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Analysis and Simulation of a Neutralizing Epitope of Transmissible Gastroenteritis Virus

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The amino acid sequences recognized by monoclonal antibodies (MAbs) specific for the antigenic site IV of the spike protein S of transmissible gastroenteritis virus were analyzed by PEPSCAN. All MAbs of group IV recognized peptides from the S region consisting of residues 378 to 390. In addition, the neutralizing MAbs (subgroup IV-A) also bound to peptides from the region consisting of residues 1173 to 1184 and to several other peptides with a related amino acid composition. The contribution of the individual residues of both sequences to the binding of a MAb was determined by varying the length of the peptide and by a consecutive deletion or replacement of parental residues by the 19 other amino acids. The sequence consisting of residues 326 to 558, tested as part of a cro-β-galactosidase hybrid protein, was antigenic, but the sequence consisting of residues 1150 to 1239 was not. Furthermore, antibodies raised in rabbits against the peptide SDSSFFSYGEIPFGN (residues 377 to 391), but not those raised against the peptide VRASRQLAKDKVNEC (residues 1171 to 1185), recognized the virus and had neutralizing activity. We infer that the epitope of the neutralizing MAbs is composite and consists of the linear sequence SFFSYGEI (residues 380 to 387) with contributions of A, D, K, N, Q, or V residues from other parts of the S molecule. The complex epitope was simulated by synthesizing peptides in which the sequences consisting of residues 380 to 387 and 1176 to 1184 were combined. MAbs of subgroup IV-A recognized the combination peptides two to six times better than the individual sequences. These results may offer prospects for the development of an experimental vaccine.

Transmissible gastroenteritis virus (TGEV) is a member of the *Coronaviridae* family and causes enteric disease in pigs of all ages, with high mortality for newborn piglets (19, 20). The spike protein S (1,447 amino acids), located on the surface of the virus, elicits antibodies that can neutralize a virus infection (3, 11, 14). Mutual competition of neutralizing monoclonal antibodies (MAbs) results in a total of five different groups of MAbs (3, 4). The antigenic site of one group of MAbs (site I or A; see Table 1) is dominant in TGEV neutralization (3). This site has been mapped within the first 543 N-terminal amino acids of the S protein (2). The binding sites of the second and third competition groups of MAbs have been located within the region consisting of residues 17 to 325, and the fourth site has been located within the region consisting of residues 379 to 529. These localizations were based on the antigenicity of proteolytic fragments of the S protein and of procaryotic expression products of S-gene fragments (2).

Garwes et al. (6) described six mutual noncompeting groups of MAbs directed against the S protein, but it is not clear how these MAbs correlate with the aforementioned five groups.

In this study, we measured the binding of antibodies to short overlapping peptides (the PEPSCAN method) derived from the S amino acid sequence (10, 18) to locate accurately the epitopes of the MAbs specific for the fourth site (group IV; Table 1). The binding patterns of the MAbs to overlapping nonapeptides and to peptides that vary in length, have an amino acid deletion(s), or have replaced amino acids were determined. As expected, the neutralizing MAbs of group IV recognized peptides within the region consisting of residues 379 to 529. Because these MAbs also recognized peptides within a second region of the S protein, we conclude that the neutralizing determinant is composite. We found that a combination peptide containing the amino acids of both regions is a better antigen than the single peptides tested.

MATERIALS AND METHODS

MAbs. Four research groups have isolated MAbs directed against the peplomer protein S of TGEV (3, 4, 6; A. P. van Nieuwstadt, unpublished data). By mutual competition among the MAbs, Correa et al. (3) and Delmas et al. (4) designated their noncompeting groups A, B, C, and D; Garwes et al. (6) designated their groups A, B, C, D, E, and F; and van Nieuwstadt (unpublished data) designated his groups A, B, C, X, and Y. Competition between exchanged MAbs of Correa et al. (3), Delmas et al. (4), and van Nieuwstadt (unpublished data) resulted in a total of five corresponding groups. A new designation of the groups is given in Table 1. In this study, 13 MAbs of group IV were used, three of which (8D.H8 and 1D.G3 [3] and CVI-TGEV-57.51 [van Nieuwstadt, unpublished data]) are non-neutralizing. The other 10 MAbs (CVI-TGEV-57.02, -57.06, -57.09, -57.12, -57.17, -57.24, -57.56, -57.57, -57.99, and -57.75) have neutralizing antibody titers ranging from 3.1 to 5.9 (van Nieuwstadt, unpublished data).

