Article

Immune Responses to Six Synthetic Peptides of Capsid Protein with Sera from HIV-1 Infected Individuals

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Many B cell epitopes within p24 of human immunodeficiency virus type 1 (HIV-1) were identified, while most of them were determined by using murine monoclonal antibodies reacting with overlapping peptides of p24. Therefore these epitopes may not represent the actual epitopes recognized by the HIV-1 infected individuals. In the present study, immune responses of 67 HIV-1 positive sera from Yunnan Province, China to five peptides on p24 of HIV-1 and one of HIV-2 were analyzed. All of 67 sera did not recognize peptide GA-12 on HIV-1 and peptide AG-23 on HIV-2, which indicated that GA-12 was not human B cell epitope and AG-23 did not cross-react with HIV-1 positive serum. Except 13 sera (19.4%), all remaining sera did not recognize peptides NI-15, DR-16, DC-22 and PS-18, which indicated that these four peptides represented B cell linear epitopes of HIV-1 p24 in some HIV-1 infected individuals but not the immuno-dominant epitopes in most individuals. *Cellular & Molecular Immunology*. 2005;2(4):289-293.

Key Words: HIV-1, human serum, immune response, p24, synthetic peptide

Introduction

The capsid protein (CA) p24 is one of the main structural proteins of human immunodeficiency virus type 1 (HIV-1). There are about 1,500 p24 molecules composing virus capsid in a mature virion (1, 2). The p24 is the most abundant protein produced during virus replication. It not only induces an early and strong immune response but also is the earliest marker of HIV-1 infection. Additionally, p24 is one of the most conserved viral proteins and shows very little antigenic variations among the strains of HIV-1 isolated. These attributes contribute to marker of infection and potential candidate of vaccine. So it is significant to identify B cell and T cell epitopes on p24 for guiding the development of HIV-1 detecting reagents and vaccines.

Many B cell epitopes in p24 have been identified (3). However, most of them were determined by using murine anti-p24 monoclonal antibodies (mAbs) which reacted with overlapping peptides covering the entire CA sequences of HIV-1 (4-10). Sera from immunized mice (11), rabbits (7, 12) or sheep (7) were used in some studies, but human antibodies were used in few reports. Langedijk and colleagues studied the epitopes on HIV-1 p24 using mAbs of mouse and sera from a broad range of species, including mouse, rabbit, sheep and human (7). Their results indicated that the epitope spectra of HIV-1 p24 were obviously different not only between individuals of the same species but also between species. The epitopes identified by other mammals may not represent the actual epitopes recognized by HIV-1 infected human beings. It is practically useful to identify these actual epitopes. One of the methods is using positive sera from HIV-1 infected individuals to react with peptides of p24 rather than murine mAbs or other mammalian sera against p24.

Several peptide studies were showed rather strong antigenicity. Rabbit serum immunized with GQMREPRGSDIA (GA-12 in this study) linked resin can react with p24 of HIV-1 by Western blot (13). GA-12 coupled to bovine serum albumin can react with 26 of 31 HIV-1 positive sera from Australia (14). Murine mAb EB1A9 can recognize peptide

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Received Aug 9, 2005. Accepted Aug 20, 2005.

Abbreviations: HIV, human immunodeficiency virus; CA, capsid protein; mAb, monoclonal antibody; rp24, recombinant HIV-1 p24; ELISA, enzyme linked immunosorbent assay; OD, optical density.

Table 1. Peptides and their sequences

Strain	Peptide	Sequence	Position in Gag
HIV-1 (HXB2)	GA-12	GQMREPRGSDIA (C)*	226-237
	NI-15	NPPIPVGEIYKRWII (C)	253-267
	DR-16	DIRQGPKEPFRDYVDR (C)	284-299
	DC-22	DCKTILKALGPAATLEEMMTAC	329-350
	PS-18	PGHKARVLAEAMSQVTNS (C)	356-373
HIV-2 (ROD)	AG-23	AEWDVQHPIPGPLPAGQLREPR G (C)	211-233

* C in parentheses was cysteine residue added by synthesis.

NPPIPVGEIYKRWII (NI-15) as an epitope of p24 (4). PGHKARVL and AEAMS which lie in PS-18 were two epitopes of p24 and p2 respectively (5). According to these results and other references, we synthesized six peptides of HIV-1/2 CA and investigated the immune responses of 67 HIV-1 positive human sera with them, and attempted to determine whether or not these peptides were human immuno-dominant epitopes of CA and to evaluate their diagnostic usefulness.

