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Bovine coronavirus spike glycoprotein: localization of an immunodominant region at the amino-terminal end of S2

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We have identified the binding site of monoclonal antibodies (MAbs) against the S2 subunit of the bovine coronavirus spike (S) glycoprotein. The location of this site was first investigated by using prokaryotic expression of DNA restriction fragments covering the entire S gene. The amino acid sequence containing the antibody binding site was shortened from 70 to 20 amino acids by digestion of plasmid DNA with exonuclease III, followed by sequencing of the smallest digestion product encoding an immunoreactive fusion protein. Finally we synthesized a set of nonapeptides covering the 20 amino acid sequence extending from the N-terminal residue of the S2 subunit (Ala 769 to Tyr 798). MAbs reacted mainly with six consecutive overlapping peptides with the sequence TTGYRFTN-FEPFTV. Polyclonal antibodies from hyperimmunized or convalescent animals reacted only with the recombinant proteins identified by MAbs, and the hyperimmune serum bound to the same set of peptides. This suggests that this highly conserved linear antigenic determinant corresponds to an immunodominant region. This region resembles both in location and immunodominance the linear determinant defined on the infectious bronchitis virus S2 subunit. The presence of similar regions in the N-terminal region of the S2 subunit of other coronaviruses is discussed.

Bovine coronavirus (BCV), an enteric coronavirus, causes acute enteritis in newborn calves and chronic infections in adult cattle (Stair *et al.*, 1972; Crouch *et al.*, 1985). When examined by electron microscopy, the virus displays two fringes of spikes (King & Brian, 1982; Dea & Tijssen, 1989) formed by two different glycoproteins, spike (S) and haemagglutin–esterase (HE). Both proteins display typical features of glycoproteins forming the peplomers of enveloped viruses. S and HE are implanted in the viral membrane at their C terminus (Boireau *et al.*, 1990; Kienzle *et al.*, 1990), trigger the immune system eliciting the production of neutralizing antibodies (Vautherot *et al.*, 1984, 1992; Deregt *et al.*, 1987) and interact with receptors on the cell surface (Vlasak *et al.*, 1988; Schultze *et al.*, 1991*a*, *b*; Stortz *et al.*, 1991).

The S glycoprotein, which presumably trimerizes to form the 'club-shaped' peplomers (De Groot *et al.*, 1987; Delmas *et al.*, 1990), is post-translationally cleaved to yield two subunits, S1 and S2, corresponding respectively to the N- and C-terminal parts of the S protein (Luytjes *et al.*, 1987; Abraham *et al.*, 1990). Cell fusion-from-without induced by murine hepatitis virus (MHV) has been shown to depend on the complete cleavage of the S molecule (Sturman *et al.*, 1985). The S1 subunit presumably forms the bulbous upper part of the projections (Cavanagh, 1983) and bears type-specific neutralization determinants (Cavanagh *et al.*, 1988; Yoo *et al.*, 1991; Vautherot *et al.*, 1992). The S2 subunit has been predicted to contain two long α helices which are involved in the formation of the stalk of the peplomer (De Groot *et al.*, 1987; Boireau *et al.*, 1990), and elicits the production of group-specific monoclonal antibodies (MAbs) (Talbot *et al.*, 1988; Lenstra *et al.*, 1989).

With the prospect of designing new immunogens containing conserved immunodominant protective epitopes, several investigations have been aimed at defining with precision the location of B and T cell epitopes on viral proteins. Conformation-independent determinants have been found on the N-terminal part of transmissible gastroenteritis virus (TGEV) (Delmas *et al.*, 1990; Correa *et al.*, 1990) and the MHV S1 subunits (Routledge *et al.*, 1991), but the MHV and infectious bronchitis virus S2 subunits have also been reported to bear conserved linear determinants recognized by neutralizing MAbs (Luytjes *et al.*, 1989; Talbot *et al.*, 1988; Routledge *et al.*, 1991; Kusters *et al.*, 1990). Two of these B cell epitopes have been tested for their immunogenicity and found to protect mice against lethal MHV infection (Talbot *et al.*, 1988; Koolen *et al.*, 1990). In the present study, we used prokaryotic expression of parts of the S gene together with pin-based peptide synthesis to precisely locate the binding site of previously characterized anti BCV S protein MAbs (Vautherot *et al.*, 1992). A linear and highly conserved determinant

corresponding to an immunodominant region was identified at the N terminus of the S2 subunit.

We have described the production and characterization of MAbs to the S1 and S2 subunits, and HE, and their use to construct an operational map of B cell epitopes on these glycoproteins (Vautherot *et al.*, 1984, 1992). The polyclonal anti-BCV antibodies were obtained from two sources: (i) a specific pathogen-free, coronavirus seronegative New Zealand White rabbit hyperimmunized with BCV isolate G_{110} (L'Haridon *et al.*, 1981) emulsified in Freund's complete adjuvant and (ii) a colostrum-deprived calf experimentally infected with a field isolate, bled 3 weeks after recovering from diarrhoea (Gouet *et al.*, 1978).

