Biological activity, binding site and affinity of monoclonal antibodies to the fusion protein of respiratory syncytial virus


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The neutralizing activity and fusion-inhibition activity per unit weight of immunoglobulin were determined for each of a panel of 20 monoclonal antibodies (MAbs) to the fusion (F) protein of respiratory syncytial (RS) virus. Neutralization did not correlate with fusion-inhibiting activity, suggesting that the F protein plays at least two independent, antibody-sensitive roles in viral infection. Antibodies with the highest biological activity against A2, a subgroup A strain of RS virus, neutralized a subgroup B strain (8/60) poorly, suggesting a degree of antigenic variation that may be important in human infection.

All but one fusion-inhibiting MAb bound to protein blots and binding was mapped to two areas on overlapping F protein fragments. One MAb with relatively poor fusion-inhibiting activity bound only to fragments C-terminal of amino acid 384, the remainder bound only to fragments containing residues 253 to 289. MAbs directed to the latter site were heterogeneous in neutralizing activity, subgroup specificity and fusion-inhibiting activity. These variations between MAbs could not be accounted for by differences in their binding avidities. We suggest that this binding site is not the complete antibody epitope which probably includes conformation-dependent elements.

Human respiratory syncytial (RS) virus belongs to the genus Pneumovirus of the Paramyxoviridae. Isolates fall into two, co-circulating, closely related subgroups, A and B (Mufson et al., 1985; reviewed by Anderson, 1990). The negative-strand RNA genome encodes ten proteins of which three, the attachment glycoprotein (G), the fusion glycoprotein (F) and a third glycoprotein of unknown function (SH), are expressed on the surface of virions and infected cells (McIntosh & Chanock, 1990). Antibodies to F and G can neutralize the virus in vitro and also protect mice (Taylor et al., 1984). However, antibodies to F are of potentially greater prophylactic value as G is highly variable and tends to induce subgroup-specific immunity (Johnson et al., 1987; Muelenaer et al., 1991).

The F glycoprotein mediates fusion of viral and cell membranes during penetration (Walsh & Hruska, 1983) but the mechanism of this process is obscure. The structure and synthesis of the protein is similar to that of other paramyxovirus F proteins (Spriggs et al., 1986; Collins & Mottet, 1991; Fig. 1). The mature F protein spike is probably a homotetramer and may be disulphide-linked to the G glycoprotein (Collins & Mottet, 1991; Arumugham et al., 1989).

A number of panels of monoclonal antibodies (MAbs) to the F protein of RS virus have been characterized according to their ability to neutralize the virus, inhibit fusion, react with a range of virus isolates and compete with each other in binding to immobilized antigen (Beeler & Coelingh, 1989; Anderson et al., 1986; Trudel et al., 1987b; Garcia-Barreno et al., 1989; Taylor et al., 1992). Beeler & Coelingh (1989) categorized antibodies capable of neutralizing the virus into three non-competitive groups. Group A had a low ratio of neutralization to fusion-inhibition activity and contained some broadly cross-reactive antibodies but also some with a narrower specificity. Group B did not inhibit fusion efficiently and largely contained antibodies of restricted specificity. Group C had a high ratio of neutralization titre to fusion-inhibition titre and was broadly cross-reactive. Other authors have found patterns similar to these. Although the binding sites of a number of neutralizing and fusion-inhibiting antibodies have been mapped to two regions of the protein, involving or close to the amino acids 265 to 272 (Arbiza et al., 1992; Lounsbach et al., 1993; Martin-Gallardo et al., 1991) and amino acids 422 to 438 (Taylor et al., 1992) the relationship between the biological characterization

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of MAbs and their binding site remains unclear. Here we have assembled a panel of MAbs and measured both their neutralizing and fusion-inhibiting capacity for viruses of subgroups A and B and attempted to explain differences in the biological activity of antibodies in terms of their binding sites and avidity.

MAbs (Table 1) were derived from Balb/c mice immunized intranasally with 10⁴ p.f.u. of live RS virus (strain A2) under ether anaesthesia. In subsequent immunizations the mice were boosted with different antigens; immunoaffinity purified A2 strain F protein in Freund’s adjuvant (Routledge et al., 1988) was used to generate MAbs 5A8, 1C1 and 2D7; a live recombinant vaccinia virus (v-F) expressing F from RS virus strain A2 (kindly provided by Dr Gail Wertz; Wertz et al., 1987) was used to generate MAb 5E2 and a β-galactosidase chimeric protein containing A2-strain F protein residues 253 to 389 (Lounsbach et al., 1993) in Freund’s adjuvant was used to generate MAbs 5A6 and 10D4. At 2 weeks and at 3 days prior to hybridoma production all mice were inoculated subcutaneously and intravenously, respectively with sucrose gradient-purified A2 strain virus (Ueba, 1978). Spleen cells were fused with SP2/0 myeloma cells (Schulman et al., 1978) and hybridomas were selected for by standard protocols in medium containing hypoxanthine and azaserine. Cultures secreting anti-RS virus F protein MAbs were detected by ELISA using RS virus and v-F-infected cell lysates. Positive cultures were cloned at least twice by limiting dilution in the presence of mouse peritoneal macrophage feeder cells.

