Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) antibodies detected by peptide microarrays

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Introduction

- ✓ The identification of HBV and HIV epitopes is important for the development of novel diagnostics and vaccines
- ✓ In this study, we have developed HBV and HIVenv chips with overlapping oligopeptides encompassing the full amino acid sequences of different HBV and HIV polypeptides. In addition, a random peptide library composed of 4608 15-mers was prepared.
- ✓ The chips were used for analyzing monoclonal antibodies and sera from HIV and HBV infected individuals.

Results

1. Targets of monoclonal antibodies

aa 733 of HIV gp160
I
#244: PEGIEEEGGERDRDR
#245: IEEEGGERDRDRSIR

aa 475 of HIV gp160
I
#157: RPGGGDMRDNWRSEL
#158: GGDMRDNWRSELYKY
#159: MRDNWRSELYKYKVV

aa 376 of HIV gp160
I
#123: GDPEIVTHSFNCGGE
#124: EIVTHSFNCGGEFFY
#125: THSFNCGGEFFYCNS
#126: FNCGGEFFYCNSTQL

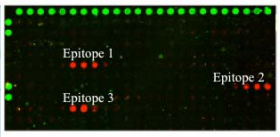
aa 736 of HIV gp160
I
#242: PRGPDRPEGIEEEGG
#243: PDRPEGIEEEGGERD
#244: PEGIEEEGGERDRDR
#245: IEEEGGERDRDRSIR
#246: EGGERDRDRSIRLVN

Figure 1: Target sequences of four monoclonal antibodies to HIVgp160 comprise 12, 9, 6, or 3 amino acids.

2. Analysis of sera from patients recovering from HBV infection and of human sera neutralizing HIV-1

Sequence recognized	Position
NSNNPD	aa 37-43 of preS1, genotype A
LGFFPD PLGFFP	aa 22-27 of preS1, genotype A aa 10-16 of preS1, genotype D
PHGGVLGWSPQA PPHGGLLGW	aa 69-84 of preS1, genotype A aa 58-66 of preS1, genotype D
DPKVRGLYF RVRGLY	aa 13-21 of preS2, genotype A aa 15-20 of preS2, genotype D
PISSIFSRIGD	aa 39-49 of preS2, genotype D
PYKMDIDPY	aa 7-15 of HBcAg, genotype A
SVRDLLDNASALYRE PSVRDLLDTASALYR	aa 26-40 of HBcAg,genotype D aa 25-39 of HBcAg,genotype A

Table 1: Epitopes detected by 5 sera of patients recovering from HBV infection.



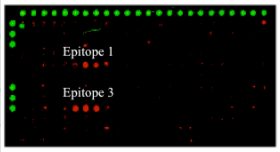


Figure 2: Three epitopes detected with two HIV-1 neutralizing human sera.

3. Screening the random peptide library with monoclonal antibody MA18/7

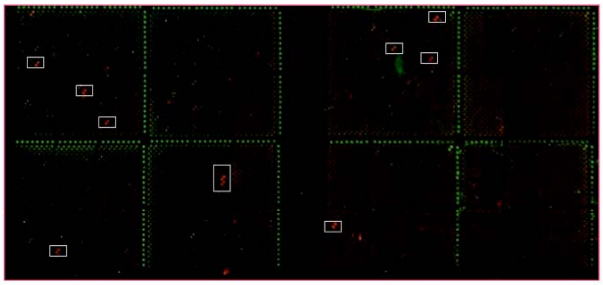


Figure 3: MA18/7 detected 10 strong responders (white squares) and 7 weak responders.

Q31B08	L	Q	L	Q	G	Y	M	M	H	R	V	E	W	C	Y
Q38G10	T	Y	W	D	R	G	F	Q	G	W	Y	G	M	I	N
Q17D08	V	D	Q	A	F	K	T	F	Q	A	E	R	K	H	T
Q13D02	G	R	V	A	L	Y	T	E	P	G	F	R	V	Q	Y
Q45D11	V	M	P	Y	P	E	T	E	P	A	F	L	D	K	C
Q31B07	C	W	F	N	C	M	K	M	D	P	G	F	K	T	I
Q24G04	T	M	Q	H	D	K	C	W	Q	Y	W	F	L	C	S
Q45F01	S	A	Y	Q	I	F	M	E	M	W	S	D	K	A	F
Q42C04	M	A	S	E	F	T	Q	A	L	D	A	A	F	F	K
Q44H09	S	N	F	P	D	A	A	F	N	N	Q	E	G	I	D

Table 2: Sequence of the ten strongly responding peptides. Common motif highlighted in yellow.

D (7)	P (3)	A (5)	F (8)
E (3)	A (2)	G (3)	Y
	K (2)	C (2)	W
	R		
	Q		
	W		

Consensus: DPAF

Table 3: The profile

aa 31 of the preS1 of HB surface antigen
I
#08: LGFFPDHQLDPAFGA
#09: FPDHQLDPAFGANSN
#10: HQLDPAFGANSNNPD
#11: DPAFGANSNNPDWDF

Figure 4: Verification of the target sequence of mAb MA18/7 using HBV scanning chip.

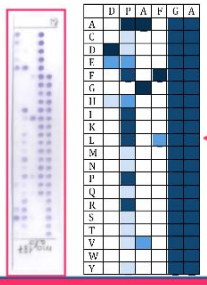


Figure 5: Single residue substitution analysis revealing the range of residues permissible at each motif position.

Conclusions

- ✓ HBV and HIVenv chips are powerful tools to identify and map humoral immune responses against HBV and HIV.
- ✓ The random peptide library has proven potential for identifying B-cell epitopes without prior knowledge of immunizing antigen.
- ✓ Using the random peptide library, studies have been initiated to identify discontinuous epitopes.

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