LOCATION OF ANTIGENIC SITES OF THE S-GLYCOPROTEIN OF TRANSMISSIBLE GASTROENTERITIS VIRUS AND THEIR CONSERVATION IN CORONAVIRUSES

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INTRODUCTION

Transmissible gastroenteritis virus (TGEV) has a single-stranded, positive-sense RNA genome of more than 20 kb (Brian et al., 1980; Rasschaert et al., 1987) and three structural proteins: S, N and M, with 1447, 382 and 262 amino acids, respectively (Kapke and Brian, 1986; Laude et al., 1987; Rasschaert and Laude, 1987; Jacobs et al., 1987).

The S glycoprotein is responsible for the induction of neutralizing antibodies (Garwes et al., 1978). On the S protein a minimum of four antigenic sites have been defined, site A being the major inducer of neutralizing antibodies (Jiménez et al., 1986). This site has been subdivided into three antigenic subsites (Correa et al., 1988). In the S glycoprotein we have defined 16 epitopes, of which 10 are involved in virus neutralization (Sánchez et al., 1989).

In this chapter, we locate the antigenic sites on the protein sequence by studying the binding of monoclonal antibodies (MAbs) to S-protein fragments and to recombinant products of pEX-TGEV vectors, and by identifying nucleotide sequence differences between TGEV genome and MAb resistant (**mar**) mutants. In addition, we studied the conservation of the antigenic sites in coronaviruses, and defined type, group and interspecies

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TABLE 1

S-GENE[a] FRAGMENTS EXPRESSED BY pEX-TGEV RECOMBINANTS

FRAGMENT NUMBER	NUCLEOTIDE[b] FRAGMENT	S PROTEIN FRAGMENT
1	-8 - 1136	1 - 378
2	976 - 1674	326 - 558
3	1588 - 2021	530 - 673
4	1675 - 2021	559 - 673
5	1819 - 2238	607 - 746
6	2022 - 2760[c]	675 - (920)[c]
7	2622 - 3477	875 - 1159
8	3447 - 3717	1150 - 1239
9	3478 - 4255	1160 - 1418

a. The S-gene from TGE virus has 4341 nucleotides, encoding a protein of 1447 residues (Rasschaert et al., 1987; Jacobs et al., 1987).

b. Numbers are relative to the start of the coding sequence.

c. Insert 6 was derived from clone B1 (Jacobs et al., 1987) using the Pst I site from the polylinker. It was checked that contains the Hpa I site at nucleotide 2619. The S-fragment coded by this insert includes, at least, the residues 675-919.

epitopes, which permitted the differentiation between enteric and respiratory porcine coronaviruses, and to classify the human coronavirus (HCV) 229E in an antigenic cluster distinct from the one formed by TGEV, canine coronavirus (CCV), feline infectious peritonitis virus (FIPV), and feline enteric coronavirus (FECV).

MATERIALS AND METHODS

Viruses, cell lines, MAbs and mar mutants

The viruses, cell lines, MAbs, and mar mutants used, have been described previously (Jiménez et al., 1986; Correa et al., 1988; Bullido et al., 1989; Sánchez et al., 1989). The purification of the virus and the S-glycoprotein has been described elsewhere (Correa et al., 1989).

Analysis of peplomer fragments

S-protein was digested with **Staphylococcus aureus** V8 protease, and the fragments analyzed by immunoblotting or fractionated by HPLC on gel filtration columns (Correa et al., 1989). A 28-kDa fragment separated by HPLC was purified by polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE). Antiserum to this fragment was produced in mice (Correa et al., 1989).

A peptide (Cys-Asp-Asn-Phe-Pro-Cys-Ser-Lys-Leu-Thr-Asn-Arg-Thr-Ile-Gly-Asn-Gln-Trp-Asn) with the sequence of the N-terminal 18 residues of the mature S protein of the TGEV strain PUR46.CC120-MAD (Gebauer et al., 1989) was synthetized and its recognition by serum analyzed by dot-blot as previously described (Correa et al., 1989).

Expression of S-gene fragments in pEX vectors

S-gene fragments were cloned in pEX expression plasmid (Stanley & Luzio, 1984; Lenstra et al., 1989). The cro-β-galactosidase hybrid proteins were extracted and analyzed by immunoblotting (Correa et al., 1989).

DNA and RNA sequencing

S-gene cDNA was cloned on Bluescript phagemid as described (Gebauer et al., 1989). Three DNA fragments, which included the nucleotides -8 to 1587, 1135 to 3329, and 3330 to 4628, of TGEV strain PUR46.CC120-MAD (Sánchez et al., 1989) were cloned. DNA purified from Bluescript plasmid, and RNA from purified virions were sequenced by oligodeoxynucleotide primer extension and dideoxy-nucleotide chain termination procedure (Sanger et al., 1977; Zimmern & Kaesberg, 1978). The sequence nucleotide differences between the PUR46.CC120.MAD **wt** and the **mar** mutants were detected using the primer complementary to nucleotides 1980 to 2000 of S-gene: 3'-TCTGTTGTATCACCCACATG-5'. Sequence data were assembled and analyzed by using the computer programs by Genetic Computer Group (Wisconsin University).

Binding of MAb to virus and antigenic homology

The binding of MAbs was determined by RIA, and the percentage of antigenic homology of a particular virus isolate, relative to the reference virus PUR46.CC120-MAD, was estimated by the formula [(a + 2b)/2n] x 100, where a and b are the number of MAbs with binding percentage values equal to 31 to 50, and 51 to 100, respectively, for the considered virus isolate, and n=42, the total number of MAbs (García-Barreno et al., 1986; Sánchez et al., 1989).

