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Localization of Antigenic Sites of the S Glycoprotein of Feline Infectious Peritonitis Virus Involved in Neutralization and Antibody-Dependent Enhancement

WAYNE V. CORAPI, RAPHAEL J. DARTEIL, JEAN-CHRISTOPHE AUDONNET, AND GILLES E. CHAPPUIS*

Rhône Mérieux, Laboratoire IFFA, 69342 Lyon Cedex 07, France

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The S glycoprotein of feline infectious peritonitis virus (FIPV) has been shown to contain the antigenic sites responsible for eliciting both neutralization and antibody-dependent enhancement. To determine the region of S responsible, overlapping DNA fragments spanning the entire S gene were cloned and expressed as fusion proteins by in vitro transcription and translation. Fusion proteins containing relevant epitopes were identified by radioimmunoprecipitation with neutralizing and enhancing FIPV-specific monoclonal antibodies (MAbs). A region spanning residues 509 to 673 reacted with most MAbs tested. Translation in the presence of microsomal membranes did not enhance reactivity, suggesting that glycosylation is not essential for recognition by the MAbs. To localize the antigenic sites further, several MAb-resistant (*mar*) mutants of FIPV were cloned and sequenced. Amino acid residues that contribute to the neutralizing and enhancing epitopes were localized to two regions, designated A1 and A2, which show partial overlap with the homologous antigenic site A of transmissible gastroenteritis virus. Site A1 contains residues 568 and 591 and is homologous with part of subsite Aa of transmissible gastroenteritis virus. Site A2 contains residues 643, 649, and 656. Double mutations in sites A1 and A2 were found in *mar* mutants derived from neutralizing and enhancing MAbs 23F4.5 and 18A7.4, while a single mutation in site A2 was found in a *mar* mutant derived from MAb 24H5.4, which is neutralizing but not enhancing. The data suggest that site A2, which includes residues 643 to 656, is a dominant neutralizing site of FIPV and that sites A1 and A2 may act in concert to induce antibody-dependent enhancement.

Feline infectious peritonitis virus (FIPV) is part of an antigenic cluster of positive-stranded RNA viruses within the *Coronaviridae* family that includes feline enteric coronavirus, canine coronavirus, transmissible gastroenteritis virus (TGEV), and porcine respiratory coronavirus (22). It causes a complex and fatal disease in cats and is unique among the coronaviruses in causing antibody-dependent enhancement (ADE). Cats with FIPV neutralizing antibody from previous natural exposure to virus, passive transfer of antibody, or vaccination frequently develop disease far more rapidly and more severely than cats that have not been previously exposed (17, 27, 28). The binding of virus-antibody immune complexes to Fc receptors on the cell surface of macrophages is believed to be the mechanism that facilitates the enhanced uptake and spread of virus (19, 29).

There are three structural proteins of FIPV. The largest of these is the spike (S) protein, which has been shown to be responsible for inducing both neutralizing antibodies and ADE (3, 7, 16, 25). Although the membrane protein (M) and nucleocapsid (N) do not play a role in ADE, there is some evidence that vaccination with M alone may provide some protection against disease (26). In vitro studies with neutralizing monoclonal antibodies (MAbs) to FIPV have shown that the major neutralizing epitopes are confined to S and that they correspond, to a large degree, with the epitopes involved in ADE (3, 16).

Successful vaccination against FIPV without the development of neutralizing antibodies that can lead to ADE has never been consistently achieved. Recombinant vaccines that do not contain any S protein may provide the best alternative for further vaccine studies, but the localization of antigenic sites on S responsible for neutralization and ADE is needed in order to better understand the phenomenon of ADE and whether its apparent linkage to neutralization can be blocked or circumvented. FIPV is perhaps the best-known animal model for studying ADE and may also provide clues for understanding this phenomenon in other virus systems.

It was previously shown that neutralizing and enhancing MAbs could be divided into six major groups according to their abilities to recognize different strains of FIPV and MAb-resistant (*mar*) mutants (3). In the present study, we have attempted to localize the antigenic sites recognized by some of those MAbs. Overlapping fusion proteins that spanned the entire length of S were used to determine the general vicinity of the relevant epitopes. RNA from *mar* mutants, each lacking a single MAb binding site, was then isolated, cloned as double-stranded DNA, and sequenced for comparison with wild-type virus. The nucleotide differences resulted in amino acid substitutions at locations that correspond well with antigenic site A, the dominant neutralizing region previously described for TGEV (4, 8–10), and suggest a possible mechanism for the induction of ADE.