PEPSCAN analysis. All 1,439 consecutive overlapping nonapeptides derived from the sequence of the spike protein S of TGEV (10, 18) were synthesized on polyethylene rods and tested as described previously (7, 8). The first peptide consisted of amino acids 1 to 9, the second consisted of amino acids 2 to 10, the third consisted of amino acids 3 to 11, etc. The binding of a MAb to each peptide was tested in an enzyme-linked immunosorbent assay (ELISA) and is

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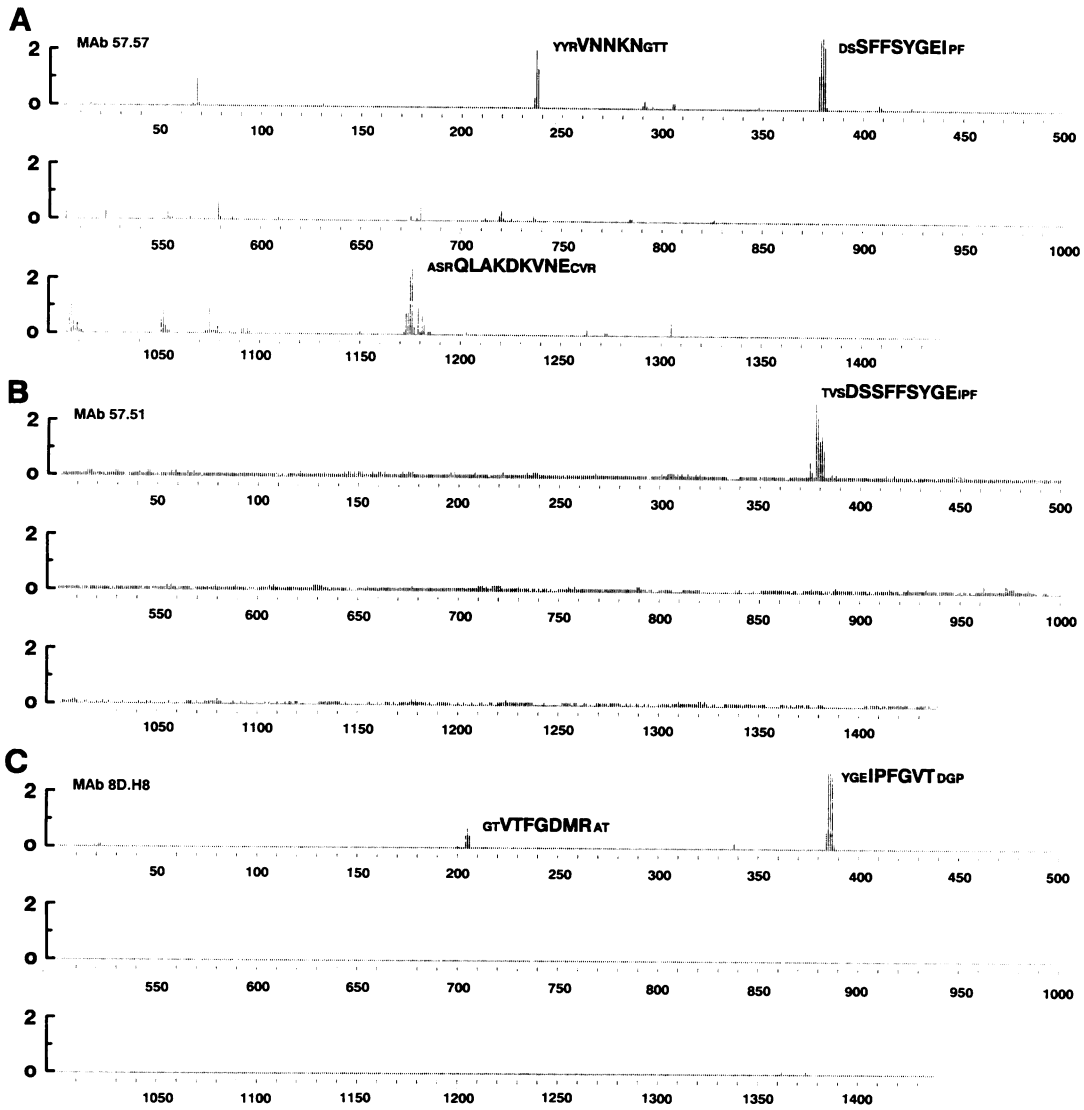