Materials and Methods

HIV-1 positive human sera

The positive sera were collected during 1996 from 67 HIV-1 infected individuals in Lincang, Gengma, Zhenkang, Fengqing, Yunxian and Jinggu of Yunnan Province, China. All sera were HIV-1 positive demonstrated by ELISA and Western blot. Triton X-100 was added to a final concentration of 0.5% to inactivate virus before test.

Peptides

Five peptides within HIV-1 Gag p24 (4-6, 13, 16) and one within HIV-2 Gag was synthesized according to Merrifield's solid-phase synthesis method by Shanghai Institute of Organic Chemistry, Chinese Academic of Sciences (Table 1). A cysteine residue was added to each peptide that was not terminated with cysteine residue (the C in parentheses).

HIV-1 lysate and recombinant HIV-1 p24

After 72 h culturing, the H9/HIV-1_{IIIB} supernatants were collected and concentrated by PEG. The viral pellets were lysed in PBS supplemented with 0.5% Triton X-100. Recombinant HIV-1 p24 (rp24) was produced as GST-p24 fusion protein in *Escherichia coli* transformed with pGEX-6p-3/p24 subtype B. According to manufacture's instruction, the fusion protein was purified by Glutathione Sepharose 4B (Amersham Pharmacia) affinity purification, and then p24 was cleaved from the fusion protein by PreScission protease (Amersham Pharmacia).

Enzyme-linked immunosorbent assay (ELISA)

Table 2. Reactivity of human sera to HIV-1_{IIIB} lysate, rp24 and peptides

Strain	Coating antigen	Positive sera (OD value)	Number of positive	Reactive frequency (%)
HIV-1 (HXB2)	GA-12	None	0	0.0
	NI-15	$22^{\#}$ (0.264)	2	3.0
		$61^{\#}$ (0.247)		
	DR-16	$13^{\#}$ (0.328)[a]	4	6.0
		$21^{\#}$ (0.328)		
		$39^{\#}$ (0.328)		
		$46^{\#}$ (0.303)		
	DC-22	$2^{\#}$ (0.203)	8	11.9
		$9^{\#}$ (0.238)		
		$11^{\#}$ (0.337)		
		$18^{\#}$ (1.168)		
		$21^{\#}$ (0.209)		
		$39^{\#}$ (0.338)		
		$56^{\#}$ (0.249)		
		$60^{\#}$ (0.204)		
	PS-18	$11^{\#}$ (0.203)	3	4.5
		$13^{\#}$ (0.219)		
		$54^{\#}$ (0.403)		
HIV-2 (ROD)	AG-23	None	0	0
HIV-1_{IIIB}	rp24	All	67	100
	Virus lysate	All	67	100

Responses of antibodies to lysate, rp24 and peptides were detected by ELISA as previously described (17). Briefly, peptide (5 μg/ml), rp24 (1 μg/ml) and HIV-1 lysate (1:400) dissolved in 0.05 M sodium carbonate/bicarbonate buffer (pH 9.6) were used to coat a 96-well plate, respectively. The plates were blocked with 5% milk, 0.02% NaN_3 in PBS-T (PBS-TMN). Fifty-fold dilution of positive sera samples in PBS-TMN were added into each coated wells and uncoated wells (as the background) and incubated for 2 h at 37°C. Then HRP conjugated goat anti-human IgG, substrate o-Phenylenediamine were added sequentially. The reaction was stopped by 2 M H_2SO_4. The wells coated with rp24 and HIV-1 lysate were used as positive control and uncoated wells as background. The corresponding background optical density (OD) values were subtracted from OD values of each sample. The cut-off absorbance was designated as 0.2.

Results

Antibody responses of 67 human sera to peptides and proteins were carried out by ELISA. As shown in Figure 1 and Table 2, all sera strongly reacted with both HIV-1 lysate and rp24. No serum recognized and reacted with both peptides GA-12