MATERIALS AND METHODS

Virus, cell cultures, and MAbs. The wild-type 79-1146 strain (18) and all *mar* mutants of FIPV were grown in CRFK cells cultured in Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum, 100 IU of penicillin per ml, and 100 μg of streptomycin per ml (Gibco BRL). All *mar* mutants were derived from the 79-1146 strain. Virus was biologically cloned three times

* Corresponding author. Mailing address: Rhône Mérieux, Laboratoire IFFA, 254 rue Marcel Mérieux, 69342 Lyon Cedex 07, France. Phone: (33) 72 72 34 47. Fax: (33) 72 72 34 98.

by plaque purification, concentrated, and partially purified by sucrose-gradient centrifugation. Production and characterization of FIPV-specific MAbs has been described previously (3). Only neutralizing or enhancing MAbs were used in the present study.

***mar* mutants.** *mar* mutants of FIPV, derived from the parental stock of FIPV 79-1146, were selected by the ability to escape neutralization by neutralizing FIPV-specific MAbs as previously described (3). Mutants were cultured several times in the presence of the selecting MAb, biologically cloned by plaque purification, and cultured again in the presence of the selecting MAb.

Isolation of RNA. RNA was isolated from the FIPV 79-1146 by using the guanidinium thiocyanate-phenol-chloroform extraction method of Chomczynski and Sacchi (2). Total RNA was isolated from *mar* mutants by using a commercially available RNA extraction buffer (Bioprobe Systems, Montreuil-sous-Bois, France) according to the manufacturer's instructions.

DNA cloning. All DNA primers were synthesized with a DNA synthesizer (PCR-Mate 391; Applied Biosystems) or purchased commercially (Eurogentec, Seraing, Belgium). Primers were designed to cover the entire coding region of the S gene of FIPV in 3 large fragments of approximately 1,600 bp and 12 smaller subfragments of approximately 400 to 500 bp each. Reverse transcription of RNA and amplification of cDNA by PCR were done by using standard techniques (21) with a DNA thermal cycler (Perkin-Elmer). Amplified DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1), digested with relevant restriction enzymes, and purified by binding to a silica matrix (Geneclean II; Bio 101 Inc., La Jolla, Calif.). All primers contained either *Xba*I, *Bam*HI, or *Xho*I restriction sites for digestion and ligation of DNA fragments into plasmid pBluescript SK+ (Stratagene, La Jolla, Calif.). Cloned FIPV sequences were later removed from pBluescript at the *Not*I and *Xho*I restriction sites and transferred to plasmid pTOPE-SX (see below) for in vitro transcription and translation. All plasmids were grown in *Escherichia coli* NM522 or NovaBlue(DE3) (Novagen, Madison, Wis.). Bacterial colonies were lysed by the alkaline lysis procedure and purified by equilibrium centrifugation in CsCl-ethidium bromide gradients (21) or by selective binding to an anion-exchange resin (Qiagen-tips; Qiagen Inc., Chatsworth, Calif.).

Construction of plasmid pTOPE-SX. Plasmid pTOPE-1b(+) (Novagen) contains the T7 promoter and part of T7 gene 10 followed by a polylinker region. The entire polylinker region was removed by digestion with restriction enzymes *Sac*II and *Xho*I and replaced with an 82-bp fragment taken from the polylinker region of pBluescript at the *Sac*II and *Xho*I sites. An extra nucleotide was added to this short piece of DNA in order to produce a frameshift mutation immediately after the *Sac*II site that allowed all downstream restriction sites to be in the same phase with restriction sites present in pBluescript-cloned FIPV sequences. The new plasmid was designated pTOPE-SX to indicate the altered sequence. FIPV sequences were then removed from the pBluescript host at the *Not*I and *Xho*I sites and ligated into pTOPE-SX. In vitro transcription and translation of pTOPE-SX inserts with T7 RNA polymerase resulted in the production of fusion proteins containing 260 amino acids of the T7 gene 10 protein along with the translated FIPV sequence.

