FEBS Letters 345 (1994) 159–161

FEBS 14041

Synthetic peptides in the determination of hepatitis A virus T-cell epitopes

Vadim S. Ivanov[a,*], Liudmila N. Kulik[a], Andrei E. Gabrielian[b], Leonid D. Tchikin[a], Alexander T. Kozhich[a,**], Vadim T. Ivanov[a]

[a]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya 16/10, 117871, Moscow, GSP-7, Russian Federation*

[b]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str. 32, 117984 Moscow B-334, Russian Federation*

Received 1 April 1994

Abstract

Computer search for probable T-epitopes of hepatitis A virus capsid proteins was performed using an integrated set of programs. Eight segments of the VP1, VP2, VP3 and VP4 proteins were chosen and synthesised. Five peptides previously examined as probable B-epitopes were used as well. All the peptides were tested for their ability to stimulate proliferation of lymph node T-cells primed with synthetic peptides. Almost all predicted T-epitopes affected the T-cell proliferation. None of the peptides had mitogenic activity. We demonstrated that regions 17–33 and 276–298 of VP1 are possible immunodominant promiscuous sites activating lymphocytes of all mouse haplotypes.

Key words: Amphipathicity prediction; T-cell proliferation; Immunodominant promiscuous site

1. Introduction

Hepatitis A virus (HAV) belongs to the picornavirus family with a host range restricted to certain primates [1]. Hygienic measures alone will not suffice to control the occurrence of hepatitis A; rather, well-targeted vaccination programs will be necessary to prevent epidemics. A number of approaches to the development of live or attenuated vaccines have been described [2]. However, the special features of HAV growing in cell cultures place limitations on the application of such vaccines. Attempts to design a subunit HAV vaccine have also been described [3]. Synthetic peptide vaccine may be another alternative, but so far there are no clear data about the localisation of immunogenic and antigenic determinants on the HAV capsid [4,5]. Results of priming the virus neutralising response after immunisation with HAV recombinant proteins suggest that the VP2 protein contains an epitope directly activating T helper cells; other epitopes must be present in VP3 and VP1 proteins [3]. Recent studies showed a T-cell response to recombinant HAV capsid proteins containing sequences from the carboxy-terminal of VP1 [6].

The present study dealt with a search for T-epitopes of HAV capsid proteins. Localization of T-epitopes could be important for developing a synthetic vaccine. We have also considered that synthetic T-epitopes alone are able to prime cellular immunity against the viral infection [7,8]. By computer sequence analyses of HAV capsid proteins we predicted a number of T-epitopes, synthesised and tested them for their ability to stimulate proliferation of lymph node T-cells primed with synthetic peptides.

2. Materials and methods

2.1. Synthetic peptides

Peptides were synthesised by a solid-phase method on a modernised Beckman 990 peptide synthesiser using PAM resin [9] and HOBt esters of Boc amino acids as described earlier [5]. The synthesised peptides were cleaved from resin and deprotected by treatment with liquid HF containing *p*-cresol, and purified by reverse-phase HPLC. The amino acid analysis of peptides was consistent with their expected compositions. The molecular masses of peptides were confirmed by FAB mass spectra.

2.2. Mice

BALB/c, CBA, F_1(BALB/c × $C_{57}Bl_6$) and F_1(CBA × $C_{57}Bl_6$) strains were obtained from the breeding colony at the Central Nursery of Laboratory Animals (Russian Academy of Medical Sciences, Krasnogorsk, Russian Federation). Mice were used at 6–8 weeks of age and were of either sex.

2.3. Lymph node T-cell proliferation assays

Mice were immunized with 200 μg of peptide antigen in 0.20 ml complete Freund's adjuvant (CFA) in two foot pads and in the base of the tail. Eight days later, the draining lymph nodes were removed, and a single-cell suspension was assayed in triplicate cultures with appropriate doses of antigen (0.1–100 μg/ml) at 4×10^5 cells per well in 96-well U-bottom Titertek plates in complete RPMI 1640 medium as described in [10,11]. [^{3}H]Thymidine incorporation into DNA during the last 16 h of a 5-day culture was determined as a measure of proliferation. Stimulation indices (SI) of proliferation were calculated by the following formula:

$$SI = \frac{\text{X cpm [}^3\text{H]thymidine incorporated with peptide}}{\text{X cpm [}^3\text{H]thymidine incorporated without peptide}}$$

An SI of 2 or more was considered positive.