To characterize the putative linear epitopes on BCV S protein we chose the pUEX expression system (Bressan & Stanley, 1987), in which the foreign genes are inserted at the 3′ end of a *cro–lacZ* hybrid gene. Expression of the fusion protein is under the control of the λr promoter, itself repressed by a thermolabile cI857 repressor inactivated by a temperature shift from 25 °C to 42 °C. cDNA clones G7, PG7-8, PG7-8-12 and P33-23 (Boireau *et al.*, 1990) were used to generate 10 partially overlapping restriction fragments covering the entire S gene (Fig. 1, Table 1). The main steps in the construction of BCV pUEX clones are summarized in Table 1. Ligations were carried out by standard procedures (Sambrook *et al.*, 1989) and recombinant plasmids were transfected into *Escherichia coli* strain RR1 by the procedure of Hanahan (1983).

Subsequent screening of ampicillin-resistant bacterial clones by hybridization was performed by following standard procedures (Sambrook *et al.*, 1989). For each construct, 10 positive clones were screened for expression of a chimeric protein of known M_r by gel electrophoresis under denaturing conditions (Laemmli, 1970).

Recombinant proteins were expressed as inclusion bodies which were purified as described (Lenstra *et al.*, 1989). Immunoscreening using MAbs or polyclonal antibodies was done by Western blotting (Vautherot *et al.*, 1992) or dot-blotting of solubilized inclusion bodies. For testing with recombinant fusion proteins, we selected MAbs from our panel which reacted with the denatured viral S1 (non-neutralizing MAb I16, antigenic site S1-D) or S2 (non-neutralizing MAbs I1 and I22, antigenic site S2-A) subunit (Vautherot *et al.*, 1992). Only MAbs to S2-A bound to fusion proteins encoded by overlapping plasmids S2 1-2 and S2 3-4 (Fig. 1 and 2*a*). The same fusion products, and only these, were recognized by the rabbit hyperimmune serum (Fig. 2*a*) and by the calf antiserum which was reacted with 8 M-urea-solubilized inclusion bodies dotted on nitrocellulose (data not shown).

The size and identity of all inserts were first checked by restriction endonuclease digestion of pUEX plasmids (data not shown). We then sequenced the recombinant plasmids using two oligonucleotide primers located on each side of the cloning site (5′ CGACTCCTGGAGCCCGTCAG 3′ and 5′ CTAGAGCCGGATCGATCCGGTC 3′). Sequencing was by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) using alkali-denatured purified plasmid DNA (Lim & Pène, 1988) and the Sequenase (T7 modified DNA polymerase) sequencing system (US Biochemicals). This allowed us to verify that (i) each plasmid contained the S gene fragment fused in frame with the *cro-β*-galactosidase gene and (ii) no mutation had occurred in the 200 bases at the 3′ and 5′ ends of the DNA fragments. From these results it appeared that the S2 subunit had a unique linear immunodominant antigenic site located in a stretch of 70 amino acids at the N terminus of the S2 subunit, from Phe 776 to Asn 850 (Fig. 1).

To determine the location of this antigenic site more precisely, we generated a library of deletion plasmids from pUEX S2 3-4 by using the strategy of Henikoff (1984). Plasmid DNA (5 μg) was linearized using *Sal*I, which was 10 bases downstream from the 3′ end of the insert, made blunt by filling with the Klenow fragment of DNA polymerase I, and finally cleaved with *Pst*I to generate a four base protrusion at the 3′ end of the polylinker region, to protect the stop codons from exonuclease III digestion. After 45 s incubation, samples were taken every 10 s over a 200 s period; the samples were treated with nuclease S1 after which the plasmids were repaired, ligated and transfected as described (Henikoff, 1984). MAb I22 (S2-A) was utilized to screen 400 bacterial clones (20 clones/digestion time) resulting from the transfection of the digested plasmid. As expected, a longer digestion time yielded a smaller number of immunoreactive clones (data not shown).

To determine which clone(s) expressed the smaller recombinant proteins, 24 fusion products were analysed by PAGE followed by Western blotting (data not shown). The deletion of the C terminus of the fusion protein did not cause a gradual loss of immunoreactivity with the rabbit polyclonal antiserum, suggesting that this polyclonal reagent recognized a single determinant (Buckland *et al.*, 1989). The 3′ and 5′ ends of the region encoding antigenic site S2-A were determined by sequencing a set of seven plasmids encoding the shortest fusion proteins detected using MAb I22 (Fig. 2*b*). The region containing this site apparently extended from Phe 779 to Pro 793.