In vitro transcription and translation. Coupled transcription and translation reactions using rabbit reticulocyte lysate and T7 RNA polymerase were performed with the TNT reticulocyte lysate system (Promega, Madison, Wis.) according to the manufacturer's instructions. Reactions included 40 μCi of translation-grade [^{35}S]methionine (Amersham France, Les Ulis, France) per 50 μl of reaction mixture for labeling of translation products. To examine the effect of posttranslational processing of proteins, 5 equivalents of canine pancreatic microsomal membranes (Promega) was added per 25 μl of reaction mixture in separate reactions. Reactions were performed at 30°C for 90 min in a thermal cycler. Translation products were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and examined by autoradiography.

Radioimmunoprecipitation and electrophoresis. Radioimmunoprecipitation assays (RIPA) were performed by mixing 5 μl of translated fusion protein with 5 μl of MAb or cat serum in 200 μl of TNE Triton buffer (150 mM NaCl, 50 mM Tris [pH 8.0], 5 mM EDTA, 0.1% Triton X-100) and mixing at 0°C for 1 h on a rocking platform. FIPV-positive and -negative cat sera and T7 Tag MAb (Novagen), which is directed against the first 10 amino acids of the T7 gene 10 fusion protein, were used as controls. Immune complexes were absorbed by adding 50 μl of recombinant protein G-agarose to samples containing MAbs or 50 μl of recombinant protein A-agarose to samples containing cat serum (Boehringer Mannheim, Mannheim, Germany). Samples were incubated for an additional 1 h at 4°C on a rotating platform.

The agarose-bound immune complexes were gently pelleted for 30 s and washed twice in RIPA buffer (150 mM NaCl, 50 mM Tris [pH 8.0], 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) and one time with Tris-Triton buffer (10 mM Tris [pH 8.0], 0.1% Triton X-100). Pelleted samples were resuspended in an equal volume of 2× sample buffer (0.125 M Tris-HCl [pH 6.8], 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.005% bromophenol blue) and boiled for 3 min. Samples were centrifuged at 10,000 × *g* for 3 min, loaded on to SDS–10% polyacrylamide gels, and subjected to electrophoresis overnight at 50 V. Gels were fixed and treated with Amplify (Amersham) for fluorographic enhancement, dried, and exposed with Hyperfilm-βmax (Amersham) for 3 to 5 days at −70°C.

DNA sequencing. *mar* mutants and clones containing FIPV inserts were sequenced in both directions, using the dideoxynucleotide chain termination method on double-stranded DNA (21, 23) and the modified T7 DNA polymerase (Sequenase; U.S. Biochemical, Cleveland, Ohio) (24) according to the manufacturer's instructions. Primers used for sequencing pBluescript SK+ inserts were (−40) reverse primer and M13 (−40) primer (U.S. Biochemical); those used for pTOPE-SX inserts were T7 gene 10 primer and T7 terminator primer (Novagen). Sequencing was done on separate *mar* mutants derived from the same MAb as well as separate clones derived from the same *mar* mutant. DNA and protein sequence data were compiled and analyzed by using the PC/GENE computer software package.

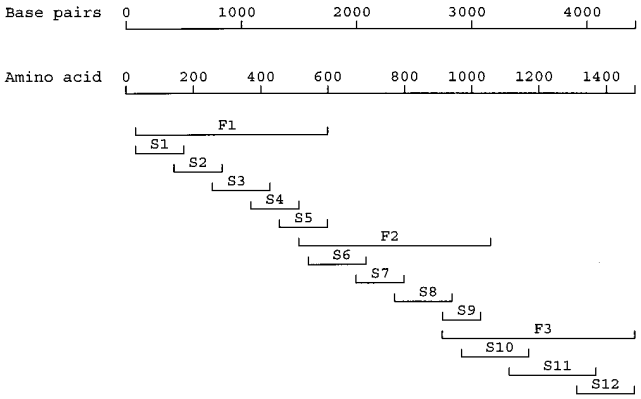

FIG. 1. FIPV S gene fragments cloned into pBluescript and pTOPE-SX vectors. Locations of the 15 overlapping fragments from the 5′ to the 3′ end of S are shown. *Xba*I or *Bam*HI restriction endonuclease sites were added to the 5′ ends and *Xho*I sites were added to the 3′ ends of all fragments for insertion into pBluescript.