RESULTS

Location of antigenic sites on S-protein fragments

S-protein was digested with **Staphyloccocus aureus V8** protease, the digestion products fractionated by gel filtration HPLC, and the antigenic sites located on the fragments by immunoblotting using MAbs as probes (**Figure 1**). Five fractions (a, b, c, d, and e) were separated by HPLC. Site-A and -C specific-MAbs recognized a peptide of 28-kDa (present in fraction d) and other partially digested peptides of higher molecular weight. Site-B specific-MAbs did not bind any peptide, while site-D specific-MAbs recognized a 50-kDa fragment (present in fraction c). The 28-kDa fragment selected by HPLC was separated from a 18-kDa component by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE), and its homogeneity analyzed by reverse phase HPLC. A single protein band was detected, although the presence of a second component accounting for less than 10% of the total protein could not be excluded (not shown).

To locate the antigenic sites on S-protein, S-gene fragments (**Table 1** and **Figure 2**) were expressed by pEX-TGEV vectors. Nine overlapping inserts, numbered 1 to 9, accounted for

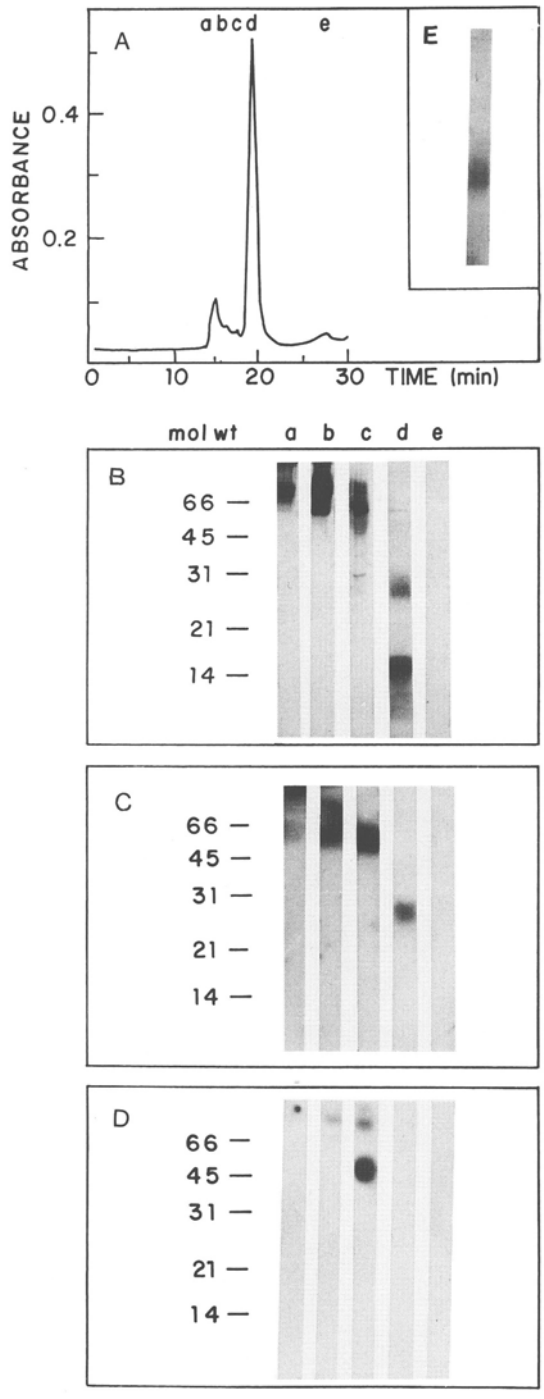

Fig. 1. Fractionation by HPLC of peptides from V8-protease digested S-protein. V8-protease fragments of S glycoprotein were separated by gel filtration HPLC on two Protein Pak columns (Fig. 1A). Five major fractions, a, b, c, d, and e, were analyzed by SDS-PAGE and silver staining (Fig. 1B), or by immunoblotting with site-A (Fig. 1C), or site-D (Fig. 1D) specific MAbs (1G.A7 and 1D.G3, respectively). Site-C specific MAbs gave the same pattern as the site-A specific MAbs (not shown). Figure 1E shows the 28-kDa fragment selected by preparative SDS-PAGE.

98% of S-gene. On immunoblots, the site-specific MAbs recognized only the expression products of inserts 1 and 2. **Figure 3** shows representative results of the binding of different MAbs to TGEV expression products. Four site-A specific MAbs (1G.A7, 1D.E7, 1A.F10, and 6A.C3) tested did not bind to the expression products. One site-B specific-MAb (1B.H11) and two site-C specific MAbs (5B.H1 and 6A.A6) bound to the product of insert 1. In contrast, two MAbs (1D.G3 and 8D.H8), which are site-D specific, recognized the product of plasmid containing insert 2, but not any of the other products.

The 28-kDa fragment was coded by the 5'-end of S-gene, as: i) polyvalent antiserum induced by this fragment(s) only recognized the product of insert 1 (**Figure 4A**), and ii) the antiserum to the 28-kDa fragment(s) recognized a synthetic peptide which included the 18 N-terminal amino acids, coupled to KLH, but not KLH alone, in a dot-blot assay (**Figure 4B**).

Amino acid sequence of a TGEV epitope present in serum proteins

The binding sequence of site-C specific-MAbs (Correa et al., 1988) has been identified using synthetic nonapeptides in a PEPSCAN and random pEX libraries (Enjuanes et al., submitted for publication; Geysen et al., 1984; Stanley and Luzio, 1984; Lenstra et al., 1989). Two sets of nonapeptides were recognized by site-C specific-MAbs (**Figure 5**), but residues 48-Pro-Pro-Asn-Ser-Asp-52 gave higher absorbance in the PEPSCAN, compared with residues 164-Pro-Ser-Asn-Ser-Glu-168. In addition, residues 48-53 have a higher surface probability based on Chou and Fasman algorithms (Enjuanes et al., submitted for publication). These data suggest that the sequence from residue 48 to 52 is the one recognized in the native virus. Interestingly, these sequences are present in proteins of sera from different species (Correa et al., 1988; Enjuanes et al., submitted for publication).

