Identification of antigenic sites mediating antibody-dependent enhancement of feline infectious peritonitis virus infectivity

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We have previously demonstrated antibody-dependent enhancement of feline infectious peritonitis virus (FIPV) infection of macrophages using both virus-specific antisera and monoclonal antibodies (MAbs) to the spike (S) protein of FIPV. To increase our understanding of this phenomenon, six representative MAbs from a previously documented group of 12 enhancing MAbs were used to identify epitopes that mediate antibody-dependent enhancement of FIPV infectivity. Analysis of the results of kinetics-based competitive ELISA (K-cELISA) among these six enhancing MAbs grouped the epitopes into two clusters. Because transmissible gastroenteritis virus (TGEV) and FIPV are so closely related antigenically, we also conducted K-cELISA experiments between the FIPV MAbs and TGEV S protein-specific MAbs for which the epitopes had previously been mapped to specific sites on the TGEV S protein. Results of these assays indicated that the two FIPV epitope clusters are homologues of the previously defined TGEV S protein sites A and E/F. In addition, two TGEV S protein-specific MAbs also induced antibody-dependent enhancement of FIPV infection of macrophages. This functional cross-reactivity provides further support for the close antigenic relationship between FIPV and TGEV. Our results provide a preliminary localization of several enhancing epitopes within the amino acid sequence of the FIPV S protein.

Feline infectious peritonitis virus (FIPV) is a member of the Coronaviridae and is antigenically related to feline enteric coronavirus, transmissible gastroenteritis virus (TGEV) and canine coronavirus (Sanchez et al., 1990). FIPV infection produces an ultimately fatal disease called feline infectious peritonitis (FIP) in domestic and exotic cats (Barlough & Stoddart, 1990). Virus-specific antibody (Ab) is involved in the pathogenesis of FIP at several levels. An accelerated, more fulminant form of FIP occurs frequently upon experimental FIPV challenge of seropositive as compared to seronegative cats (Pedersen & Boyle, 1980; Weiss & Scott, 1981a, b, c). In addition, immune complex and complement deposition incite the pyogranulomatous lesions of FIP (Jacobs-Geesl et al., 1980, 1982) and virus-specific Ab can enhance FIPV infection of macrophages (Corapi et al., 1992; Hohdatsu et al., 1991a; Olsen et al., 1992). Antibody-dependent enhancement (ADE) of FIPV infection of macrophages in vitro has been documented using antiserum from FIPV-infected cats (Olsen et al., 1992) and using monoclonal antibodies (MAbs) (Corapi et al., 1992; Hohdatsu et al., 1991a; Olsen et al., 1992). Our previous work has demonstrated that ADE of FIPV infection of primary feline peritoneal macrophages is mediated by MAbs to the spike (S) protein of FIPV (Corapi et al., 1992; Olsen et al., 1992). Analysis of 67 MAbs raised against two strains of FIPV and one strain of TGEV revealed a distinct association between the ability of a MAb to enhance FIPV infectivity for macrophages and to neutralize FIPV infectivity for Crandell feline kidney cells (Corapi et al., 1992; Olsen et al., 1992). A third study has demonstrated ADE of FIPV infectivity using MAbs specific for either the S or the membrane (M) proteins of FIPV (Hohdatsu et al., 1991a); however, enhanced disease in cats in vivo has been demonstrated only after vaccination with a recombinant vaccinia virus expressing the FIPV S protein (Vennema et al., 1990) and not with a similar vaccine expressing the FIPV M protein (Vennema et al., 1991). Enhancement has not been shown to occur with MAbs specific for the third FIPV structural protein, the nucleocapsid protein.

ADE of virus infectivity for macrophages in vitro has
been demonstrated across a wide range of virus families (Porterfield, 1986). Furthermore, a substantial body of epidemiological information suggests that ADE of dengue virus (DV) infection of macrophages underlies the development of a more severe form of dengue in vivo called dengue haemorrhagic fever or dengue shock syndrome (Halstead, 1989), analogous to the development of accelerated FIP (Weiss & Scott, 1981a). The presence of enhancing Abs in human immunodeficiency virus (HIV)-infected patients has also been correlated with the progression of AIDS (Homsy et al., 1990). There is, therefore, concern that ADE of virus infectivity may confound the development of vaccines against DV (Halstead, 1989; Morens & Halstead, 1990) and HIV (Bolognesi, 1989; Homsy et al., 1990; Robinson et al., 1988). Since ADE of disease has been clearly demonstrated following vaccination of cats with a variety of candidate vaccines (Olsen & Scott, 1991; Vennuma et al., 1990), a more specific understanding of ADE of FIPV infectivity is needed. Toward this end, we sought to begin to localize the epitopes on the S protein of FIPV 79-1146 recognized by enhancing MAbs.

