LINEAR NEUTRALIZING EPITOPES ON THE PEPLOMER PROTEIN OF CORONAVIRUSES

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Introduction

Three years ago, we reported a comparison of the primary structures of
the S peplomer proteins of three coronaviruses - mouse hepatitis virus (MHV,
strain A59), infectious bronchitis virus (IBV, strain M41), and feline infec-
tious peritonitis virus (FIPV, strain 79-1146) - which represent the three
antigenic clusters in the coronavirus family (De Groot *et al.*, 1987a, b). A
periodicity in the C-terminal part of the S sequence indicated the presence
of a coiled-coil structure, which forms the stalk of the peplomer. The non-
conserved N-terminal sequence probably forms the bulbous part of the peplo-
mer.
In the work reported here, we used the sequence information to locate epi-
topes that mediate virus neutralization. We will report the results of an
approach that focuses on linear epitopes, i.e., epitopes that depend only on
the sequence and not on the native conformation of the protein.

Approach

The large size of the peplomer proteins (1162 to 1452 residues) clearly
prohibits classical methods of synthesizing peptides to search for antigenic
sequences. Instead, we used a combination of two methods, prokaryotic expres-
sion and PEPSCAN peptide synthesis. Insertion of peplomer cDNA fragments into
the prokaryotic expression plasmid pEX (Stanley & Luzio, 1984) leads to an
attachment of a peplomer fragment to the C terminus of a cro-β-galactosidase
hybrid protein. The fragments were generated by restriction enzymes (Correa
et al., 1989; De Groot *et al.*, unpublished; Kusters *et al.*, 1989b; Lenstra
et al., 1989; Luytjes *et al.*, 1989), by random action of DNase I (Lenstra *et
al.*, 1989), or by synthetic oligonucleotides (Kusters *et al.*, 1989b). The an-
tigenicity of the expression products was assayed by Western blotting. With
only a few exceptions, antibodies that recognized the peplomer protein on a
Western blot also bound to one of the hybrid proteins.
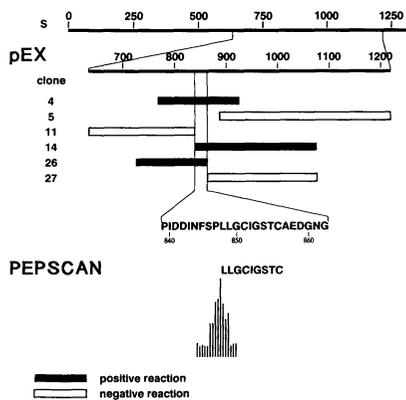
Figure 1. Binding of MAb 19.2 to pEX expression and PEPSCAN products of MHV.
S: The 1324 amino acids of the MHV-A59 peplomer protein.
pEX: Antigenicity of pEX expression products of clones 4, 5, 11, 14, 26, and 27, which delineate the epitope to the residues 839-862.
PEPSCAN: Analysis of nonapeptides from the S region 839-862, synthesized on a solid support and tested in an ELISA. Vertically is plotted the extinction, measured at 405 nm. The positive binding narrows down the epitope to nine amino acids (Luytjes *et al.*, 1989).

The length of the identified antigenic sequences varied from 6 to 200 residues. Within such an antigenic sequence, the localization of the epitope was narrowed down by PEPSCAN analysis (Geysen *et al.*, 1984). A few antibodies (see below) did not bind to any of the peptides, probably because of the unusual length of the epitopes.

Mouse hepatitis virus

Five antigenic sites were identified on the S peplomer protein of MHV (Talbot *et al.*, 1984b; Talbot & Buchmeier, 1985). Three sites, designated A, B, and C, are involved in neutralization. Site A was found to be conformation independent (Talbot *et al.*, 1984a) and is thought to be involved in cell-to-cell fusion (Collins *et al.*, 1982). Various cDNA restriction fragments were expressed in pEX (Luytjes *et al.*, 1989) and the epitope of the site A-specific MAb 19.2 was located within the sequence 839-862, 121 residues from the N terminus of the S2 subunit (Fig. 1, pEX). PEPSCAN analysis of nonapeptides from this region (Fig. 1, PEPSCAN) delineated the epitope within the 848-856 sequence.