To establish which amino acid sequence is recognized by MAbs or polyclonal antibodies, we tested these antibodies for binding to a set of 22 synthetic consecutive, overlapping nonapeptides (Geysen *et al.*, 1984)

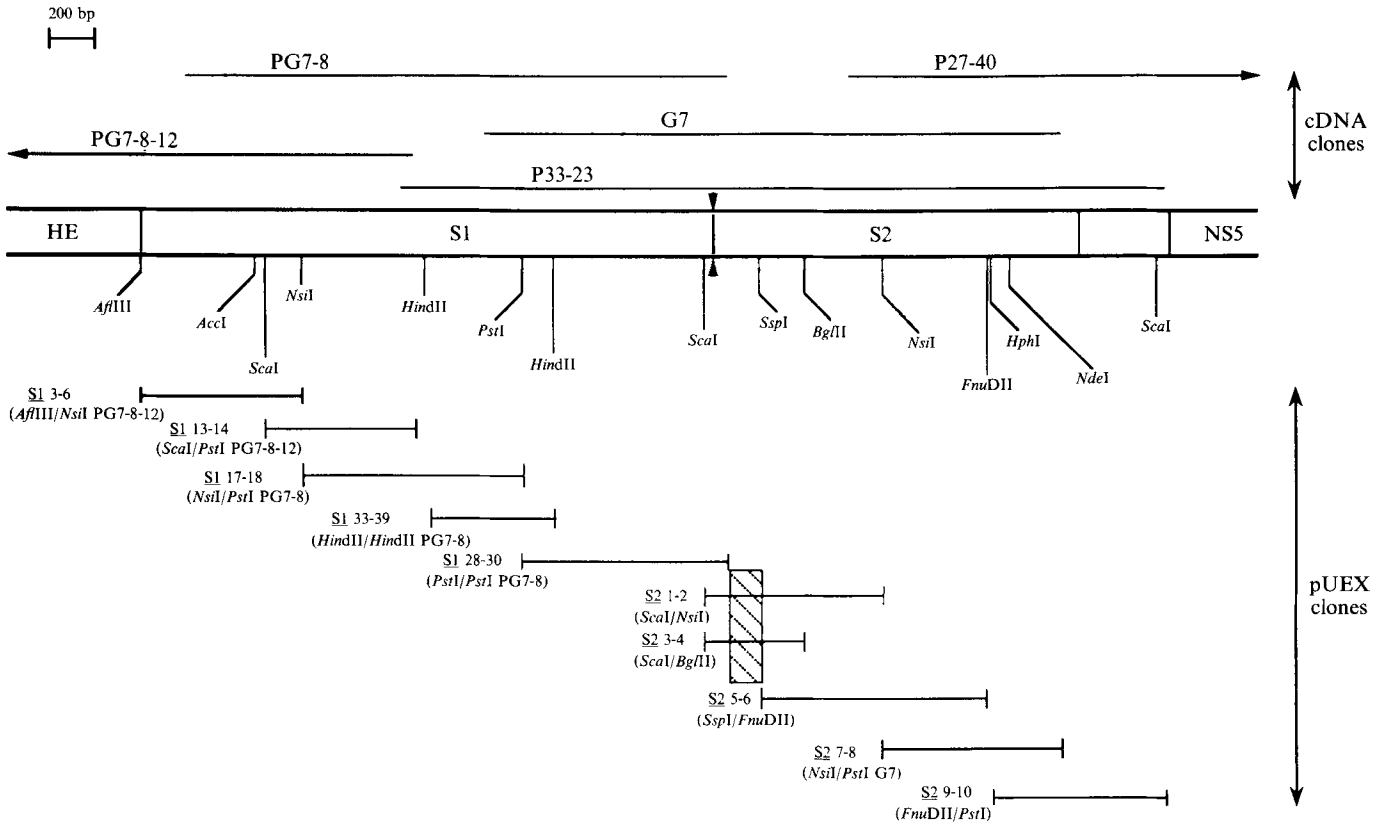

Fig. 1. Schematic representation of BCV S gene expression products. The upper lines represent the cDNA clones used to generate the restriction fragments (Boireau *et al.*, 1990). The restriction endonuclease sites are indicated under the middle bar representing the S gene and parts of its flanking sequences in HE and NS5. Arrowheads point to the cleavage site between S1 and S2. Horizontal bars show the restriction fragments fused in-frame with the *cro–lacZ* gene of the pUEX vector. The vertical shaded box defines the S2 region to which anti-S/gp95 MAbs bound, extending from amino acids 779 to 850.