Six MAbs raised against FIPV 79-1146 (3G7, 17E1.6, 18A7.4, 18H9.1, 23F4.5, 23F8.1) were selected from a previously documented (Olsen et al., 1992) group of 12 FIPV S protein-specific enhancing MAbs. These six provide a representative cross-section of the larger pool of enhancing MAbs as regards the relative levels of ADE and neutralization of FIPV they induced (Olsen et al., 1992) and IgG subclass (Corapi et al., 1992). We developed a kinetics-based competitive ELISA (K-cELISA) which could be used to determine the degree of overlap among the epitopes defined by these six enhancing MAbs. The Ig fraction of ascites fluids for each MAb was partially purified by ammonium sulphate precipitation and equilibrated to 1 mg protein per ml (Biuret method). A portion of each purified MAb was conjugated to biotin (Zymed Laboratories).

To prepare antigen (Ag) for K-cELISA, FIPV 79-1146 was grown in NLFK cells which had been adapted to growth in L-15 and McCoy's 5a media (Gibco) supplemented with glutamine, gentamicin (Gibco), and gammaglobulin-free serum (γGFS) (Gibco). [The use of γGFS in place of fetal bovine serum and NLFK cells in place of A72 cells dramatically reduced background levels of MAb binding to uninfected cell preparations (data not shown).] Thirty hours after infection, virus was harvested by freezing and thawing the cell cultures, centrifuged, and resuspended at a concentration of 1 mg protein per ml (Biuret method) of distilled H2O. A mock-infected control Ag preparation was diluted identically in parallel with the virus-containing Ag preparation.

To perform K-cELISA, Ag in 100 μl volumes was dried onto the wells of Immunulon 2 Removawell strips (Dynatech Laboratories). A concentration of 1 μg of virus-containing Ag per well gave optimal results. Antibodies to be assayed were diluted in PBS containing 0.05 % Tween-20 (PBS-T). Binding of biotinylated MAb (bMAb) was detected following a 30 min incubation at room temperature with horseradish peroxidase (HRPO)-conjugated streptavidin (Zymed) at an optimal dilution of 1:2500, followed by washing and addition of tetramethylbenzidine peroxidase substrate solution (Kirkgaard & Perry Laboratories). The optimal concentration of bMAb was 2 μg of protein per ml. This concentration produced the greatest differential in KELA slopes when tested on virus-containing Ag compared to control Ag. After such optimization, all K-cELISAs were conducted solely on virus-containing Ag because virtually no reactivity was detected using control Ag at these concentrations.

For our initial K-cELISAs, each bMAb was mixed 1:1 (v/v) with various dilutions of unlabelled FIPV MAbs (or PBS-T to assess binding in the absence of competitor) and added to triplicate wells in a total volume of 100 μl per well. After binding for 18 h at 4 °C, plates were washed with PBS-T using an automated plate-washer and assayed as above. The percentage competition was defined as the ability of a given dilution of unlabelled MAb to block the binding of bMAb. Competition was calculated as the mean KELA slope value in the presence of competitor (n = 3 wells) divided by the mean KELA slope value in the absence of competitor × 100, expressed as a percentage. Slope units are defined by the linear regression of the reaction rate between HRPO and substrate solution, and are directly proportional to the quantity of bound Ab (Barlough et al., 1983).