Figure 2. Binding of MAbs to pEX expression and PEPSCAN products of IBV.
The listed sequence of the S protein from strain D207 (residues 544-574) spans the junction of the two subunits between the residues 545 and 546. From strains D1466 and M41 the differences with D207 are indicated. The horizontal bars indicate the S sequence fragments synthesized by expression of cDNA fragments from strain D207 (pXD207-Rsa and -XP), D1466 (pXD1466- B, -NB, and -SN), and M41 (pXM41- 13.4, -15.1, and -2.1) or by expression of synthetic oligonucleotides (pXOligo-1 to -7). Dashed ends indicate continuation beyond the listed sequence. Vertical lines indicate the delineation of the epitopes. The PEPSCAN figure (analysis as described in Fig. 1) shows the binding of MAb 54.5 and 30.6 to nonapeptides from the region 550-578 (Kusters *et al.*, 1989). In the SUMMARY figure dashed ends indicate that epitope boundaries were not located exactly.
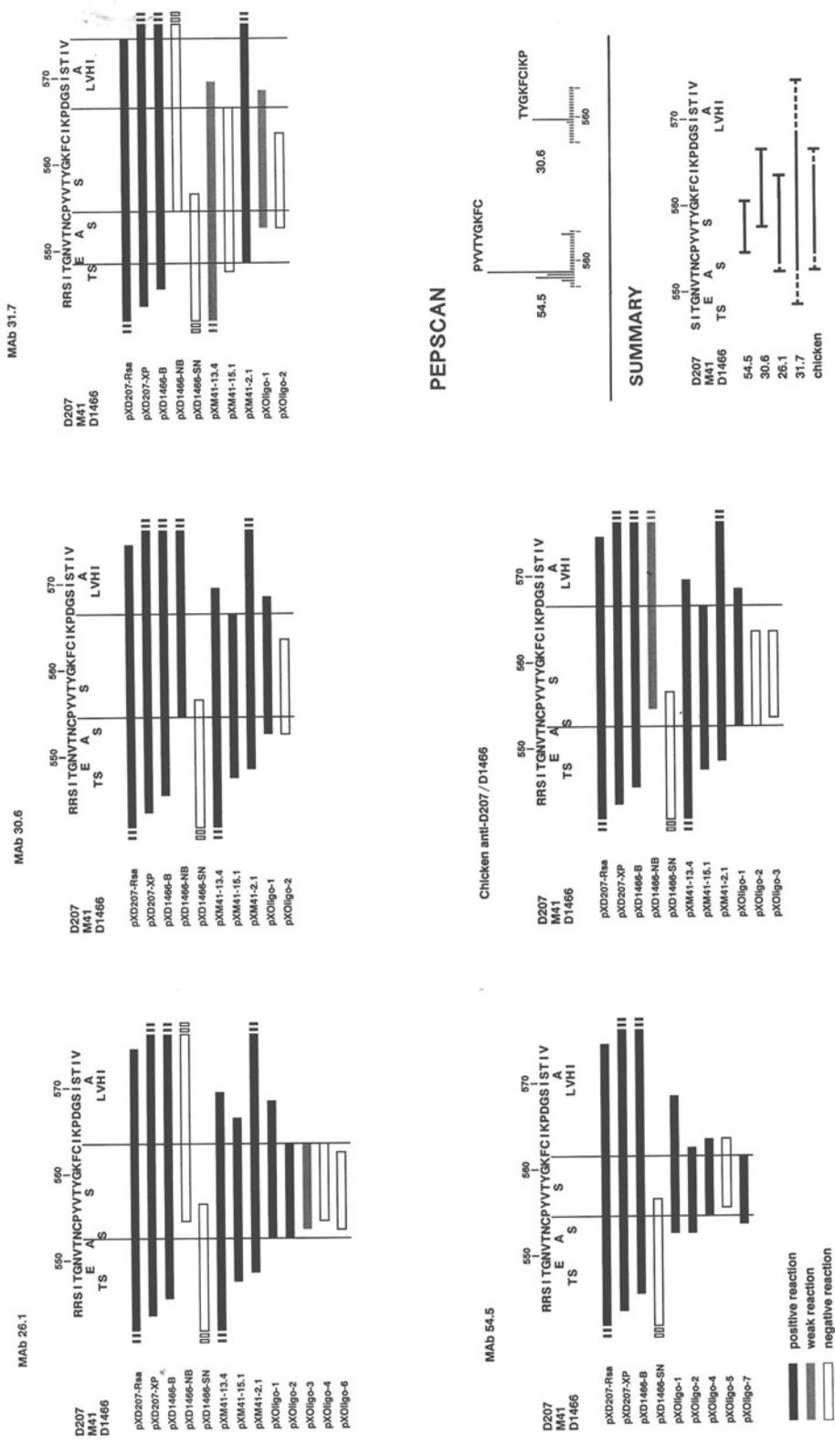
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Figure 2.

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Infectious bronchitis virus

The peplomer protein of IBV contains two subunits - S1 consisting of the residues 1-545 (Kusters *et al.*, 1989a) and S2 containing the residues 546-1180 (Kusters *et al.*,1989c). The S1 subunit contains most of the epitopes that are involved in neutralization (Cavanagh *et al.*, 1986). By mutual competition of monoclonal antibodies, Koch *et al.*, (1986, 1989) identified four antigenic sites. Three sites, located on S1, are conformation-dependent and induce the serotype-specific neutralizing response. The fourth site (site D) is located on S2; judged from the recognition of the antigen on Western blots it is conformation-independent. Antibodies specific for site D are not serotype-specific and have a weak but significant neutralizing activity (Koch *et al.*, 1986, 1989). Further, Lenstra *et al.* (1989) reported that this site is recognized strongly by rabbit, mouse, and chicken antisera.

