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New virus-specific T-helper epitopes of foot-and-mouth disease viral VP_1 protein

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Immunogenicity studies of synthetic peptides from different regions of VP_1 protein of foot-and-mouth disease virus strain A_{22} revealed the following active fragments: 39–61, 50–69, 135–159, 175–189, 170–189 and 197–213. Testing of virus neutralizing antibody production in rabbits primed by peptides and then inoculated by the virus showed that only peptides 135–159 and 170–189 were able to induce the functional T-cell helper activity. Localization of virus-specific T-cell recognition sites in sequences 135–159 and 170–189 was confirmed in in vitro recognition experiments of the virus by peptide activated mice lymphocytes.

Foot-and-mouth disease; Synthetic peptide immunogenicity; T-cell recognition site

1. INTRODUCTION

The creation of purely peptide foot-and-mouth disease vaccine meets with a number of problems, starting with identification of virus specific B- and T-cell epitopes. Over the past years considerable advances in B-epitope identification have been made [1,2]. The epitopes recognized by T-cell so far attract less attention. Recently the 21–40 VP_1 fragment of foot-and-mouth disease virus (FMDV) O_1K strain was estimated as immunodominant for vaccinated cattle [3]. However other T-epitopes can not be excluded from the VP_1 sequence, particularly bearing in mind that peptide from the C-terminal part of the protein showed pronounced T-cell stimulatory activity [3]. Moreover, VP_1 sequences from different FMDV strains might differ in the location of their T-epitopes. In the present study we identified new virus specific T-cell epitopes for VP_1 of FMDV strain A_{22}.

2. EXPERIMENTAL

2.1. *Peptide synthesis*

Peptides were synthesized using solid–phase chemistry on the PAM-polymer [4]. Boc-derivatives of trifunctional amino acids were used as Thr(Bzl), Ser(Bzl), Glu(OBzl), Asp(OBzl), Tyr($BzlCl_2$), Arg(Tos), His(Bom), Cys(Acm), Lys(ZCl). The cleaved peptides were purified by ion-exchange chromatography. The yield of peptides was 60–70% (counting on the first amino acid). The homogeneity of peptides was confirmed by amino acid analysis, mass-spectral data and by HPLC on a TSK-ODS column (4.6 × 250 mm) in acetonitril gradient in 0.05% TFA (from 0 to 70%).

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Abbreviations: PAM, 4-(oximethyl)phenylacetamidomethyl; TPA, titer of antipeptide antibodies; TVA, titer of virus neutralizing antibodies; CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant.

2.2. *Immunization*

Animals were inoculated with peptides twice, with 44 days between immunizations. The inoculation dose was 200 μg for rabbits or 100 μg for guinea pigs and mice. The dose of inactivated purified virus A_{22} [5] was 20 μg. CFA was used for the first injection and IFA was employed for the booster one. On the 55th day after the first inoculation samples of blood were obtained, and guinea pigs received 500 half-infectious doses of A_{22} FMDV. The protection was tested on the 5th day after the challenge [6]. Serum neutralizing activity was determined in the pig kidney cell culture containing 100–320 ID_{50} of virus. TVA (in $-\log_2$ scale) was calculated as serum dilution at which the 50% tissue cytopathic effect was depressed [6]. An indirect ELISA technique was used to assay the TPA (in $-\log_{10}$ scale). The plates (Costar, Cambridge) were coated with uncoupled peptides at concentration of 20 μg/ml. Antibody titers were calculated as serum dilution at which the A_{492} value exceeded 0.1, the value being at least 2-times higher than the negative standard.

2.3. *Virus specificity of antipeptide T-helper response*

Rabbits were inoculated with free peptides (200 μg) with CFA, 28 days later the animals received the inactivated purified virus A_{22} (5 μg) with IFA. On the 4th day after the virus injection the blood samples were obtained, TPA and TVA were measured. The T-cell proliferation was assayed as described [7]. Briefly, BALB/c mice were inoculated in the base of the tail and in the hind footpads (100 μg of peptide with CFA). After 7 days lymph node cells were obtained. Dilutions of peptides or inactivated purified virus A_{22} were added to cells. After the incubation of cells (37°C, 5% CO_2, 90% H_2O) for 3 days 1 μCi [^{3}H]thymidine was added and pulsed cells harvested 18 h later.