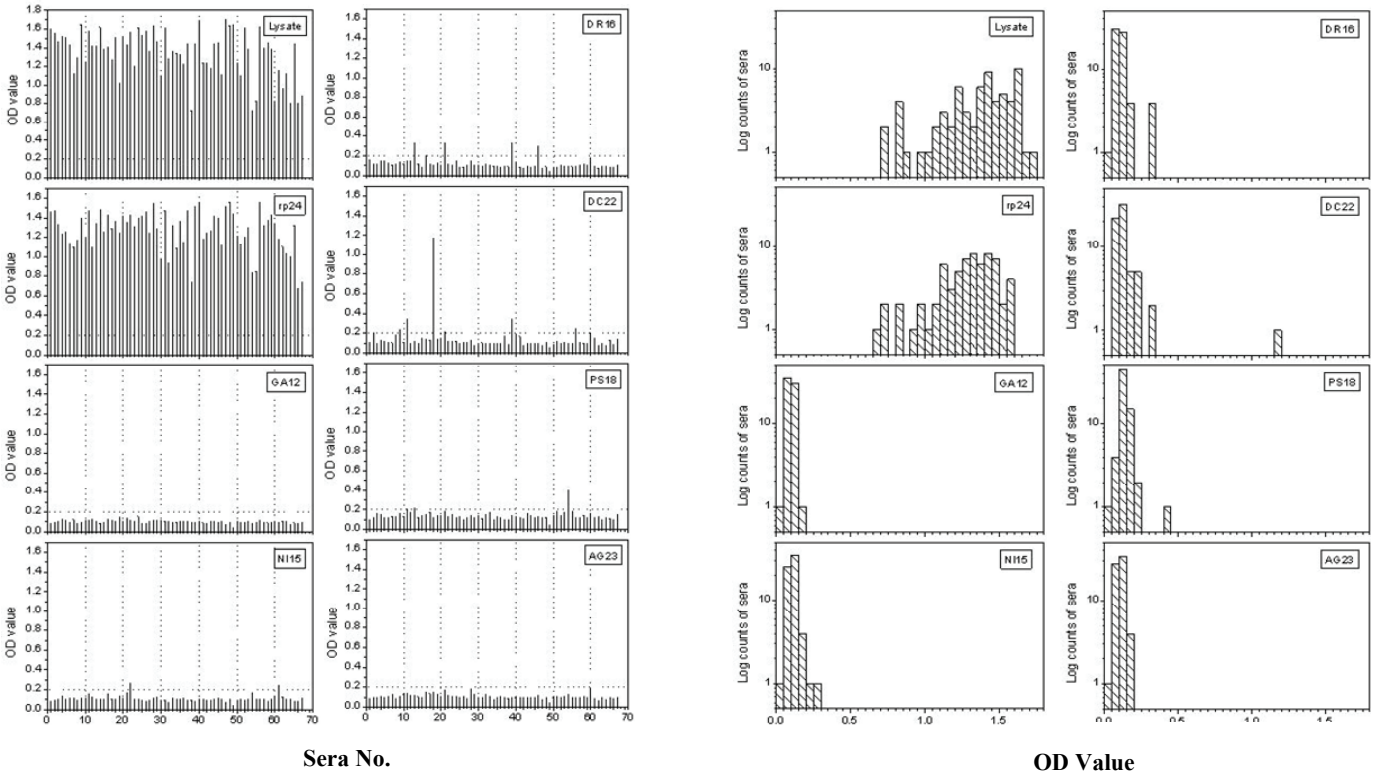

Figure 1. Left: reactivity of 67 human sera from HIV-1 infected individuals to rp24, HIV-1_{IIIB} lysate and six peptides derived from HIV-1_{HXB2} Gag (GA-12, NI-15, DR-16, DC-22 and PS-18) and from HIV-2_{ROD} Gag (AG-23), dot line indicates cut-off. Right: histogram for response of 67 human sera to rp24, HIV-1_{IIIB} lysate and peptides.

(HIV-1) and AG-23 (HIV-2). The result indicates that GQMREPRGSDIA (GA-12) is not the B cell epitope on p24. The HIV-1 positive sera did not serologically cross-react with the sequence AEWDVQHPIPGPLPAGQLREPRG (AG-23) on HIV-2 Gag.

There were 13 sera (19.4%) among 67 sera tested recognizing and reacting with peptides NI-15, DR-16, DC-22 and PS-18. The others did not react to these four peptides. The reactivity frequencies of peptides NI-15, DR-16 and PS-18 with sera were 3%, 6% and 4.5% respectively. The reactivity frequency of peptide DC-22 was rather high, up to 12%. Among these 13 peptide-positive sera, No.21 and 39 sera can react to peptides DR-16 and DC-22, No.11 upon DC-22 and PS-18, No.13 on DR-16 and PS-18 simultaneously, while the others can only recognize one of the four peptides. Weak immune responses of most peptide-positive sera to the peptides were observed. OD values of 9 sera (69%) were between 0.20-0.30 and 6 sera (46%) between 0.30-0.40. Only 2 sera (15%) were above 0.4. No.54 serum moderately reacted with PS-18 (OD 0.403), and No.18 serum strongly reacted to DC-22 (OD 1.168). These results indicated that these four peptides represented as B cell linear epitopes of HIV-1 p24 in some HIV-infected individuals but not the immuno-dominant epitopes in most individuals. All 13 peptide-positive sera were randomly distributed geographically.