Restriction enzyme fragments as well as random DNase fragments from IBV strain M41 were used to generate hybrid proteins in pEX (Lenstra *et al.*, 1989) and to locate site D within the 37 N-terminal residues from S2. Subsequently, Kusters *et al.* (1989b) made a detailed map of the site D epitopes by testing the antigenicity of (i) expression products of cDNA fragments from the strains D207, M41, and D1466; (ii) expression products of synthetic oligonucleotides that encoded the epitope sequence (Fig. 2); and (iii) peptides of different lengths synthesized by the PEPSCAN method. This dissection of the antigenic site revealed the location of overlapping epitopes with lengths varying from 6 to at least 17 residues (Fig. 2, SUMMARY).

Despite extensive sequence divergence in both the S1 and the S2 subunits, the antigenic sequences has been conserved among the different serotypes (Kusters *et al.*, 1989b, c). Antibodies specific for site D bound to denatured virus, to pEX expression products, and to synthetic peptides. This indicates a segmental mobility on the peplomer surface (Westhof *et al.*, 1984). Presumably, the same mobility is essential for a specific molecular recognition during one of the stages of the virus life cycle.

Transmissible gastroenteritis virus and feline infectious peritonitis virus

Transmissible gastroenteritis virus (TGEV) and FIPV, both representatives of the third antigenic cluster, are generally recognized by the same antibodies. Three groups of investigators (Correa *et al.*, 1988; Delmas *et al.*, 1986; Garwes *et al.*, 1986) have inventoried the antigenic sites on the TGEV peplomer protein, using mutually competing monoclonal antibodies. For the sake of clarity we designated the sites found by Correa *et al.*, (1988) and Delmas *et al.*, (1986) by roman numerals (Table 1). Sites I and V contain most determinants mediating virus neutralization. The monoclonal antibody COR5 raised against FIPV (S.A. Fiscus, unpublished) neutralized both FIPV and TGEV and probably bound to a sixth antigenic site.

Antibodies specific for site I did not bind to any pEX products or peptides. Monoclonal antibody 1D.B12, which is specific for site II, can discriminate between TGEV and the closely related porcine respiratory coronavirus (PRCV) and bound to a hybrid protein from a pEX-TGEV recombinant containing the 1-

Table 1. Noncompetitive groups of MAbs directed against TGEV.

This paper	Correa *et al.*, 1988.	Delmas *et al.*, 1986.
I	A	A
II	B	D
III	C	
IV	D	C
V		B
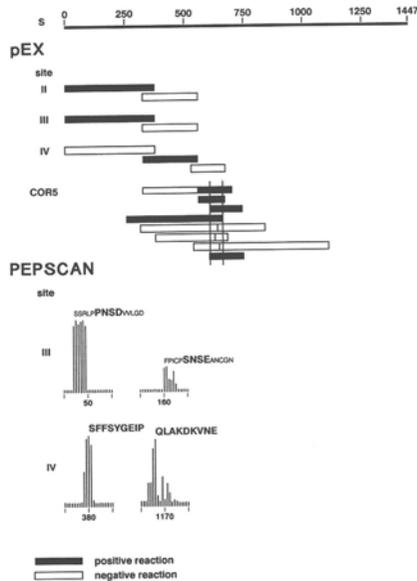
Figure 3. Binding of MAbs to TGEV/pEX and PEPSCAN products.
S : The 1447 amino acids of the TGEV peplomer protein.
pEX: Antigenicity of MAbs of group II, III, and IV with pEX expression products of cDNA clones (TGEV: Correa *et al.*, 1989; FIPV: De Groot *et al.*, *unpublished*).
PEPSCAN: Analysis is as described in Fig 1. For MAbs of site III the common amino acids from the recognized nonapeptides from the regions 40- 60 and 140-160 are indicated in bold. For MAb 57.57, a representative for MAbs of site IV, the sequence of the most antigenic nonapeptide from the regions 370-390 and 1160-1180 (Posthumus *et al.*, 1989) is indicated.

