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Use of substituted and tandem-repeated peptides to probe the relevance of the highly conserved RGD tripeptide in the immune response against foot-and-mouth disease virus

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Antigenic site A of foot-and-mouth disease virus (FMDV) is an exposed, mobile loop which includes a central, highly conserved Arg-Gly-Asp tripeptide (RGD, VP1 residues 141–143 in serotype C) thought to be part of the cell attachment site. We have analyzed the contribution of RGD to the interaction of site A with antibodies by incorporating selected amino acid replacements at RGD into synthetic peptides representing site A, and analyzing the reactivity of substituted peptides with site A-specific monoclonal antibodies (MAbs). Replacement of Arg-141, Gly-142 or Asp-143 by alanine resulted in the loss of one, three and five epitopes, respectively, out of seven epitopes probed. Other replacements resulted in the loss of even larger numbers of epitopes, suggesting that the amino acids of the RGD region are either directly involved in interaction with antibodies or that they exert an important influence on the interaction of surrounding residues with antibodies. Thus, we explored the ability of tandem repeats of the RGDL sequence (corresponding to FMDV C-S8c1) to evoke neutralizing antibodies in rabbits and guinea pigs. Neutralizing activity was generally low but with a broad specificity for different FMDV serotypes and variants. Significant decreases in neutralizing titers were observed with boosting, suggesting a possible suppression of those anti-peptide antibodies which may also be directed to cellular RGD sequences. The results point to an involvement of RGD in the antigenic structure of site A, and open the possibility that broadly neutralizing antibodies might be induced by tandem repeats of the critical, conserved domain.

Immunopeptide: RGD: Cross-neutralization: Antigenic variability

1. INTRODUCTION

Foot-and-mouth disease virus (FMDV), responsible for the economically most important viral disease of cattle and other cloven-hooved animals [1–3], shows extreme genetic variability and antigenic diversity, as do most other RNA viruses [3–7]. Analysis of the reactivity of monoclonal antibodies (MAbs) with FMDV has shown that a few capsid regions, which include multiple overlapping epitopes, are involved in virus neutralization [3,8–11]. The main antigenic site of FMDV (site A) is a flexible loop on protein VP1 exposed on the virus surface [12,13]. B-cell epitopes located on this site A (VP1 residues 138–150 in serotype C, isolate C-S8c1) map at residues surrounding a conserved RGD tripeptide (residues 141–143) [3,10] thought to be a cell-attachment site [14]. Serotypes C, most type O, and some type A viruses also show a conserved Leu at position 144 (Table I).

Frequent amino acid substitutions occur within antigenic site A of field FMDV variants and of laboratory mutants, including antibody-escape mutants. The effect of observed amino acid replacements within site A on the reactivity of FMDV C-S8C1 with MAbs has been faithfully reproduced by synthetic peptides which included the relevant substitutions [15]. The extremely variable sequences at positions 138–140 and 146–150 seem to attract immune responses which might be detoured from RGDL [3,7]. The practical absence of mutants in this RGDL region is a significant hurdle to the assessment of its contribution to the overall immunogenicity and antigenicity of site A. We have approached this problem by evaluating the effect of replacements at each of the three positions of the RGD tripeptide on the interaction of synthetic peptides with MAbs. We have also evaluated the immune response of rabbits and guinea pigs to several synthetic peptides composed of three RGDL tandem repeats. Our results show that the RGD tripeptide is an important determinant of the interaction of antigenic site A with antibodies, and that

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Abbreviations: EID, enzyme-linked immunodot assay; EITB, enzyme-linked immunoelectrotransfer blot assay; FMDV, foot-and-mouth disease virus; TFA, trifluoroacetic acid, MBS, 3-maleimidobenzoyl-*N*-hydroxysuccinimide ester; SPDP, *N*-succinimidyl-3-(2-pyridyldithio)-propionate.

a significant anti-peptide and FMDV-neutralizing response can be obtained with peptides based on RGD. However, a potent neutralizing anti-viral response requires the highly variable residues surrounding the RGD. Appropriate combinations of peptides representing most residues of site A, along with tandem repeats of the conserved residues around the RGD core, may be useful in the formulation of anti-FMDV synthetic vaccines with broader antigenic spectra than formulations assayed so far [16–18].

