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Antigenic Homology among Coronaviruses Related to Transmissible Gastroenteritis Virus

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The antigenic homology of 26 coronavirus isolates, of which 22 were antigenically related to transmissible gastroenteritis virus (TGEV), was determined with 42 monoclonal antibodies. Type, group, and interspecies specific epitopes were defined. Two group specific MAbs distinguished the enteric TGEV isolates from the respiratory variants. An antigenic subsite involved in neutralization was conserved in porcine, feline, and canine coronavirus. The classification of the human coronavirus 229E in a taxonomic cluster distinct from TGEV group is suggested. © 1990 Academic Press, Inc.

INTRODUCTION

Transmissible gastroenteritis virus (TGEV) belongs to one of the two major antigenic groups of coronaviruses (Siddell *et al.*, 1982; Sturman and Holmes, 1983; Spaan *et al.*, 1988), which includes feline infectious peritonitis virus (FIPV), feline enteric coronavirus (FECV), canine coronavirus (CCV), human coronavirus 229E (HCV 229E), and, recently, the porcine respiratory coronavirus (PRCV) (Pensaert *et al.*, 1986; Callebaut *et al.*, 1988).

The genome and protein composition of TGEV (Garwes and Pocock, 1975; Laude *et al.*, 1986; Jiménez *et al.*, 1986), FIPV (Horzinek *et al.*, 1982), FECV (Boyle *et al.*, 1984; Fiscus and Teramoto, 1987), and HCV 229E (Schmidt and Kenny, 1982; Kemp *et al.*, 1984; Screiber *et al.*, 1989) have been defined. Less information is available for CCV (Garwes and Reynolds, 1981; Horzinek *et al.*, 1982) and PRCV (Callebaut *et al.*, 1988). The virions contain a single-stranded, positive sense RNA molecule (molecular weight $6–8 \times 10^6$ Da) (Brian *et al.*, 1980; Lai, 1987). Between TGEV and FIPV a high degree of homology in the primary structure of the peplomer protein has been described (39% from amino acids 1 to 274 and 93% from residues 275 to 1447). Between TGEV and HCV 229E nucleocapsid genes little nucleotide sequence homology has been shown, although a region of 105 amino acids with 46% residue homology was found (Schreiber *et al.*, 1989).

Three major structural proteins: the peplomer (S, 180–200 kDa), the membrane (M, 21–30 kDa), and the nucleoprotein (N, 45–50 kDa) have been described for TGEV, PRCV, FIPV, and FECV (see above references). For HCV 229E two other proteins of approximately 39 and 16–18 kDa have been reported, although it is not clear if they are structural proteins (Schmidt and Kenny, 1982; Kemp *et al.*, 1984). In addition, the peplomer glycoprotein is processed in two proteins of 107 and 92 kDa, which were not described for the other coronaviruses of the TGEV group (Kemp *et al.*, 1984).

Although PRCV, FIPV, FECV, CCV, and HCV 229E have been classified in the TGEV group, the precise antigenic homology among these viruses has not been established, nor the origin of the new emerging strain PRCV. In addition, no antigenic homology with other coronaviruses has been found for porcine epidemic diarrhea virus (PEDV), which causes almost the same pathology as TGEV.

In the peplomer protein of TGEV four antigenic sites (A, B, C, and D) were defined, site A being the major inducer of neutralizing antibodies. This site was subdivided into three antigenic subsites (Aa, Ab, and Ac) by characterizing MAb resistant (*mar*) mutants (Correa *et al.*, 1988).

In this communication we describe the antigenic homology among 26 coronavirus strains and define type, group, and interspecies specific epitopes. It is described that an antigenic subsite (Correa *et al.*, 1988), which is a major inducer of neutralizing antibodies, is shared by TGEV, PRCV, CCV, FIPV, and FECV. In addition, the classification of HCV 229E in a taxonomic cluster distinct from that of TGEV is strongly suggested.

MATERIALS AND METHODS

Virus and cells

The characteristics of the viruses and the cells on which they were grown are summarized in Table 1.