Results of a representative K-cELISA among the six FIPV MAbs assayed against MAb 17E1.6 are graphically shown in Fig. 1(a). Assays were similarly conducted using each of the six enhancing FIPV MAbs as the bMAb. Results of these six-way cross-competitions are diagrammatically shown in Fig. 1(b). The epitopes represented by these six enhancing MAbs could be grouped into two major clusters on the S protein. The cluster of epitopes represented by MAbs 3G7, 18H9.1, 23F4.5 and 23F8.1 is hereafter referred to as site A, with the restricted overlap between the 3G7 and 23F4.5 epitopes referred to as subsite A', and the cluster recognized by MAbs 17E1.6 and 18A7.4 referred to as site E/F. The epitope linkages within these clusters are based upon the assumption that competition for binding between two MAbs reflects steric hindrance between MAbs that are specific for the same or closely related epitopes. Competition between any two MAbs was always reciprocal regardless of which of the two was assayed as bMAb.

An association may exist between IgG subclass and the ability to mediate ADE and/or neutralization of
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• 3G7
• 17E1.6
• 18A7.4
• 18H9.1
• 23F4.5
• 23F8.1

Fig. 2. Summary of K-cELISA between TGEV-specific and FIPV-specific MAbs. Unlabelled FIPV-specific competing MAbs are listed in the left hand column and TGEV-specific bMAbs are listed across the top row. Black boxes indicate that the unlabelled MAb blocked > 80% of the binding of the bMAb (> 80% competition). Grey stippled boxes similarly indicate 20 to 80% competition and white boxes indicate < 20% competition. When the K-cELISA were performed in reciprocal fashion (unlabelled TGEV-specific MAbs were used to compete with the binding of biotinylated FIPV-specific MAbs), the same overall pattern of competition was found but the competitions depicted as 20 to 80% were > 80% as well.

FIPV infectivity. The majority of MAbs that can mediate both ADE and neutralization of FIPV infectivity are of the IgG2a subclass, whereas those which are neutralizing but not enhancing are IgG1 MAbs (Corapi et al., 1992). The site A/A' epitope cluster is particularly interesting in this regard since MAbs 3G7 and 18H9.1 are two exceptions to the subclass pattern. Both are IgG1 MAbs (Baines, 1988; Corapi et al., 1992), yet 3G7 is both neutralizing and enhancing and 18H9.1 is enhancing but not neutralizing (Olsen et al., 1992). MAbs 23F4.5 and 23F8.1 are IgG2a MAbs (Corapi et al., 1992) which are both neutralizing and enhancing (Olsen et al., 1992). The grouping of these four enhancing epitopes in a single epitope cluster in this study suggests that enhancement may be epitope-dependent, as well as Ab subclass-dependent.

To localize the enhancing epitopes more specifically within the S protein of FIPV, we took advantage of the fact that there is a substantial degree of antigenic homology between the FIPV and TGEV S proteins (Sanchez et al., 1990) as well as 93% amino acid sequence identity for residues 275 to 1447 (Jacobs et al., 1987). Considerable work has been done to characterize and physically to map epitopes on the S protein of TGEV (Correa et al., 1988, 1990; Delmas et al., 1986, 1990; Garwes et al., 1987; Gebauer et al., 1991; Posthumus et al., 1990; Simkins et al., 1989, 1992). Although there is some confusion regarding site designations (see Posthumus et al., 1990, for clarification), most investigators agree that there are four major and one or two minor antigenic sites on the S protein of TGEV. The presence of multiple epitope clusters on the S protein of FIPV as shown here and elsewhere (Corapi et al., 1992; Hohdatsu et al., 1991b) is, therefore, consistent with the antigenic structure of the TGEV S protein. TGEV MAbs 25C9.3 and 25E4.3/26F4.3, which have previously been mapped to TGEV S protein sites A and E/F, respectively (Simkins et al., 1992), were assayed by K-cELISA against the six
Table 1. TGEV-specific MAbs tested for ADE of FIPV infection of primary feline peritoneal macrophages

<table>
<thead>
<tr>
<th>MAb*</th>
<th>TGEV protein specificity</th>
<th>Virus neutralization titre</th>
<th>FIPV enhancement factor†</th>
</tr>
</thead>
<tbody>
<tr>
<td>25C9.3</td>
<td>S</td>
<td>47000</td>
<td>48:1</td>
</tr>
<tr>
<td>25E4.3</td>
<td>S</td>
<td>4000</td>
<td>37:3</td>
</tr>
<tr>
<td>25H4.3</td>
<td>S</td>
<td>18000</td>
<td>≤1:2</td>
</tr>
<tr>
<td>42E4.3</td>
<td>M</td>
<td>4</td>
<td>≤1:2</td>
</tr>
</tbody>
</table>