2. MATERIALS AND METHODS

2.1. *Cells and viruses*

Procedures for cell culture were as previously described [19]. Viral antigens were prepared by FMDV infection of BHK-c2 cell monolayers, and recovered virus was partially purified by sedimentation through a sucrose cushion [20]. FMDV from three serotypes were utilized: C-S8c1 {C1 Santa Pau; Spain, 1970, wild type and its MAb SD6-resistant mutant HR [11]}, A_5-Westerwald and O_1-Kaufbeuren

2.2. *Synthetic peptides*

The peptides used in this study ware shown in Table II. They were synthesized as C-terminal carboxamides by solid-phase procedures [21,22], as recently described [15]. A Cys residue was added to the C-terminus of all sequences to allow conjugation to carriers. After HF cleavage and deprotection, purification was done by reversed-phase liquid chromatography on C_{18}-silica using linear acetonitrile-into-water gradients (both solvents containing 0.1% trifluoroacetic acid). Fractions of satisfactory (> 95%) purity by HPLC were pooled and characterized by amino acid analysis (6 N HCl, 110°C, 24 h) and electrospray mass spectrometry. Peptides were coupled to keyhole limpet hemocyanin (KLH) through MBS [23] for immunizations or to BSA through SPDP [24] for immunological assays.

2.3. *Immunological procedures*

Two rabbits (New Zealand or Blanco Buscat) were immunized with 400 μg of RGDL peptides coupled to KLH in complete Freund adjuvant. Boosting was carried out with the same amount of peptide in incomplete Freund adjuvant three times every three weeks, except for rabbits receiving $RGDL_3$ and $RGDL_3X$, which were boosted only twice. Animals were bled before the first inoculation and 10 days after each inoculation, and the sera obtained were kept at −70°C until use. The same protocol was followed with two to six Dunkin Hartley guinea pigs, except that peptide A24 was included as a control, and the amount of peptide inoculated was 200 μg of $RGDL_3AA$ and the equivalent molar amounts of the other peptides. Sera were labeled s_n, n being the number of inoculations received before bleeding. Immunoglobulins of s_1 and s_3 samples (2 ml) were concentrated with 50% ammonium sulfate, resuspended in 0.5 ml PBS and loaded on a Sephadex G-200 column (75 × 1.6 cm). Proteins were eluted with PBS (20 ml/h) and 3 ml-fractions were collected and subjected to PAGE to determine those corresponding to IgG and IgM. Samples were pooled, concentrated with 50% ammonium sulfate, resuspended in 0.5 ml PBS and dialyzed against PBS.

Table I

Sequence alignment of residues 136–151 of FMDV isolates

Virus	FMDV loop[a]
C-S8c1	^{136}YTASA....**RGD**LAHLTTTH^{151}
HR	YTASA....**RGD**LARLTTTH
O1-K	YNRNAVPNL**RGD**LQVLAQKV
A5-W	YSTGGP..R**RGD**MGSAAARA

[a] Dots represent amino acid deletions needed to align the sequences keeping the conserved Y(136) and the RGD motif at equivalent positions.

Table II

Synthetic peptides[a] and their carrier protein conjugates

Peptide	Amino acid sequence[b]	MW[c]	Peptide-carrier protein ratio[d]	
			KLH	BSA
RGD(A19)	ASA**RGDL**AHLTTTHARHLP	2023.7	–	7
AGD	ASA**AGDL**AHLTTTHARHLP	1938.6	–	–
KGD	ASA**KGDL**AHLTTTHARHLP	1995.7	–	–
SGD	ASA**SGDL**AHLTTTHARHLP	1954.6	–	–
RAD	ASA**RADL**AHLTTTHARHLP	2037.7	–	–
RED	ASA**REDL**AHLTTTHARHLP	2081.7	–	–
RGA	ASA**RGAL**AHLTTTHARHLP	1979.7	–	–
RGK	ASA**RGKL**AHLTTTHARHLP	2036.8	–	–
RGE	ASA**RGEL**AHLTTTHARHLP	2037.7	–	–
HR	YTASA**RGDL**ARLTTTHARHLP	2307.0	–	–
A24	TTTYTASA**RGDL**AHLTTTHARHLP.C	2768.0	740	–
$RGDL_3AXA$	C.A**RGDL**AXA**RGDL**AXA**RGDL**A	2099.2	2775	18
$RGDL_3AA$	CVA**RGDL**A.A**RGDL**A.A**RGDL**A	1971.7	2413	15
$RGDL_3A$	C..**RGDL**A..**RGDL**A..**RGDL**	1588.2	3003	15
$RGDL_3X$	C..**RGDL**X..**RGDL**X..**RGDL**	1672.6	1130	15
$RGDL_3$	C **RGDL**...**RGDL**...**RGDL**	1446.0	3504	16