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Monoclonal antibodies and sera

The characteristics of the MAbs have been described previously (Jiménez *et al.,* 1986; Correa *et al.,* 1988). The anti-TGEV antisera were collected in farms where TGEV was detected. The control anti-PRCV antisera were obtained in gnotobiotic pigs and were kindly provided by M. Pensaert (Gent, Belgium). The field sera from farms potentially infected with PRCV (that is, from controlled farms where no TGE was detected, but which were positive for TGEV or an antigenically related virus), were obtained from Segovia (Spain). The anti-HCV 229E antiserum was raised in guinea pig and kindly provided by J. Hierholzer (CDC, Atlanta, Georgia).

The antisera to TGEV and to HCV 229E were adsorbed twice with each cell line used to grow the viruses [swine testicle (ST) cells (McClurkin and Norman, 1966) or human embryo lung fibroblast (HELF) cells (Hierholzer, 1976)] by incubating the serum at 4° for 1 hr with 1 vol of packed cells. The serum was recovered after centrifuging first at low speed (1×10^3 rpm in a Sorvall GS3 rotor for 10 min) and then at 1×10^4 rpm in a Sorvall SS-34 rotor for 15 min. The serum used for the neutralizations was decomplemented.

Virus neutralization and purification, radioimmunoassay (RIA) for antibody binding, and competitive radioimmunoassay (cRIA)

The procedures for the neutralization, the RIA, and the cRIA, have been described previously (Correa *et al.,* 1988). Optimum amounts of the antigen (between 0.2 and 1.0 μg of protein per well) were used in the RIA and the cRIA. The antigen was purified (Correa *et al.,* 1988) or partially purified virus, which was obtained from supernatants of infected cultures, collected before cell lysis was observed, by clarification at 6×10^3 rpm (Sorvall GS3 rotor) for 20 min and sedimentation of the virus at 25×10^3 rpm (Sorvall AH627 rotor) for 1 h at 4°.

Antigenic homology

The percentage of antigenic homology of a particular virus isolate, relative to the reference virus PUR46-CC120-MAD, was estimated by the formula $[(a + 2b)/(2n)] \times 100$, 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). The MAbs with relative binding percentages between 0 and 30 were arbitrarily given no contributions to the antigenic homology, and they are not considered in the formula. The MAbs with relative binding percentages between 31 and 50, and 51 to 100, were given a contribution of 1 and 2, respectively, in the formula to estimate the antigenic homology.

RESULTS

The binding (Fig. 1) of 42 MAbs specific for the Purdue strain of TGE virus (PUR46-CC120-MAD) (Jiménez *et al.,* 1986; Correa *et al.,* 1988) to 26 strains of coronaviruses (Table 1) indicated that PRCV, FIPV, FECV, and CCV have conserved determinants in the three major structural proteins (S, M, and N). These viruses, isolated from three continents and collected, at least, throughout 42 years have conserved, in the peplomer protein, the antigenic subsite Ac, an inducer of TGEV neutralizing antibodies (Correa *et al.,* 1988). In contrast, the antigenic sites B, C, and D, which showed a high variability, particularly sites B and C, which are in general present in TGEV isolates, are completely absent in PRCV isolates and in the other coronaviruses analyzed (Fig. 1).

By neutralization (Table 2) five MAbs (three specific for subsite Ac, and the other two of an unknown subsite) neutralized TGEV, PRCV (not shown), FIPV, FECV, and CCV.

In contrast, none of the 42 MAbs bound to the other two porcine coronaviruses, PEDV and hemagglutinating encephalomyelitis virus (HEV), nor to the HCV 229E or the mouse hepatitis virus (MHV) (Fig. 1). In agreement with these results, none of the 42 MAbs, including those which recognized the epitopes conserved in TGEV, PRCV, FIPV, FECV, and CCV, neutralized the HCV-229E (Table 2).

On the basis of the percentage of antigenic homology of each particular virus isolate, relative to the reference virus (PUR46-CC120-MAD), (Fig. 1 and Materials and Methods), the coronaviruses studied (Fig. 1) could be classified into four different clusters, with homology percentages between 93 and 100%, 69 and 83%, 26 and 30%, and 0%, which include the TGEVs, the PRCVs, the canine and feline isolates, and the other viruses, respectively. While the members of the three first clusters are closely related to one another, an antigenic relationship among the members of the fourth cluster has not been described.