3. RESULTS AND DISCUSSION

The VP_1 (A_{22} strain) fragments selected for synthesis

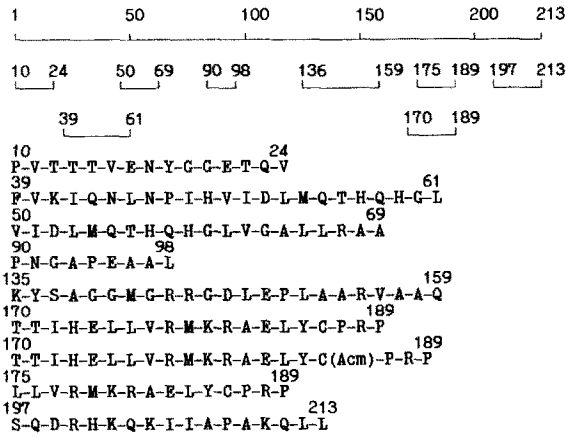

Fig. 1. Synthetic peptides from VP_1 of the FMDV A_{22} strain.

are shown in Fig. 1. The choice was based on standard theoretical predictions of antigenic determinants [8]. Data on VP_1 variability were also taken into account [9]. Peptide 10–24 is hydrophilic and contains a β-bend. The 39–61 peptide represents a highly variable VP_1 region; 50–69 forms an amphipathic α-helix. Peptide 135–159 from the main immunogenic site includes 'Tyr^{136} residue' exposed on the virion surface [10], the variable 136–144 region and an amphipathic α-helix. The 170–189 region is both conservative and hydrophilic. The peptide with the 170–189 VP_1 sequence was prepared in two forms: with the free SH-moiety of the Cys^{186} residue (170–189) and with Acm group on that moiety (170–189Acm). Peptide 197–213 was selected because of the known antigenicity of the 200–213 section [11]; it includes a hydrophilic 197–199 sequence.

The antigenic properties of synthetic peptides were estimated from ELISA data of peptide binding with antivirus rabbit sera (Table I). Only peptides 135–159 and 197–213 bind to the virus antibodies, the rest of the peptides being inactive in that test. These two fragments have been reported as the only virus-neutralizing B-epitope containing sites [1,2].

Immunogenicity of synthetic peptides in different mice strains, rabbits and guinea pigs was studied (Table II). The results from Table II for peptides 10–24, 50–69, 135–159, 175–189 and 197–213 were partially described [12,13]. Judging from the antipeptide titers peptides 39–61, 50–69, 135–159, 175–189, 170–189 and 197–213 are immunogenic. However, these peptides were unable to induce formation of the virus neutralizing antibodies in rabbits and in guinea pigs and to protect guinea pigs against the disease, the only exception being the earlier described protective peptide 135–159 from the main immunogenic region [13]. Analysis of antipeptide titers showed that peptides 39–61, 135–159 and 170–189 were the most potent immunogens. Peptide 39–61 was able to induce a high level of peptide antibodies in mice BALB/c and CBA/J, rabbits and guinea pigs. Peptide 135–159 was immunogenic in mice BALB/c, C57BL/6, rabbits and guinea pigs. Fragment 170–189 induced formation of antibodies in all investigated animals with high titers. Peptide 197–213 was immunogenic only in rabbits and guinea pigs. Antibodies against this peptide were not virus neutralizing despite the presence of a minor virus neutralizing B-epitope in this VP_1 part [2]. We believe that antibody-inducing site of the free peptide 197–213 is not a virus-neutralizing B-epitope.

Of the peptides studied, the fragments outside the main and the minor immunogenic sites did not contain virus-neutralizing B-epitopes. However, the immunogenicity of these peptides indicates the presence of T-helper epitopes capable of stimulating the antipeptide T-cell immunity. In this work we studied the ability of the most potent immunogenic peptides 39–61, 135–159,

Table I

Binding of rabbit antivirus serum to synthetic VP_1 fragments

Peptide	Titer of antivirus antibodies (−lg)
10– 24	< 1.0
39– 61	< 1.0
50– 69	< 1.0
90– 98	< 1.0
175–189	< 1.0
170–189	< 1.0
197–213	1.6
135–159	3.1

Table II

Immunogenicity of synthetic VP_1 fragments

Peptide	Mice			Rabbits		Guinea pigs		
	BALB/c TPA	CBA/J TPA	C57BL/6 TPA	TPA	TVA	TPA	TVA	Protection (%)
10– 24	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	0
39– 61	5.5	3.1	< 1.0	5.0	< 1.0	4.1	< 1.0	0
50– 69	< 1.0	< 1.0	< 1.0	3.1	< 1.0	2.5	< 1.0	0
90– 98	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	0
135–159	3.4	< 1.0	4.8	3.8	4.2	3.4	3.7	100
175–189	2.6	3.2	2.8	4.9	< 1.0	< 1.0	< 1.0	0
170–189	5.8	5.8	5.5	5.2	< 1.0	4.1	< 1.0	0
197–213	< 1.0	< 1.0	< 1.0	3.5	< 1.0	2.8	< 1.0	0

Table III

Enhancement of virus-neutralizing antibodies by priming with synthetic peptides

First immunization	Second immunization	TPA	TVA
39– 61	virus	4.4	1.2
39– 61	–	4.4	< 1.0
135–159	virus	3.4	4.4
135–159	–	3.4	1.5
170–189	virus	4.9	4.0
170–189	–	4.9	< 1.0
197–213	virus	3.6	1.5
197–213	–	3.6	< 1.0
CFA	virus	< 1.0	1.3

170–189 and fragment 197–213 from the minor immunogenic site to activate the virus-specific T-helper cells and to induce antiviral T-cell immunity. The rabbits were primed with different peptides, the second immunization was performed with a suboptimal dose of the inactivated and purified virus (Table III). Priming with CFA or inoculation with the peptides without second immunization with the virus was employed as a control.