MW values of 5×10^6 and 67,000 for KLH and BSA, respectively, were used in the calculations

[a] X=ε aminohexanoic acid.

[b] Some peptides carry an additional Cys residue at the N- or C-terminus to allow specific conjugation to the carrier. Underlined residues indicate replacements in the original sequence of site A.

[c] Calculated molecular weight in Da. For all peptides, mass spectra gave the expected MW values (± Da).

[d] Determined by amino acid analysis of conjugates after extensive dialysis.

2.4. *Immunological and neutralization assays*

Enzyme-linked immunodot (EID) and enzyme-linked immunoelectrotransfer blot (EITB) protocols were as described [19], except that MAbs were replaced by sera at various dilutions, and goat anti-mouse IgG-peroxidase by goat anti-rabbit IgG (H+L)-horseradish peroxidase (Bio-Rad, 1:2,500 dilution, 1 h, r.t.) or goat-anti-guinea pig (H+L)-horseradish peroxidase (Nordic, 1:1,000 dilution, 1 h, r.t.). For the antigen competition assays with peptides substituted at RGD, ELISA plates were coated with 5 pmol of peptide A19 (Table II) coupled to KLH, incubated overnight at 4°C and saturated with 5% BSA. Mixtures of a fixed non-saturating amount of MAb and variable amounts (1.5, 5, 15, 45, 135, 405 and 1215 pmol) of competitor peptides (in 0.5% BSA in PBS) were preincubated for 2 h at r.t., added to the ELISA plates and further incubated for 1 h at r.t. After washing with 0.05% Tween 20, 0.1% BSA in PBS, peroxidase-conjugated goat anti-mouse IgG (Bio-Rad, 1:3,000 dilution) was added and incubated for 1 h at r.t. The plates were thoroughly washed with 0.05% Tween 20, 0.1% BSA in PBS, and the reaction developed using *o*-phenylenediamine as a substrate. Absorbance was read at 492 nm.

Neutralization of infectivity was performed on BHK-c2 cell monolayers after incubation of sera or fractionated Ig dilutions with virus as described [19], except that neutralization values are expressed as plaque reduction percentage (PRP).

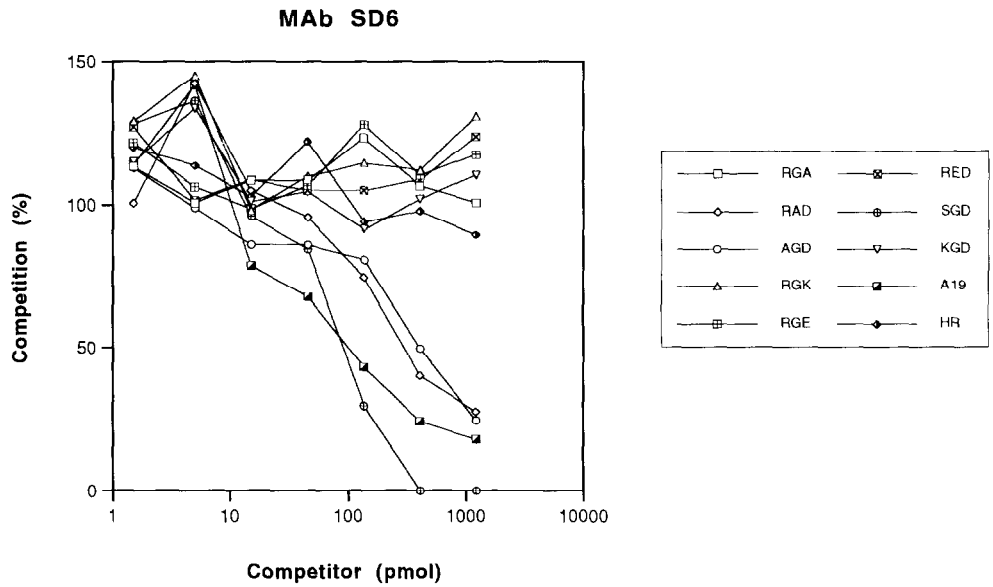

Fig. 1. Antigen-competition assay of monoclonal antibody SD6 and selected peptides.