RESULTS

Cloning and translation of FIPV fragments. To localize the general region of the S gene of FIPV responsible for neutralization and ADE, overlapping clones of S that could be translated as fusion proteins and tested for their reactivity with various MAbs were needed. Three large fragments of S (Fig. 1, F1 to F3) and 12 smaller subfragments (S1 to S12), all derived from the parental stock of FIPV 79-1146, were cloned into the plasmid pBluescript SK+. The sequences of all clones were determined and compared with the published sequence of S for FIPV 79-1146 (5) to ensure against PCR error. FIPV inserts were then removed and recloned into plasmid pTOPE-SX at the *Not*I and *Xho*I restriction sites for in vitro transcription and translation (Fig. 2). Plasmid pTOPE-SX was constructed from plasmid pTOPE-1b(+) by changing the polylinker region between *Sac*II and *Xho*I and adding an extra base after the *Sac*II site as described in Materials and Methods. Translation products consisted of fusion proteins containing the first 260 amino acids of the T7 gene 10 protein, with a calculated molecular size of approximately 28 kDa, followed by FIPV S sequences. The larger FIPV peptides, F1, F2, and F3, had a calculated molecular size of approximately 62 kDa, resulting in fusion proteins of about 90 kDa, which corresponded well with the observed results (Fig. 3A). Smaller FIPV peptides S1 to S12, with an average calculated molecular size of approximately 18 kDa, resulted in fusion proteins of about 46 kDa (Fig. 3B).

Cotranslational processing with pancreatic microsomal membranes. To optimize conditions for recognition of translated proteins by MAbs, fusion proteins were also translated in the presence of canine pancreatic microsomal membranes. Core glycosylation at the N-terminal end of S resulted in an increase in the molecular size of the fusion protein F1 from 90 kDa to either 98 or 145 kDa, an increase of 8 or 55 kDa, respectively (Fig. 3A). A corresponding 8-kDa increase in mo-

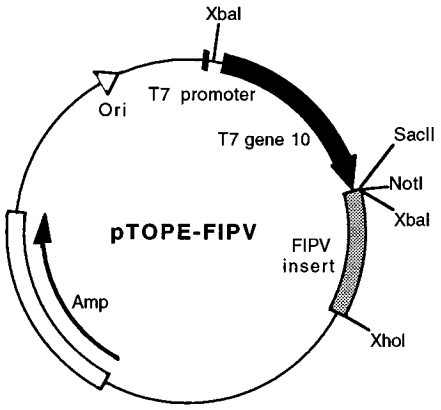

FIG. 2. Construction of plasmid pTOPE-SX with FIPV insertion for expression of the T7 gene 10-FIPV fusion protein. FIPV fragments were removed from pBluescript plasmid and inserted at *Not*I and *Xho*I restriction sites of pTOPE-SX. Expression of fusion proteins was driven by T7 RNA polymerase in the presence of rabbit reticulocyte lysate.

lecular size was seen in subfragment S1, which increased from 46 to 54 kDa (Fig. 3B). Translation in the presence of microsomal membranes did not have any effect on the other remaining FIPV fragments.

Recognition of FIPV peptides by MAbs. Neutralizing and enhancing MAbs were tested by RIPA for the ability to recognize radiolabeled fusion proteins. FIPV-positive and -negative cat sera and T7 Tag MAb were used as controls. The FIPV-positive serum and T7 Tag MAb recognized fusion proteins, while the negative serum did not (Fig. 4A). Overloading of immunoglobulin molecules sometimes produced a curved band at about 46 kDa when proteins were precipitated with whole cat serum (lane 3), but this band did not appear when MAbs were used (lane 1). FIPV-specific MAbs 23F4.5, 18A7.4,