378 sequence (Correa *et al.*, 1989), but not to any fragment of the sequence 326-1449 (Fig. 3, pEX). This indicates a location of the epitope within the N-terminal region 1-325. Because the PEPSCAN pattern derived with nonapeptides from the TGEV peplomer sequence was inconclusive (not shown), the epitope is probably longer than nine residues. Site III is not involved in neutralization and cross-reacts with components of nonimmune porcine serum (Correa *et al.*, 1988). Recognition by MAbs, specific for site III, of pEX hybrid proteins and PEPSCAN peptides (Fig. 3) led to the identification of the tetrapeptide motive P/S-N-S-E/D, which occurs not only twice in the peplomer protein (residues 49-52 and 165-168) but also in several serum proteins (Posthumus *et al.*, 1989b). Thus, the epitope mapping may explain the cross-reaction with serum components.
Site IV is recognized by neutralizing as well as non-neutralizing monoclonal antibodies (Delmas *et al.*, 1986; Correa *et al.*, 1988; Van Nieuwstadt *et al.*, 1989). MAb 57.57 and all other antibodies specific for site IV recognized nonapeptides around residue 380 (Fig. 3, PEPSCAN). Only neutralizing antibodies specific for site IV bound to a second group of peptides around residue 1180 (Posthumus *et al.*, 1989). However, only the 380 region was antigenic when tested as part of a pEX hybrid protein. Further, antisera against a peptide with the sequence 377-390 were positive in a virus-ELISA and TGEV neutralization assay, but antisera against a 1176-1184 peptide were not (Posthumus *et al.*, 1989). We propose that site IV is complex and that the recognition of nonapeptides from the region 1176-1193 (and nonapeptides with a similar amino acid composition) reflects the surrounding of the region 380-388 in the tertiary structure of the peplomer. Thus, the neutralizing MAbs specific for site IV may bind to a linear part of a discontinuous epitope.

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The epitope of COR5 was located in the region 607-660 (in the numbering of the TGEV sequence) by using pEX-FIPV and pEX-TGEV recombinants (Fig. 3, pEX; De Groot, unpublished). The region encoding this epitope appeared to encompass three restriction enzyme cleavage sites at positions corresponding to the amino acid residues 629, 640 and 648. These data indicate that the linear epitope of COR5 is unusually long and probably can explain the negative results obtained with PEPSCAN peptides.
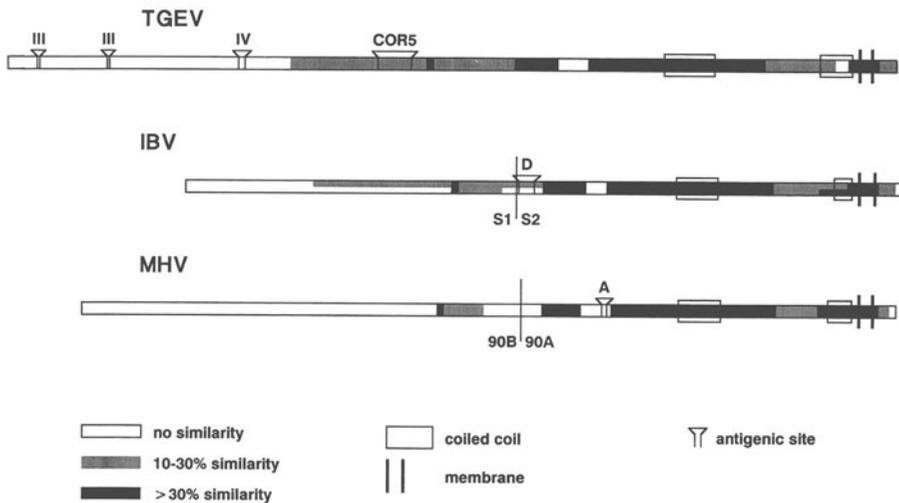

Figure 4. Linear antigenic sites on aligned peplomer sequences.

Survey of linear epitopes of the peplomer proteins

Fig. 4 shows a schematic alignment of the amino acid sequences (De Groot *et al*., 1987a, b) of the peplomer proteins from MHV, IBV and TGEV/FIPV with the locations of the linear epitopes. The length of the epitopes varied from 5 (TGEV site III) to at least 20 (COR5) residues. Antigenic sites appear to be located in regions without or with only moderate sequence similarity. In contrast to the finding with homologous picornaviruses (Rossman *et al*., 1985), no homology is observed with regard to the locations of the epitopes. For instance, in IBV nearly all linear epitopes of monoclonal antibodies (and most of the linear epitopes of polyclonal antibodies, Lenstra *et al*., 1989) are mapped in site D, but different patterns are observed for MHV and TGEV/ FIPV. If the linear epitopes are located within relatively flexible surface

loops (Westhof *et al.*, 1984), our observations demonstrate the degree of structural divergence of the peplomer proteins of the different coronaviruses. This divergence is probably caused by differences in host range. It would be interesting to extend these observation to conformation-dependent epitopes. However, this awaits not only the localization of these epitopes by analyzing mutants that escape neutralization, but also a convincing alignment of the regions with a low apparent sequence similarity (de Groot *et al.*, 1987a).

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