Ascites fluids were prepared for each MAb by standard protocols and immunoglobulin concentrations were determined by isotype-specific ELISA in comparison with a standard serum of known isotype content (The Binding Site). ELISA plates (Nunc Immnoplate II) were coated with isotype-specific goat anti-mouse immunoglobulin (Sigma) in carbonate buffer pH 9-6. Mouse ascites were titrated in the coated plates and bound immunoglobulin was detected with rabbit anti-mouse IgG peroxidase conjugate (Dako). Optimal dilutions of all reagents were determined by checkerboard titrations. Repeated assays of identical aliquots of ascites fluid gave results varying by ±40% of the mean. The major isotype in the ascites fluid was assumed to be that of the MAb. Irrelevant immunoglobulin in ascites
Table 1. Western blotting, virus neutralization and fusion inhibition by a panel of MAbs to the F protein of RS virus

<table>
<thead>
<tr>
<th>MAb*</th>
<th>Western blot</th>
<th>Neutralizing titre/mg†</th>
<th>Neutralizing titre ratio (subgroup A/B)‡</th>
<th>Fusion inhibiting titre/mg</th>
<th>Affinity (log K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS348</td>
<td>+</td>
<td>8599</td>
<td>782</td>
<td>1538</td>
<td>8.0</td>
</tr>
<tr>
<td>RS2B8</td>
<td>+</td>
<td>6680</td>
<td>607</td>
<td>1791</td>
<td>8.5</td>
</tr>
<tr>
<td>BD51</td>
<td>−</td>
<td>1409</td>
<td>31</td>
<td>64</td>
<td>nd</td>
</tr>
<tr>
<td>B151</td>
<td>+</td>
<td>1104</td>
<td>33</td>
<td>255</td>
<td>9.7</td>
</tr>
<tr>
<td>7C2</td>
<td>+</td>
<td>2100</td>
<td>2.5</td>
<td>1405</td>
<td>9.6</td>
</tr>
<tr>
<td>47F</td>
<td>+</td>
<td>1452</td>
<td>1.4</td>
<td>402</td>
<td>9.2</td>
</tr>
<tr>
<td>RS18B2</td>
<td>+</td>
<td>1068</td>
<td>2.4</td>
<td>1394</td>
<td>9.3</td>
</tr>
<tr>
<td>1C1</td>
<td>+</td>
<td>765</td>
<td>3.4</td>
<td>225</td>
<td>9.0</td>
</tr>
<tr>
<td>2H9</td>
<td>+</td>
<td>275</td>
<td>1.3</td>
<td>200</td>
<td>9.3</td>
</tr>
<tr>
<td>1E3</td>
<td>+</td>
<td>81</td>
<td>3.0</td>
<td>79</td>
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</tr>
<tr>
<td>19</td>
<td>+</td>
<td>3164</td>
<td>4.9</td>
<td>327</td>
<td>ND</td>
</tr>
<tr>
<td>5A8</td>
<td>−</td>
<td>2435</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>5E2</td>
<td>−</td>
<td>3059</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>4E5</td>
<td>−</td>
<td>1854</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>2D7</td>
<td>−</td>
<td>2719</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>5A6</td>
<td>+</td>
<td>-ve</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>1A12</td>
<td>−</td>
<td>-ve</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>B81</td>
<td>−</td>
<td>-ve</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>10D4</td>
<td>−</td>
<td>-ve</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7E10</td>
<td>−</td>
<td>-ve</td>
<td>NA†</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* The sources of the MAbs (except 1C1, 5A8, 5E2, 2D7, 5A6 and 10D4, first described in this study) are as follows: 1E3, 4E5 and 1A12, Routledge et al. (1985); BD51, Yazici (1993); RS348, RS2B8 and RS18B2, Bourgeois et al. (1991); 19, Taylor et al. (1984); 7E10, Richards (1994); 47F, Garcia-Barreno et al. (1989); 7C2, Trudel et al. (1987a) and B151 and B81, Mufson et al. (1985).
† The 60% plaque reduction neutralization titre per mg immunoglobulin against RS virus strain A2.
‡ The subgroup A RS virus strain A2 and the subgroup B strain 8/60 were used.
§ ND, Not done.
|| NA, Not applicable; less than 60% neutralization of the subgroup B strain obtained with an ascites dilution of 1/20.