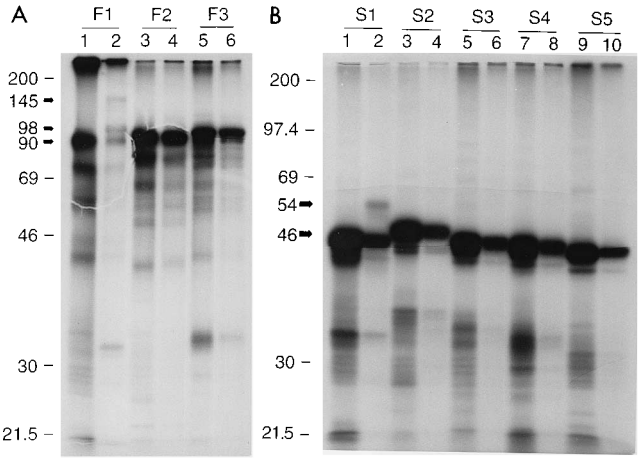

FIG. 3. SDS-PAGE analysis of ^{35}S-labeled FIPV fusion proteins. Proteins were expressed by in vitro transcription and translation of cloned FIPV fragments in plasmid pTOPE-SX and then subjected to SDS-PAGE and fluorography. (A) Ninety-kilodalton fusion proteins of FIPV fragments F1 to F3 in the absence (lanes 1, 3, and 5) or presence (lanes 2, 3, and 6) of canine microsomal membranes. Core glycosylation of F1 resulted in additional bands at 98 and 145 kDa. (B) Fusion proteins of smaller FIPV fragments S1 to S5 in the absence (lanes 1, 3, 5, 7, and 9) and presence (lanes 2, 4, 6, 8, and 10) of canine microsomal membranes. Core glycosylation of S1 resulted in increase of molecular size from 46 to 54 kDa. Positions of molecular weight markers are shown in kilodaltons. Arrows indicate calculated molecular sizes of fusion proteins F1 and S1.

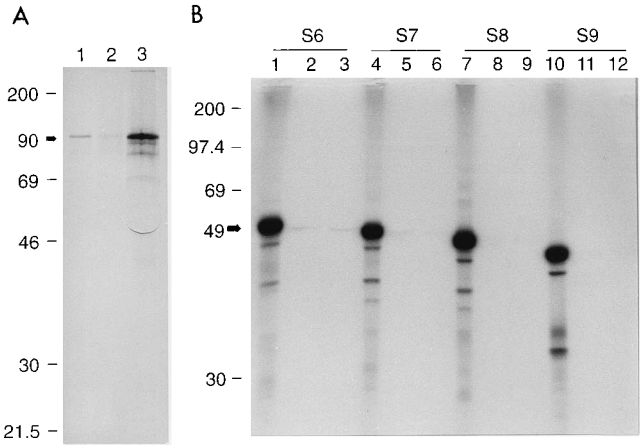

FIG. 4. Immunoprecipitation of ^{35}S-labeled FIPV fusion proteins with MAbs or cat serum. (A) Fusion protein F1 (90 kDa) was immunoprecipitated with T7 Tag MAb, recognizing the first 10 amino acids of the T7 gene 10 (lane 1), FIPV-negative cat serum (lane 2), or FIPV-positive cat serum (lane 3). (B) Immunoprecipitation of fusion proteins S6, S7, S8, and S9 with neutralizing and enhancing MAbs 23F4.5 (lanes 2, 5, 8, and 11) and 18A7.4 (lanes 3, 6, 9 and 12). Fusion protein controls are shown in lanes 1, 4, 7, and 10. Only S6 (49 kDa) was recognized by the MAbs. Positions of molecular weight markers are shown in kilodaltons. Arrows indicate calculated molecular sizes of fusion proteins F1 and S6.

and 24H5.4 were found to recognize fragment F2 and subfragment S6, which have in common a 165-amino-acid region that begins at amino acid residue 509 and ends at residue 673. Recognition of fusion proteins F2 and S6 by the MAbs was weak but could be detected by comparison with the complete absence of immunoprecipitation of all remaining fusion proteins with the same MAbs (Fig. 4B). Recognition was not improved by using fusion proteins that had been translated in the presence of canine pancreatic microsomal membranes, suggesting that glycosylation is not essential for MAb recognition of the relevant epitopes.

Sequencing of *mar* mutants. To localize the antigenic sites located on subfragment S6 more precisely, the S6 regions (nucleotides 1594 to 2089) of several FIPV *mar* mutants were cloned in plasmid pBluescript SK+ and sequenced. Observed sequences were then compared with the previously obtained homologous sequence of the parental 79-1146 strain. *mar*23F4.5 was selected by its ability to escape neutralization by MAb 23F4.5, a MAb that was previously shown to have a neutralization titer of 20,480 (3) and enhanced FIPV infection as much as 100-fold above normal (16). When *mar*23F4.5 was sequenced, it was found to have base substitutions at positions 1840 and 2014, which resulted in amino acid changes at residues 591 (Asp) and 649 (Arg) (Table 1). MAb 18A7.4 had a neutralization titer of 5,120 and also enhanced FIPV infection at least 100-fold above normal. *mar*18A7.4 had base substitutions at positions 1772 and 2036, which resulted in amino acid

TABLE 1. Predicted amino acid changes of FIPV S mutants

Clone	Nucleotide substitution[a]	Amino acid change	Position
*mar*23F4.5	GAC→TAC	Asp→Tyr	591
	AGA→GGA	Arg→Gly	649
*mar*18A7.4	GAT→GTT	Asp→Val	568
	AGA→AAA	Arg→Lys	656
*mar*24H5.4	GAT→TAT	Asp→Tyr	643

[a] Mutants were derived from FIPV 79-1146.