FIG. 1. PEPSCAN of overlapping nonapeptides from the sequence of the TGEV spike protein S. The MAb binding, measured as the extinction at 405 nm by an ELISA, is plotted against the sequence position of the N-terminal amino acid of the peptide (1 to 1439). The common amino acids of the sequence of the recognized nonapeptides are indicated in large type. (A) Subgroup IV-A MAb 57.57 (diluted $1:10^3$); (B) subgroup IV-B MAb 57.51 (diluted $1:5 \times 10^3$); (C) subgroup IV-C MAb 8D.H8 (diluted $1:10^5$).

expressed as the extinction at 405 nm. Ascitic fluid dilutions varied from $1:10^3$ to $1:5 \times 10^3$. Binding is plotted vertically against the sequence position of the N-terminal amino acid of the peptide (Fig. 1). Overlapping tri- to nonapeptides from the regions consisting of residues 372 to 389 and 1166 to 1201 (Fig. 2) and peptides in which each amino acid of two parent sequences (SFFSYGEI and QLAKDKVNE) was consecutively replaced by all 19 other amino acids were synthesized and tested in the same way. The amino acid replacements are plotted in alphabetical order: A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y (Fig. 3).

Synthesis of peptides on a milligram scale. The peptides acetyl (Ac)-CSDSSFFSYGEIPFG-amide and Ac-VRASRQ LAKDKVNEC-amide were prepared by conventional solid-phase peptide synthesis with benzotriazole-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP, Castro reagent) as a coupling reagent (5). Briefly, 600 mg of 4-methylbenzhydrylamine resin (1% divinylbenzene; 0.5 meq/g) was swelled, washed, and suspended in dimethylformamide. The coupling of an amino acid onto the resin was performed in 3 ml of dimethylformamide containing the *t*-butyloxycarbonyl (Boc)-amino acid (1 mmol), BOP (1 mmol), and diisopropylethylamine (3 mmol). After 1 h of shaking, the resin was successively washed with dimethylformamide (3×), isopropanol (3×), and dichloromethane (3×). If the reaction was not complete, as judged by the test of Kaiser et al. (12), the coupling was repeated. After coupling took place, the Boc group was removed with 50% trifluoroacetic acid–dichloromethane and the resin was successively washed with dichloromethane (3×), 7% diisopropylethylamine-dichloromethane (3×), and dimethylformamide (3×). This procedure was repeated for all consecutive residues. After the synthesis was completed, the N-terminal amino group was acetylated with acetic anhydride. The peptides were deprotected by treating part of the resin with 10 ml of 10% anisole in anhydrous hydrogen fluoride for 1 h at 0°C. After removal of hydrogen fluoride in vacuo, the crude products were dissolved in 5 ml of 10% acetic acid.