These results (Fig. 1 and Table 2) permitted us to define (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 coronavirus antigenically related to TGEV from other species (feline and canine)—e.g., MAbs 1B.B5, 1A.F10, 6A.C3, and 8B.E3.

As no TGEV-specific MAb recognized the HCV 229E, it was studied (Fig. 2) if unadsorbed or cell adsorbed (see Materials and Methods) polyvalent anti-

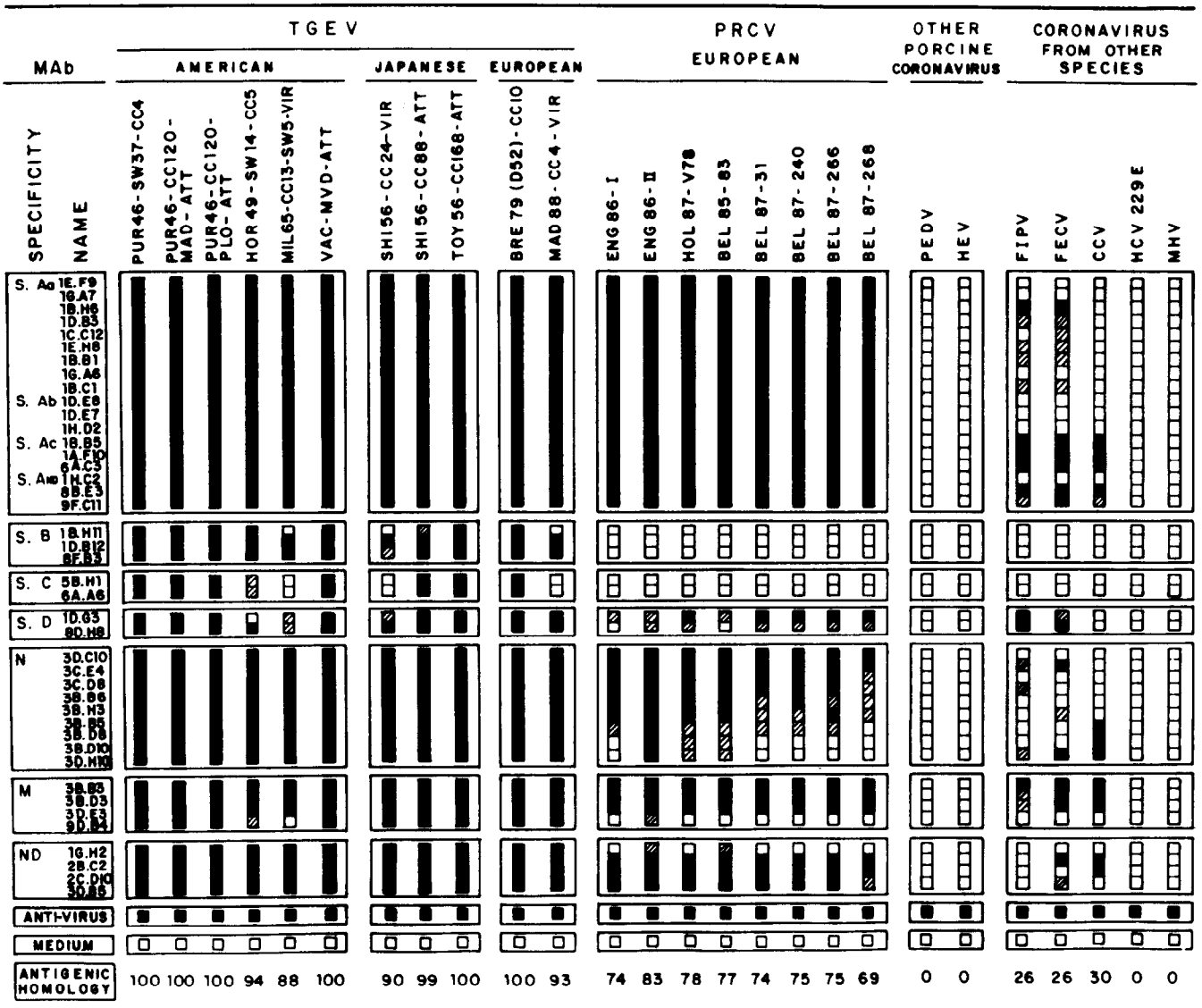


FIG. 1. Binding of MAbs to coronaviruses. The value of the MAb binding to the PUR46-CC120-MAD, determined by RIA, was taken as the reference value (100). The characteristics of the viruses used are summarized in Table 1. The specificity of the MAbs is named according to Correa *et al.* (1988). Symbols: □, 0 to 30; ▨, 31 to 50; ■, 51 to 100. The antigenic homology of each virus isolate relative to the reference virus PUR46-CC120-MAD (see Materials and Methods) was expressed in percentage. The anti-virus sera were TGEV specific in the case of TGEV, PRCV, FIPV, FECV, and CCV, and specific for the homologous virus, in the case of PEDV, HEV, HCV 229E, and MHV.

sera, specific for the structural proteins of each virus, bound to both virions. In addition, the recognition of the serologically unrelated MHV by these antisera was determined. The TGEV-specific antiserum strongly bound to the homologous virus but it did not recognize, or recognized weakly, the HCV 229E and the MHV, respectively (Figs. 2A, 2B, and 2C). The HCV 229E specific antiserum recognized with high and low titer the homologous and the murine virus, respectively, and, to a minor extent, the TGEV (Fig. 2D). By preadsorbing the serum with ST cells, or with these cells and the HELF cells (Figs. 2E and 2F, respectively), the recognition of TGEV was extensively diminished and the binding to the MHV decreased, while the homologous reactivity was conserved. In agreement with the binding studies, the unadsorbed or the preadsorbed antisera specific for TGEV and HCV 229E neutralized the homologous virus more than 10^3-fold, while the heterologous virus was neutralized less than 3-fold (results not shown).

As TGEV showed antigenic variability *in vivo,* it was determined if a virus variant could become predominant in the virus population by passage in cells in culture. Three clones of TGE virus (PUR46-CC120-MAD, PUR46-CC120-PLO, and SHI56-CC83) were passaged