Neutralizing activity ranged over four orders of magnitude. Eleven of the MAbs were able to neutralize both strains of virus, commonly achieving greater than 95% plaque reduction. Titres were uniformly higher on the A strain than the B, but the ratio of titres on these two viruses varied widely. For six MAbs the ratio fell between 1 and 3-4. The remaining MAbs showed a more marked preference and the two MAbs with the highest neutralization titres on the A strain, RS348 and RS2B8, achieved a 60% plaque reduction of the 8/60 virus only at the highest concentration of ascites tested, 1/20.

Four MAbs (5A8, 5E2, 4E5 and 2D7) neutralized the subgroup A virus to high titre but showed no neutralizing activity against 8/60. The neutralization curves for these MAbs were flat and never exceeded 75%.

The five remaining MAbs showed very low or undetectable levels of neutralizing activity against both viruses.

Fusion-inhibition assays were performed in microtitre plates (Gibco-Nunc) seeded with 10^4 HeLa cells in growth medium [containing 10% fetal calf serum (FCS)]. After incubation at 37 °C overnight the monolayers were washed in PBS and inoculated with 2 x 10^4 p.f.u./well of RS virus strain A2. After incubation at 37 °C for 1 h the residual inoculum was washed off with PBS and the cells were estimated by calculating the mean concentration of all isotypes other than the MAb isotype for six ascites preparations. The values obtained (IgG1, 0.2±0.13 mg/ml; IgG2a, 0.25±0.03 mg/ml; IgG2b, 0.07±0.06 mg/ml) gave a quantitative estimate of the irrelevant immunoglobulins in ascites to be assayed. Estimates of MAb isotype concentration were thus corrected for host mouse contamination by subtracting the appropriate figure.
MAbs

<table>
<thead>
<tr>
<th>F1-574</th>
<th>F18-212</th>
<th>F214-574</th>
<th>F190-289</th>
<th>F253-384</th>
<th>F190-255</th>
<th>F253-289</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS348</td>
<td>RS2B8</td>
<td>7C2</td>
<td>47F</td>
<td>RS18B2</td>
<td>1C1</td>
<td>2H9</td>
</tr>
<tr>
<td>4E5</td>
<td>1D3</td>
<td>1E3</td>
<td>1D4</td>
<td>1E3</td>
<td>5A8</td>
<td>5E2</td>
</tr>
<tr>
<td>2D7</td>
<td>1A2</td>
<td>1E3</td>
<td>5A8</td>
<td>5E2</td>
<td>4E5</td>
<td>2D7</td>
</tr>
<tr>
<td>Anti-β-gal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Gal</td>
<td>F protein</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Fig. 2. Dot-blotting of a panel of MAbs to the F protein of RS virus against β-galactosidase chimeras containing amino acid sequences from the F protein gene. 1G9, a control MAb ascites to the capsid protein of feline calicivirus. β-Gal, purified native β-galactosidase from E. coli. Anti-β-gal, polyclonal mouse antibody to native β-galactosidase. F protein, affinity purified native RS virus F protein.

were overlaid with twofold dilutions of the MAb under test in maintenance medium (containing 2% FCS). Syncytia were allowed to develop for 32 h at 37 °C and then fixed and stained (Diffquick; Baxter Dade). Syncytia were scored 1+ (uninfected control cells) to 4+ (virus controls) by two independent observers and the lowest dilution of serum reducing syncytia to 2+ was taken as the endpoint. Repeated titrations gave a variation in titre of up to twofold.

The ability of the MAbs to inhibit fusion of infected cells showed no overall correlation with their ability to neutralizing the virus. MAbs exhibiting subgroup-specific neutralization failed to inhibit fusion and fusion-inhibition activity was also low, relative to neutralizing activity, in those MAbs showing a marked preference for the A2 virus strain. This suggests the existence of a crucial function for the F protein in the infectious process, in addition to the induction of fusion, that is highly sensitive to blocking by subgroup-specific antibodies.

Based on these properties it would seem that the panel of MAbs studied here contains representatives of all three groups defined by Beeler & Coelingh (1989). Six MAbs (7C2, 47F, RS18B2, 1C1, 2H9 and 1E3) have low neutralization/fusion inhibition ratios, neutralize viruses of both subgroups and correspond to group A antibodies. MAbs 7C2 and 47F have been shown not to compete with MAb 19 (Taylor et al., 1992) which, with a high neutralization to fusion inhibition ratio, corresponds to group C antibodies. Group B antibodies, distinctively lacking in fusion-inhibition activity, are probably represented by four MAbs; 5A8, 5E2, 4E5 and 2D7.