Nucleotide differences between the sequences of TGEV **wt** and **mar** mutants selected from this virus

The S-gene of TGEV strain PUR46.CC120-MAD and of the double **mar** mutant **dmar** 1B.B5-1B.B1 were cloned in the Bluescript vector. Three cDNA fragments covering the S-gene of each virus were inserted on the plasmid, using the strategy described previously (Gebauer et al., 1989). The first 1950 nucleotides of the 5'-end of each gene were sequenced on these plasmids. Three nucleotide differences were detected at the DNA level (**Table 2**), which corresponded to residues 538, 543 and 631 of the S-protein. To determine if these nucleotide differences were present in the consensus population of the genomic RNA, direct RNA sequencing of the **wt** virus, of four **dmar** mutants, and of 11 single **mar** mutants, selected by MAbs specific for the three antigenic subsites Aa, Ab and Ac, was performed. The sequence differences detected by DNA sequencing (**Table 2**) were confirmed by sequencing of consensus genomic RNA populations. All **mar** mutants selected with subsite-Aa specific-MAbs showed nucleotide differences which caused a change in residue 538 of S-protein. One **mar** mutant selected with a subsite-Ac specific-MAb had the two other residue changes (543 and 631), also detected in the two **dmar** mutants derived from it. The change in residue 631 may have been incidental during the cloning of the **mar** mutant 1B.B5, which was used to select the **dmar** 1B.B5-1B.B1 and **dmar** 1B.B5-1D.E7, as synthetic nonapeptides that contain residue 543 were recognized by the subsite-Ac specific-MAb 1A.F10 (L. Enjuanes, W.P.A. Posthumus, and R. Meloen, unpublished results). Two mutants (**mar** 1D.E7 and **mar** 1H.D2) selected with subsite-Ab specific-MAbs, one mutant (**mar**

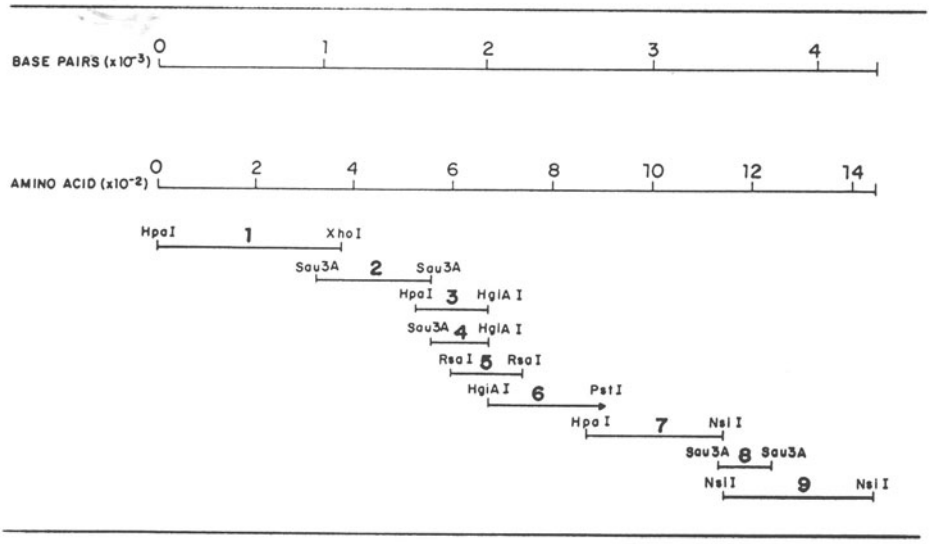

Fig. 2. S-gene fragments cloned in pEX vector. Location of the nine partially overlaping S-gene fragments named 1 to 9, from 5'-end to 3'-end, inserted in the expression vector pEX (Stanley and Luzio, 1984), using the indicated restriction endonuclease insertion sites. For insert 6, a **Pst I** site from the polylinker region of the cDNA clone B1 (Jacobs et al., 1987) was used.

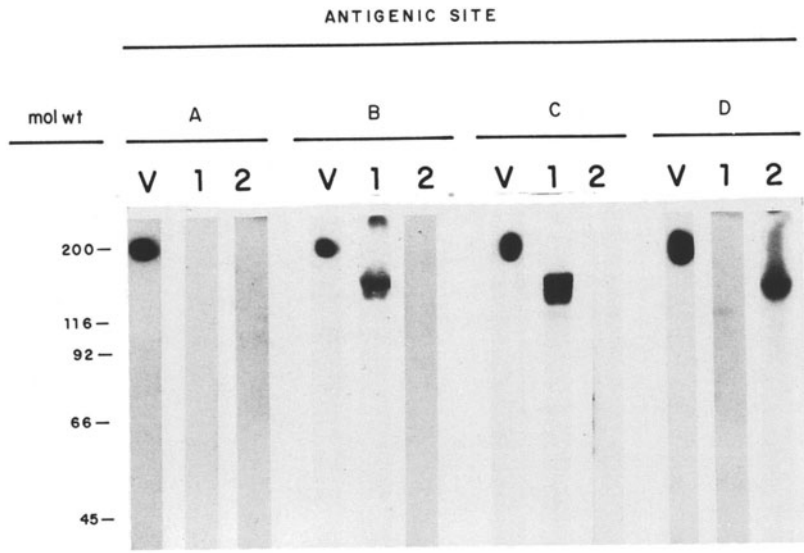

Fig. 3. Immunoblotting of pEX-TGEV expression products with MAbs. Lane V contains TGEV proteins; lanes 1 and 2 the expression products of insert 1 and 2, respectively. Antigens had been incubated in the presence of 2.5% SDS and 5% 2-ME. A, site-A specific MAbs (1G.A7, 1D.E7, 1A.F10, and 6A.C3); B, site-B specific MAb (1B.H11); C, site-C specific MAbs (5B.H1 and 6A.A6); and, D, site-D specific MAb (1D.G3).