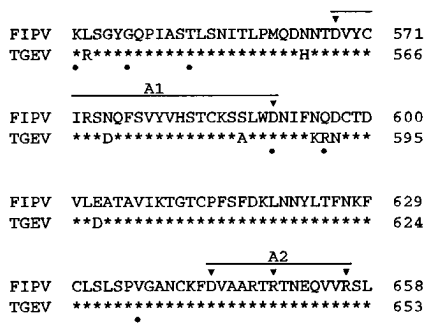

FIG. 5. Comparison of antigenic sites in FIPV S primary structure with those of TGEV. A portion of the amino acid sequence of the cloned subfragment recognized by FIPV neutralizing MAbs is shown. Consensus between the two sequences is shown by an asterisk. The position of amino acid changes resulting in resistance to neutralization is shown by an arrowhead for FIPV and a solid circle for TGEV (10, 12). The proposed FIPV antigenic sites A1 and A2 are indicated.

changes at residues 568 (Asp) and 656 (Arg). MAb 24H5.4 had a neutralization titer of 96 but did not enhance FIPV infection. *mar*24H5.4 had a single base substitution at position 1996, which resulted in an amino acid change at residue 643 (Asp).

Comparison of FIPV and TGEV antigenic sites. The amino acid sequence of the S protein of FIPV shows extensive homology with that of TGEV (5, 20), particularly in the carboxy-terminal portion (amino acids 278 to 1452), where the two sequences are 93% homologous (14). Previous studies have shown that the dominant neutralizing sites of TGEV are found on the S protein within antigenic site A (4, 8–10). Site A has been localized to an area that includes amino acid residues 538 through 591 of TGEV (10) and corresponds to residues 543 through 596 of FIPV (Fig. 5).

FIPV *mar*23F4.5 and *mar*18A7.4 each had one amino acid substitution within this region at residues 591 and 568, respectively. The mutation at residue 591 (Asp) in *mar*23F4.5 occurred at the same position as that reported for TGEV *mar*1B.H6 (10) and *mar*20.9 (9), both of which are site A specific. The additional amino acid changes seen with *mar*23F4.5 and *mar*18A7.4 at positions 649 and 656 and the single mutation seen with *mar*24H5.4 at position 643 occurred in the same general vicinity as that seen in TGEV *mar*1B.B5 (TGEV residue 631) (4, 10).

When the hydrophilicity profile of the 165-amino-acid sequence of the S6 fragment was plotted, the cluster of three mutations at amino acid residues 643 to 656 matched the calculated position of the best antigenic determinant. This region was designated A2 and most likely corresponds to an important site for neutralization of FIPV. The mutation in *mar*23F4.5 occurred precisely at the region of highest hydrophilicity. The second mutations in *mar*23F4.5 and *mar*18A7.4 occurred in a less hydrophilic region designated A1, which represents part of the homologous Aa region of TGEV (10).

DISCUSSION

Recent published reports have demonstrated that ADE of FIPV infection can be mimicked in vitro by using MAbs (3, 13, 16). Although there has been some confusion as to whether antibodies directed against M can also produce ADE (13), the general consensus has been that neutralizing antibodies directed against the S protein are mainly responsible for this phenomenon. While much work has been done to develop a vaccine for FIPV that lacks the ability to induce ADE, no published reports have yet identified the antigenic sites on S that are responsible for stimulating the production of enhancing antibodies. A competitive assay using FIPV and TGEV MAbs suggests that there may be some overlap between enhancing epitopes on FIPV and antigenic sites A, E, and F of TGEV (15).