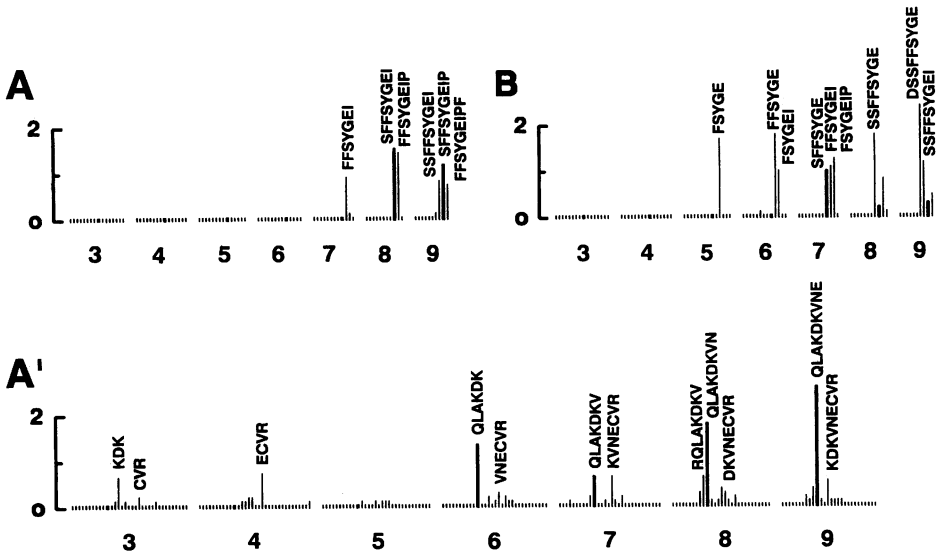

FIG. 2. Effect of length variation of peptides from the sequence regions consisting of residues 372 to 389 and 1166 to 1201 on the recognition of MAbs from subgroups IV-A and IV-B. Recognition pattern of MAb 57.57 (A and A') and MAb 57.51 (B) with all overlapping peptides of three to nine residues from residues 372 to 389 (A and B) and from residues 1166 to 1201 (A'). The MAb binding, measured as the extinction at 405 nm by an ELISA, is plotted vertically, and the numbers below the horizontal axes correspond to the numbers of the residues of the peptides tested. Boldface lines indicate the peptides with N-terminal amino acid 380 (A and B) or 1176 (A'). (A) Subgroup IV-A MAb 57.57 (diluted 1:3 × 10^4); (B) subgroup IV-B MAb 57.51 (diluted 1:5 × 10^5); (A') subgroup IV-A MAb 57.57 (diluted 1:5 × 10^3).

The fluoride ions were removed on a column (3 by 1 cm) with an ion-exchange resin (AG 2-X8; Bio-Rad Laboratories; acetate form). The products were lyophilized in 1% acetic acid.

Immunogenicity of peptides. One milligram of solubilized peptide (Ac-CSDSSFFSYGEIPFG-amide or Ac-VRASRQL AKDKVNEC-amide) was coupled via *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) to keyhole limpet hemocyanin. After dialysis, both peptide-keyhole limpet hemocyanin complexes were used for the immunization of two rabbits (rabbits R850 and R851 and rabbits R961 and R962, respectively) as described by Meloen et al. (16). The binding of antibodies in antipeptide sera to whole TGEV (Purdue strain) was measured by ELISA (van Nieuwstadt, unpublished data). The neutralization of infection of SK6 cells by TGEV was assayed in a microdilution system, starting at a serum dilution of 1:8 (van Nieuwstadt, unpublished data). Binding of antipeptide antibodies to the peptides was measured as described elsewhere (16).

RESULTS

Classification of MAbs. Western blot (immunoblot) analysis of proteolytic fragments and cro-β-galactosidase–peplomer hybrid proteins (pEX products) allowed approximate localizations of sites I, II, III, and IV (2). Epitopes localized in this way are likely to be sequential (13, 15). Therefore, we tried to obtain more accurate locations by PEPSCAN analysis. All 1,439 consecutive overlapping nonapeptides derived from the S-protein sequence were synthesized, and the binding of MAbs was tested in an ELISA. MAbs from group I (CVI-TGEV-57.16, -57.20, -57.30, -57.55, -57.73, and -57.90 [van Nieuwstadt, unpublished data] and 1G.A7, 1D.E7, 1A.F10, and 6A.C3 [3]), group II (1D.B12 and 1B.H11 [3]), and group V (57.97 [van Nieuwstadt, unpublished data]), which probably bind only to conformation-dependent epitopes, did not yield consistent results. Binding to the nonapeptides of the nonneutralizing MAbs of group III (5B.H1 and 6A.A6 [3]), which cross-react with swine sera devoid of anti-TGEV activity, will be described elsewhere (L. Enjuanes, unpublished data).

In this study, we focus on 13 MAbs that belong to competition group IV (Table 1) and were isolated in two different laboratories (3, 11; van Nieuwstadt, unpublished data). On the basis of their PEPSCAN patterns (see below), the MAbs were assigned to three subgroups: IV-A, the ten neutralizing MAbs; IV-B, the nonneutralizing MAb 57.51; and IV-C, the two nonneutralizing MAbs 8D.H8 and 1D.G3. We have checked that MAbs from all three subgroups recognized the procaryotic expression (pEX) product that contains the peplomer residues 326 to 559 (2), but none recognized any of the other cro-β-galactosidase–peplomer hybrid proteins (not shown).