3. RESULTS

3.1. *Reactivity of antibodies with FMDV peptides substituted at the RGD domain*

To explore the possible contribution of the RGD tripeptide to the interaction of antigenic site A with antibodies we replaced each of the three residues by Ala in synthetic peptides comprising VP1 residues 138 to 156 of VP1 of FMDV C-S8c1 (Table II). We chose to study the binding to substituted peptides of seven MAbs that recognize different continuous epitopes located within site A [10,11,19,20]. An antigen competition ELISA was used, in which KLH-conjugated peptide A19 was attached to plates and the binding to MAbs was quantitated in the presence of increasing concentrations of the free peptide to be tested. The results obtained with one of the MAbs (SD6) are shown in Fig. 1. The complete results (Table III) show that tolerance to replacement by alanine was greatest for Arg-141, resulting in the loss of only the epitope defined by MAb 6D11. In contrast, Asp-143 was the least tolerant to Ala replacement, with only two of the probed epitopes remaining positive. To test whether amino acids other than Ala, in particular those leading to gross side chain and/or charge alteration and that could occur as a result of a point mutation of the C-S8c1 nucleotide sequence, resulted in comparable or different antigenic modification, several additional substituted peptides were tested in the same competition ELISA (Fig. 1 and Table III). Any of the replacements resulted in a larger alteration of MAb recognition than that caused by Ala. In particular, the presence of Glu at position 142 led to loss of all epitopes probed, an alteration as drastic as that induced by the His-146→Arg replacement [11,25]. Thus, each of the amino acids in the RGD triplet has an important contribution to the recognition of site A by antibodies.

Table III

Antigen-competition ELISA with RGD-substituted peptides

	Monoclonal antibody						
Peptide	SD6	4C4	6D11	7FC12	7AH1	7JD1	7CA11
RGD(A19)	+	+	+	+	+	+	+
AGD	+	+	−	+	+	+	+
RAD	+	+	+	−	−	−	+
RGA	−	−	−	−	−	+	+
SGD	+	−	−	−	−	−	+
KGD	−	−	−	−	−	±	+
RED	−	−	−	−	−	−	−
RGK	−	−	−	−	−	+	+
RGE	−	−	−	−	−	+	+
HR	−	−	−	−	−	−	−

Amino acid sequences of the peptides are shown in Table II. Competition ELISA was performed as described in section 2. + indicates that a 20-fold molar excess of the competing antigen over plate-bound peptide A19 caused an inhibition in Mab binding greater than 30%; − indicates that such inhibition was lower than 30%; ± indicates inhibition close to 30%.

3.2. *Immunogenicity of peptides containing tandem repeats of the RGDL sequence*

Since the RGD sequence is involved in the recognition of antigenic site A by antibodies, we considered the possibility that synthetic peptides based on such sequence could induce neutralizing antibodies which might define a broader antigenic spectrum than peptides involving variable residues around RGD. We chose to build synthetic peptides made of tandem repeats of the basic unit RGDL because Leu-144 is strictly conserved in all type C viruses sequenced to date and

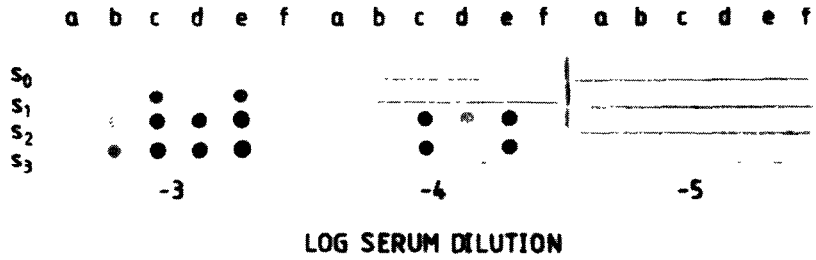

Fig. 2. Reactivity of synthetic peptide ($RGDL_3X$) including the RGD sequence with sera raised against homologous peptide (rabbit 742). This result is representative for both rabbits and guinea-pigs, except that the latter showed lower anti-KLH responses. s_n are sera extracted 10 days after immunization number n (see section 2). Antigens applied to nitrocellulose were a and f, PBS; b, KLH (4.4×10^{-3} pmol, amount equivalent to that in 5 pmol of the corresponding conjugate); c, KLH (4.4×10^{-2} pmol); d, peptide $RGDL_3X$ (5 pmol); e, peptide $RGDL_3X$ (50 pmol). Sera dilutions are indicated

