Biopanning of HGV epitope from a phage displayed library*

KE Shen (柯 山)[1], QI Zhongtian (戚中田)[1], YANG Ziyi (杨子义)[2], SHI Ming (施 明)[2]
WANG Jianan (王建安)[2], YAO Zhijian (姚志建)[3] and SHEN Beifen (沈倍奋)[2]**
(1. Second Military Medical University, Shanghai 200433, China; 2. Beijing Institute of Basic Medical Science, Beijing 100850, China; 3. National Center of Human Genome Research, Beijing 100176, China)

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Abstract Three mouse monoclonal antibodies (mAb) specific to E2 antigen of human hepatitis G virus (HGV) were used to bio-panning of a phage displayed random peptide library of 15 amino acid residues. After 3 rounds of screening, the ratio of output to input increased to 1.1×10^{-3} and the false positive rate reduced to 0.2%, which means the enrichment was effective. At the third round of screening, 15 plage clones were selected for the use in binding and competitive inhibition tests. Thirteen of them could specifically react with the mAb M6. The inhibition rates of phage 10 clones out of 15 were over 60%. From the deduced insert sequence in the coat protein VIII, the core sequence NPLWP was found in 6 phage clones which are homologeous to the amino acids 301—305 of HGV E2. The sera from the mice immunized with the phage clone C2 containing motif sequence were found positive for anti-HGV. These indicate the possibility that NPLWP motif in the short peptide is the mimic of HGV E2 epitope that can be recognized by HGV mAb M6.

Keywords: phage-displayed random peptide library, antigen epitope, hepatitis G virus.

A new kind of positive-sense single-stranded RNA virus designated hepatitis G virus (HGV) was isolated in 1995. The genetic organization of HGV resembles hepatitis C virus (HCV). Similar to HCV, HGV is transmitted by blood and can cause persistent infection. Up to date, few HGV antigen epitopes similar to the core or C33c antigens of HCV which can induce host immune response in most of the infected individuals have been identified[1]. In order to find out the short peptides that can be used in HGV diagnosis and vaccine development in future, we used the HGV E2-specific monoclonal antibodies (mAbs) M6, M13 and M30 as the ligates to bio-panning of the epitope from a phage-displayed random peptide library to find the short peptides that can specifically react with the mAbs and inhibit the antigen-antibody binding. We obtained a core sequence of NPLWP which can be used in further studies on the characteristics of HGV-induced immunization.

1 Materials and methods

1.1 Materials

HGV-specific mAbs M6, M13 and M30 were obtained from Professor A. M. Engel's laboratory[1]. The phage-displayed random peptide libraries of 15 amino acid were kindly provided by

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** Corresponding author.

Professor G. P. Smith[2]. *E. coli* strain K91/kana was kanamycin-resistant, F^+ strain. HGV E2 antigen was previously expressed in and purified from an insect expression system in our laboratory[1]. HRP-conjugated anti-M13 antibody was purchased from Pharmacia.

1.2 Methods

1.2.1 Bio-panning. The bio-panning was performed as described in ref. [3]. The mAbs M6, M13 and M30 were mixed and used to coat the 96-well plates for screening of antigen epitope from the phage-displayed random peptide library. The amount of antiboby coating, TBST (Tris buffered saline with Tween -20) concentration and washing conditions used in 3 rounds of secreening are listed in table 1.

Table 1 Washing conditions of bio-panning

	1st round	2nd round	3rd round
Amount of Ab coating	500 ng	50 ng	50 ng
Concentration of TBST	0.1%	0.2%	0.5%
Washing conditions	5s, 10 times	15s, 20 times	25s, 30 times

1.2.2 Enrichment analysis. The unbinding phages, the phage from the last washing and the eluted phage were respectively considered as input, wash and output. The diluted phages were transfected into *E. coli* K91/kana and the transduction unit (TU) was detected[4]. The rate of false positive in output was regarded as false positive rate. In each round, the ratio of output to input and the false positive rate were analyzed[5].

1.2.3 Binding test. The 96-well plates were respectively coated with 0.5 μg of mAb M6, M13 or M30. Binding test was performed as described in ref. [6]. The results were measured on an ELISA reader at 450 nm.