Binding of MAbs to nonapeptides. The binding patterns of the MAbs 57.57, 57.51, and 8D.H8, which are representative for the three group IV subgroups, to the 1,439 nonapeptides of the S protein are shown in Fig. 1. They all bound to peptides from the region around residue 380. The neutralizing MAbs of subgroup IV-A, represented by MAb 57.57, bound to nonapeptides from the region consisting of residues 378 to 389 (DSSFFSYGEIPF), which contains a sequence present in all peptides recognized, the core sequence FFSYGEI (Fig. 1A). In addition, these MAbs recognized nonapeptides starting at residues 68, 236 to 238, 579, 680, 1005 to 1010, 1051 to 1053, 1075, and 1173 to 1182 (Fig. 1A). These antigenic regions are compared in Fig. 4. The peptides all contain the amino acid D, K, N, or V and may therefore be considered as mutual antigenic analogs. Dilution of the MAbs to 1:10^6 reduced the binding to these nonapeptides 10 to 100 times more than to the nonapeptides from the region consisting of residues 378 to 389 (results not shown).

The nonneutralizing MAb 57.51 of subgroup IV-B bound only to nonapeptides from the region consisting of residues 375 to 389 (TVSDSSFFSYGEIPF) with a core sequence of DSSFFSYGE (Fig. 1B). MAb 8D.H8 of subgroup IV-C

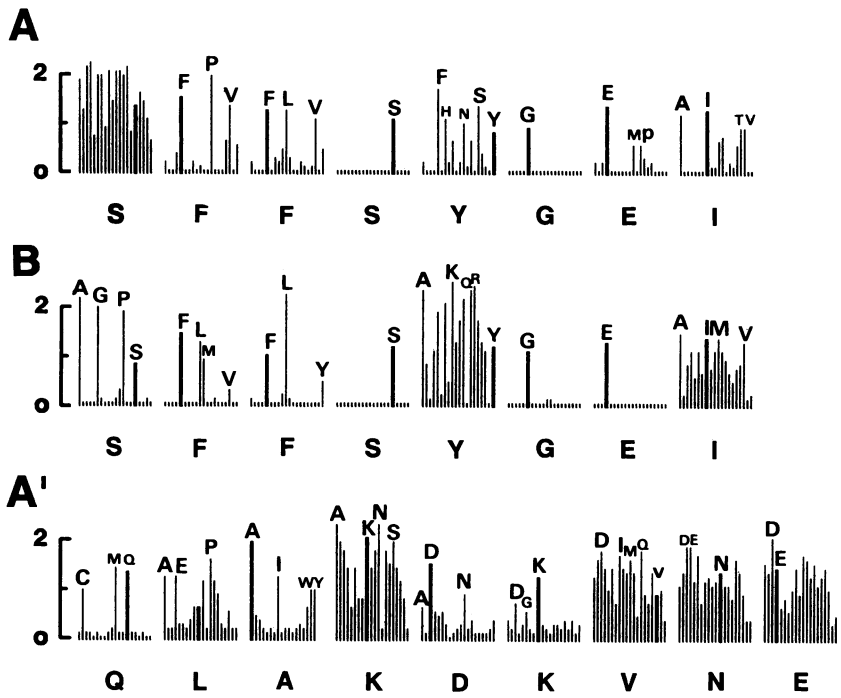

FIG. 3. Effect of amino acid substitutions in the peptides SFFSYGEI and QLAKDKVNE. In both sequences, each parental residue was consecutively replaced by all 20 amino acids, plotted in the alphabetical order of the one-letter code. This resulted in eight sets of 20 peptide analogs for the sequence SFFSYGEI and nine sets for the sequence QLAKDKVNE. The MAb binding, measured as the extinction at 405 nm by an ELISA, is plotted vertically, and the capital letters below the horizontal axes indicate the parental residues substituted. Boldface lines indicate the peptides with the native sequence. (A) Subgroup IV-A MAb 57.57 (diluted $1:10^4$) and (B) subgroup IV-B MAb 57.51 (diluted $1:10^5$), both with the 160 analogs of SFFSYGEI; (A') subgroup IV-A MAb 57.57 (diluted $1:5 \times 10^3$) with the 180 analogs of QLAKDKVNE.

recognized nonapeptides from the region consisting of residues 384 to 395 (YGEIPFGVTDGP) with a core sequence of IPFGVT. This MAb also recognized nonapeptides from the region consisting of residues 204 to 214. Comparison of both sequences suggests that peptides from residues 204 to 214 (GT-VT-FG-D-MRAT) are antigenic analogs of the peptides from residues 384 to 395 (YGEIP-FG-VT-D-GP).