in nearly all type O isolates [7]. Table I shows the amino acid sequence alignment of the FMDV loop of representatives of FMDV serotypes C, O, and A. The RGDL repeats were separated by different spacer residues as shown in Table II. Anti-peptide antibody titers in rabbits and guinea pigs were generally high. A representative EID assay is shown in Fig. 2. All sera reacted with heterologous RGDL-containing antigens, though to a much lesser extent than with the homologous peptide (not shown), suggesting a low proportion of antibodies with RGDL as the sole target. As expected, the first boost improved anti-peptide antibody levels, though further inoculations had no effect. Some sera reacted with entire FMDV C-S8c1 and/or its HR mutant, but with other sera this reaction was not observed. EITB did not show which was the viral protein recognized by anti-peptide antibodies, even when highly concentrated sera (1 : 4 dilution) were used, again suggesting that RGDL was too short a target for efficient binding to antibodies.

3.3. *FMDV neutralization by anti-peptide sera*

Anti-peptide sera were tested for their ability to neutralize C-S8c1 FMDV (Table IV). Values showed considerable variation depending on the peptide antigen and the individual animal inoculated. Even the highest neutralization values (PRP = 74 for guinea pig 1 at 1:5 dilution and 78 for rabbit 747 at 1:20 dilution) were two to three orders of magnitude lower than those attained with anti-peptide A24 sera. In spite of the mentioned variations, neutralization values for rabbits and guinea pigs were of the same order. Dilutions higher than 1:20 rendered undetectable levels of specific neutralization. No correlation was found between viral particle recognition by EID and neutralizing ability. In most animals, neutralizing values dropped significantly with the number of boosts. To study the underlying phenomenon, IgGs and IgMs corresponding to s_1 and s_3 of rabbit 740 (s_1PRP = 70, s_3PRP = 20) were fractioned and assayed for their neutralization ability. Only the IgM fraction of s_1 was found to neutralize C-S8c1 to a significant extent (PRP = 58%).

Neutralizing activity elicited in rabbits was higher for peptides including more amino acids of the consensus C-S8c1 sequence. Guinea pig sera gave better results when shorter peptides were used as immunogens. Another difference, which might be significant, between the two animal systems was the effect of including ε-aminohexanoic acid as a spacer. Comparison of results obtained with the $RGDL_3$/$RGDL_3X$ and $RGDL_3AA$/$RGDL_3AXA$ pairs is illustrative: in rabbits, no differences were found among sera against peptides with or without ε-aminohexanoic acid, while in guinea pigs neutralizing activity was completely lost when this amino acid was present as spacer in the immunogen (Table IV).

In addition to C-S8c1, two representatives of other serotypes (FMDV O_1-Kaufbeuren and A_5-Westerwald) were also tested for cross-neutralization by the anti-peptide sera. Again, low but significant neutralization was noted with several of the tested sera. It must be emphasized that peptide A24, which includes the RGDL sequence surrounded by serotype-specific C sequences, did not induce any detectable neutralizing activity against O_1K or A_5W (Table IV). It is also noteworthy that mutant HR, resulting from the His-146→Arg substitution in VP1, and which led to the loss of all tested site A epitopes involved in neutralization of infectivity [10,25], was neutralized at least as efficiently as its wild-type counterpart C-S8c1 (Table IV). These results point to a relaxation of the specificity of the immune response against FMDV when sequences responsible for serotype specificity are excluded from the peptide immunogens. However, encephalomyocarditis virus (EMCV), the phylogenetically closest relative to aphthoviruses, was not neutralized to any extent by each serum tested (Table IV).