In this study, we have used a series of neutralizing and enhancing MAbs to FIPV to identify fragments of S containing relevant epitopes by expressing them in *E. coli* as fusion proteins, using a modified pTOPE expression system. Only fusion proteins containing amino acids 509 to 673, located in the amino-terminal half of S, were recognized by the MAbs in this system. Translation of fusion proteins in the presence of canine microsomal membranes resulted in core glycosylation only in those fragments that contained the first 465 amino acids at the N-terminal end of S and caused an increase in the molecular sizes of those fragments of either 8 or 55 kDa, depending on the length of S sequence translated. Since the native S protein has a predicted molecular size of about 160 kDa (5), the addition of carbohydrate totaling 55 kDa to the N-terminal third would account for the observed molecular size of 210 kDa measured in the mature protein (1, 6). Whether this is the only site of N-linked glycosylation in FIPV is not known, but repeated attempts to demonstrate glycosylation in other regions of S were unsuccessful. The addition of microsomal membranes reduces the translation efficiency as much as 75%, and it is possible that the detection method used was not sensitive enough to recognize shifts in molecular size of the remaining fusion proteins. Glycosylation of fusion proteins derived from the N-terminal end of S, however, did not enhance their recognition by any of the MAbs.

Once the general region containing the relevant antigenic sites was identified, the antigenic sites were localized more precisely by sequencing the 495 nucleotides of the S gene coding for the S6 region in *mar* mutants and comparing the results with those found in the parental 79-1146 strain. The antigenic sites were localized within two small regions, designated A1 (residues 568 to 591) and A2 (residues 643 to 656). The A1 site is homologous with a portion of the antigenically dominant site A of TGEV and overlaps part of the TGEV subsite Aa. A1-specific *mar*23F4.5 had a mutation at the same amino acid (residue 591) as that reported for two different Aa-specific TGEV *mar* mutants (9, 10). The A2 site is located in one of the more highly hydrophilic regions of S and was the primary antigenic determinant calculated for cloned fragment S6.

*mar*23F4.5 and *mar*18A7.4 had mutations in A1 and A2, while *mar*25H5.4 had only a single mutation in A2. The double mutations were seen both in independently derived *mar* mutants and in separate clones derived from the same *mar* mutant. Mutations at two different sites in a single TGEV *mar* mutant have been reported, one of which occurred in the TGEV Aa region and the other of which occurred in an area close to the FIPV A2 site (Fig. 5, TGEV residues 543 and 631, respectively) (4, 10). Because the S6 region was the only area sequenced in the FIPV *mar* mutants, it is possible that additional mutations were present outside this region. The lack of reactivity of MAbs with the other fusion proteins, however, suggests that the relevant mutations were confined to this area.

The presence of two mutations in *mar*23F4.5 and *mar*18A7.4 in the same general areas suggests that both sites A1 and A2 contribute to the formation of epitopes recognized by MAbs 23F4.5 and 18A7.4. Both MAb 23F4.5 and MAb 18A7.4 are highly neutralizing and enhancing, which suggests that a cross-linking of these two sites by antibodies may play a role in the development of ADE. The finding that MAb 24H5.4 is neutralizing but not enhancing and that *mar*24H5.4 had only a

single mutation in site A2 supports this view. The hydrophilic nature of A2 and the fact that all three MAb binding sites were localized within such a narrow region suggests that A2, which includes amino acid residues 643-DVAARTRTNEQVVR-656, is a dominant neutralizing site of FIPV. The close homology between FIPV site A1 and part of TGEV subsite Aa suggests that A1, which includes amino acid residues 568 to 591, may also, on its own, be an important neutralizing site of FIPV. Assuming an average group length of six amino acids per epitope, A1 and A2 could perhaps be extended another five amino acids in either direction.

If cross-linking or close apposition of A1 and A2 is necessary to induce ADE, then immunization with a synthetic peptide containing only A2 should result in the production of antibodies capable of neutralizing FIPV but not causing enhancement. Alternatively, A1 and A2 may simply represent two independent neutralizing sites on S, with the magnitude of ADE observed being merely a reflection of the degree of affinity of antibodies for either region combined with the efficient presentation of antibody Fc fragments to Fc receptors. In the latter case, immunization with a peptide containing antigenic site A1 or A2 should instead produce neutralizing antibodies capable of inducing high levels of ADE.

Attempts have been made to develop a vaccine against FIPV by immunizing cats with FIPV antigen that does not contain S (26) or with virus that produces only low levels of neutralizing antibodies (11, 12). Both strategies have been attempts to avoid the production of antibodies that appear to be inseparably linked to both neutralization and ADE. If two separate antigenic sites are in fact linked and play a role together in the development of ADE, while neutralization is solely dependent on a response to independent antigenic sites, of which A2 plays a major role, then a third strategy may become available that would allow vaccination with a recombinant vaccine or synthetic peptide that contains antigenic site A2.

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