Minimal size of antigenic peptides required for recognition by MAbs. Tri- to nonapeptides from the regions consisting of residues 372 to 389 and 1166 to 1201 were tested with MAb 57.57 (Fig. 2A and A'). The sequences SFFSYGEI (residues 380 to 387) and QLAKDKVNE (residues 1176 to 1184) were judged to contain the amino acids of the antigenic site necessary for binding the neutralizing MAbs from subgroup IV-A. Further, amino acid deletions within peptides with the sequence SFFSYGEI abolished the MAb recognition (data not shown).

Again, the nonneutralizing MAb 57.51 showed a different recognition pattern (Fig. 2B). The highest binding activity was found with the nonapeptide DSSFFSYGE, but this sequence was only slightly more antigenic than the pentapeptide FSYGE. Internal deletions in longer peptides were tolerated only if the FSYGE sequence remained intact (data not shown). The core sequence recognized by the nonneutralizing MAbs 1D.G3 and 8D.H8 (residues 387 to 392) was outside the range of the peptides shown in Fig. 2.

Essential amino acids in the regions SFFSYGEI and QLAKDKVNE. The recognition of the individual residues by the MAbs of subgroups IV-A and IV-B was determined by the consecutive replacement of each amino acid in the sequence SFFSYGEI. All neutralizing MAbs gave a binding pattern like that of MAb 57.57 (Fig. 3A). The residues essential for binding were the second S, G, and E. Both F's and I probably contributed to the binding.

A similar recognition pattern was found with the nonneutralizing MAb 57.51 (Fig. 3B). However, the first S also contributed to the binding. Fig. 3A' shows the replacement pattern of the sequence QLAKDKVNE with the neutralizing MAb 57.57. The most relevant residues for binding were the amino acids Q, A, and D and the second K, but none was irreplaceable.

Immunogenicity of the two antigenic sequences. Two peptides, Ac-CSDSSFFSYGEIPFG-amide (residues 377 to 390)

TABLE 1. Noncompetitive groups of neutralizing MAbs directed against TGEV

Designation used in this paper	Designation used by:	
	Correa et al. (3)	Delmas et al. (4)
I	A	A
II	B	D
III	C	
IV	D	C
V		B

S region	Sequence alignment
1173 - 1190	ASR Q L A K D K V N E CVRSQS
68 - 76	CIR N NSN D L
236 - 246	YYR V N N K N GTT
1005 - 1018	G VANAD K MTMYTAS
1051 - 1061	Q T D VLN K N QQI
1076 - 1083	SFG K V N D A

FIG. 4. Comparison of sequences recognized by MAbs of subgroup IV-A with the region comprising residues 1173 to 1190.

TABLE 2. Immunogenicity of peptides derived from the TGEV S protein

Peptide[a]	Titer[b]		
	Peptide ELISA	Virus ELISA	Neutralization
Ac-C*SDSSFFSYGEIPFG-NH2	5.1/4.8	4.1/4.1	2.1/1.2
Ac-VRASRQLAKDKVNEC-NH2	5.3/5.3	—[c]/—	—/—

[a] *, Nonsequential cysteine residue used for peptide coupling to keyhole limpet hemocyanin via MBS.

[b] Antipeptide serum titers of two rabbits, immunized with the immunogen indicated, are expressed as the $-\log_{10}$ of the serum dilution. Antipeptide sera were collected 8 weeks after immunization.

[c] —, Titer less than 0.8.

and Ac-VRASRQLAKDKVNEC-amide (residues 1171 to 1185), were used to raise rabbit antisera. In an ELISA, antibodies against Ac-CSDSSFFSYGEIPFG-amide bound to the immunogen and to whole virus. In addition, these two antisera neutralized an infection of SK6 cells by the attenuated Purdue strain of TGEV at a 1:16 and a 1:128 dilution (Table 2). Antibodies against the peptide Ac-VRASRQLAK DKVNEC-amide bound to the peptide used as the immunogen. However, they did not bind to whole virus and had no neutralizing activity (Table 2).