Discussion

In this study, six peptides derived from HIV-1/2 CA were synthesized according to available results and serological reactivities with Chinese HIV-1 positive sera were studied to determine whether or not these peptides were epitopes of p24 recognized by human B cells. Our results indicated that these five peptides were not the immuno-dominant B-cell epitopes. The peptides may lie in the interior of p24 tertiary structure and could not be recognized by B cells. It also suggests that those peptides were useless in p24 antibodies diagnosis. At least, that is not applicable to HIV-1 infected individuals in China.

To identify the epitopes of p24 is very significant for making specific and sensitive diagnostic reagents of detecting p24. We had prepared the murine mAbs against NI-15 and DC-22 and goat sera against DR-16, PS-18, GA-12 and AG-23 previously (17). The different combinations of these antibodies in the sandwich ELISA were failed to detect p24 antigen in culture supernatant. It could be explained by the results in this study.

Many B-cell epitopes of p24 has been identified (3). The five HIV-1 peptides used were studied previously by using

murine mAbs or sera, and identified as epitopes or overlapped with a epitope (3, 18). Except three murine mAbs reactive to sequence overlapping with GA-12 (7), there were no other reports to identify the sequence of GA-12 as an epitope. The sequence was just within or overlapping some epitopes determined. NI-15 (NPPIPVGEIYKRWII) was identified by murine mAbs EB1A9 as an epitope of p24 (4, 15). DR-16 (DIRQGPKEPFRDYVDR) overlaps a highly conserved epitope of p24 among the strains of HIV-1. Furthermore, QGPKEPFRDYVDRFY of HIV-1 is similar to simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV) (8). Truong et al. (11) identified four epitopes in Balb/c immunized rp24 and p55 respectively. SP27 (aa 285-304), which overlapped with DR-16 (aa 284-299), can be recognized by sera against rp24 and p55 simultaneously. The amino acid sequence (KTILKALGPAA TLEEMMTACQGVG) overlapping DC-22 was determined as an epitope by murine mAbs (19). There was one mAb recognized an epitope within DC-22 (7). Sequence of PS-18 overlapped the last eight amino acids of p24 through the first ten amino acids of p2. Its p24 portion (PGHKARVL) and p2 portion (AEAMS) were two epitopes of p24 and p2 respectively (5).

A few studies had been done using human sera. But it seems that there are more contradictions than similarities between previous results and ours. Langedijk and colleagues used seven murine mAbs and sera from two rabbits, two sheep and twelve human individuals to react with synthetic nonapeptide coupled to the solid supports. Four patient sera (33%) reacted with the sequence within GA-12, four sera (33%) with NI-15, five sera (42%) with DR-16, two sera (17%) with DC-22 and none with PS-18 (7). The positive frequencies of sera with GA-12, NI-15 and DR-16, DC-22 were higher than ours (0%, 3%, 6% and 12% respectively).

In this study, GA-12 did not react with any of 67 sera. Rabbit serum immunized with GA-12 linked resin can react with p24 of HIV-1 by Western blot (13). GA-12 coupled to bovine serum albumin can react with 26 of 31 HIV-1 positive sera from Australia (14).

Previous study (16) showed that only one peptide (LQEQIGWMTNNPPIP) reacted with all positive sera and displayed highest reactivity, while the others did not. These non-reactive peptides included five peptides: No.13 peptide was identical to NI-15; No.10 (aa 223-242), No.16 (aa 283-302), No.21 (aa 333-347) and No.22 (aa 253-263) were overlapping with GA-12, DR-16, DC-22 and PS-18 respectively.

The inconsistent results may result from several factors. Firstly, the use of peptides with different lengths and sequences leads to different specificity and sensitivity. Studies in which overlapping peptides were used indicated that two peptides with even only one amino residual shift could show significant reactivity with antibody. The use of short synthetic peptides may reduce sensitivity. Secondly, the methods of immobilizing peptides to solid phase are one of the key factors affecting the serological reactivity of peptides. The antibodies could react better with resin- or BSA-linked peptides than free peptides (13, 14).

Acknowledgements

We greatly appreciate recombinant p24 expression *E. coli* kindly provided by Dr. Kazuyoshi Ikuta of Osaka University, Japan. The work was supported by grants from the National Natural Science Foundation of China (39500137), the Natural Science Foundation of Yunnan (95C0099Q), Key Scientific and Technological projects of China (2004BA719A14) and Yunnan (2004NG12), CAS Projects (STZ-01-17; KSCX2-SW-216; KSCX1-SW-11), and National 863 Program (2003AA219142).

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