Table 1. *Recombinant pUEX clones containing fragments of BCV S gene*

Recombinant plasmid (see also Fig. 1)	Vector preparation	Dephosphorylation*	cDNA clone	Enzymes used to cleave DNA fragments	Probes used for screening†
pUEX S1 3-6	pUEX2; *Sma*I/*Pst*I		PG7-8-12	*Afl*III/*Nsi*I	PG7-8-12
pUEX S1 13-14	pUEX3; *Sma*I/*Pst*I		PG7-8-12	*Sca*I/*Pst*I	PG7-8-12
pUEX S1 17-18	pUEX2; *Pst*I	+	PG7-8	*Nsi*I/*Pst*I	PG7-8-12
pUEX S1 28-30	pUEX2; *Pst*I	+	PG7-8	*Pst*I	PG7-8-12
pUEX S 33-39	pUEX3; *Sma*I	+	PG7-8	*Hin*dII	PG7-8-12
pUEX S2 1-2	pUEX3; *Sma*I/*Pst*I		PG33-23	*Sca*I/*Nsi*I	PG33-23
pUEX S2 3-4	pUEX3; *Sma*I/*Bam*HI		PG33-23	*Sca*I/*Bgl*II	PG33-23
pUEX S2 5-6	pUEX3; *Sma*I	+	G7	*Ssp*I/*Fnu*DII	PG33-23
pUEX S2 7-8	pUEX2; *Pst*I		G7	*Nsi*I/*Pst*I	PG33-23
pUEX S2 9-10	pUEX1; *Sma*I/*Pst*I	+	P33-23	*Fnu*DII/*Pst*I	

* Where indicated, the vector was treated with calf intestine alkaline phosphatase (Boehringer Mannheim).
† α-^{32}P-labelled random-primed inserts.

extending from Ala 769 to Tyr 798 of the S protein sequence (Boireau *et al.*, 1990). Amino acids were coupled to activated polyethylene pins (C.R.B. Ltd) by using Fmoc-based chemistry under the conditions described by the manufacturer. After completion of synthesis, the antibodies were tested for binding to peptides by standard procedures (Geysen *et al.*, 1984). MAb I1 bound strongly and in a reproducible way to six consecutive nonapeptides covering the sequence TTGYRFTNFEPFTV (Fig. 3). In contrast, MAb I22