Combination of the two antigenic sequences. To determine whether a combination of two antigenic sequences could enhance the binding of neutralizing MAbs, the two combination peptides QLAKDKVNE-SFFSYGEI and SFFSY GEI-QLAKDKVNE and the control peptides SFFSYGEI, QLAKDKVNE, SFFSYGEI-SFFSYGEI, and QLAKDKV NE-QLAKDKVNE were synthesized on polyethylene rods and tested by ELISA. At a dilution of $1{:}10^7$, MAb 57.57 bound to the peptide SFFSYGEI at twice the background level and to the peptide QLAKDKVNE at background level. However, a much stronger binding was found to the combination peptide SFFSYGEI-QLAKDKVNE (Fig. 5). Similar patterns were obtained with the MAbs 57.12 and 57.02 and with the other neutralizing MAbs but not with the nonneutralizing MAb 57.51 (Fig. 5). We conclude that at least some residues of QLAKDKVNE contribute to the binding of the MAb and mimic one or more components of the neutralizing epitope.

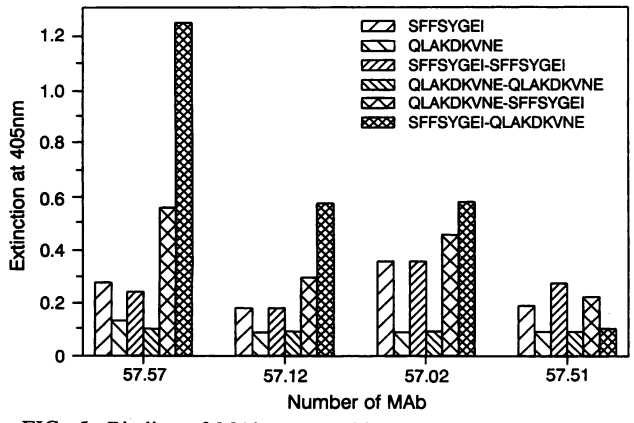

FIG. 5. Binding of MAbs to combination peptides of two core sequences. Monomer, dimer, and combination peptides from the sequences SFFSYGEI (residues 380 to 388) and QLAKDKVNE (residues 1176 to 1184) were tested with the neutralizing MAbs 57.57 (diluted $1{:}10^7$), 57.12 (diluted $1{:}5 \times 10^5$), and 57.02 (diluted $1{:}10^6$) and with the nonneutralizing MAb 57.51 (diluted $1{:}10^6$). An average background extinction level of 0.1 was measured with the nonbinding peptides AAAAAAAAA and KKLFVVLVV (N-terminal sequence of the signal peptide of the S protein).

DISCUSSION

Binding to peptides from the region consisting of residues 378 to 396. In the PEPSCAN of the S-protein sequence, all MAbs specific for site IV recognized nonapeptides in the region consisting of residues 378 to 396. On the basis of the fine specificity within this region, the MAbs could be divided into three subgroups: IV-A, the neutralizing MAbs; IV-B, the nonneutralizing MAb 57.51; and IV-C, the nonneutralizing MAbs 8D.H8 and 1D.G3. The MAb fine specificities illustrate the distinction between an antigenic site, which is recognized by a group of mutually competing MAbs, and an epitope, the actual amino acids within an antigenic site required for binding an individual antibody (13, 15a).

The neutralizing MAbs all recognized three nonapeptides that share the core sequence FFSYGEI (Fig. 1A). Systematic length variation and replacement of residues showed that the preceding serine enhances the antigenicity but can be replaced by most other amino acids (Fig. 2 and 3). All residues of the core sequence clearly contribute to or are essential for the binding of the neutralizing antibodies. The core sequence FFSYGEI is therefore assigned as the primary determinant of the antigenic site of the set of neutralizing MAbs. Polyclonal antibodies raised against whole TGEV in pigs did not recognize this epitope (data not shown). However, a peptide that contained the core sequence FFSYGEI elicited neutralizing antibodies in rabbits.