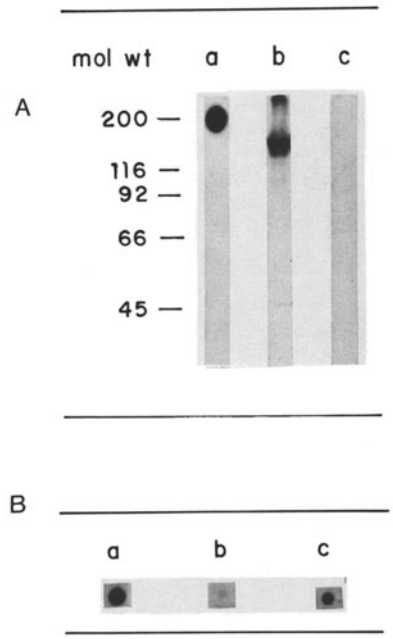

Fig. 4. Binding of antiserum against the 28-kDa S protein fragment to pEX-TGEV expression products or to a synthetic peptide from the N-terminal end of S protein. Figure 4A shows an immunoblot of the TGEV proteins (lane a) or pEX-TGEV expression products of insert 1 (lane b) and 2 to 9 (lane c), incubated with a murine antiserum against the 28-kDa fragment. Figure 4B shows an immunodot analysis of the 28-kDa specific antiserum with TGEV (a), KLH (b), and with a synthetic peptide containing the 18 N-terminal residues of the S protein, conjugated to KLH (c).

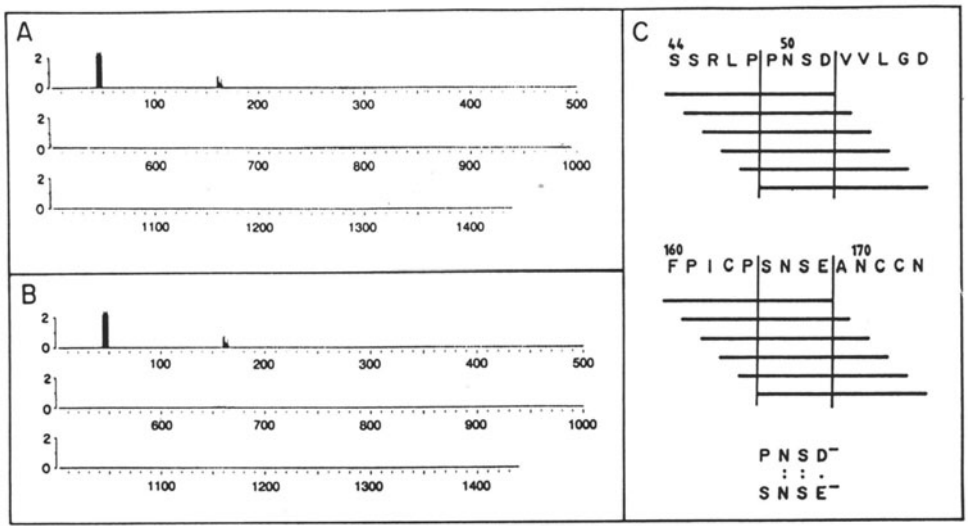

Fig. 5. PEPSCAN results of site-C specific MAbs tested on overlapping nonapeptides covering the S-protein. Binding activities of MAb 6A.A6 (A) and 5B.H1 (B), measured in an ELISA, were plotted on the vertical axis and on the horizontal axis the sequence position of the N-Terminal amino acid of the peptide. The position and sequence of the two sets of overlapping peptides recognized by both MAbs was the same and it is shown in panel C.

1B.H6) that has altered subsites Aa and Ab, and three double mutants (**dmar** 1B.B5-1D.E7, **dmar** 1D.E7-1G.A6, and **dmar** 1D.E7-1B.B1) have nucleotide differences at positions 586 or 591 (**Table 2**), indicating that these residues are involved in subsite Ab.

Variability of the antigenic sites

The binding of 42 MAbs specific for the strain PUR46.CC120-MAD of TGEV to 26 coronavirus strains (**Figure 6**) indicated that TGEV, PRCV, FIPV, FECV, and CCV had conserved determinants in the three major structural proteins. These viruses had in common, in the peplomer protein, the antigenic subsite Ac, an inducer of TGEV neutralizing antibodies. In contrast, the antigenic sites B, C and D, showed a high variability, particularly sites B and C, which were generally present in TGEV isolates, were absent in PRCV strains, and in the other coronaviruses analyzed. None of the 42 MAbs bound to HCV 229E, porcine epidemic diarrhea virus (PEDV), hemagglutinating encephalomyelitis virus (HEV), and mouse hepatitis virus (MHV).

Five MAbs, three specific for antigenic subsite Ac an two other site-A specific-MAbs, neutralized TGEV, PRCV, FIPV, FECV and CCV (Sánchez et al., 1989). None of the 42 MAbs, including those which recognized the conserved epitopes, neutralized either the HVC 229E or the serologically unrelated coronaviruses: PEDV, HEV and MHV (Sánchez et al., 1989).