TABLE 1

CORONAVIRUS

Designation[a]	Origin (Year of isolation)	Characteristics	Source (Reference)
TGEV			
PUR46-SW37-CC4	Purdue University (1946)	Purdue isolate passaged 37 times in pigs and 4 times in ST cells.	R. Wesley and R. Woods (Doyle and Hutchings, 1946; Haelterman, 1962; Lee *et al.*, 1954; Saif and Bohl, 1986).
PUR46-CC120-MAD	Purdue University (1946)	Purdue isolate passaged 115 times in ST cells at Dr. Bohl's Laboratory, and 5 times in our laboratory. Previously denominated PUR 46. C1.P1 (Bullido *et al.*, 1989). Attenuated.	E. H. Bohl (Bohl, 1972; Bohl and Kumagai, 1965; Bullido *et al.*, 1989).
PUR46-CC120-PLO	Purdue University (1946)	As PUR46-CC120-MAD but passaged at Dr. P. Vannier's laboratory. Attenuated.	P. Vannier (Bohl, 1972; Bohl and Kumagai, 1965; Bullido *et al.*, 1989).
HOR49-SW14-CC5	Minnesota (1949)	Received after being passaged 14 times in young pigs. Passaged 5 times on ST cells.	R. Wesley (Young *et al.*, 1955).
MIL65-CC13-SW5	USA (1965 or early)	ATCC VR-743. Passaged 13 times in PK cells and 5 times in gnotobiotic pigs. Virulent.	American Type Culture Collection (Bohl, 1972; Bohl and Kumagai, 1965).
VAC-MVP	USA (unknown)	Attenuated live vaccine.	Modern Veterinary Products, Inc.
SHI56-CC24	Japan (1956)	Received at passage 19 in swine kidney cells. Passaged 5 times on ST cells. Virulent.	H. Sazawa (Furuuchi *et al.*, 1976; Harada *et al.*, 1969).
SHI56-CC88	Japan (1956)	Received at passage 83 in swine kidney cells. Passaged 5 times on ST cells. Attenuated.	H. Sazawa (Furuuchi *et al.*, 1976; Jiménez *et al.*, 1986).
TOY56-CC168	Japan (1956)	Received at passage 163 in swine kidney cells. Passaged 5 times on ST cells. Attenuated.	H. Sazawa (Furuuchi *et al.*, 1976; Jiménez *et al.*, 1986).
BRF79(D52)-CC10	France (1979)	Passaged 10 times in ST cells.	P. Vannier (Aynaud *et al.*, 1984).
MAD88-CC4	Spain (1988)	Isolated in a farm after receiving pigs from Belgium. Passaged four times on ST cells. Virulent.	J. M. Escribano (Laviada *et al.*, 1988).
PRCV			
ENG86-I-CC5	England (1986)	Isolate PVC-135308, originally grown on primary pig kidney cells and passaged 5 times on ST cells.	I. H. Brown (Brown and Cartwright, 1986; Garwes *et al.*, 1988).
ENG86-II-CC5	England (1986)	Isolate PVC-137004, originally grown on primary pig kidney cells and passaged 5 times on ST cells.	I. H. Brown (Brown and Cartwright, 1986; Garwes *et al.*, 1988).
HOL87-V78-CC5	The Netherlands (1987)	Originally isolated on ST cells and passaged 5 times on this cell line.	M. B. Pensaert (Pensaert *et al.*, 1986, 1987)
BEL85-83-CC5	Belgium (1985)	idem	idem
BEL87-31-CC5	Belgium (1987)	idem	idem
BEL87-240-CC5	Belgium (1987)	idem	idem
BEL87-266-CC5	Belgium (1987)	idem	idem
BEL87-268-CC5	Belgium (1987)	idem	idem
Other porcine coronavirus			
PEDV (CV777)	Belgium (1987)	Causes 50% mortality up to the age of 7 days. Grown *in vivo*.	M. Pensaert (Pensaert and de Bouck, 1978).
HEV (67N)	Iowa (1970)	ATCC VR-741. Passaged 29 times on primary PEK cells.	American Type Culture Collection (Mengeling *et al.*, 1972).
Coronavirus from other species			
FIPV		ATCC VR-2004. Grown on CRFK cells.	American Type Culture Collection (Evermann *et al.*, 1981).
FECV		ATCC VR-989. Strain 79-1683. Grown on CRKF cells.	F. V. Scott (Pedersen *et al.*, 1984).
CCV		Strain S3786. Grown on A72 cells.	L. E. Carmichael (Cornell University)
HCV229E		ATCC VR-740. Grown on HELF cells.	J. C. Hierholzer (Hierholzer, 1976).
MHV		A59 strain. Grown on 3T3 cells.	K. Holmes (Sturman and Holmes, 1983).

[a] The first three letters of the virus nomenclature generally indicate the geographical origin or have been taken from its widely accepted name. The two first numbers indicate the year of isolation. The numbers after SW or CC indicate the number of passages in swine or in cells in culture, respectively.