* TGEV-specific MAbs were synthesized and characterized as previously described (Simkins et al., 1989, 1992)
† Enhancement factors reflect the maximum degree of enhancement of FIPV 79-1146 infection of primary feline peritoneal macrophages induced by each MAb. Enhancement factors were calculated as previously described (Olsen et al., 1992)

FIPV-enhancing MAbs. These results of K-cELISA between the TGEV- and FIPV-specific MAbs are shown in Fig. 2. TGEV MAb 25C9.3 largely competed against the FIPV MAbs mapped to clusters A/A' while TGEV MAbs 25E4.3 and 26F4.3 mainly competed for binding with the FIPV MAbs of cluster E/F. These results suggest that the FIPV epitope clusters defined as site A/A' and site E/F are homologous to site A and site E/F of TGEV, respectively. TGEV site A MAbs were previously shown to cross-react with FIPV strain 79-1146 (Delmas et al., 1990). The homology between the S proteins of FIPV and TGEV is further substantiated by the fact that TGEV MAbs 25C9.3 and 25E4.3 were also capable of mediating ADE of FIPV infection of primary feline macrophages (Table 1). This functional cross-reactivity further supports the hypothesis that FIPV and TGEV may simply be host-range mutants of one another (Horzinek et al., 1982). Assessment of the ability of the TGEV MAbs to induce ADE of FIPV infectivity was conducted using a previously described assay (Olsen et al., 1992).

Previous work has demonstrated that TGEV site E and F MAbs compete with each other’s binding (Simkins et al., 1989, 1992), so the FIPV grouping of MAbs 17E1.6 and 18A7.4 into a single cluster is not unexpected. Likewise, the ability of the TGEV MAbs to compete partially with MAbs from both of the FIPV epitope clusters (stippled boxes, Fig. 2), and thereby bridge these clusters, is also consistent with the previously demonstrated overlap between TGEV sites A, B and E/F (Simkins et al., 1992). TGEV site A has been mapped to specific amino acids on the TGEV S protein. Sequencing of MAb-resistant mutants (Correa et al., 1990; Gebauer et al., 1991) and epitope scanning using the PEPSCAN method (Gebauer et al., 1991) have been used to identify amino acids 538, 541 to 543, 586 and 591 as being specifically involved in the formation of TGEV site A. Analysis of a comparison of the TGEV and FIPV S protein sequences (Jacobs et al., 1987) indicates that TGEV amino acids 538 (lysine), 541 (glycine), 542 (tyrosine), 543 (glycine) and 586 (aspartate) are strictly conserved in FIPV. These homologies provide a basis for future investigations to localize the FIPV enhancing epitopes at site A/A' more specifically. To our knowledge, the TGEV E/F site has not been mapped to specific amino acids. Thus, delineation of the position of the corresponding FIPV site E/F is not yet possible. However, the overlap between TGEV sites A/B and E/F (Simkins et al., 1989, 1992) and the bridging between FIPV clusters A and E/F demonstrated here suggests that the E/F site may be topographically related to the A site on the FIPV S protein as well.

In summary, ADE of FIPV infection of macrophages may play a critical role in the immunopathogenesis of FIP and should be an important consideration for future FIPV vaccine development. We have previously shown that ADE of FIPV infectivity is mediated by MAbs to the S protein, and we now present results demonstrating that the epitopes delineated by six enhancing MAbs are grouped into two major epitope clusters on the S protein of FIPV and that these clusters are homologous to sites A and E/F of TGEV. We have also determined that epitope recognition by these six FIPV infection-enhancing MAbs is dependent upon the conformational integrity of the epitopes (as determined from Western immunoblotting analyses), but is independent of their glycosylation (as determined by radioimmunoprecipitation of FIPV proteins which were produced in the presence of tunicamycin or treated with N-glycosy clave) (data not shown). The preliminary locations of the enhancing epitopes of FIPV defined in our work by comparison to TGEV can now be confirmed through sequencing of MAb escape mutants and/or synthetic peptide analyses.

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References


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