Based on the percentage of antigenic homology of each particular virus isolate, relative to the reference virus (PUR46.CC120-MAD) (**Figure 1**), the studied coronaviruses could be classified into four different clusters, with homology percentages between 90-100, 69-83, 26-30, and 0, which included the TGEVs, PRCVs, the canine and feline isolates, and the other viruses, respectively. No antigenic relationship has been described among the members of the fourth cluster. These results (**Figure 1**, and Sánchez et al., 1989), defined: i) **type specific epitopes**, which were common to enteric TGEV isolates -e.g., those recognized by MAbs 1D.B12 and 8F.B3; ii) **group specific epitopes**, which were common to enteric TGEV and respiratory PRCV isolates -e.g., those defined by MAbs 1D.E8, 1D.E7 and 1H.D2; and, iii) **interspecies specific epitopes**, which were the ones shared by TGEV, PRCV, and coronaviruses from other species (feline and canine), antigenically related to TGEV -e.g., MAbs 1B.B5, 1A.F10, 6A.C3, and 8B.E3.

As no TGEV-specific MAb recognized the HCV 229E, it was studied if polyvalent antisera, specific for the structural proteins of each virus, bound or neutralized to both viruses. The results (Sánchez et al., 1989) showed that there was no crossneutralization. Anti-TGEV serum did not bind to the HCV 229E. The anti-HCV 229E serum apparently bound to MHV and, into a minor extent, to TGEV, but these reactivities were extensively diminished by preadsorbing the serum with the cells used to grow TGEV and HCV 229E.

In order to differentiate sera from animals infected with TGEV or PRCV, two type specific MAbs, 1D.B12 and 8F.B3, could be used in a competitive RIA, as these MAbs bound to all strains of TGEV tested, but not to the PRCV isolates (Sánchez et al., 1989).

DISCUSSION

In this chapter, we describe a correlation between the

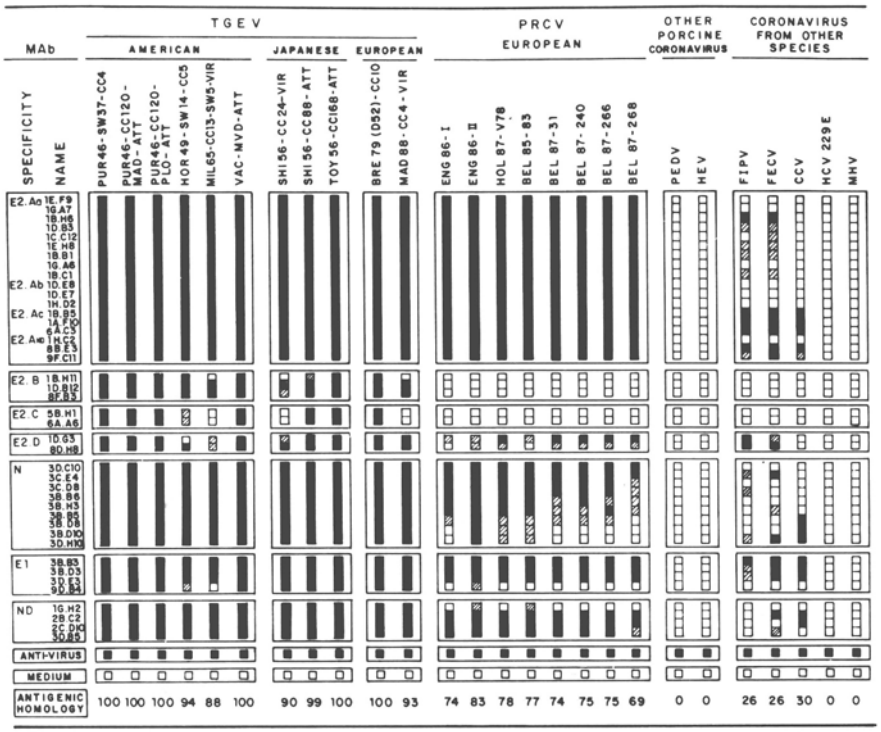

Fig. 6. Binding of MAbs to coronaviruses. The value of the MAb binding to the PUR46.CC120-MAD, determined by RIA, was taken as the reference value (100). The characteristics of the viruses used were summarized previously (Sánchez et al., 1989). The specificity of the MAbs is named according to Correa et al. (1988). Symbols □, 0 to 30; ▨, 31 to 50; ■, 51 to 100. The antigenic homology of each virus isolate relative to the reference virus PUR46.CC120-MAD, was expressed in percentage. The anti-virus sera were TGEV specific in the case of TGEV, PRCV, FIPV, FECV, and CCV, and epecific for the homologous virus, in the case of PEDV, HEV, HCV 229E, and MHV.

antigenic structure of the TGE virus S-protein with its physical map. MAbs specific for four antigenic sites were used to screen protein fragments, as well as the expression products of S-gene sequences. In addition, the sequencing of **mar** mutants, selected from TGEV stocks with site-A specific-MAbs was used to locate this site on S-gene. The antigenic homology among 26 coronavirus strains has been studied with a collection of 42 MAbs, which recognized a minimum of 25 epitopes, and polyvalent antisera. This analysis identified type, group and interspecies epitopes, provided MAbs which differentiate members of the TGEV antigenic types, particularly TGEV and PRCV isolates, and revealed that the inclusion of the HCV 229E in this taxonomic cluster should be reconsidered.