*Corresponding author. Fax: (7) (095) 335 5844.
E-mail: IVANOV@PEPSYN.SIOBC.MSK.SU.

***Present address:* Department of Health and Human Services, Public Health Service, NIH, Bldg. 10, Rm. 10N208, Bethesda, MD 20205, USA.

SSDI 0014-5793(94)00427-W

3. Results and discussion

The known protein T-epitopes are limited in number and share several common features. A high percentage of these regions is found within the helical segments of the intact protein and/or can be presented as amphipathic helices [12]. Therefore methods for prediction the amphipathic helices are the most widespread techniques for T-epitope searches. One such technique employs the method known as the 'power spectrum' procedure: the segment is considered an optimal T-epitope if the hydrophobicity of residues alternates with a frequency similar to the number of residues per turn in the α-helix [12,13].

We used a standard AMPHI program [13] to predict sites with periodic alterations in hydrophobic and hydrophilic residues as probable T-epitopes. Fig. 1 presents a plot of the amphipathicity calculated for the VP1 capsid protein: the dominant peaks correspond to the most probable T-epitopes. Regions overlapped by the synthethic peptides are shown as stretches on the top of the plot. Also shown is the helical wheel representation of some of the peptides to demonstrate their amphipathic structure (Fig. 2). It can be seen that the hydrophobic residues are located on one side and the hydrophilic residues on the opposite side of the wheel.

The second technique of T-epitope prediction proposed by Rothbard and Taylor [14] is based on sequence pattern recognition. The proposed pattern is composed of a charged residue or a glycine followed by two or three hydrophobic residues and the polar or charged residue at the C-terminus, and fits well to the amphipathic helix model. In contrast to the first technique the latter is based on a typical 'all-or-none' principle. Searching for T-cell epitopes using Rothbard's characteristic fragments usually leads to an over-prediction of probable epitopes. The second disadvantage of this method is that an estimation of the preference of any site (i.e. ranging of sites) is impossible. We used this method together with the amphipathic helices searching program in order to select sites both amphipathic and containing the characteristic pattern. We included the standard Garnier-Robson method [15] in our program set for estimation of conformational preference of sites in question. Fragments with high turn and/or coil preferences were excluded from further analysis because our previous investigations had indicated the low probability of these conformations for T-epitopes.

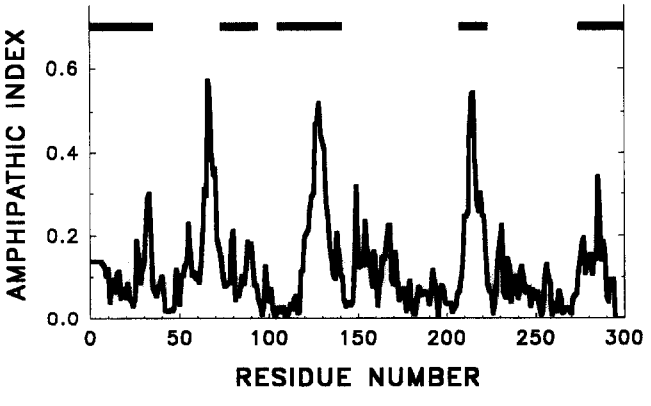

Fig. 1. Amphipathicity profile of HAV VP1 capsid protein. Dashes on the top correspond to the regions overlapped by synthetic peptides.

Once calculations had been performed the following segments of HAV capsid proteins were selected for synthesis: 75–92, 115–139 and 209–221 of VP1, 69–99 and 80–99 of VP2, 45–57 and 137–150 of VP3 and 1–23 of VP4 (Table 1).

In addition we used peptides synthesised earlier as probable B-epitopes [5] that would be T-epitopes according to calculations (10–33 and 276–298 of VP1) or were overlapping with them (1–17, 11–25 and 75–85 of VP1).