TABLE 2

NEUTRALIZATION OF CORONAVIRUSES BY TGE VIRUS SPECIFIC MAb's

MAb		Neutralization index[b] for				
Specificity[a]	Name	TGEV	FIPV	FECV	CCV	HCV 229E
S. A.a	1E.F9	>4.0	0.1	0.3	0.6	0.0
	1G.A7	>4.0	0.0	0.3	0.5	0.0
	1B.H6	>4.0	0.2	1.0	0.4	0.0
	1D.B3	>4.0	0.4	0.7	0.4	0.0
	1C.C12	>4.0	0.3	0.5	0.4	0.0
	1E.H8	>4.0	0.0	0.5	0.5	0.0
	1B.B1	>4.0	0.1	0.3	0.6	0.0
	1G.A6	>4.0	0.0	0.2	0.5	0.0
	1B.C1	>4.0	0.0	0.3	0.5	0.0
S. A.b	1D.E8	>4.0	0.0	0.2	0.4	0.0
	1D.E7	>4.0	0.0	0.3	0.9	0.0
	1H.D2	>4.0	0.0	0.3	0.7	0.0
S. A.c	1B.B5	>4.0	0.7	2.2	2.5	0.0
	1A.F10	>4.0	3.5	>4.0	4.0	0.0
	6A.C3	>4.0	2.0	>4.0	3.5	0.0
S. A.ND	1H.C2	3.6	0.2	0.1	1.0	0.0
	8B.E3	>4.0	4.0	>4.0	4.0	0.0
	9F.C11	>4.0	1.0	>4.0	3.5	0.0
S. D	8D.H8	0.3	0.0	0.2	0.5	0.0
N	3B.D8	0.3	0.0	0.2	0.5	0.0
	3B.D10	0.3	0.2	0.2	0.7	0.0
	3D.H10	0.3	0.0	0.2	0.7	0.0
M	3B.B3	0.3	0.0	0.3	0.4	0.0
	3B.D3	0.3	0.0	0.3	0.5	0.0
	3D.E3	0.3	0.0	0.1	0.7	0.0
	9D.B4	0.3	0.2	0.4	0.4	0.0
ND	2B.C2	0.1	0.2	0.1	0.4	0.0
	2C.D10	0.2	0.2	0.1	0.7	0.0
MoLV	6D2	0.0	0.3	0.3	0.5	0.0

[a] The first letter refers to the protein (S, peplomer; M, membrane; and, N, nucleoprotein), the second to the antigenic site, and the small letters to the subsite (Correa *et al.*, 1988). MoLV, Moloney leukemia virus; ND, not determined.

[b] The neutralization index is the $\log_{10}$ of the ratio of the PFU after incubating the virus in the presence of medium or the indicate MAb.

30 times on ST cells (m.o.i. = 10^{-2}). The binding of the 42 MAbs listed in Fig. 1, to purified virus from passages 1 and 30, showed no antigenic change (results not shown).

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 (Fig. 1). Sera from animals infected with any of these two viruses inhibited the binding (Fig. 3A) of a ^{125}I-labeled MAb (6A.C3), directed to an interspecies specific epitope (Fig. 1), while only the sera from animals infected with TGEV inhibited the binding of the TGEV specific MAb 1D.B12 (Fig. 3A). When 93 field sera samples were studied, all were positive for PRCV virus in the RIA (titers between 3×10^2 and 10^4) and by neutralization (decreased $10^{2.8}$ to $>10^5$-fold virus infectivity), and also were positive against TGEV, both by RIA (titers between 3×10^2 and 9×10^3) and by neutralization (decreased $10^{3.3}$- to $>10^6$-fold virus infectivity) (results not shown). In contrast, only the field sera from TGEV positive farms inhibited both the site-A and -B specific MAbs (Fig. 3B), confirming that MAb 1D.B12 could be used to distinguish TGEV and PRCV isolates.

DISCUSSION

The antigenic homology among 26 strains of coronavirus 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 specific epitopes (Fig. 1 and Table 2), provided MAbs which differentiate among members of the TGEV antigenic types, particularly TGEV and PRCV isolates, and revealed that the exclusion of the HCV 229E from this taxonomic cluster should be considered.

The antigenic subsite S.Ac is interspecies specific, as it is conserved in porcine, feline, and canine coronaviruses (Fig. 1 and Table 2). This subsite is defined