HPLC fractionation of the peptides obtained after degradating S-protein with staphyloccocal V8 protease, resulted in the isolation of a 28-kDa fragment, which was identified as being N-terminal, since an antiserum to this peptide, only reacted with the recombinant product of insert 1 (**Figure 4A**), and because of the recognition by this antiserum of a synthetic peptide containing the 18 N-terminal residues of S-protein (**Figure 4B**). The presence of a second 28-kDa fragment formed by residues different from the first 325 amino acids, which could have been copurified by HPLC and SDS-PAGE with the other 28-kDa fragment, can not be completely ruled out. Nevertheless, this possibility seems unlikely, since the polyvalent antiserum induced by the 28-kDa fragment recognized recombinant products of insert 1.

Figure 7 summarizes the location of the antigenic sites. Site A must be complex and discontinuous. To the formation of this site residues around positions 538, 543, 586, and 591, changed in the **mar** mutants selected with site-A specific-MAbs must be essential. The residue 538 must be implicated in the formation of subsite Aa (or influences its conformation), as seven **mar** mutants selected with MAbs specific for this subsite showed nucleotide differences in this residue. Amino acids 543 and 631 may be involved in the formation of subsite Ac, as these two residues were changed in **dmar** 1B.B5-1B.B1, **dmar** 1B.B1-1D.E7, and **mar** 1B.B5, from which the **dmars** were derived. Recognition of synthetic nonapeptides, that contain residue 543, by the subsite-Ac specific-MAb 1A.F10 (L. Enjuanes, W.P.A. Posthumus, and R. Meloen, unpublished results), suggests that the relevant residue difference which facilitated the escape of the **mar** 1B.B5 mutant from the neutralization by the corresponding MAb, is in position 543. The change in residue 631 may have been incidental during the cloning of **mar** 1B.B5 mutant. Subsite Ab must be formed by amino acids around residues 586 and 591, as nucleotide differences corresponding to either one of these residues were detected in two single mutants (**mar** 1D.E7 and **mar** 1H.D2), or in three double mutants (**dmar** 1B.B5-1D.E7, **dmar** 1D.E7-1G.A6, and **dmar** 1D.E7-1B.B1). In addition, site-A specific-MAbs bound to a 28-kDa fragment, identified as N-terminal, which suggest that amino acids within the first 325 residues may contribute to the formation of site A. The antigenic site A of TGEV may resemble one of the neutralization epitopes of foot-and-mouth-disease virus, shown recently to be formed by two separated antigenic regions (Thomas et al., 1988; Parry et al., 1989).

The precise location of site C was determined by PEPSCAN between residues 48 and 52, and we have also obtain data on the locations of site D by using the same technology (Posthumus et al., this volume), which confirmed the results presented here. Interestingly, the four antigenic sites A, B, C, and D, are in the 40% N-terminal residues of S-protein, and they are the target

TABLE 2

NUCLEOTIDE SEQUENCE DIFFERENCES BETWEEN TGEV-wt AND TGEV-mar MUTANTS

CLONE	NUCLEIC ACID SEQUENCED	ANTIGENIC SUBSITE SPECIFICITY OF MAb[c]	NUCLEOTIDE SEQUENCE IN wt VIRUS	NUCLEOTIDE SEQUENCE IN mar MUTANT	BASE CHANGED	AMINO ACID CHANGE RESIDUE	FROM	TO
BLUESCRIPT.PUR46.dmar 1B.B5-1B.B1[a]	DNA	c/a	AAG	CAG	1612	538	Lys	Gln
			GGT	GAT	1628	543	Gly	Asp
			GTT	GCT	1892	631	Val	Ala
PUR46.dmar 1B.B5-1B.B1[b]	RNA	c/a	AAG	CAG	1612	538	Lys	Glu
			GGU	GAU	1628	543	Gly	Asp
			GUU	GCU	1892	631	Val	Ala
PUR46.dmar 1B.B5-1D.E7	RNA	c/b	GGT	GAT	1628	543	Gly	Asp
			GAC	AAC	1756	586	Asp	Asn
			GTT	GCT	1892	631	Val	Ala
PUR46.dmar 1D.E7-1G.A6	RNA	b/a	CGA	CAA	1772	591	Arg	Gln
PUR46.dmar 1D.E7-1B.B1	RNA	b/a	CGA	CAA	1772	591	Arg	Gln
PUR46. mar 1B.B5	RNA	c	GGU	GAU	1628	543	Gly	Asp
			GUU	GCU	1892	631	Val	Ala
PUR46. mar 1G.A7	RNA	a	AAG	AUG	1613	538	Lys	Met
PUR46. mar 1B.C1	RNA	a	AAG	ACG	1613	538	Lys	Thr
PUR46. mar 1D.B3	RNA	a	AAG	ACG	1613	538	Lys	Thr
PUR46. mar 1G.A6	RNA	a	AAG	ACG	1613	538	Lys	Thr
PUR46. mar 1C.C12	RNA	a	AAG	ACG	1613	538	Lys	Thr
PUR46. mar 1E.H8	RNA	a	AAG	ACG	1613	538	Lys	Thr
PUR46. mar 1E.F9	RNA	a	AAG	AUG	1613	538	Lys	Met
PUR46. mar 1D.E7	RNA	b	CGA	CAA	1772	591	Arg	Gln
PUR46. mar 1H.D2	RNA	b	CGA	CCA	1772	591	Arg	Pro
PUR46. mar 1B.H6[d]	RNA	a/b	GAC	AAC	1756	586	Asp	Asn

a. The sequences of the 5'-ends $2x10^3$ nucleotides of the PUR 46.C1 strain of TGE virus and of the double mar mutant dmar 1B.B5-1B.B1 were obtained using cDNAs cloned on the Bluescript plasmid.

b. RNA sequencing was performed on RNA from purified virions.