To determine whether a peptide could be considered a T-epitope, mice were primed with the free peptide and then proliferation of lymph node T-cells was estimated by measuring the incorporation of [^{3}H]thymidine. Each peptide was also tested for its mitogenic activity. For this purpose, serial peptide dilutions were added to a suspension of lymphocytes from lymph nodes of mice that had not been primed with peptide but were immunised with an emulsion containing PBS and CFA. In this case, there was no increase in thymidine incorporation by cell cultures after applying the tested peptide (data not shown). Of fifteen tested peptides, nine induced proliferation of primed lymphocytes with a SI from 2 to 6 (Table 2). As a rule, stimulation of lymphocyte proliferation depended upon mouse haplotype. This fact is observed for all the peptides with the exeption of peptides 10–33 and 276–298 of VP1 protein, that stimulated lymphocyte proliferation of all the mouse strains used. The shortened analogue of VP1-(10–33), peptide VP1-(11–25) did not induce proliferation of CBA mice lymphocytes, whereas peptide VP1-(1–17) did not influence proliferation of lymphocytes from any of the tested strains of mice. On

Table 1
Synthetic peptides from the HAV capsid proteins

Protein	Segment	Amino acid sequence
VP1	1–17	VGDDSGGFSTTVSTEQN
	10–33	TTVSTEQNVPDPQVGITTMRDLKG
	11–25	TVSTEQNVPDPQVGI
	75–85	GESRHTSDHMS
	75–92	GESRHTSDHMSIYKFMGR
	107–126	YTFPITLSSTSNPPHGLPST
	115–126	STSNPPHGLPST
	115–139	STSNPPHGLPSTLRWFFNLFQLYRG
	209–221	YAVSGALDGLGDK
	276–298	MSRIAAGDLESSVDDPRSEEDRR
VP2	69–99	LTTHALFHEVAKLDVVKLLYNEQFAVQGLLR
	80–99	KLDVVKLLYNEQFAVQGLLR
VP3	45–57	GIKITHFTTWTSI
	137–150	PGNELIDVTGITLK
VP4	1–23	MNMSKQGIFQTVGSGLDHILSLA

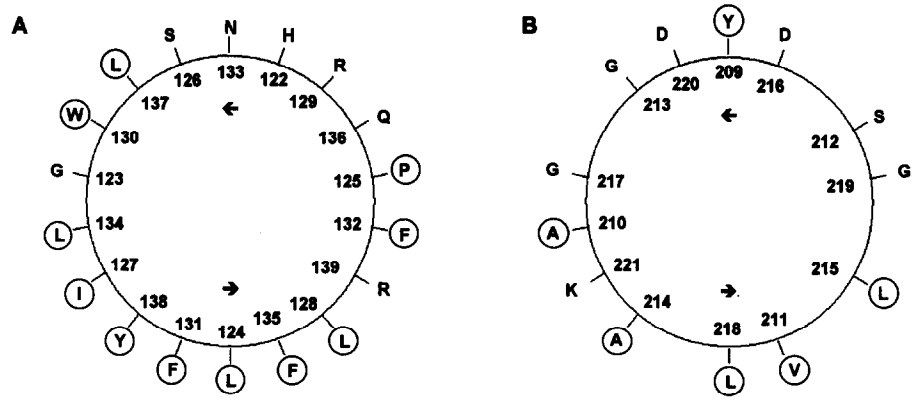

Fig. 2. Helical wheel presentation of peptides VP1-(115–139) (A) and VP1-(209–221) (B). Amino acid side chains are projected onto a plane perpendicular to the axis of the α-helix. Adjacent side chains in the sequence are separated by 100° of an arc on the wheel. Hydrophobic residues are circled.

the basis of the results obtained, we can assume localisation of the immunoactive site in region 17–33 of VP1 protein inducing lymphocyte proliferation independent of testing strains of mice. There are possible T-epitopes in this region and in the region 276–298 of VP1 protein recognising distinct molecules of Ia proteins, as was determined for the malaria T-cell epitopes [16].

Peptide VP1-(115–139) induced a proliferative response in F_1(BALB/c × $C_{57}Bl_6$) and F_1(CBA × $C_{57}Bl_6$) mice (data not shown). Its analogues VP1-(107–126) and VP1-(115–126), did not induce a proliferative response in any of the strains of mice primed with these peptides, however, both peptides initiated an increase in SI up to 4 and 5, respectively, in F_1(CBA × $C_{57}Bl_6$) mice primed with VP1-(115–139) peptide (data not shown). A similar effect was not detected with lymphocytes from CBA and BALB/c mice.