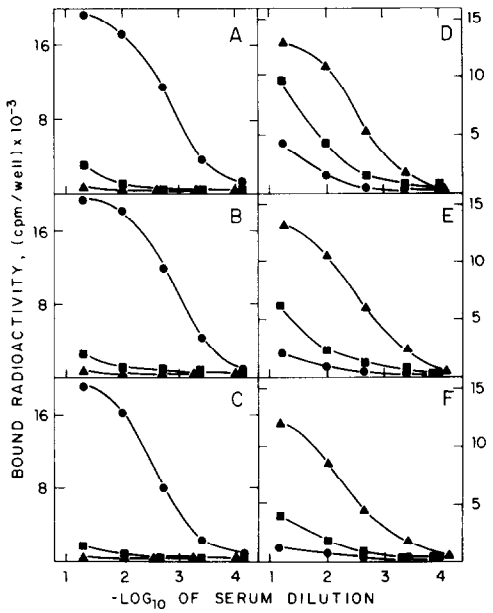
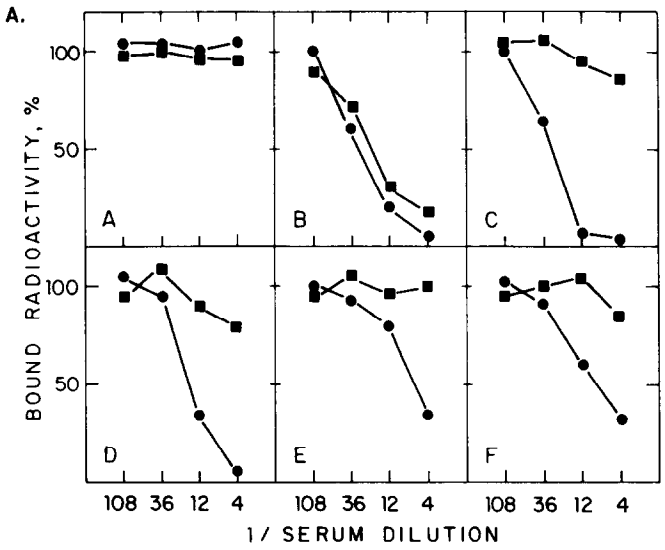


FIG. 2. Binding of antisera to TGEV and HCV 229E, to coronaviruses. The binding of antisera to TGEV and HCV 229E unadsorbed (A and D, respectively), or adsorbed with HELF cells (B) or ST cells (E), respectively, or with both cell lines (C and F, respectively), to purified TGEV (●), HCV 229E (▲), and MHV (■) was determined by RIA.



B.

SERA ORIGIN	NUMBER of SAMPLES	BINDING INHIBITION ANTIGENIC SITE	PERCENTAGE 25 50 75 100
NEGATIVE	2	A B	
ANTI-TGEV	5	A B	
ANTI-PRCV	3	A B	
FIELD SERA FROM TGEV POSITIVE FARMS	6	A B	
FIELD SERA FROM FARMS POTENTIALLY INFECTED WITH PRCV	87	A B	

FIG. 3. Binding inhibition of site A and B specific MAbs to TGEV by homologous antiserum or serum against the PRCV. Panel A shows the inhibition of the binding of ^{125}I-labeled MAbs 6A.C3 (Site A) (●) and 1D.B12 (Site B) (■) to TGEV by serum from nonimmune animals (A), serum against TGEV (B), PRCV (C), or field serum of animals infected with PRCV from three farms (D, E, and F, respectively), determined by cRIA. B shows the binding inhibition of site A and B specific MAbs by field sera from farms where TGE was detected or undetected (potentially infected with PRCV). The bracketed lines represent the standard deviation. The field sera from farms potentially infected with PRCV were TGEV positive sera, collected in well-controlled farms where no TGE was detected.

by three MAbs, of which MAb 6A.C3 did not permit the isolation of MAb resistant (*mar*) mutants (Jiménez *et al.*, 1986). Anti-idiotypic antibodies of the β-type (i.e., internal image), specific for this MAb, could induce protection against coronaviruses of the three species.

The three structural proteins of different isolates of TGEV and PRCV are highly conserved (Fig. 1), indicating that PRCV, which was detected for the first time in 1984 (Pensaert *et al.*, 1986), could be derived from TGEV. Nevertheless, PRCV showed major antigenic differences in sites B and C of S peplomer protein (Fig. 1) and could derive by recombination between TGEV 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 favored, versus the accumulation of point mutations, as two antigenic sites are different among these viral strains and mechanisms of genetic recombination have been demonstrated in coronavirus (Makino *et al.*, 1986). The most likely candidates among the coronaviruses, which could provide the new sites B and C to the respiratory variants of TGEV, should infect the same cells as TGEV, i.e., PEDV, CCV, and FIPV (Woods *et al.*, 1981). The production of PRCV variants by dele-

tion in sites B, C, and D could also be the generation mechanism of the PRCV variants.