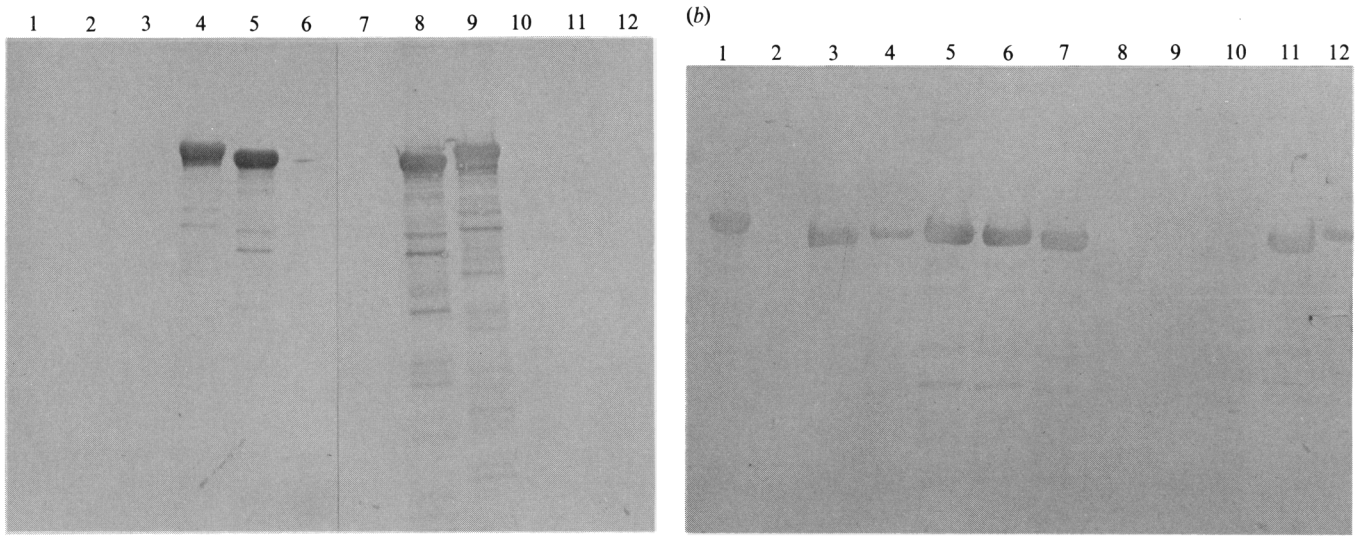

Fig. 2. (*a*) Immunostaining of recombinant fusion proteins with anti-S2 MAb I1 (lanes 1 to 6), and a rabbit polyclonal antiserum to BCV (lanes 7 to 12). Denatured recombinant proteins were run on a 10% SDS–polyacrylamide gel and blotted onto nitrocellulose. The fusion proteins were from pUEX clones S2 9-10 (lanes 1 and 12), control β-galactosidase (lanes 2 and 11), S2 7-8 (lanes 3 and 10), S2 1-2 (lanes 4 and 9), S2 3-4 (lanes 5 and 8) and S2 5-6 (lanes 6 and 7). (*b*) Western blot immunoscreening of deleted pUEX S2 3-4 inserts. After initial screening, seven immunopositive (lanes 3 to 7, 11 and 12) and three negative (lanes 8 to 10) recombinant proteins were denatured, separated on a 10% SDS–polyacrylamide gel and blotted onto nitrocellulose. MAb I22 was used to detect the recombinant proteins synthesized by the original pUEX clone S2 3-4 (lane 1), and the deleted clones 4·78 (lane 3), 4·25 (lane 4), 4·12 (lane 5), 4·8 (lane 6), 3·72 (lane 7), 3·48 (lane 8), 2·98 (lane 9), 2·65 (lane 10), 2·46 (lane 11), 2·21 (lane 12). A negative control consisted of a recombinant non-structural protein treated under the same conditions (pUEX NS5, lane 2).

bound only to nonapeptide YRFTNFEPF (Fig. 3), although competition binding assays showed that MAbs I1 and I22 recognized determinants mapping to site S2-A. As expected, the polyclonal rabbit antiserum recognized five nonapeptides spanning the sequence TTGYRFTNFEPFT (Fig. 3). The same polyclonal antibodies and MAbs did not bind to any of 65 consecutive, overlapping hexapeptides covering the sequence from Phe 779 to Asp 850, synthesized before the nonapeptide set (data not shown). Taken together, our data indicated that a major immunoreactive region of the S2 subunit, delineated by anti-S2-A MAbs and polyclonal antibodies, is located at the N terminus, beginning two residues downstream from the S protein cleavage site. The absence of binding of MAbs to any other fusion protein or hexapeptide downstream from this sequence makes the existence of a composite linear determinant unlikely (Posthumus *et al.*, 1990).

By analysing the data from pUEX sequencing together with the results from antibody binding to peptides, we tried to define the S2-A epitope more accurately. We found that the 3′ end of insert S1 28-30, encoding an immunologically non-reactive fusion protein, ended at Asn 778 (data not shown). The size of the recombinant protein indicates that the 3′ end sequences are fully translated and this result suggests that Phe 779 and possibly the two following amino acids (Glu 780 and Pro

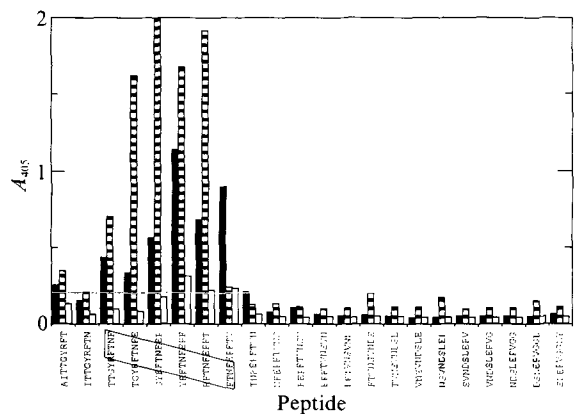

Fig. 3. Antigenic profile for the N-terminal region of the S2 subunit (epitope scanning). Twenty-two overlapping nonapeptides, amino acids 769 to 798, of the S glycoprotein were synthezised on a solid phase and tested in an ELISA for binding with MAb I1 (■), I22 (□) and polyclonal rabbit IgG (▤). The horizontal dotted line denotes the cut-off value (absorbance of 0·2). The consensus sequence present in peptides best recognized by the antibodies is boxed.

781) are necessary for the conservation of immunoreactivity; this is in good agreement with the results of antibody binding experiments, which showed an absence of reactivity with the nonapeptide ending at Asn 778 and a progressive increase in binding of MAbs to the following nonapeptides (Fig. 3). The FTNF tetrapeptide

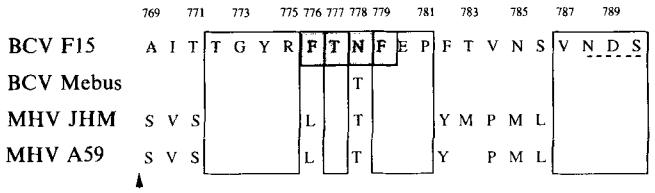

Fig. 4. Comparison of the N-terminal amino acid sequences of BCV and MHV S2. The sequences of BCV F15 (Boireau *et al.*, 1990), BCV Mebus (Abraham *et al.*, 1990), and of MHV JHM (Schmidt *et al.*, 1987) and MHV A59 (Luytjes *et al.*, 1987) were aligned starting at the cleavage site (black arrow). Conserved sequences are boxed and the four amino acid sequence recognized by MAbs to the S2-A epitope is outlined by a shaded box. A potential *N*-glycosylation site is underlined by a dotted line.

was present in all nonapeptides yielding the highest binding values, and the presence of Phe 776 appeared to be necessary for the binding of MAbs (Fig. 3).