We therefore conclude that the highly conserved RGD tripeptide located within antigenic site A is an important determinant of the interaction of site A with neutralizing antibodies. Peptides based on tandem repeats of the RGDL tetrapeptide were capable of inducing neutralizing antibodies with highly relaxed serotype and variant specificity. Although the neutralization levels were low, the observations reported in this paper

Table IV
Neutralizing ability of anti-RGDL peptide sera

Peptide	Animal	Virus[a]				
		C-S8c1	HR	O_1K	A_5W	EMCV
	A. Guinea pigs					
$RGDL_3$	1	74.3	45.0	20.6	32 4	ND[b]
	2	47.5	97.6	33 9	38.3	ND
	3	70.5	66.1	83.8	24 1	0
	4	72.5	92.7	56.3	8.9	ND
	5	43.8	80.9	8.9	0	0
	6	29.2	47.5	0	0	ND
	Mean	*56 3*	*71.6*	*33 9*	*17.3*	
$RGDL_3A$	7	70.5	88.0	8.9	42.4	ND
	8	62.0	59.3	32.4	0	0
	9	48.7	95.2	4.5	0	ND
	10	35.4	90.4	6.7	65.3	ND
	11	30.8	95.2	0	4.5	ND
	12	36.9	90.4	0	18 7	ND
	Mean	*47 4*	*86.4*	*8.6*	*21.8*	
$RGDL_3AA$	13	22.4	76.0	8.9	56.3	ND
	14	20.6	73.7	10.9	69 1	ND
	15	39.7	95.2	22.4	65 3	ND
	16	56.3	80.9	2.3	30.8	ND
	17	0	88.0	0	16.8	ND
	18	10.9	20.6	0	0	< 2.5
	Mean	*25 0*	*72.4*	*7 4*	*39 7*	
$RGDL_3X$	19	0	4.5	0	2.3	ND
	20	8.9	27.5	25 9	12.9	ND
$RGDL_3AXA$	21	0	0	25.9	0	ND
	22	0	42.4	16.8	0	ND
A24	23	100	100	0	0	ND
	23[c]	66.1	33.9	0	0	ND
	24	100	100	0	0	ND
	24[c]	55.3	76.6	0	0	ND
	B. Rabbits					
$RGDL_3$	744	16.8	27.5	9.0	0	ND
	745	47.5	73 7	76.0	0	ND
$RGDL_3A$	732	51.0	84.5	0	4 5	0
	738	80.9	100	0	4.5	ND
$RGDL_3AA$	746	93.4	95.1	0	0	ND
	747	89.3	96.6	0	0	ND
$RGDL_3X$	742	20.6	38.3	0	6.7	ND
	743	30.8	66.1	14.9	0	ND
$RGDL_3AXA$	739	77.1	97.5	14.9	6.7	ND
	740	62.8	77.6	6.7	6.7	ND

[a]Values are given as plaque reduction percentage (PRP) of s_3 diluted 1:5 and 1:4 for rabbits and guinea pigs, respectively.
[b]ND = not determined.
[c]PRPs comparable to those obtained for 1:5 dilution, which corresponded to a 1:6250 dilution.

open the way to engineer synthetic constructs capable of inducing broader antiviral immune responses than peptides based on the authentic amino acid sequences of natural isolates.

4. DISCUSSION

Previous studies have shown that neutralizing anti-FMDV activity can be induced by peptides corresponding to the 140–160 VP1 loop [16–18], but the possible implication of the conserved RGD(L) residues in recognition of antigenic site A by neutralizing antibodies has not been established, except for one reported case of G→E substitution in serotype A which conferred resistance to one neutralizing MAb [8]. The implication of RGD in antibody binding has been investigated by minimally disrupting the peptide sequence, and presumably the peptide chain conformation, as a result of single substitutions by Ala [26]. Severeal epitopes were lost, most of them as a result of a replacement of Asp-143 (Table III). Other more drastic replacements involving charge alterations resulted in the loss of additional epitopes (Table III). Our previous observations [10,15] have firmly establised that synthetic peptides representing antigenic site A of FMDV C-S8c1 faithfully mimic the behaviour of entire virus particles in their interaction with antibodies. It may be significant that the epitopes defined by MAbs 7CA11 and 7JD1, which were mapped to the carboxy-side of RGD by analysis of escape mutants and binding to peptides [11], were the least affected by replacements at the RGD tripeptide (Table III). Each of the other MAbs tested mapped at both sides of RGD, suggesting that this tripeptide is either directly involved in interactions with the MAbs or participates in delineating the local conformation(s) recognized by MAbs. The effect of Ala replacements would seem to favor the first interpretation.