c. The antigenic subsites were defined as described by Correa et al. (1988).

d. mar 1B.H6 has altered part of both subsites a and b (Gebauer et al., 1989)

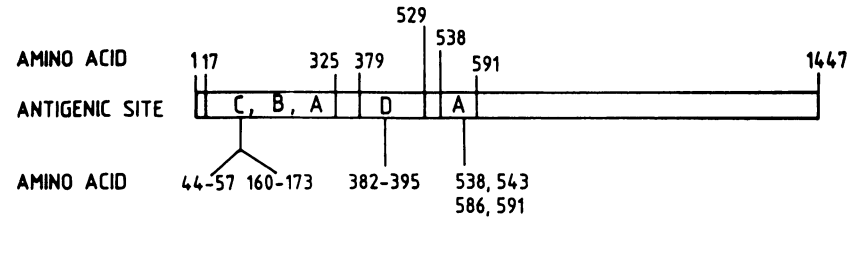

Fig. 7. Location of the antigenic sites of S glycoprotein. Site A is discontinuous and formed by residues around positions 538 to 591 and, probably, by residues between 1 and 325. Site B is located between 1-325. The precise position of site D has been determined by Posthumus et al. (This volume). The relative order of sites C and B and part of site A is not defined.

of 1015 independently derived TGE virus hybridomas selected in our laboratory (Correa et al., 1988). This indicates that the S-peplomer has a relatively small immunogenic area, which in the model of De Groot et al. (1987) is located in the globular part of the peplomer.

While antigenic site A is higly conserved, sites B, C, and D, showed variability. Particularly, sites B and C were not present in the respiratory isolates. The interspecies specific subsite Ac is conserved in porcine, feline and canine coronaviruses. Anti-idiotypic antibodies of the β-type, specific for MAb 6A.C3 which recognized this subsite, could have a role in protection against coronaviruses of the three species.

PRCV was detected for the first time in 1984 (Pensaert et al., 1986; Callebaut et al., 1988) and could be derived from TGEV, by recombination between this virus and other porcine (PEDV or HEV), feline (FIPV or FECV), or canine (CCV) coronaviruses, which would provide the modified antigenic sites B and C. This hypothesis is favoured, versus the accumulation of point mutations, as two antigenic sites are different between these viral strains, and mechanisms of genetic recombination have been demonstrated in coronavirus (Makino et al., 1986). The production of PRCV variants by deletion in sites B, C, and D, could also be the mechanism of PRCV generation, as the mRNA coding the S-protein of PRCV is 5% smaller than the corresponding mRNA of TGEV (P. Britton, personal communication). Since PRCV provides some protection against TGEV (Hooyberghs et al., 1988), it could be predicted that the presence of TGEV will decrease in countries where PRCV is present, while other transmissible gastroenteric coronaviruses serologically unrelated to TGEV will be prevalent.

HCV 229E has been previously included in the TGEV group (Mcnaughton, 1981), based on a weak crossreactivity of antisera against TGEV and HCV 229E with these viruses, detected by immunofluorescence. In contrast, none of the 42 MAbs tested, bound or neutralized HCV 229E. Furthermore, polyvalent antisera to TGEV or HCV 229E did not show an antigenic homology between the viral proteins, as the crossreactivity observed could be adsorbed by uninfected cells. There are two other issues which differentiate the TGEV group viruses, from HCV 229E. First, while TGEV related isolates have three major structural proteins, HCV 229E has two other proteins (Kemp et al., 1984). Secondly, while the peplomer protein is not processed in TGEV related coronaviruses, the S-protein of the HCV 229E is processed in certain cell lines (Schmidt and Kenny, 1982; Kemp et al., 1984). Althought a minor antigenic relationship among HCV 229E and TGEV related strains can not be excluded, based on some homology detected by genome sequencing (Schreiber et al., 1989), HCV 229E does not make a uniform antigenic cluster with TGEV, FIPV, FECV, and CCV, and we strongly suggest reconsidering the inclusion of the human virus in the same taxonomic group.

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REFERENCES

Brian, D.A., Dennis, D.E., and Guy, J.S. 1980. Genome of porcine transmissible gastroenteritis virus. **J. Virol.**, 34:410.

Bullido, M.J., Correa, I., Jiménez, G., Suñe, C., Gebauer, F., and Enjuanes, L. 1988. Induction of transmissible gastroenteritis coronavirus neutralizing antibodies "in vitro" by virus-specific T helper cell hybridomas. **J. Gen. Virol.**, 40:659.

Callebaut, P., Correa, I., Pensaert, M., Jiménez, G., and Enjuanes, L. 1988. Antigenic differentiation between transmissible gastroenteritis virus of swine and a related porcine respiratory coronavirus. **J. Gen. Virol.**, 69:1725.

Correa, I., Jiménez, G., Suñé, C., Bullido, M.J., and Enjuanes, L. 1988. Antigenic structure of the E2 glycoprotein from transmissible gastroenteritis coronavirus. **Virus Res.** 10:77.

Correa, I., Gebauer, F., Bullido, M.J., Suñé, C., Baay, M.F.D., Zwaagstra, K.A., Posthumus, W.P.A., Lenstra, J.A., and Enjuanes, L. 1989. Localization of antigenic sites of the E2 glycoprotein of transmissible gastroenteritis coronavirus. **J. Gen. Virol.**, in press.

De Groot, R.J., Luytjes, W., Horzinek, M.C., Van der Zeijst, B.A.M., Spaan, W.J.M., and Lenstra, J.A. 1987. Evidence for a coiled-coil structure in the spike proteins of coronaviruses. **J. Mol. Biol.** 196:963.