We have shown that MAbs to S2-A delineate a group-specific antigenic determinant, reacting with all BCV strains, MHV, porcine haemagglutinating encephalomyelitis virus and human coronavirus strain OC43 (Vautherot *et al.*, 1984, 1992). The alignment of the sequences (Fig. 4) shows that the conservative changes of Phe to Leu at position 776 and Asn to Thr at 778 do not affect the antibody binding ability. The effect of a single amino acid change on the antigenicity of a sequence representing a linear epitope has been well documented by replacement studies Getzoff *et al.*, 1987). In such sequences, amino acids are either critical for antibody binding and, as such, cannot be substituted by any other amino acid, or may be replaced either by any other amino acid or only by an amino acid with similar chemical properties. In amino acid sequences homologous to epitopes defined either on the TGEV S (Posthumus *et al.*, 1990) or the respiratory syncytial virus F (Scopes *et al.*, 1990) proteins, the Phe residues could be replaced by Leu and/or other hydrophobic amino acids without any loss in immunoreactivity. This is in good agreement with our results on the conservation of antigenicity despite a naturally occurring replacement of a Phe by a Leu residue. When tested against MHV, none of the anti-S2-A MAbs displayed any neutralizing activity (data not shown).

The site defined in this study differs from all the other epitopes already delineated on this protein (Luytjes *et al.*, 1989; Talbot *et al.*, 1988; Routledge *et al.*, 1990). However, the location of epitope S2-A strikingly resembles that of neutralizing antigenic site S2-G delineated on the IBV S2 subunit (Kusters *et al.*, 1990). The S2-A epitope appears to be a moderately hydrophilic region at the tip of the S2 subunit, in a good position for eliciting the production of antibodies (Westhof *et al.*, 1984). Indirect evidence for this was given by Gallagher *et al.* (1990) who, by immunizing with peptides homologous to the amino acid sequences flanking the cleavage site in MHV S protein, showed that the SVSTGYRLTTC sequence, partially overlapping epitope S2-A (Fig. 4), is immunogenic in the rabbit, eliciting the production of monospecific anti-S2 polyclonal antibodies. In contrast to the BCV S2 subunit, neither the S1 subunit nor HE fragments expressed in prokaryotic vectors are recognized by MAbs or polyclonal antibodies (J. F. Vautherot, unpublished data). We are currently verifying whether peptides containing the sequence defined above or recombinant proteins are immunogenic, and might elicit antibodies which inhibit virus functions.

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References

ABRAHAM, S., KIENZLE, T. E., LAPPS, W. & BRIAN, D. A. (1990). Deduced sequence of the bovine coronavirus spike protein and identification of the internal proteolytic cleavage site. *Virology* **176**, 296–301.

BOIREAU, P., CRUCIERE, C. & LAPORTE, J. (1990). Nucleotide sequence of the glycoprotein S gene of bovine enteric coronavirus and comparison with the S proteins of two mouse hepatitis virus strains. *Journal of General Virology* **71**, 487–492.

BRESSAN, G. M. & STANLEY, K. K. (1987). pUEX, a bacterial expression vector related to pEX with universal host specificity. *Nucleic Acids Research* **15**, 10056.

BUCKLAND, R., GIRAUDON, P. & WILD, F. (1989). Expression of measles virus nucleoprotein in *Escherichia coli*: use of deletion mutants to locate the antigenic sites. *Journal of General Virology* **70**, 435–441.

CAVANAGH, D. (1983). Coronavirus IBV: structural characterization of the spike protein. *Journal of General Virology* **64**, 2577–2583.

CAVANAGH, D., DAVIS, P. J. & MOCKETT, A. P. (1988). Amino acids within hypervariable region 1 of avian coronavirus IBV (Massachusetts serotype) spike glycoprotein are associated with neutralization epitopes. *Virus Research* **11**, 141–150.

CORREA, I., GEBAUER, F., BULLIDO, M. J., SUÑÉ, C., BAAY, M. F. D., ZWAAGSTRA, K. A., POSTHUMUS, W. P. A., LENSTRA, J. A. & ENJUANES, L. (1990). Localization of antigenic sites of the E2 glycoprotein of transmissible gastroenteritis coronavirus. *Journal of General Virology* **71**, 271–279.

CROUCH, C. F., BIELEFELDT OHMANN, H., WATTS, T. C. & BABIUK, L. A. (1985). Chronic shedding of bovine enteric coronavirus antigen–antibody complexes by clinically normal cows. *Journal of General Virology* **66**, 1489–1500.