Four of the MAbs studied here do not fit comfortably into this scheme as they share high neutralization/fusion inhibition ratios and a marked preference for the subgroup A virus strain. Two of these antibodies, RS348 and RS2B8, are particularly noteworthy for their exceptionally high, strain-specific neutralizing activity. The existence of a variable epitope exquisitely sensitive to neutralization may be important in human susceptibility to re-infection and thus may provide an explanation for the co-circulation of two virus subgroups.

The binding of the MAb panel was mapped by dot-blotting on overlapping fragments of the F protein polypeptide expressed as β-galactosidase chimeras in Escherichia coli (Lounsbach et al., 1993) (Fig. 1). The results are presented in Fig. 2. Dot-blots confirmed the results obtained with Western blots of strain A2-infected cell lysates denatured in reducing sample buffer (Samson et al., 1986) (Table 1) with all Western blot-negative antibodies proving unable to bind to the F protein/β-galactosidase chimeras. Only one of the fusion-inhibiting antibodies, BD51, failed to bind but the binding of all four fusion inhibition-negative, group B antibodies (5A8, 5E2, 4E5 and 2D7) was conformation dependent.

Of the 10 neutralizing antibodies that showed binding in blots, all bound to the chimera carrying the full F polypeptide amino acid sequence, F1-574. The six group B MAbs and three unclassified MAbs bound to all fragments containing the amino acid sequence residues 253 to 289 including a chimera containing only this peptide. None of the MAbs bound to fragments containing only sequences N-terminal of residue 256. The binding of group A MAbs to amino acids 253 to 289...
is broadly in agreement with the findings of Arbiza (1992) who mapped neutralization escape mutants derived with four MAbs, including MAbs 47F and 7C2. One of these MAbs, B4, also bound to residues 226, 268 or 272. One of these MAbs, B4, also bound to residues 265 to 275. This region is conserved with the binding site of MAbs 47F and 7C2, also determined to be residues 255 to 275. This region includes the binding site of the N-terminal, a helix-rich half of the cysteine-rich domain of the F protein. This region contains the only known structural features hitherto associated with fusion and terminal hepadn repeat sequence (residues 57 to 199).

Thus MAbs binding to the amino acid 253 to 289 site, including two that bind to peptides sharing the conserved amino acid 265 to 272, exhibit diverse functional properties differing in their strain specificity, neutralizing activity, and fusion-inhibiting capacity. Differences in specificity and fusion-inhibiting capacity were determined by the greater or lesser involvement of non-conserved amino acid residues in the binding site. The 8/60 strain has not been sequenced, however, among all available F protein sequences, from RS viruses of both human subgroups and bovine strains, there was no clear correlation between MAb avidity and neutralization titre. Surprisingly, the two MAbs with the highest neutralizing titres were the only two MAbs with MAbs binding to the amino acid 253 to 289 site, including two that bind to peptides sharing the conserved amino acid 265 to 272, exhibit diverse functional properties differing in their strain specificity, neutralizing activity, and fusion-inhibiting capacity. Differences in specificity and fusion-inhibiting capacity were determined by the greater or lesser involvement of non-conserved amino acid residues in the binding site. The 8/60 strain has not been sequenced, however, among all available F protein sequences, from RS viruses of both human subgroups and bovine strains, there was no clear correlation between MAb avidity and neutralization titre. Surprisingly, the two MAbs with the highest neutralizing titres were the only two MAbs with
that of the group A antibodies. It may be that the full epitope recognized by all of these antibodies involves other elements, possibly distant in primary structure, and that recognition of these additional sites, not amino acids 253 to 289, determines the level of functional activity of the antibody. This would be in agreement with our previous observation that F protein fragments containing the 253 to 289 amino acid sequence fail to induce antibodies capable of neutralizing the virus (Lounsbach et al., 1993). A major conformation-dependent element in the epitope of these antibodies may also explain their apparent low avidity as conformational changes may occur on binding of proteins to plastic adversely affecting antibody binding to non-linear epitopes (Darst et al., 1988).

The further delineation of such conformation-dependent epitopes is problematic. Bourgeois et al. (1991), following studies with synthetic peptides, reported binding of MAb RS348 to amino acids 205 to 225, which lies immediately adjacent to the N-terminal leucine zipper-like structure of F1. However, this observation was not confirmed in the current study as F190-255, containing this sequence, failed to bind the antibody. Further studies with peptides (Trudel et al., 1987a) and antibody escape mutants (Arbiza et al., 1992) have suggested the involvement of additional sites in the binding of some putative group A antibodies. Unfortunately findings of different studies have not always been consistent or easily interpreted and a more powerful means of analysis is required to further elucidate these antibody-antigen interactions. Such studies, based perhaps on site-directed mutagenesis and X-ray crystallography, will provide insights not only into the mechanisms of virus neutralization but also help to resolve the way in which virus-induced membrane fusion is mediated.

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References


Short communication


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