Enjuanes, L., Posthumus, W.P.A., Correa, I., Erkens, J.H.F., Meloen, R., Lenstra, H.A., and Stanley, K.K. 1989. Amino acid sequence of a transmissible gastroenteritis coronavirus epitope present in serum proteins. Submitted.

García-Barreno, B., Sanz, A., Nogal, M.L., Viñuela, E., and Enjuanes L. 1986. Monoclonal antibodies of African swine fever virus: antigenic differences among field virus isolates and viruses passaged in cell culture. **J. Virol.**, 58:385.

Garwes, D.J., Lucas, M.H., Higgins, D.A., Pike, B.V., and Cartwright, S.F. 1978. Antigenicity of structural components from porcine transmissible gastroenteritis virus. **Vet. Microbiol.** 3:179.

Gebauer, F., Correa, I., Bullido, M.J., Suñé, C., Smerdou, C., and Enjuanes L. 1989. Sequences of two major epitopes of Transmissible gastroenteritis coronavirus. Virology.

Geysen, H.M., Meloen, R.H., and Barteling, S.J. 1984. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. **Proc. Nat. Acad. Sci. USA.** 81:3998.

Hooyberghs, J., Pensaert, M.B., and Callebaut, P. 1988. Transmissible gastroenteritis: outbreaks in swine herds previously infected with a TGEV-like porcine respiratory coronavirus. 10th Int. Pig. Veter. Soc. Congress. Rio Janeiro, p.200.

Jacobs, L., De Groot, R., Van der Zeijst, B.A.M., Horzinek, M.C. and Spaan, W. 1987. The nucleotide sequence of the peplomer gene of porcine transmissible gastroenteritis virus (TGEV): comparison with the sequence of the peplomer protein of feline infectious peritonitis virus (FIPV). **Virus Res.** 8:363.

Jiménez, G., Correa, I., Melgosa, M.P., Bullido, M.J., and Enjuanes, L. 1986. Critical epitopes in transmissible gastroenteritis virus neutralization. **J. Virol.** 60:131.

Kapke, P.A., and Brian, D.A. 1986. Sequence analysis of the porcine transmissible gastroenteritis coronavirus nucleocapsid protein gene. **Virology** 151:41.

Kemp, M.C., Hierholzer, J.C., Harrison, A., and Burks, J.S. 1984. Characterization of viral proteins synthesized in 229-E infected cells and effect(s) of inhibition of glycosylation and glycoprotein transport. **Adv. Exp. Med. Biol.** 173:65.

Laude, H., Rasschaert, D., and Huet, J.C. 1987. Sequence and N-terminal processing of the transmembrane protein E1 of the coronavirus transmissible gastroenteritis virus. **J. Gen. Virol.** 68:1687.

Lenstra, J.A., Kusters, J.G., Koch, G., and Van Der Zeijst, B.A.M. 1989. Antigenicity of the peplomer protein of infectious bronchitis virus. **Mol. Immunol.** 26:7.

Macnaughton, M.R. 1981. Structural and antigenic realtionships between human, murine and avian coronaviruses. **Adv. Exp. Med. Biol.** 142:19.

Makino, S., Stohlman, S.A., and Lai, M.M.C. 1986. Leader sequences of murine coronavirus RNA can be freely reassorted. Evidence for the role of free leader RNA in transcription. **Proc. Natl. Acad. Sci. USA**, 83:4204.

Parry, N.R., Barnett, P.V., Ouldridge, E.J., Rowlands, D.J., and Brown, F. 1989. Neutralizing epitopes of type O foot-and-mouth disease virus. II. Mapping three conformational sites with synthetic peptide reagents. **J. Gen. Virol.** 70:1493.

Pensaert, M., Callebaut, P., and Vergote, J. 1986. Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis. **Vet. Quart.** 8, 257.

Rasschaert, D., Gelfi, J. and Laude, H. 1987. Enteric coronavirus TGEV: partial sequence of the genomic RNA, its organization and expression. **Biochimie**, 69:591.

Rasschaert, D., and Laude, H. 1987. The predicted primary structure of the peplomer protein E2 of the porcine coronavirus transmissible gastroenteritis virus. **J. Gen. Virol.**, 68:1883.

Sánchez, C., Jiménez, G., Laviada, M.D., Correa, I., Suñé, C., Bullido, M.J., Gebauer, F., Smerdou, C., Callebaut, P., Escribano, J.M., and Enjuanes, L. 1989. Antigenic homology among coronaviruses related to transmissible gastroenteritis virus. **Virology.**

Sanger, F., Nicklen, S., and Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. **Proc. Natl. Acad. Sci. USA**, 74:5463.

Schmidt, O.W., and Kenny, G.E. 1982. Polypeptides and functions of antigens from human coronaviruses 229E and OC43. **Infect. Immun.**, 35:515.

Schreiber, S., Kamahora, T., and Lai, M.M.C. 1989. Sequence analysis of the nucleocapsid protein gene of human coronavirus 229E. **Virology** 169:142.

Stanley, K.K., and Luzio, J.P. 1984. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. **EMBO J.**, 3:1429.

Sturman, L.S., and Holmes, K.V. 1983. The molecular biology of coronaviruses. **Adv. Virus Res.**, 28:35.

Thomas, A.A., Woortmeijer, R.J., Puijk, W., and Barteling, S.J. 1988. Antigenic sites of foot-and-mouth disease virus type A10. **J. Virol.**, 62:2782.

Zimmern, D., and Kaesberg, P. 1978. 3'-Terminal nucleotide sequence of encephalomyocarditis virus RNA determined by reverse transcriptase and chain-terminating inhibitors. **Proc. Natl. Acad. Sci. USA.**, 75:4257.