DEA, S. & TIJSSEN, P. (1989). Antigenic and polypeptide structure of turkey enteric coronaviruses as defined by monoclonal antibodies. *Journal of General Virology* **70**, 1725–1741.

DE GROOT, R. J., LUYTJES, W., HORZINEK, M. C., VAN DER ZEIJST, B. A. M., SPAAN, W. J. M. & LENSTRA, J. A. (1987). Evidence for a coiled-coil structure in the spike proteins of coronaviruses. *Journal of Molecular Biology* **196**, 963–966.

DELMAS, B., RASSCHAERT, D., GODET, M., GELFI, J. & LAUDE, H. (1990). Four major antigenic sites of the coronavirus transmissible gastroenteritis virus are located on the amino-terminal half of spike glycoprotein S. *Journal of General Virology* **71**, 1313–1323.

DEREGT, D. & BABIUK, L. A. (1987). Monoclonal antibodies to bovine coronavirus: characteristics and topographical mapping of neutralizing epitopes on the E2 and E3 glycoproteins. *Virology* **161**, 410–420.

GALLAGHER, T. M., PARKER, S. E. & BUCHMEIER, M. J. (1990). Neutralization-resistant variants of a neurotropic coronavirus are generated by deletions within the amino-terminal half of the spike glycoprotein. *Journal of Virology* **64**, 731–741.

GETZOFF, E. D., GEYSEN, H. M., RODDA, S. J., ALEXANDER, H., TAINER, J. A. & LERNER, R. A. (1987). Mechanisms of antibody binding to a protein. *Science* **235**, 1191–1196.

GEYSEN, H. M., MELOEN, R. H. & BARTELING, S. J. (1984). Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Procedings of the National Academy of Sciences, U.S.A.* **81**, 3998–4002.

GOUET, P., CONTREPOIS, M., DUBOURGUIER, H., RIOU, Y., SCHERRER, R., LAPORTE, J., VAUTHEROT, J. F., COHEN, J. & L'HARIDON, R. (1978). The experimental production of diarrhea in colostrum-deprived axenic and gnotoxenic calves with enteropathogenic *E. coli* rotavirus, coronavirus and in combined infection of rotavirus and *E. coli*. *Annales de Recherches Vétérinaires* **9**, 433–440.

HANAHAN, D. (1983). Studies on the transformation of *Escherichia coli* with plasmids. *Journal of Molecular Biology* **166**, 577–580.

HENIKOFF, S. (1984). Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**, 351–359.

KIENZLE, T. E., ABRAHAM, S., HOGUE, B. G. & BRIAN, D. A. (1990). Structure and orientation of expressed bovine coronavirus hemagglutinin-esterase protein. *Journal of Virology* **64**, 1834–1838.

KING, B. & BRIAN, D. A. (1982). Bovine coronavirus structural proteins. *Journal of Virology* **42**, 700–707.

KOOLEN, M. J. M., BORST, M. A. J., HORZINEK, M. C. & SPAAN, W. J. M. (1990). Immunogenic peptide comprising a mouse hepatitis virus A59 B-cell epitope and an influenza virus T-cell epitope protects against lethal infection. *Journal of Virology* **64**, 6270–6273.

KUSTERS, J. G., JAGER, E. J., LENSTRA, J. A., KOCH, G., POSTHUMUS, W. P. A., MELOEN, R. H. & VAN DER ZEIJST, B. A. M. (1989). Analysis of an immunodominant region of infectious bronchitis virus. *Journal of Immunology* **143**, 2692–2698.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.

LENSTRA, J. A., KUSTERS, J. G., KOCH, G. & VAN DER ZEIJST, B. A. M. (1989). Antigenicity of the peplomer protein of infectious bronchitis virus. *Molecular Immunology* **26**, 7–15.

L'HARIDON, R., SCHERRER, R., VAUTHEROT, J. F., LA BONNARDIÈRE, C., LAPORTE, J. & COHEN, J. (1981). Adaptation d'un isolement de coronavirus entérique bovin à la culture cellulaire et caractérisation de la souche obtenue. *Annales de Recherches Vétérinaires* **12**, 243–251.

LIM, H. M. & PÈNE, J. J. (1988). Optimal conditions for supercoil DNA sequencing with the *Escherichia coli* DNA polymerase I large fragment. *Gene Analysis Techniques* **5**, 32–39.

LUYTJES, W., STURMAN, L. S., BREDENBEEK, P. J., CHARITE, J., VAN DER ZEIJST, B. A. M., HORZINEK, M. C. & SPAAN, W. J. M. (1987). Primary structure of the glycoprotein E2 of coronavirus MHV-A59 and identification of the trypsin cleavage site. *Virology* **161**, 479–487.