Among the fragments investigated only 135–159 and 170–189 enhanced the virus neutralizing antibody formation. Immunization with peptide 135–159 alone induced virus-neutralizing antibodies (titer 1.5) that was due to the presence of virus-neutralizing B-epitope in the peptide. A significant increase in the virus-neutralizing antibody titers after second inoculation with the virus (up to titer 4.4) were ascribed to recognition by the virus of B- and T-cells activated by peptide 135–159. Peptide 170–189 did not contain virus neutralizing B-epitopes and thus inoculation with that peptide did not stimulate the formation of neutralizing antibodies. However, priming with peptide 170–189 followed by inoculation with the virus induced neutralizing antibodies with titer 4.0. The virus was thus recognized by T-helper cells activated by the peptide. Peptides 39–61 and 197–213 were unable to enhance the formation of the virus induced neutralizing antibodies.

Specificity of peptide activated T-cells was investigated in vitro on BALB/c mouse lymphocytes (Table IV). Mice were immunized with peptides 39–61, 135–159 and 170–189 ('immunogens' in Table IV), and 7 days later the ability was tested of peptides 39–61, 135–159, and 170–189Acm, control peptides 90–98 and 136–152, and of the virus ('antigens' in Table IV) to induce the proliferative activity of lymphoid cells. Peptide 170–189Acm was employed in this case as an antigen because we found out that the S-unprotected peptide 170–189 non-specifically inhibits proliferation of lymphoid cells in vitro (data not shown). Uninoculated animals were employed as control.

Peptides 135–159, 170–189Acm, 39–61 were able to stimulate the proliferative activity of the cells from the animals inoculated with the same peptide. However, the virus was able to induce the T-cell proliferation only when mice were inoculated with peptides 135–159 or 170–189.

The above results prove that peptide 135–159 includes not only the virus-neutralizing B-epitope, but also the virus-specific T-epitope. Peptide 170–189 does not contain the virus-neutralizing B-epitope, but includes the virus-specific T-helper epitope. Taking into account that peptide 170–189 contains a conserved VP_1 region, we propose to apply this peptide as a T-cell carrier for FMDV protective B-epitopes in anti-FMD

Table IV

Virus induced proliferation of BALB/c mice lymphoid cells activated with the peptides

Immunogen	Antigen	c.p.m.				
		0 μM*	0.1 μM	1 μM	10 μM	100 μM
39– 61	39– 61	17,542 ± 3,037	20,039 ± 1,942	24,290 ± 796	31,408 ± 2,758	64,756 ± 6,747
	Virus**	17,542 ± 3,037	16,247 ± 2,958	16,175 ± 1,015	18,785 ± 3,351	19,113 ± 2,198
	136–152	17,542 ± 3,037	17,759 ± 1,919	17,269 ± 1,830	19,650 ± 450	18,034 ± 2,763
135–159	135–159	5,635 ± 784	6,152 ± 1,283	14,422 ± 2,529	30,401 ± 2,799	41,245 ± 5,375
	Virus**	5,635 ± 784	4,990 ± 156	9,132 ± 130	12,382 ± 667	18,280 ± 1,823
	90– 98	5,635 ± 784	5,540 ± 700	5,340 ± 758	5,308 ± 209	5,488 ± 248
170–189	170–189Acm	6,231 ± 546	5,482 ± 1,392	13,262 ± 1,550	26,465 ± 1,122	28,838 ± 2,884
	Virus**	6,231 ± 546	5,692 ± 761	10,011 ± 5,417	15,141 ± 970	18,149 ± 3,817
	136–152	6,231 ± 546	5,443 ± 698	5,915 ± 161	7,388 ± 726	6,571 ± 1,469
–	135–159	2,787 ± 625	2,391 ± 137	2,659 ± 262	2,867 ± 255	2,434 ± 158
	170–189Acm	2,787 ± 625	2,543 ± 115	2,548 ± 338	2,508 ± 177	2,505 ± 29
	39– 61	2,787 ± 625	2,829 ± 193	2,773 ± 244	2,764 ± 199	2,673 ± 368
	Virus**	2,787 ± 625	2,536 ± 216	2,271 ± 124	2,334 ± 245	2,817 ± 688

*Peptide concentration. **Purified inactivated virus A_{22} in concentrations: 0.05; 0.1; 1; 5 μg/ml.

synthetic vaccines. Incorporation of the 170–189 peptide into the synthetic construction will induce T-helper memory cells recognized by different serotypes of FMDV.

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