviour of 1E3 could arise in a limited number of ways. First, 1E3 could be biclonal and not monoclonal where the mixed clones happen to contain two different antibodies, one for F₁ and one for F₂. Second, an F₂-sized (20K) portion of F₁ might be cleaved from a proportion of F₁ molecules which bears the epitope recognized by the monoclonal antibody and mimics F₂ binding. Third, a common epitope may exist within both F₁ and F₂ and both may be recognized by the same MAb.

The cloning procedure that led to the production of MAbs in this laboratory (Routledge et al., 1985) involved re-cloning from single colony wells which were antibody-positive, which argues against the biclonal explanation. Isoelectric focusing and SDS–PAGE size analysis of the heavy
Fig. 1. Western blot analysis of RS virus-infected HeLa cell proteins and partially purified fusion protein with 1E3. Uninfected and RS virus strain A2-infected (m.o.i. 0.1 to 1.0) confluent HeLa cells were maintained in Medium 199 supplemented with 2% (v/v) foetal calf serum for approx. 72 h until c.p.e. was extensive. The cells were rinsed in phosphate-buffered saline and scraped into Laemmlli's sample buffer but without reducing agent (Laemmlli, 1970) and immediately boiled for 2 min. Proteins in these extracts together with a partially purified preparation of F1,2 fusion protein (also derived from A2-infected HeLa cells) were separated in 10% (w/v) SDS-polyacrylamide gels either under reducing [2.5% (v/v) 2-mercaptoethanol final concentration in sample buffer] (lanes 3, 4 and 6) or non-reducing conditions (lanes 1, 2 and 5) (Laemmlli, 1970). The gels were equilibrated in transfer buffer [25 mM-Tris, 192 mM-glycine, 20% (v/v) methanol] for 30 min at 4 °C and electrophoretically transferred to a sheet of nitrocellulose paper (Burnette, 1981) for 3 h at 100 V and excess sites were blocked overnight at 4 °C in rinse buffer [150 mM-NaCl, 1.5 mM-KH₂PO₄, 8 mM-Na₂HPO₄, 2.7 mM-KCl, 0.05% (v/v) Tween 20, pH 7.4] containing 1% (w/v) bovine serum albumin (BSA). After washing in rinse buffer the blot was incubated for 2 h at 37 °C with a 1/1000 dilution of 1E3 ascites fluid in rinse buffer + 1% (w/v) BSA. Bound MAb was detected by incubating the washed blot with peroxidase-conjugated rabbit anti-mouse whole IgG at 1/1000 dilution in rinse buffer + 10% (v/v) foetal calf serum for 2 h at 37 °C. The blot was extensively washed in rinse buffer and colour developed by incubating for a few min at 37 °C with a mixture of 60 mg 4-chloro-1-naphthol dissolved in 20 ml ice-cold methanol with 100 ml 10 mM-Tris·HCl pH 7.4, 150 mM-NaCl plus 60 µl 30% (w/v) hydrogen peroxide. Lanes 1 and 3, proteins from uninfected cells; lanes 2 and 4, proteins from RS virus-infected cells; lanes 5 and 6 partially purified F1,2 protein.

and light chains of 1E3 revealed by Western blotting and incubating with peroxidase-conjugated rabbit anti-mouse whole IgG supports this opinion (not shown). Nevertheless, it remained a possibility that 1E3 was biclonal and that heavy and light chains from both clones happened to be the same sizes.