In conclusion, we have demonstrated that almost all the predicted T-epitopes influenced the proliferation of lymphocytes in mice primed with the peptides and this influence depended on the strain of mouse. As an exception to this rule we found immunodominant promiscuous sites in the regions 17–33 and 276–298 of VP1 capsid protein activating lymphocytes of all mouse haplotypes.

Table 2
Ability of peptides from the HAV capsid proteins to induce a T-cell proliferative response in mice primed with these peptides

Peptide	Stimulation index*		
	BALB/c	F_1BALB/c × $C_{57}Bl_6$)	CBA
VP1-(1–17)	1.2	1.2**	1.2
VP1-(10–33)	4.2	6.0	2.1
VP1-(11–25)	2.1	2.5**	1.3
VP1-(75–92)	6.2	2.2	1.1
VP1-(75–85)	1.1	1.0	1.0
VP1-(115–139)	1.0	2.0	1.1
VP1-(107–126)	1.1	1.0	ND
VP1-(115–126)	1.0	0.9	ND
VP1-(209–221)	1.1	2.2	1.0
VP1-(276–298)	2.5	4.0	2.1
VP2-(69–99)	2.4	2.1	1.0
VP2-(80–99)	ND	1.2	1.2
VP3-(45–57)	1.2	6.1	2.0
VP3-(137–150)	1.5	1.7	1.8
VP4-(1–23)	1.1	2.2	1.5
Con A	9.1	10.2	8.1

ND, no data; Con A, concanavalin A.
*The highest SI value, obtained with optimal peptide concentrations (100–250 μg/ml), is presented.
**Results obtained with F_1(CBA × $C_{57}Bl_6$) mice.

References

[1] Lemon, S.M. (1985) N. Engl. J. Med. 313, 1059–1067.
[2] Wiedermann, G., Ambrosch, F., Kolaritsch, H., Hofmann, H., Kunz, Ch., Hondt, E.D., Delem, A., Andre, F.E., Safary, A. and Stephenne, J. (1990) Vaccine 8, 581–584.
[3] Powdrill, T.F. and Johnston, J.M. (1991) J. Virol. 65, 2686–2690.
[4] Siegl, G. and Lemon, S.M. (1990) Virus Res. 17, 75–92.
[5] Tchikin, L.D., Kozhich, A.T., Ivanov, V.S., Ivanov, V.T., Nastashenko, T.A., Kusov, Yu.Yu. and Balayan, M.S. (1991) Bioorgan. Khimia 17, 964–971.
[6] Harmon, S.A., Broman, B., Powdrill, T.F., Johnston, J.M., Ehrenfeld, E. and Summers, D.F. (1993) J. Infect. Dis. 167, 990–992.
[7] Milich, D.R., McLachlan, A., Thornton, G.B. and Hughes, J.L. (1987) Nature 329, 547–549.
[8] Boots, A.M.H., Kusters, J.G., van Noort, J.M., Zwaagstra, K.A., Rijke, E., van der Zeijst, B.A.M. and Hensen, H.J. (1991) Immunology 74, 8–13.
[9] Mitchell, A.R., Kent, S.B.H., Engelhardt, M. and Merrifield, R.B. (1978) J. Org. Chem. 43, 2845–2852.
[10] Milich, D.R., McLachlan, A., Moriarty, A. and Thornton, G.B. (1987) J. Immunol. 139, 1223–1231.
[11] Berkower, I., Matis, L.A., Buckenmeyer, G.K., Gurd, F.R.N., Longo, D.L. and Berzofsky, J.A. (1984) J. Immunol. 132, 1370– 1378.
[12] Berzofsky, J.A. (1988) Immunol. Lett. 18, 83–92.
[13] Cornette, J.L., Cease, K.B., Margalit, H., Spouge, J.L., Berzofsky, J.A. and DeLisi, C. (1987) J. Mol. Biol. 195, 659–685.
[14] Rothbard, J.B. and Taylor, W.R. (1988) EMBO J. 7, 93–100.
[15] Garnier, J., Osguthorpe, D.J. and Robson, B. (1978) J. Mol. Biol. 120, 97–120.
[16] Sinigaglia, F., Guttinger, M., Kilgus, J., Doran, D.M., Matile, H., Etlinger, H., Trzeciak, A., Gillessen, D. and Pink, J.R.L. (1988) Nature 336, 778–780.