1.2.4 Inhibition test. Each well of the plates was first coated with 0.05 μg of HGV antigen. After blocked with 1% bovine serum albumin (BSA), the wells were incubated overnight at 4℃ with 0.1 μg Ab and about 3 μg (10^{11} particles) of positive phages[7], then incubated at 37℃ for 1h with HRP labelled anti-mouse IgG. The absorbance was measured by an ELISA reader at 450 nm. The positive control was no phage contained, and the negative control was no mAb contained. The inhibition rate was calculated by the formula

$$\text{Inhibition rate}\% = \frac{A_{\text{positive control}} - A_{\text{positive phages}}}{A_{\text{positive control}}} \times 100.$$

1.2.5 DNA sequencing. The primer used in sequencing was an oligonuclectide of 5'-TGAATTTTCTGTATGAGG-3'[6]. Determination of DNA sequences was carried on an automated DNA sequencer ABI PRISM 310.

1) unpublished data.

1.2.6 Analyses of core sequence and homology. According to the sequences inserted in the random region of the positive phages, the amino acid sequences of short peptide displayed were deduced[8]. Subsequently, the core sequence was compared with the data in the EMBL database and the homology of the core sequence to the HGV antigen was analyzed.

1.2.7 Characterization of antigenicity of the positive clones. The positive clones with the core sequence were propagated and diluted to $A_{270\ nm}$ value of 2 in TBS[7,9], and then serially diluted. 40 μL of each diluted phages was incubated with 0.1 μg mAb for 2 h[10]. The plates coated with 0.05 μg per well of HGV E2 antigen were blocked with 1% BSA and then incubated overnight at 4℃ with the reaction mixture. After washing, each well was incubated with 100 μL of HRP-conjugate rabbit anti-human antibodies at 37℃ for 1 h. The absorbance was measured by an ELISA reader at 450 nm wavelength.

1.2.8 Immunogenicity determination of the positive clones. The positive clones were propagated and diluted to $A_{270\ nm}$ value of 2 in 0.9% NaCl. The Balb/c mice were immunized with 400 μL of the phages for four times. After 7 d—10 d of last immunization, the sera were collected for antibody. The titer of acquired antibody was measured as discribed in Section 1.2.7, except for no phages contained.

2 Results

2.1 Enrichment analyzing

The ratio of output/input and the false positive rate after 3 rounds of screening are shown in table 2. The results indicate that the ratio of output/input increases and false positive rate decreases after each round of secreening.

Table 2 The ratio of output/input of bio-panning

	Round 1	Round 2	Round 3
Input	2.6×10^{10}	2.2×10^{10}	9.1×10^{9}
Wash	8×10^{4}	3.0×10^{4}	5.1×10^{4}
Output	3.2×10^{5}	2.4×10^{6}	1.0×10^{7}
False positive	25%	1.2%	0.5%
Output/Input	1.2×10^{-5}	1.1×10^{-4}	1.1×10^{-3}

2.2 Binding test

Fifteen phage clones from the 3rd round screening were selected and bound with mAbs M6, M13 and M30 respectively. As shown in fig. 1, clones C1 and C15 were negative. While clones C2—C14 were found specifically binding to M6. No binding was observed with mAbs M13 or M30.

2.3 Competitive inhibition test

The competitive inhibition test was performed on phage clones C2—C14. As shown in fig. 2, the inhibition rates of clones C6, C9 and C10 were lower than 40%. The rest of the clones were able to inhibit the specific binding of HGV Ag to mAb M6, and with over 60% of the inhibition rate.

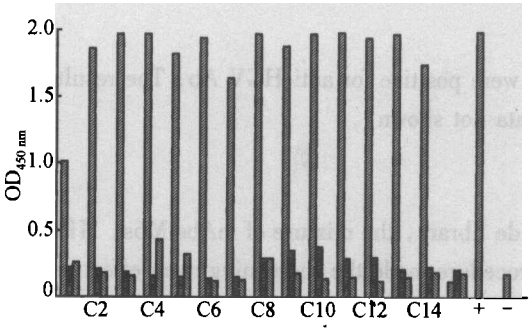

Fig. 1. The binding of phages to mAbs. □ M6; □, M13; □ , M30; C1—C15, phage clones; + , positive control; − , negative control.