In order to resolve this issue we conducted the following experiment. Antibodies derived from 1E3 which had first been bound and then eluted from (i) only the F1, and (ii) only the F2 components of partially purified F protein were tested (together with a variety of control antibodies derived from 1E3) for their subsequent ability to bind to both F1 and F2 components of partially purified F protein and to RS virus-infected HeLa cell extracts separated on SDS-polyacrylamide gels and blotted onto nitrocellulose sheets. (Details of the purification of F protein is the subject of a separate communication.)
Fig. 2. Reciprocal elution/binding analysis of proteins from RS virus-infected HeLa cells and partially purified F_{1,2} protein. A partially purified preparation of F_{1,2} protein was boiled in reducing SDS sample buffer and separated as full-width samples on a pair of 10% SDS-polyacrylamide slab gels (i and ii). Cell extracts were prepared as in Fig. 1, re-boiled in the presence of 2.5% (v/v) 2-mercaptoethanol for 2 min and proteins were separated on a third 10% SDS-PAGE slab gel (iii). All gels were electroblotted onto nitrocellulose paper and strips from the sides of all three blots were immunostained to reveal F_1 and F_2 regions as described in Fig. 1. The remaining portion of gel (i) was incubated with a 1/250 dilution of 1E3 for 2 h at 37 °C and rinsed six times in buffer A (150 mM-NaCl, 5 mM-EDTA, 50 mM-Tris-HCl pH 7.5). Horizontal strips of nitrocellulose corresponding to the F_1 and F_2 regions of the washed blot (together with a non-F_1, non-F_2 region) were excised, cut up, placed in separate 10 ml syringes and rinsed again in buffer A. Bound antibody was eluted by drawing 4.5 ml of 0.2 M-glycine–HCl pH 2.8 (elution buffer) up and down in each syringe four times over a 4 min period at 4 °C. Each blot extract was immediately neutralized with 1 M-Tris, 300 mM-NaCl, 2% (w/v) BSA, and sufficient 300 mM-NaCl, 2% (w/v) BSA solution added to bring the volumes of extract to 9 ml (modification of Talian et al., 1983). The eluted MAb 1E3 preparations anti-F_1 (lanes 1), anti-F_2 (lanes 2), a mixture of anti-F_1 and anti-F_2 eluates (lanes 3), 1E3 treated with elution buffer (lanes 4), no-antibody control (lanes 5), anti-non-F_1 or -F_2 (lanes 6) and untreated control 1E3 (lanes 7) were incubated overnight at room temperature with vertical strips from gel blot ii (a) and gel blot iii (b). The presence of bound antibody was detected as for Fig. 1.

Fig. 2 shows the results of this reciprocal elution/binding analysis. The component antibody derived from 1E3 that bound to F_1, when eluted bound to both F_1 and F_2 polypeptides. The same was true for the F_2-bound antibody; when eluted it bound to F_1 as well as to F_2. If the binding of 1E3 to both F_1 and F_2 was due to two different monoclonal antibodies (one to F_1 and the other to F_2) then the anti-F_1 eluate should only have re-bound to F_1 and the anti-F_2 eluate only to the F_2 polypeptide. Thus, these results confirmed that 1E3 was monoclonal and bound to both F_1 and F_2 polypeptides.

There remains the possibility that the 20K band we refer to as F_2 could be an F_2-sized portion of F_1 (say F_x) which bears the same epitope as F_1 which is recognized by MAb 1E3. If this was the case then F_x would have been disulphide-linked to the remaining hypothetical portion of F_1 (because we saw no 20K band in non-reduced preparations of fusion protein, Fig. 1), say F_y, of approximate size 30K [= F_1 (50K)–F_x (20K)]. Treatment of radiolabelled RS virions with
trypsin followed by immunoprecipitation with anti-F MAb reveals a resistant fraction (presumed to be derived from F₁) which migrates just above F₂ in reducing SDS-PAGE (B. F. Fernie, personal communication). Further experiments will be needed to establish whether such a fragment is indeed derived from F₁ and is also produced in infected cells and purifies with F₁₂.

Computer analysis of the recently published amino acid sequence derived from the F gene (Collins et al., 1984; Elango et al., 1985) using the program of Queen & Korn (1980) reveals only two groups of five (or more) contiguous amino acids which are repeated elsewhere in the F protein. One group (Leu–Leu–Ser–Leu–Ile) is found at residues 257 to 261 and again at 538 to 542 but these are both within the F₁ portion of the molecule. The other group (Val–Thr–Leu–Ser–Lys) is found at residues 127 to 131 and 557 to 561 which are five and 13 residues from the C termini of F₂ and F₁ respectively. In addition, there are three repeats of four amino acids within the F polypeptide, any of which might form part of the antigenic sites recognized by 1E3. Further experiments will be required to determine which are the actual sites within F₁ and F₂ which react with this antibody.

We propose the term geminiepitopic to refer to the situation when a monoclonal antibody reacts with two distinct epitopes occurring on the same protein molecule.

This work is supported by an MRC Grant to G.L.T. and A.C.R.S.

Note. Subsequent to the submission of this paper, Walsh et al. (1986, Journal of General Virology 67, 505–513) reported that one of their anti-F MAbs (L4) reacted strongly to the 48K (F₁) fragment and weakly to the 23K (F₂) fragment, suggesting some degree of cross-reactivity between these fragments.

REFERENCES


Short communication


(Received 7 March 1986)