TGEV showed *in vivo* (Fig. 1) antigenic heterogeneity in sites B, C, and D, while antigenic site A was highly conserved. The heterogeneity observed *in vivo* was not detected by virus passage in established cell lines (ST), which did not derive to a variant virus which would have overcome the original phenotype, as described for other porcine viruses (García–Barreno *et al.,* 1986). The PRCV apparently emerged in 1984 and has quickly spread to all European countries (Pensaert *et al.,* 1986). This virus showed heterogeneity in the antigenic site D of S protein, and on the N protein (Fig. 1), in contrast to the TGEV isolates, which have conserved the N protein. This could be explained if, as expected, PRCV are young viruses, not fully adapted to their ecological niche (Steinhauer and Holland, 1987). As previous infection with these viruses provide some protection against TGEV (Hooyberghs *et al.,* 1988), the prediction could be that the presence of TGEV will decrease in these countries, while other transmissible gastroenteric coronaviruses serologically unrelated to TGEV, as PEDV (Fig. 1), will be prevalent in the areas where PRCV is present.

An observation with potential interest was that all virulent TGEV studied (strains MIL65-CC13-SW5, SHI56-CC24, and MAD88-CC4) had in common a similar antigenic pattern of reactivity with site-B and -C specific MAbs (Fig. 1) not presented by the attenuated viruses. No antigenic correlation was established between the antigenic pattern and the geographical location or date of isolation.

HCV 229E has been previously included in the TGEV group (Macnaughton, 1981), based on the weak cross-reactivity of antisera against TGEV and HCV 229E with these viruses, detected by immunofluorescence using a dilution of 1 in 10 of polyvalent antiserum (Pedersen *et al.,* 1978). In contrast, none of the 42 MAbs tested (Fig. 1 and Table 2), some of which showed an antigenic relationship between porcine, feline, and canine coronaviruses, either bound or neutralized HCV 229E. Furthermore, the TGEV-specific polyvalent antisera did not recognize the HCV 229E. Although the anti-HCV 229E showed some reactivity against TGEV, this binding was practically eliminated by adsorbing the antiserum with cells of porcine and of human origin, suggesting that the observed cross-reactivity was caused by the recognition of carbohydrates or cellular material contaminating the viral preparations. In fact, the anti-HCV 229E antiserum showed a nonspecific binding with the murine coronavirus MHV (Figs. 2D, 2E, and 2F), that is antigenically unrelated to HCV 229E (Siddell *et al.,* 1982; Spaan *et al.,* 1988), which was higher than the one observed with the TGEV. Reynolds *et al.* (1980) also reported that antisera to HCV 229E failed to neutralize CCV and TGEV, even when they were screened at a dilution of 1 in 2, and Scott (1987) reported that infection of cats with HCV 229E resulted in homologous neutralizing antibody responses, but neutralizing antibodies to TGEV, FIPV, or CCV were not detected. There are two other issues that differentiate the TGEV group viruses, from HCV 229E. First, while TGEV-related isolates have three major structural proteins, HCV 229E may have two other proteins of 39 and 16–18 kDa (Kemp *et al.,* 1984). Second, while the peplomer protein is not processed in TGEV related coronaviruses, the corresponding protein of the HCV 229E is processed in certain cell lines (Schmidt and Kenny, 1982; Kemp *et al.,* 1984). To summarize, although a minor antigenic relationship among HCV 229E and the TGEV related strains, based on some homology detected by genome sequencing (Schreiber *et al.,* 1989), cannot be excluded, HCV 229E does not make a uniform antigenic cluster with TGEV, FIPV, FECV, and CCV (Fig. 1), and we strongly suggest considering the exclusion of the human virus from this taxonomic group.

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