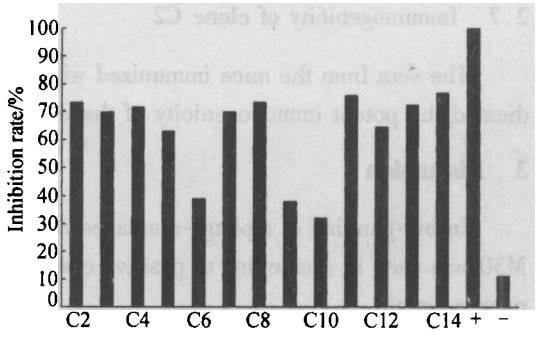

Fig. 2. The results of competitive inhibition test. C2—C14, phage clones; + , positive control; − , negative control.

2.4 Sequencing results

The sequencing results of the 10 clones which can effectively inhibit the binding of HGV Ag to mAb M6 are listed in table 3.

Table 3 Amino acid sequences of the positive phage clones

Clone	Sequence														
C2,C14	N	P	L	W	P	I	N	C	L	L	L	S	E	C	S
C3	N	P	L	W	N	H	P	C	P	N	L	D	C	S	M
C4,C8	N	P	F	W	P	L	N	A	G	N	L	D	F	S	T
C12	N	S	L	W	P	Y	P	E	L	S	L	H	S	K	G
C5	K	G	P	T	T	C	S	P	O	T	P	Q	N	C	P
C7	S	T	G	P	T	E	S	I	F	Y	M	A	H	L	N
C11,C13	H	P	T	T	F	S	S	Q	H	P	F	Y	Q	S	M

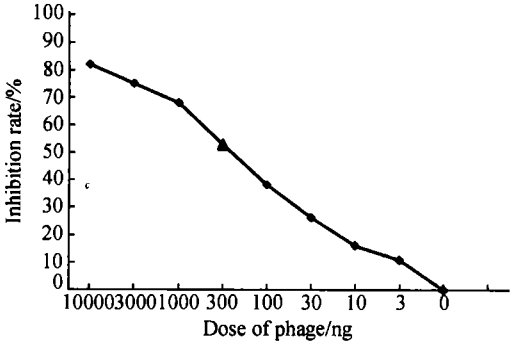

Fig. 3. Inhibition of clone C2 to HGV Ag-Ab binding. ▲ showing the inhibition is over 50% when the dose of phage is 300 ng.

2.5 Analyses of core sequence and homology

Among the 10 phage clones which could competitively inhibit the binding of HGV Ag to Ab, 6 clones were of core sequence NPLWP, which is similar to the sequence of NPVCP (amino acids 301—305) in HGV E2. It seems that the sequence NPLWP is the binding motif of mAb M6 to HGV.

2.6 Antigenicity of clone C2

The phage clone C2 with the core sequence NPLWP was tested in several concentrations for the capability of inhibiting the binding of Ag with Ab. The inhibition curve in fig. 3. shows that this clone C2 has specific antigenicity.

2.7 Immunogenicity of clone C2

The sera from the mice immunized with clone C2 were positive for anti-HGV Ab. The results indicated the potent immunogenicity of the clone C2 (data not shown).

3 Discussion

In bio-panning of a phage-displayed random peptide library, the mixture of mAbs M6s, M13 and M30 was used in secreening of positive clones. This procedure made the secreening more effective and more accurate.

The ratio of output to input is an indicator for evaluating the enrichment in bio-panning[11]. We found from our results that the ratio of output/input was considerably influenced by the elution conditions in each round of screening. Therefore, we reduced the phage number in the last wash (Wash) and regarded the ratio of "Wash" to output as false positive rate.

By the tests of affinity screening, binding, competitive inhibition and DNA sequencing, we isolated the phage clones which competitively inhibited the binding of the mAb M6 to HGV E2. Since most of the clones share the core sequence of NPLWP, we consider that NPLWP may be a motif of HGV E2 because the clone C2 with this sequence is able to competitively inhibit the binding of mAb M6 to HGV E2 and to induce immune-response in mice. Our results suggest that the sequence NPLWP with potential antigenicity and immunogenicity should be further studied for its use in the development of HGV diagnostic reagent, vaccme and peptide drug.

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