The involvement of RGD in antibody binding raised the possibility that such a sequence could become the target of an induced immune response with the added advantage that the conservation of RGD could circumvent the serious problem of antigenic variation of FMDV [3,6,19,20,25]. Perhaps not unexpectedly, these predictions have met with only partial success. Antibodies raised against tandem repeats of the RGDL tetrapeptide bound effectively homologous peptides and to a lesser extent the heterologous peptides. However, their ability to neutralize virus was limited (Table IV). It should be noted that the RGD sequence is the binding site of the integrin superfamily [27], which includes a variety of widely distributed proteins involved in cell adhesion. Although the residues surrounding RGD are important for recognition, peptides containing or mimicking the RGD motif can inhibit platelet aggregation [28,29], so that it cannot be ruled out that the immune system could limit the anti-RGD antibody production for self tolerance. The structure of the VP1 G-H loop of FMDV C-S8c1, where antigenic site A is located, is not known. However, the RGD in a reduced form of FMDV O_1BFS has been recently elucidated [13]. In this chemically modified virus the RGD tripeptide acquires a conformation very similar to γ-11-cristallin and probably also to other integrin-binding proteins. Thus, it is not unlikely that the spatial arrangement adopted by RGD on the C-S8c1 virion surface

might show some similarity to integrin-binding sites in several proteins. Indeed, our finding that neutralizing activity is essentially confined to IgMs strongly suggests that auto-antibodies (normally belonging to the M isotype [30]) are produced at an early stage of the immune response and later suppressed, thus preventing the switch to IgGs from lymphocyte clones recognizing both self antigens and FMDV virions. Dilution of the incipient IgM populations may be assumed to take place by selection and amplification of clones recognizing other RGD conformations present in our peptides. This would explain the fact that peptide recognition improvements were observed concomitant to decrease or even complete loss of neutralizing ability.

Other reasons could also contribute to the low neutralizing responses. First, tandem repeats include epitopes not present in the virus (e.g. LAXAR for $RGDL_3AXA$, etc.), which could induce anti-peptide but not anti-virus response. On the other hand, the RGDL tandem repeat peptides could adopt multiple conformations capable of inducing antibodies not recognizing the native conformation on the virus surface. Furthermore, the rather short length of the 4–6 residue target could provide insufficient anchoring points for antibodies, which would clearly limit the immunogenicity and antigenicity of the sequences despite their presentation as tandem repeats. The relatively poor results observed for ε-aminohexanoic acid-containing repeats could be attributed to the enhanced structural mobility conferred by this residue [31], resulting in the induction of antibodies against neutral or unfavourable conformations.

Of particular interest are the results of mutant HR neutralization. This variant [11,25] is resistant to MAbs directed to site A and it is neutralized less efficiently than its parental C-S8c1 by polyclonal sera. However, our data suggest that the His-146→Arg replacement does not significantly affect the RGDL conformation, as shown by the fact that peptides that elicited specific neutralizing activity were cros-reactive.

The danger of inducing FMDV outbreaks due to handling of live virus or improper inactivation of antigens [32] has stimulated the search for vaccines based on synthetic peptides [33,34]. Peptides derived from site A have been shown to elicit a protective response against FMDV in guinea pigs and cattle [16–18] and in some cases some evidence of cross-protection has been presented [35]. However, the use of these peptides may not necessarily provide coverage against many co-circulating variants. This problem might be eventually attenuated with anti-FMDV vaccine formulations that include peptides reproducing conserved sequences, so that cross-reactive response to variants would be favoured. In spite of the limited success of this initial attempt, the results show that a neutralizing response against a broad spectrum of variants is possible. Choice of appropriate adjuvants as well as the inclusion of tandem-repeated peptides with a few additional residues surrounding the RGD tripeptide may evoke the induction of immune response with greater neutralization potential yet maintaining a relaxed specificity to cause neutralization of many variants able to escape antibodies directed to authentic site A.

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