With the nonneutralizing MAb 57.51, the most antigenic peptide was the nonapeptide DSSFFSYGE. The nonapeptide pattern (Fig. 1B), the peptide length variation (Fig. 2B), and the replacement analysis (Fig. 3B) suggest that the N-terminal part of this sequence is essential for binding. However, the peptapeptide FSYGE was almost as antigenic as the nonapeptide (Fig. 2B), while deletions outside the FSYGE segment did not affect the antigenicity (not shown). These data indicate that the preceding DSSF sequence modulates the binding to the antigenic core peptide FSYGE. Similar effects of residues recognized outside the actual core sequence have been observed with epitopes of infectious bronchitis virus (13).

The epitopes of the nonneutralizing MAbs 8D.H8 and 1D.G3 overlap with the other two epitopes but are located more towards the C terminus of the spike protein S (Fig. 1C).

Binding to peptides from the region consisting of residues 1172 to 1187. All neutralizing MAbs bound significantly to peptides from a second region of the sequence consisting of residues 1172 to 1187 and to peptides from a number of other regions, with a variation in binding strength. Comparison of the sequences of the antigenic peptides outside the region consisting of residues 378 to 396 revealed various combinations of the residues D, K, N, and V (Fig. 4). Length variation of the peptides from the region consisting of residues 1166 to 1201 showed that QLAKDK is the core sequence and that its antigenicity is enhanced by attachment of the sequence VNE (Fig. 2A'). However, this enhancement was not specific, since replacement analysis of the peptide QLAKDKVNE showed that only the Q, A, D, and the second K are essential (Fig. 3A'). The relatively strong binding to peptides starting with Q at residue 1176 could depend on this residue being N terminal (Fig. 1A and 2). The core sequence QLAKDK is assigned as the second primary determinant and could be part of the antigenic site (see below).

The D, K, N, or V residues are presumably essential for the weak binding of the MAbs to the nonapeptide KDKVN ECVR (residues 1179 to 1187), and this could therefore result in the fortuitous cross-recognition of peptides with homologous sequences (Fig. 1A, 2A′, and 4).

The epitope of the neutralizing MAbs is probably discontinuous. The PEPSCAN patterns with the neutralizing MAbs suggest that the antigenic site IV is composite. We therefore assume that the two primary determinants (residues 380 to 387 and 1176 to 1183) form one complex antigenic site. For the two VP_1 segments on the viral surface of foot-and-mouth disease virus, it has been shown that these two sequence segments can form a complex determinant on the viral surface (1). It has also been shown that one constituent part of a discontinuous epitope can be sufficient for binding an antibody (9, 17). We assume that this also applies for the region consisting of residues 380 to 387 of the S protein of TGEV. Three observations indicate that the linear sequence consisting of residues 1172 to 1187 might not be part of the actual binding site of the neutralizing MAbs: (i) the recognition of peptides from the second region seems to depend on a combination of residues rather than on a defined sequence; (ii) a cro-β-galactosidase hybrid protein that contained the sequence consisting of residues 326 to 559 gave, on a Western blot, a clear signal with the neutralizing MAbs (2; results not shown), while the signal of the sequence consisting of residues 1151 to 1240 was not above the background level; and (iii) antibodies raised against a peptide consisting of residues 377 to 391 recognized whole virus particles and had neutralizing activity, while antibodies against a peptide consisting of residues 1171 to 1184 bound only to the peptide immunogen. We infer that the antigenic site IV of TGEV, recognized by the neutralizing MAbs of group IV, consists of at least two parts: the core sequence FFSYGEI (residues 381 to 387) and a combination or combinations of A, D, K, N, Q, and V residues. The presence of these additional residues in the epitope would then be reflected by the cross-recognition of peptides containing three or more of these residues.

To mimic the complex neutralizing epitope, we combined the sequences consisting of residues 380 to 387 and 1176 to 1184. Such a combination peptide is indeed more antigenic with the neutralizing MAbs than each of its constituent parts is (Fig. 5). This will allow the replacement and deletion analysis of the combination sequence to define further the requirements of antigenicity. If specific amino acids of the region consisting of residues 1176 to 1184 contribute to the induction of neutralizing antibodies in antipeptide sera, prospects for developing an experimental vaccine against TGEV could arise.

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