LUYTJES, W., GEERTS, D., POSTHUMUS, W., MELOEN, R. & SPAAN, W. (1989). Amino-acid sequence of a conserved neutralizing epitope of murine coronavirus. *Journal of Virology* **63**, 1408–1412.

POSTHUMUS, W. P. A., LENSTRA, J. A., SCHAAPER, W. M. M., VAN NIEUWSTADT, A. P., ENJUANES, L. & MELOEN, R. H. (1990). Analysis and simulation of a neutralizing epitope of transmissible gastroenteritis virus. *Journal of Virology* **64**, 3304–3309.

ROUTLEDGE, E., STAUBER, R., PFLEIDERER, M. & SIDDELL, S. G. (1991). Analysis of murine coronavirus surface glycoprotein functions by using monoclonal antibodies. *Journal of Virology* **65**, 254–262.

SAMBROOK, J., FRITSCH, E. F. & MANIATIS, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edn. New York: Cold Spring Harbor Laboratory.

SANGER, F., NICKLEN, S. & COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, U.S.A.* **74**, 5463–5467.

SCHMIDT, I., SKINNER, M. & SIDDELL, S. (1987). Nucleotide sequence of the gene encoding the surface projection glycoprotein of coronavirus MHV-JHM. *Journal of General Virology* **68**, 47–56.

SCHULTZE, B., WAHN, K., KLENK, H. D. & HERRLER, G. (1991*a*). Isolated HE protein from hemagglutinating encephalomyelitis virus and bovine coronavirus has receptor-destroying and receptor-binding activity. *Virology* **180**, 221–228.

SCHULTZE, B., GROSS, H.-J., BROSSMER, R. & HERRLER, G. (1991*b*). The S protein of bovine coronavirus is a hemagglutinin recognizing 9-*O*-acetylated sialic acid as a receptor determinant. *Journal of Virology* **65**, 6232–6237.

SCOPES, G. E., WATT, P. J. & LAMBDEN, P. R. (1990). Identification of a linear epitope on the fusion glycoprotein of respiratory syncytial virus. *Journal of General Virology* **71**, 53–59.

STAIR, E. L., RHODES, M. B., WHITE, R. G. & MEBUS, C. A. (1972). Neonatal calf diarrhea: purification and electron microscopy of a coronavirus-like agent. *American Journal of Veterinary Research* **33**, 1147–1157.

STORZ, J., HERRLER, G., SNODGRASS, D. R., HUSSAIN, K. A., ZHANG, X. M., CLARK, M. A. & ROTT, R. (1991). Monoclonal antibodies differentiate between the haemagglutinating and the receptor-destroying activities of bovine coronavirus. *Journal of General Virology* **72**, 2817–2820.

STURMAN, L. S., RICARD, C. S. & HOLMES, K. V. (1985). Proteolytic cleavage of the E2 glycoprotein of murine coronavirus: activation of cell-fusing activity of virions by trypsin and separation of two different 90K cleavage fragments. *Journal of Virology* **56**, 904–911.

TALBOT, P. J., DIONNE, G. & LACROIX, M. (1988). Vaccination against lethal coronavirus-induced encephalitis with a synthetic decapeptide homologous to a domain in the predicted peplomer stalk. *Journal of Virology* **62**, 3032–3036.

VAUTHEROT, J. F., LAPORTE, J., MADELAINE, M. F., BOBULESCO, P. & ROSETO, A. (1984). Antigenic and polypeptide structure of bovine enteric coronavirus as defined by monoclonal antibodies. In *Molecular Biology and Pathogenesis of Coronavirus*, pp. 117–131. Edited by P. J. M. Rottier, B. A. M. van der Zeijst & W. J. M. Spaan. New York & London: Plenum Press.

VAUTHEROT, J.-F., MADELAINE, M.-F., BOIREAU, P. & LAPORTE, J. (1992). Bovine coronavirus peplomer glycoproteins: detailed antigenic analyses of S1, S2 and HE. *Journal of General Virology* **73**, 1725–1737.

VLASAK, R., LUYTJES, W., SPAAN, W. & PALESE, P. (1988). Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. *Proceedings of the National Academy of Sciences, U.S.A.* **85**, 4526–4529.

WESTHOF, E., ALTSCHUH, D., MORAS, D., BLOOMER, A. C., MONDRAGON, A., KLUG, A. & VAN REGENMORTEL, M. H. V. (1984). Correlation between segmental mobility and the location of antigenic determinants in proteins. *Nature, London* **311**, 123–126.

YOO, D., PARKER, M. D., SONG, J., COX, G. J., DEREGT, D. & BABIUK, L. A. (1991). Structural analysis of the conformational domains involved in neutralization of bovine coronavirus using deletion mutants of the spike glycoprotein S1 subunit expressed by recombinant baculoviruses. *Virology* **183**, 91–98.

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